Endocrine Profiling and Prioritization of Environmental Chemicals Using ToxCast™

David Reif[1], Matt Martin[1], Shirlee Tan[2], Keith Houck[1], Richard Judson[1], Ann Richard[1], Thomas Knudsen[1], David Dix[1], Robert Kavlock[1]

[1]National Center for Computational Toxicology
Office of Research and Development

[2]Office of Science Coordination and Policy
Office of Pollution Prevention, Pesticides and Toxic Substances

Presentation outline

I. Prioritization framework
   i. Rationale for integrated prioritization scheme
   ii. Definitions and notation
   iii. Interpreting ToxScores for individual chemicals

II. Implementation for the task of endocrine prioritization
   i. EDSP prioritization
   ii. Developing a prioritization scheme for EDCs
   iii. Data sources
   iv. Results: EDSP chemicals of interest in ToxCast Phase-I
   v. Results: empirical distribution of EDSP chemicals of interest
   vi. Results: guidepost ("spike-in") chemicals
   vii. Results: exploring ToxScores in the context of in vivo results
   viii. Results: rank by specific slices (e.g. AR) in the context of in vivo results
   ix. Results: chemical classes
   x. Results: simulation studies assess sensitivity to spurious assay results

III. Future directions
   i. Alternative implementations
   ii. Incorporation of new/other data (e.g. QSAR models and other extant tools)

IV. Conclusions

Rationale for an integrated chemical prioritization scheme

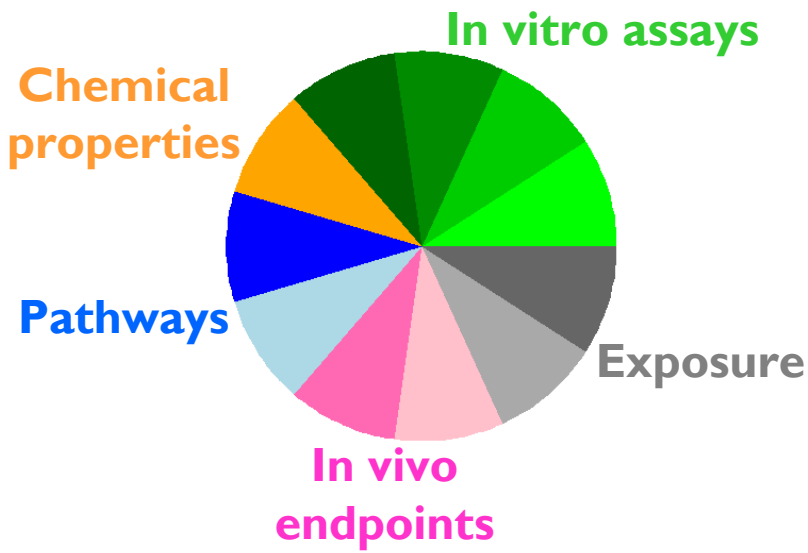
What do we know?

What are the sources of our knowledge?

Can we integrate information from disparate sources?

Does certain knowledge carry more importance?

Can we compare chemicals on an even playing field?



→ A numerical index that can be used for ranking (instead of absolute thresholds) is more flexible for different prioritization tasks and can better accommodate new data, new chemicals, data adjustments, etc.

Definitions & Notation

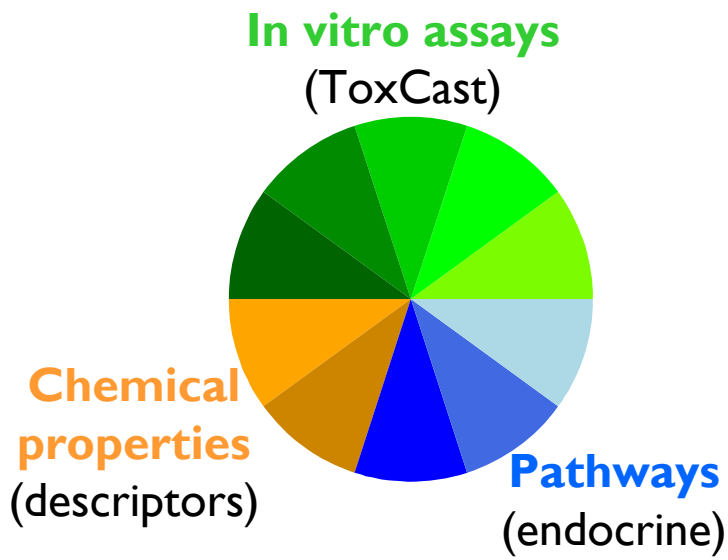
$$\text{ToxScore} = \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{P} w_p * \text{pathway}_p$$

Component: Individual in-vitro assays, chemical properties/descriptors, etc. (e.g. ER-binding assay from Novascreen)

Slice: "Pie" slices representing individual *components* or aggregrations of multiple related *components* (e.g. slice *i*=2 represents multiple in-vitro assays related to the estrogen receptor)

Domain/Axis: Domain/field of knowledge; represented by the slice(s) of a given color family (e.g. all chemical properties have slices in some shade of orange)

Example Sentence:
"For each chemical, the ToxScore™ integrates information across multiple *domains*, which are composed of one or more *slices*, which are composed of one or more *components*."

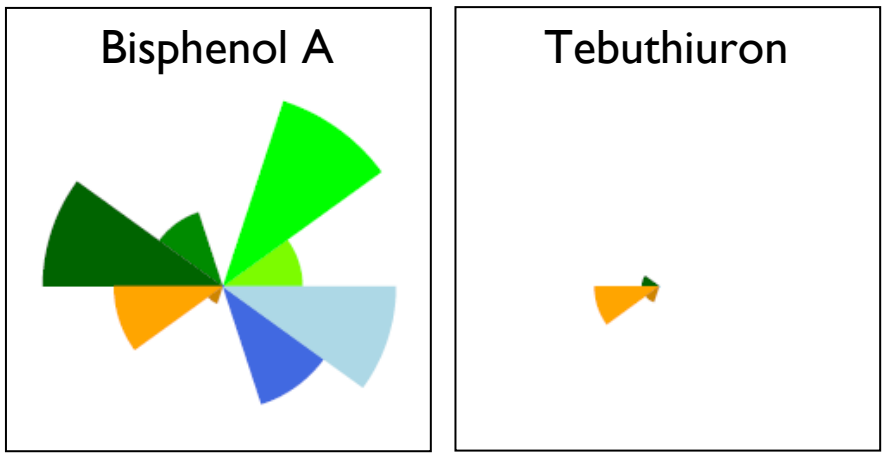


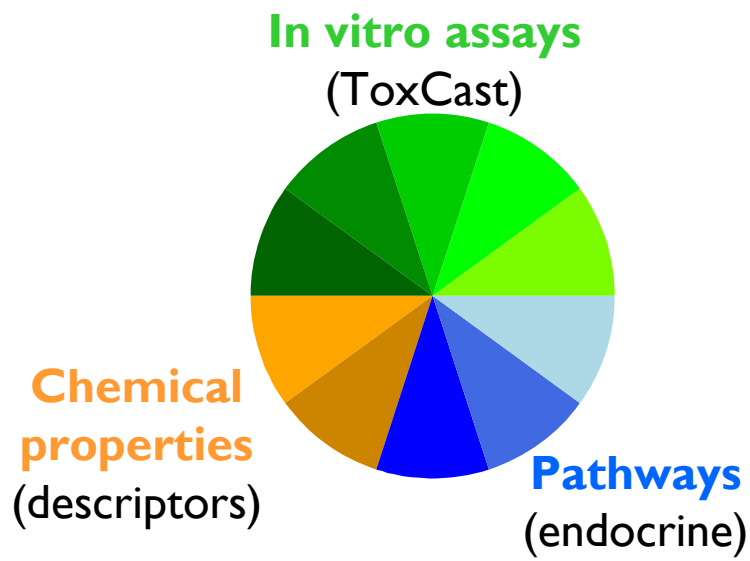
Interpreting ToxScores for individual chemicals

Each chemical profile/ signature/ fingerprint gives a score index (ToxScore) used for ranking chemicals

ToxScore = f(In vitro assays + Chemical properties + Pathways)

$$\text{ToxScore} = \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{P} w_p * \text{pathway}_p$$





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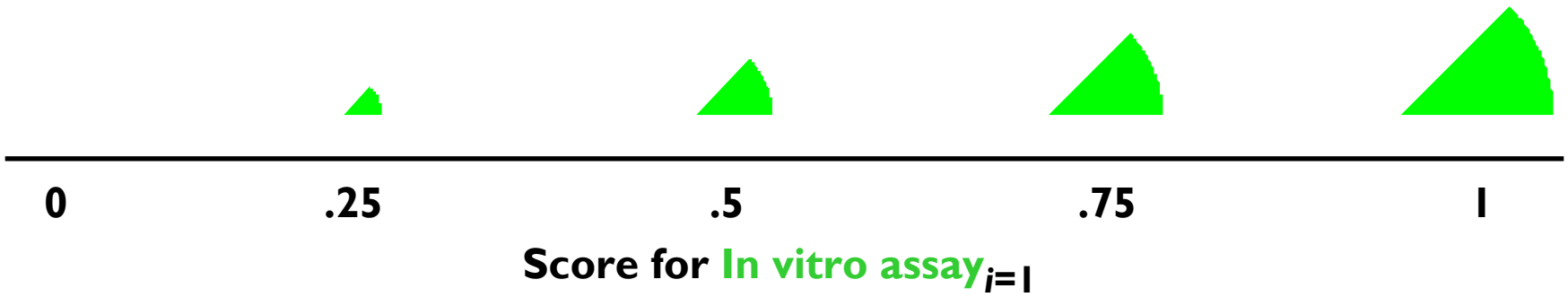

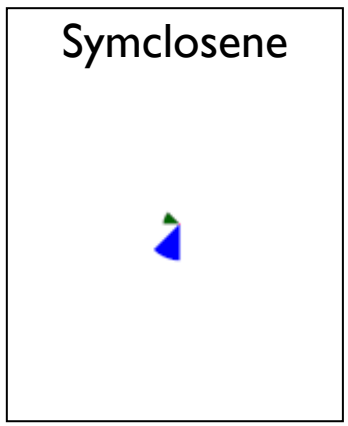

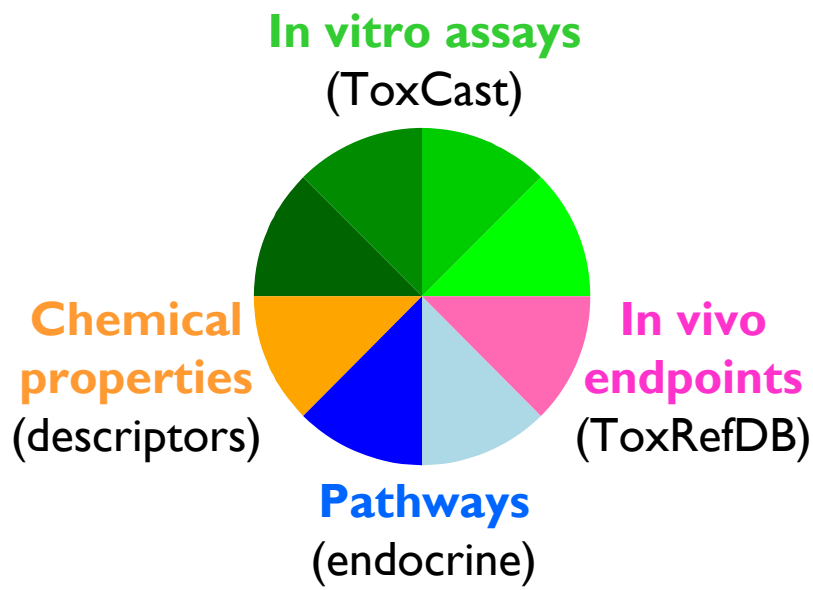

EDSP prioritization

United States Environmental Protection Agency

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Endocrine Disruptor Screening Program (EDSP)

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Chemical Selection Approach for Initial Tier 1 Screening

Highlights

Approach for Selecting the Initial List of Chemicals for Screening - Federal Register Notice [PDF file, 17pp., 125KB, About PDF]

Contact us for more information

Overview of the EDSP

The EDSP was established in response to a Congressional mandate in the Federal Food, Drug, and Cosmetic Act (FFDCA), to screen pesticides, chemicals and environmental contaminants for their potential to affect the estrogen, androgen or thyroid hormone systems. The core elements of the EDSP are:

- Assay development and validation for Tier 1 screening and Tier 2 testing;
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- Development of program policies and procedures to require testing.

For more information on endocrine disruptors and the core elements of the screening program see the "Endocrine Primer."

Priority Setting - Approach for Initial Screening

The approach used by EPA for selecting 50 to 100 chemicals for initial screening under the Federal Food, Drug and Cosmetic Act is summarized below. Nothing in the approach for selecting the initial list would provide a basis to infer that any of the chemicals selected for the list interferes with or is suspected to interfere with the endocrine systems of humans or other species. This action may be of interest to those who are involved with, or are interested in, pesticide chemicals or the topic of endocrine disruptors.

The approach includes consideration of the most current databases and priority-setting tools available. For this approach EPA:

1. Focused chemical selection for this initial list on the subset of chemicals for which testing is required (i.e., pesticide chemicals);
2. Used exposure data as the primary basis for chemical selection;
3. Deferred consideration of nominations from the public;
4. Excluded mixtures; and
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1. Substances anticipated to have low potential to cause endocrine disruption (e.g., most polymers with number average molecular weight greater than 1,000 daltons, strong mineral acids, and strong mineral bases);

2. "Positive control" chemicals used by EPA for the validation of the screening assays proposed for the Tier 1 battery.

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Subsequent Approaches for Chemical Selection

EPA anticipates that it may modify its chemical selection approach for subsequent screening lists based on experience gained from the results of testing of chemicals on the initial list, the need for a broader approach in the future to incorporate different categories of chemicals (e.g., non-pesticide substances) and additional pathways of exposure, and the availability of new priority-setting tools (e.g., High Throughput Pre-Screening (HTPS) or Quantitative Structure Activity Relationship (QSAR) models). In addition, the Agency intends to conduct a review of the data received from the screening to evaluate whether the program could be improved or optimized.

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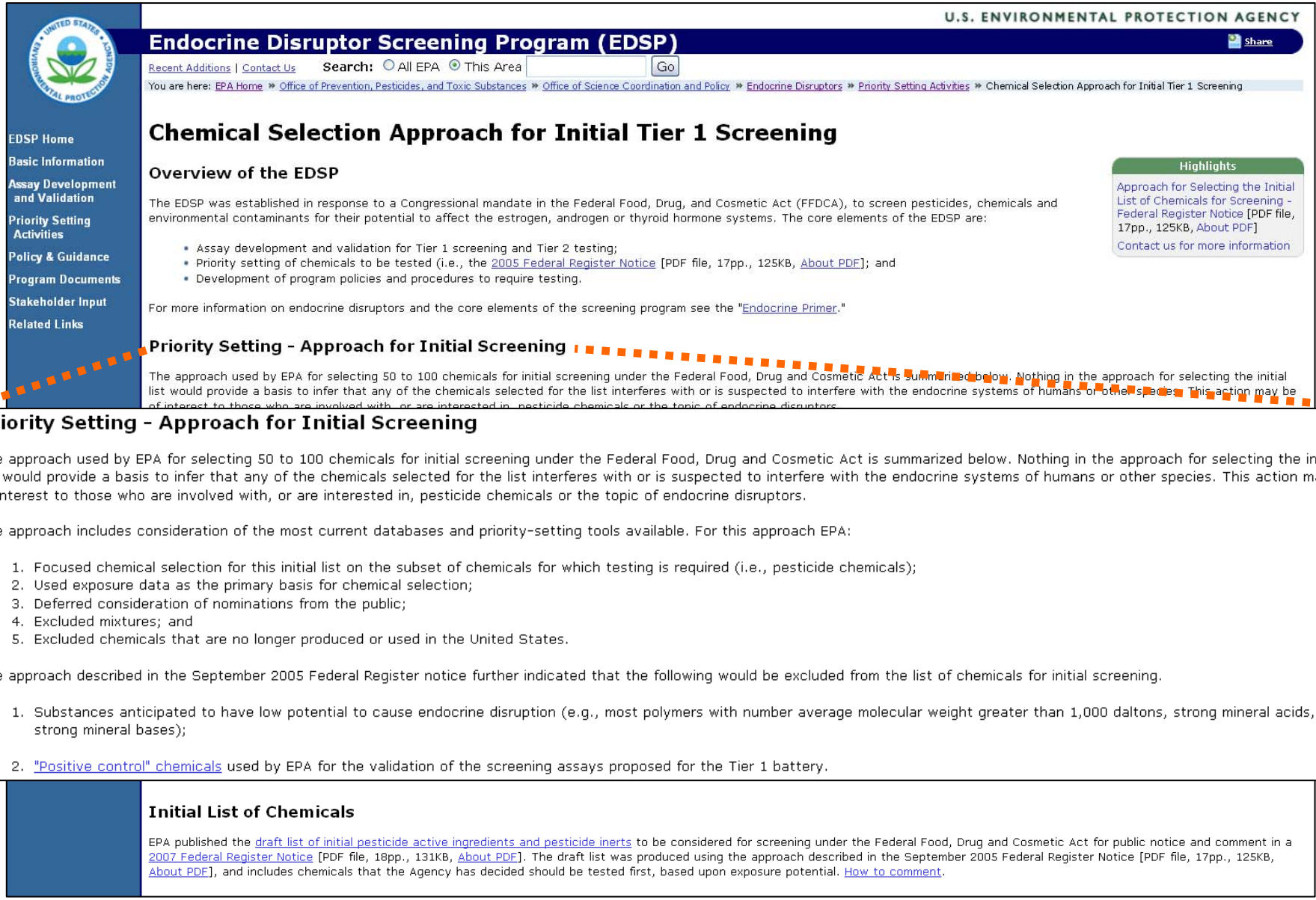
EDSP prioritization



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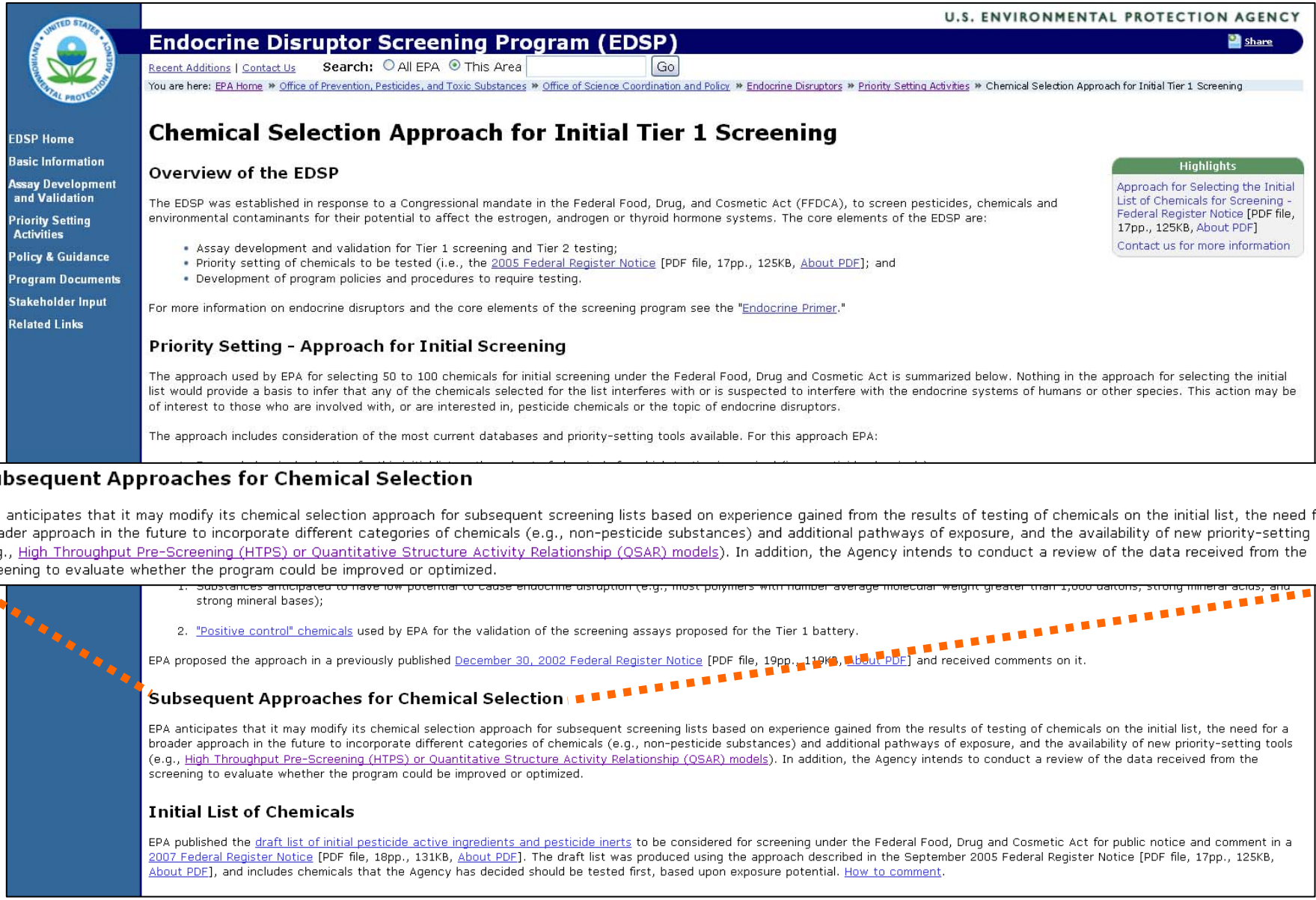

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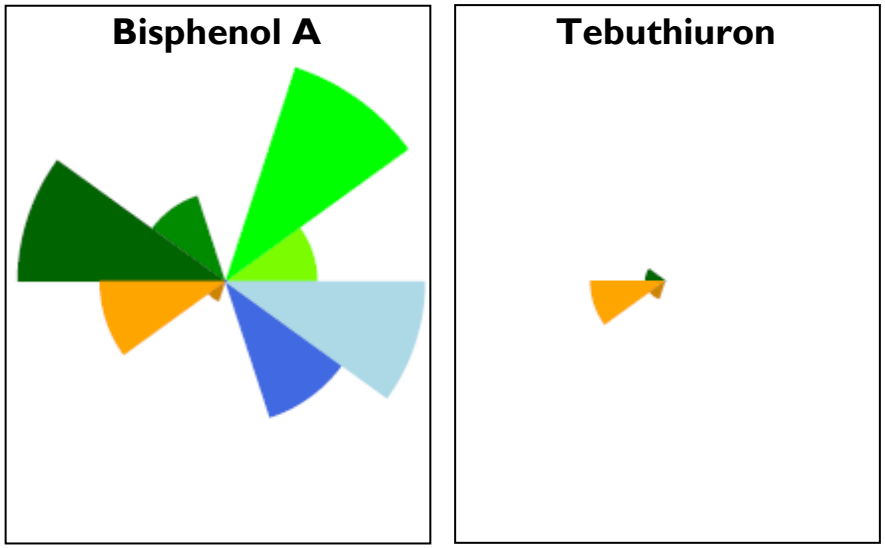
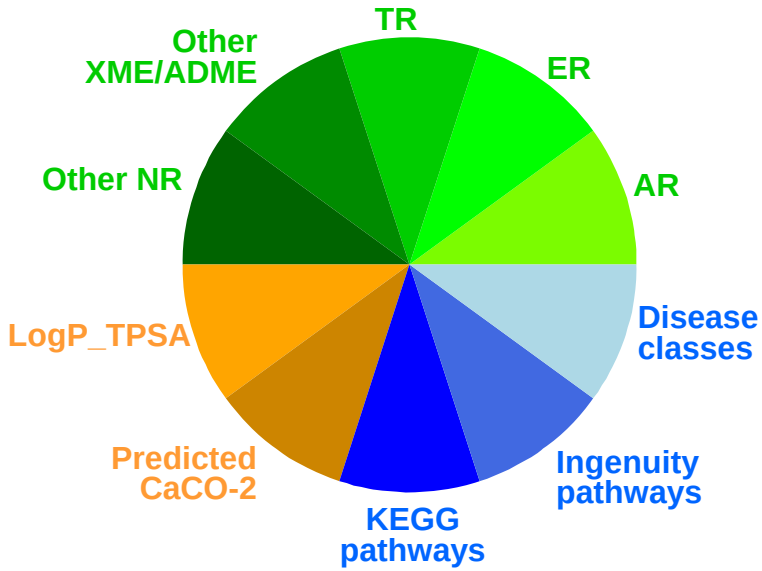
Developing a prioritization scheme for EDCs

ToxScore = f(In vitro assays + Chemical properties + Pathways)



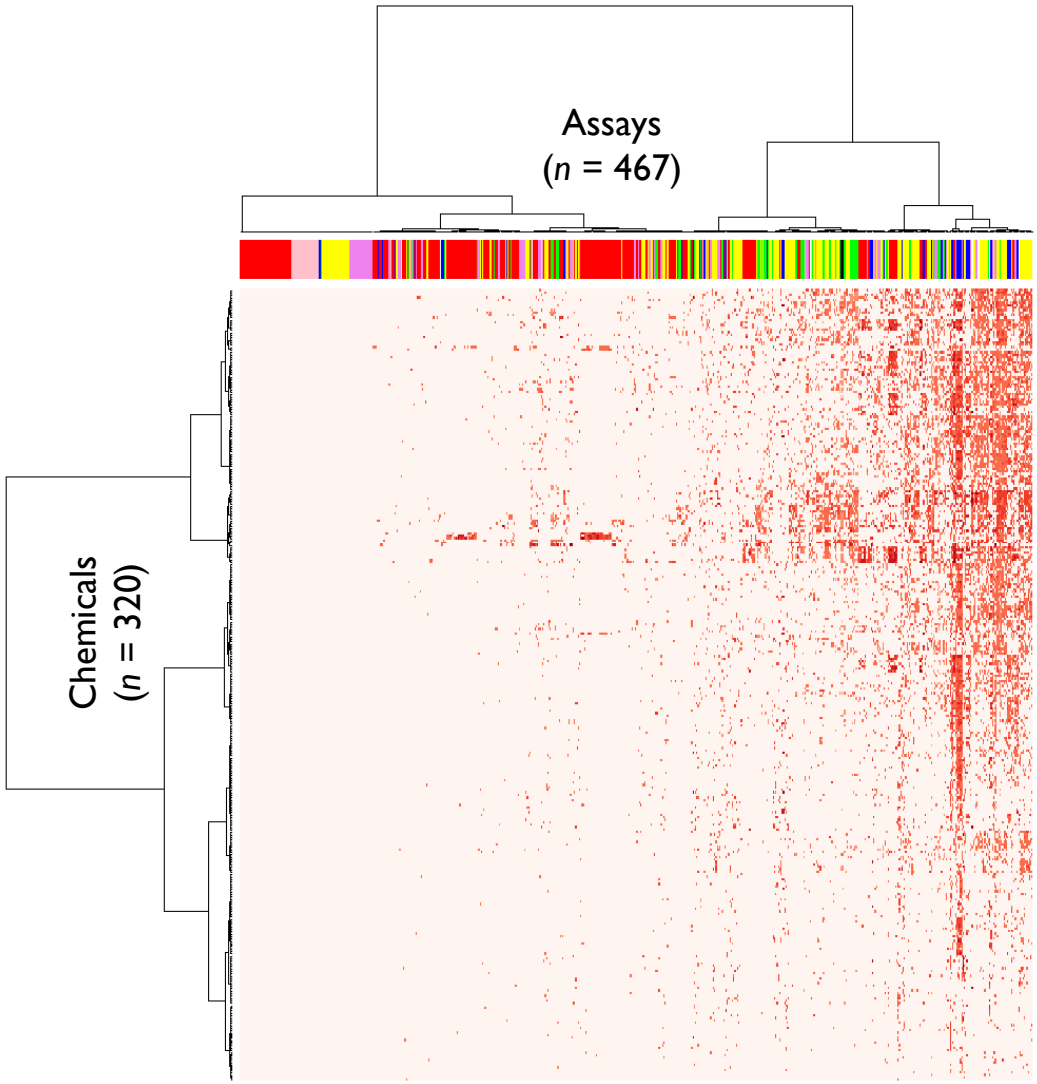


The ToxScore index is calculated from a weighted combination of all data sources for each chemical.

For each slice, distance from the origin (center) is proportional to the normalized value (e.g. assay potency or predicted permeability) of the component data points comprising that slice, and the width (in radians) indicates the relative weight of that slice in the overall ToxScore calculation.

The slices are drawn counter-clockwise from the right, so in this example, the AR slice is #1, the ER slice is #2, etc.

Data sources: ToxCast *in vitro* HTS assays



Cellular Assays

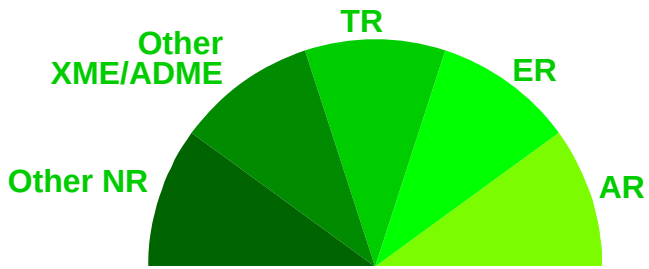
- **Cell lines**
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- **Primary cells**
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- **Biotransformation competent cells**
  - Primary rat hepatocytes
  - Primary human hepatocytes
- **Assay formats**
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Biochemical Assays

- **Protein families**
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- **Assay formats**
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

[Slide adapted from Keith Houck]

Data sources



IN VITRO ASSAYS

> AR

"ATG_AR_TRANS" "NCGC_AR_Agonist" "NVS_NR_rAR" "NVS_NR_hAR" "NCGC_AR_Antagonist"

> ER

"NVS_NR_hER" "NVS_NR_bER" "NCGC_ERalpha_Agonist" "ATG_ERa_TRANS" "ATG_ERE_CIS" "NCGC_ERalpha_Antagonist"

> TR

"NCGC_TRbeta_Agonist" "ATG_THRa1_TRANS" "NVS_NR_hTRa" "CLZD_UGT1A1_48" "NCGC_TRbeta_Antagonist"

> Other XME/ADME

"CLZD_CYP1A1_48" "CLZD_CYP1A2_48" "CLZD_CYP2B6_48" "CLZD_CYP3A4_48" "NVS_ADME_hCYP1A1" "NVS_ADME_hCYP1A2"
"NVS_ADME_hCYP1B1" "NVS_ADME_hCYP2A6" "NVS_ADME_hCYP2B6" "NVS_ADME_hCYP2C18" "NVS_ADME_hCYP2C19" "NVS_ADME_hCYP2C19_Activator"
"NVS_ADME_hCYP2C8" "NVS_ADME_hCYP2C9" "NVS_ADME_hCYP2D6" "NVS_ADME_hCYP2E1" "NVS_ADME_hCYP2J2" "NVS_ADME_hCYP3A4"
"NVS_ADME_hCYP3A5" "NVS_ADME_hCYP4F12" "NVS_ADME_hCYP4F12_Activator" "NVS_ADME_rCYP1A1" "NVS_ADME_rCYP1A2" "NVS_ADME_rCYP2A1"
"NVS_ADME_rCYP2A2" "NVS_ADME_rCYP2B1" "NVS_ADME_rCYP2C11" "NVS_ADME_rCYP2C12" "NVS_ADME_rCYP2C13" "NVS_ADME_rCYP2C6"
"NVS_ADME_rCYP2D1" "NVS_ADME_rCYP2D2" "NVS_ADME_rCYP2E1" "NVS_ADME_rCYP3A1" "NVS_ADME_rCYP3A2" "CLZD_SULT2A1_48"
"CLZD_HMGCS2_48“ "NVS_ADME_hCYP19A1"

> Other NR

"ATG_Ahr_CIS" "ATG_CAR_TRANS" "ATG_ERRa_TRANS" "ATG_ERRg_TRANS" "ATG_FXR_TRANS" "ATG_GR_TRANS" "ATG_GRE_CIS"
"ATG_LXRa_TRANS" "ATG_LXRb_TRANS" "ATG_PPARa_TRANS" "ATG_PPARd_TRANS" "ATG_PPARg_TRANS" "ATG_PXR_TRANS" "ATG_PXRE_CIS"
"ATG_RARa_TRANS" "ATG_RARb_TRANS" "ATG_RARg_TRANS" "ATG_RXRa_TRANS" "ATG_RXRb_TRANS" "NCGC_LXR_Agonist" "NCGC_PPARg_Agonist"
"NCGC_PXR_Agonist_human" "NCGC_PXR_Agonist_rat" "NCGC_RXRa_Agonist" "NVS_NR_bPR" "NVS_NR_hCAR" "NVS_NR_hCAR_Agonist" "NVS_NR_hFXR"
"NVS_NR_hGR" "NVS_NR_hPPARa" "NVS_NR_hPPARg" "NVS_NR_hPR" "NVS_NR_hPXR" "NVS_NR_hRAR"

For a complete description of all data sources and links to data, see:
Judson et al. (2009) *Environ Health Perspect*

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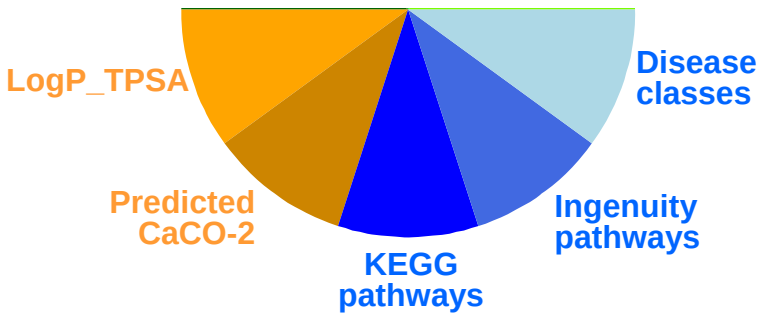
Data sources



CHEMICAL PROPERTIES

> LogP_TPSA

"LogP_TPSA"

> Predicted CaCO-2

"PCaco_QP"

PATHWAYS

> KEGG pathways

"PS_KEGG_Adipocytokine_signaling_pathway" "PS_KEGG_Androgen_and_estrogen_metabolism" "PS_KEGG_Androgen_and_estrogen_metabolism_Mus_musculus"
"PS_KEGG_Biosynthesis_of_steroids" "PS_KEGG_Biosynthesis_of_steroids_Mus_musculus" "PS_KEGG_GnRH_signaling_pathway"
"PS_KEGG_GnRH_signaling_pathway_Rattus_norvegicus" "PS_KEGG_Insulin_signaling_pathway" "PS_KEGG_Melanogenesis"
"PS_KEGG_Melanogenesis_Rattus_norvegicus" "PS_KEGG_PPAR_signaling_pathway" "PS_KEGG_Thyroid_cancer"

> Ingenuity pathways

"PS_Ingenuity_Aryl_Hydrocarbon_Receptor_Signaling" "PS_Ingenuity_Estrogen_Receptor_Signaling" "PS_Ingenuity_Glucocorticoid_Receptor_Signaling"
"PS_Ingenuity_Insulin_Receptor_Signaling" "PS_Ingenuity_PPARaRXRa_Activation" "PS_Ingenuity_PPAR_Signaling"
"PS_Ingenuity_RAR_Activation" "PS_Ingenuity_TRRXR_Activation"

> Disease classes

"PS_Disease_Goh_Endocrine" "PS_Disease_Goh_Developmental"

For a complete description of all data sources and links to data, see:
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EDSP chemicals of interest in ToxCast Phase-I

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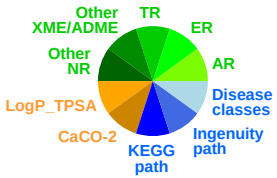
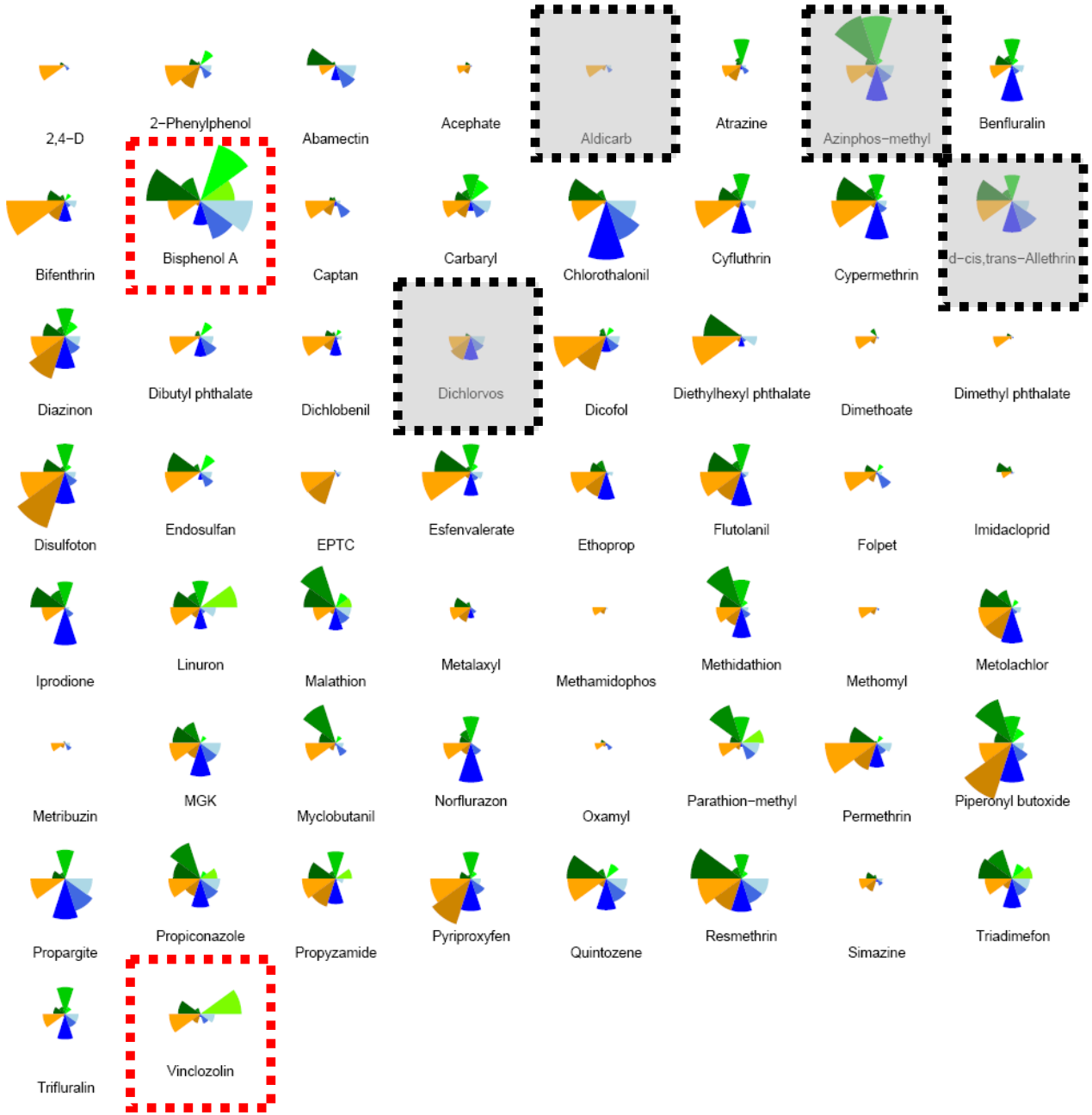


The chemicals are arranged in alphabetical order (by name)

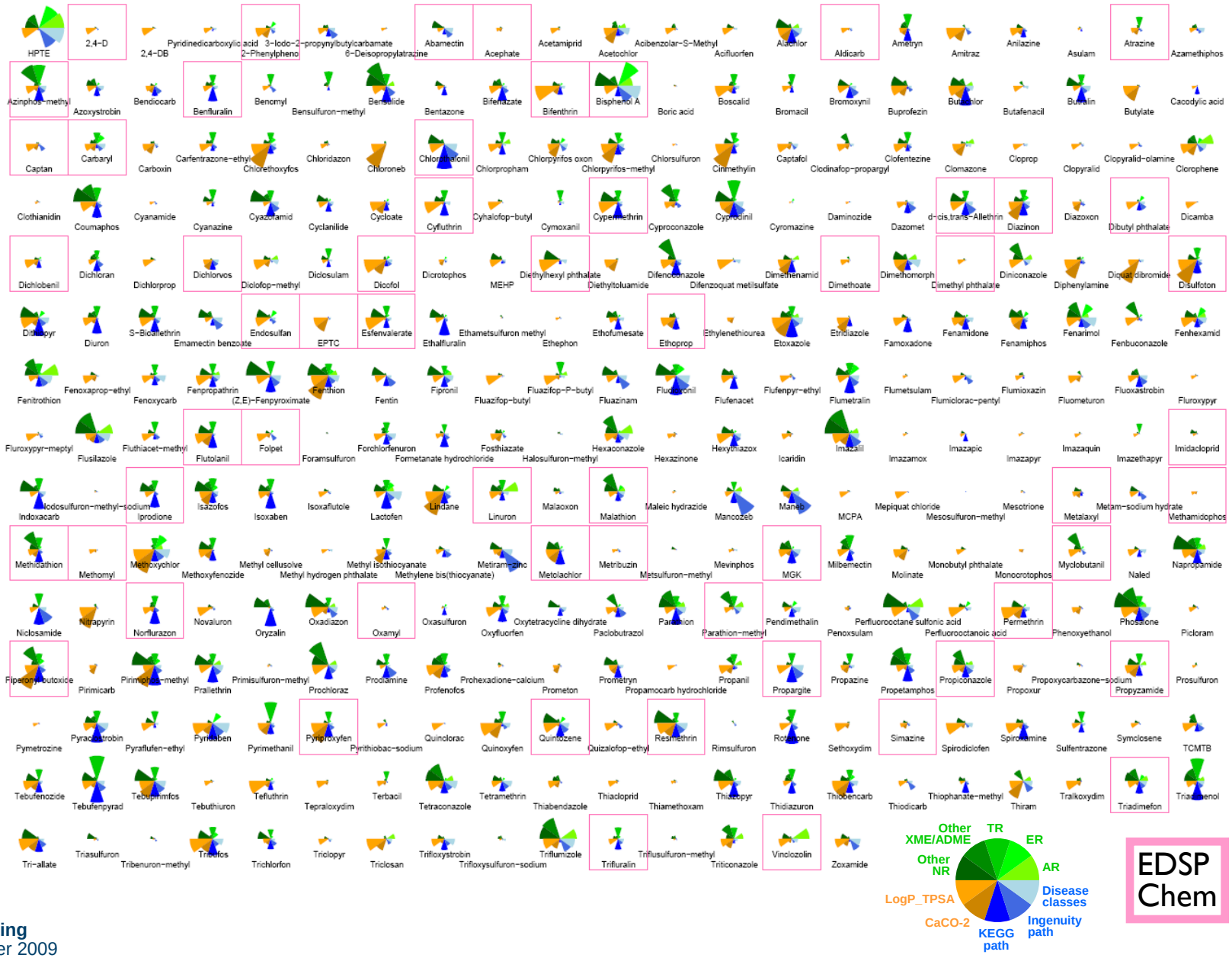
EDSP chemicals show a range of activities across the ToxScore components

Chemicals that have been dropped from the EDSP Tier-1 screening list

Of endocrine interest, but not in the official Tier-1 screening list



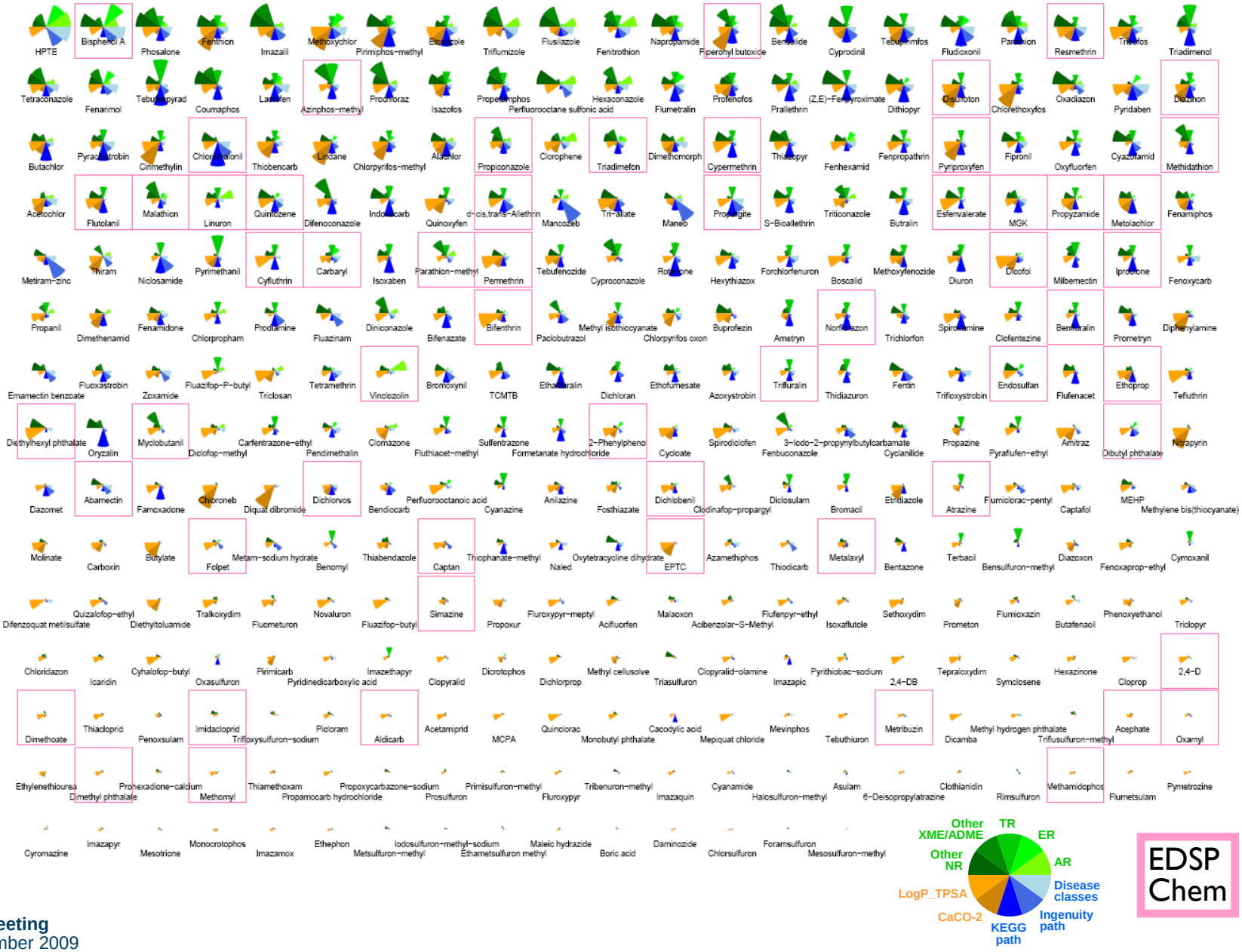
EDSP chemicals of interest in ToxCast Phase-I



Distribution of EDSP chemicals of interest

(sorted by ToxScore)

United States Environmental Protection Agency

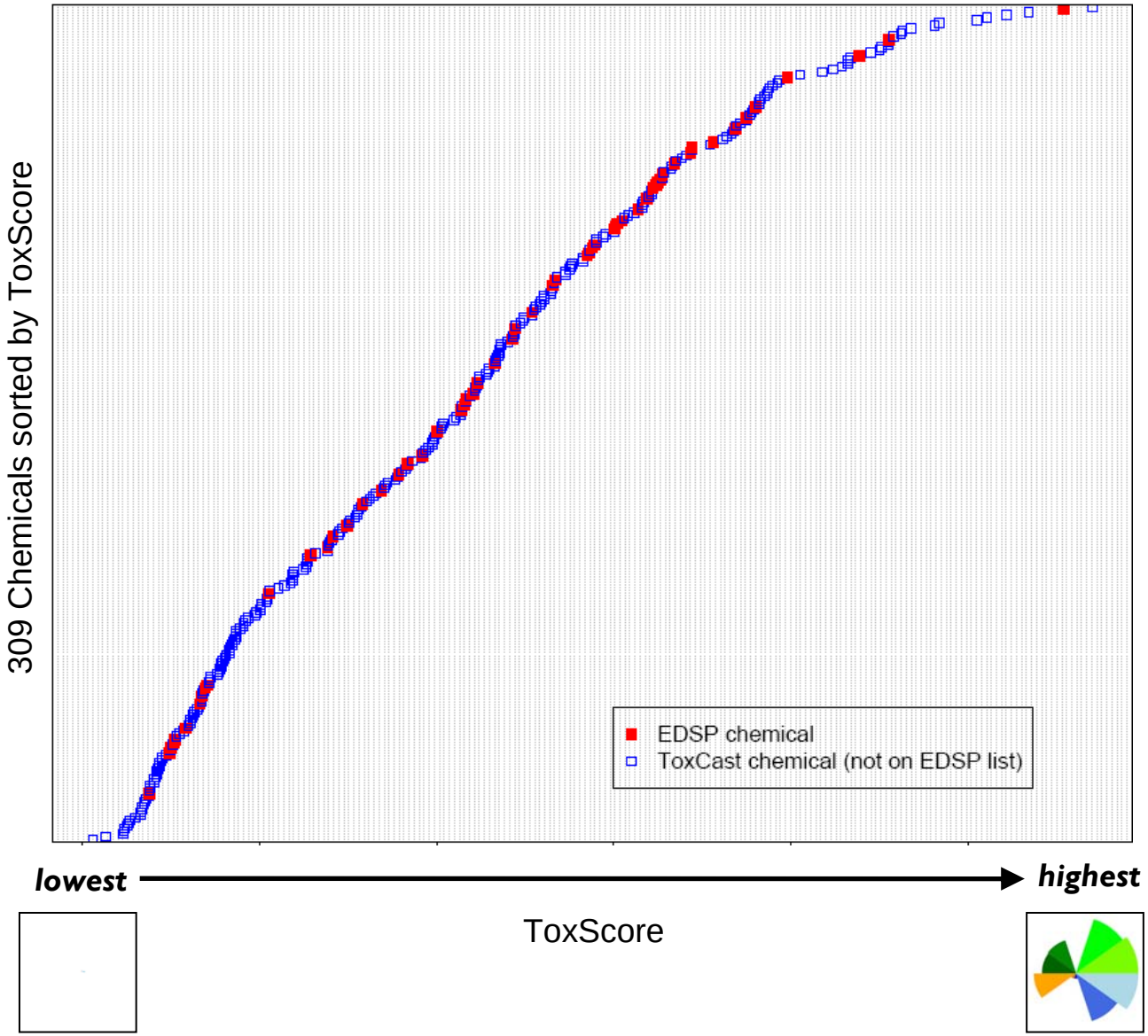


EDSP Chem

Distribution of EDSP chemicals of interest

(sorted by ToxScore)

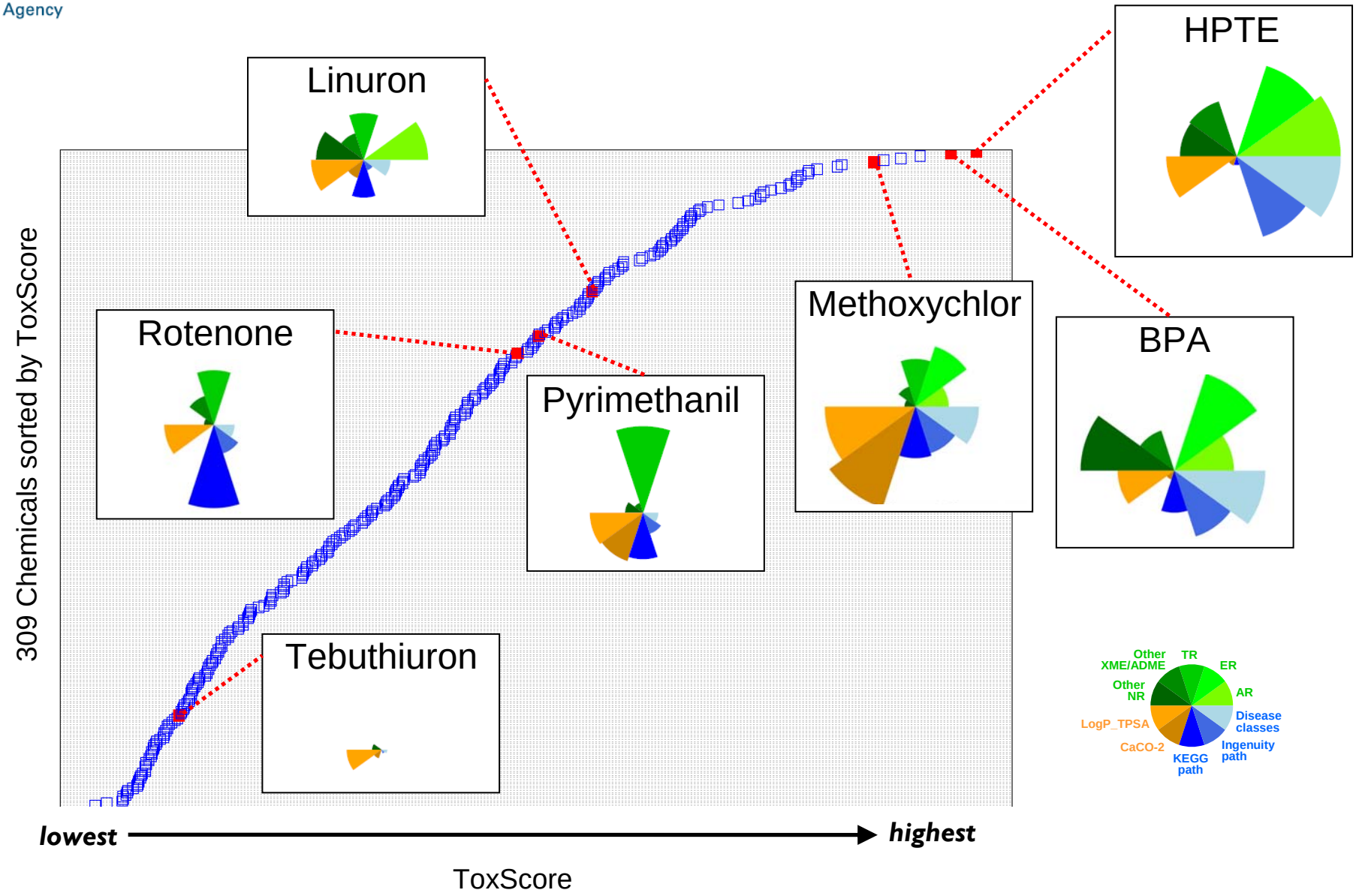
United States Environmental Protection Agency



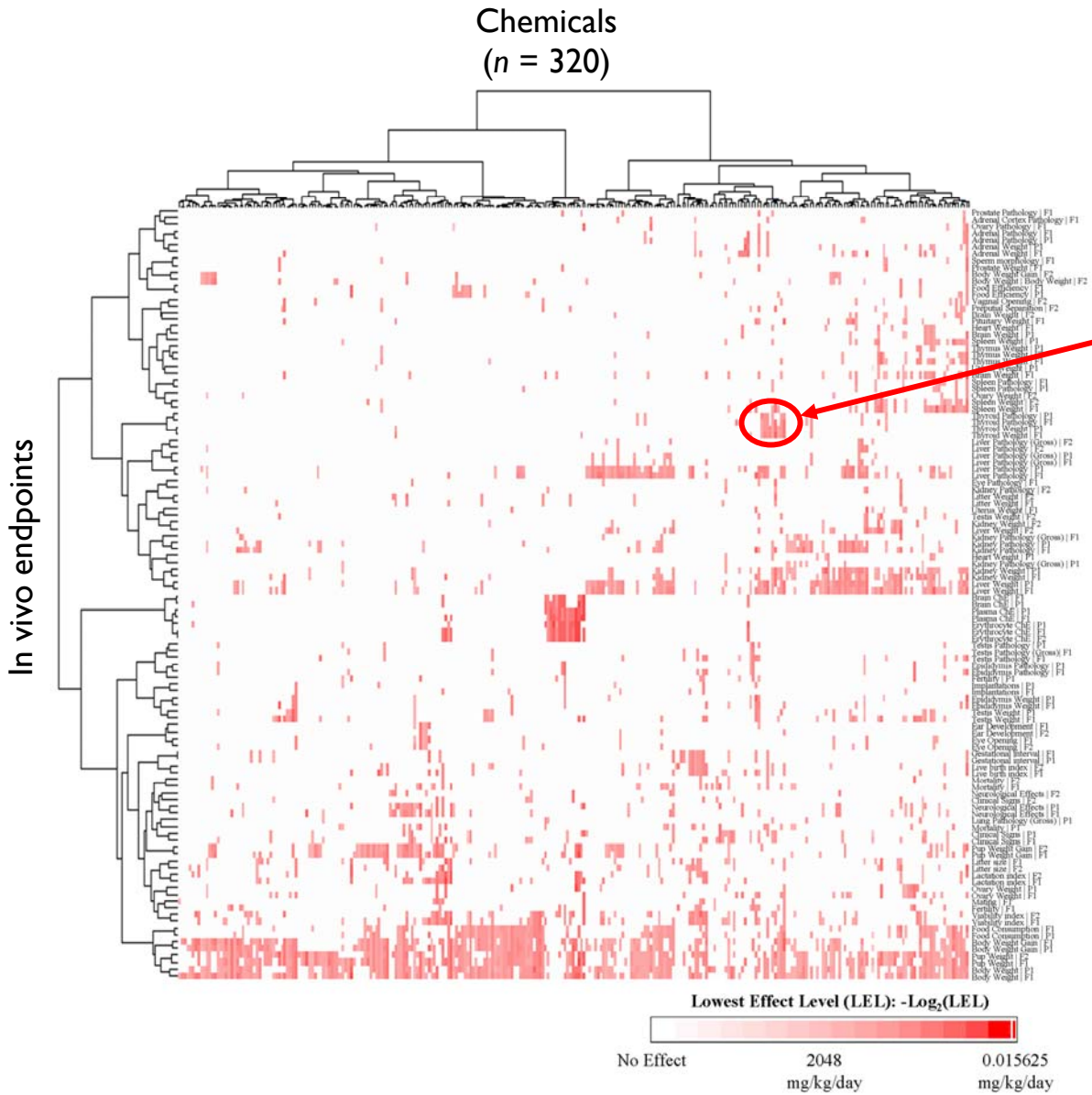
The ToxScore (horizontal axis) for each chemical (vertical axis) is symbolized by a box, sorted according to overall ToxScore.

EDSP chemicals of interest are highlighted (solid red boxes) along the sorted ToxScore distribution for all 309 ToxCast Phase-I chemicals.

Guidepost ("spike-in") chemicals



Data sources: Multigeneration Reproductive (MGR) studies captured in ToxRefDB



Thyroid Pathology (P1)
Thyroid Pathology (F1)
Thyroid Weight (P1)
Thyroid Weight (F1)

[Slide adapted from Matt Martin]

Exploring ToxScores in the context of in-vivo results

ToxScore = f(In vitro assays + Chemical properties + Pathways)

ToxScores without an in-vivo domain can be annotated according to ToxRefDB endpoint(s), but the in vivo domain does not contribute to the ToxScore calculation

[in-vivo **negative**]

Tebuthiuron

[in-vivo **positive**]

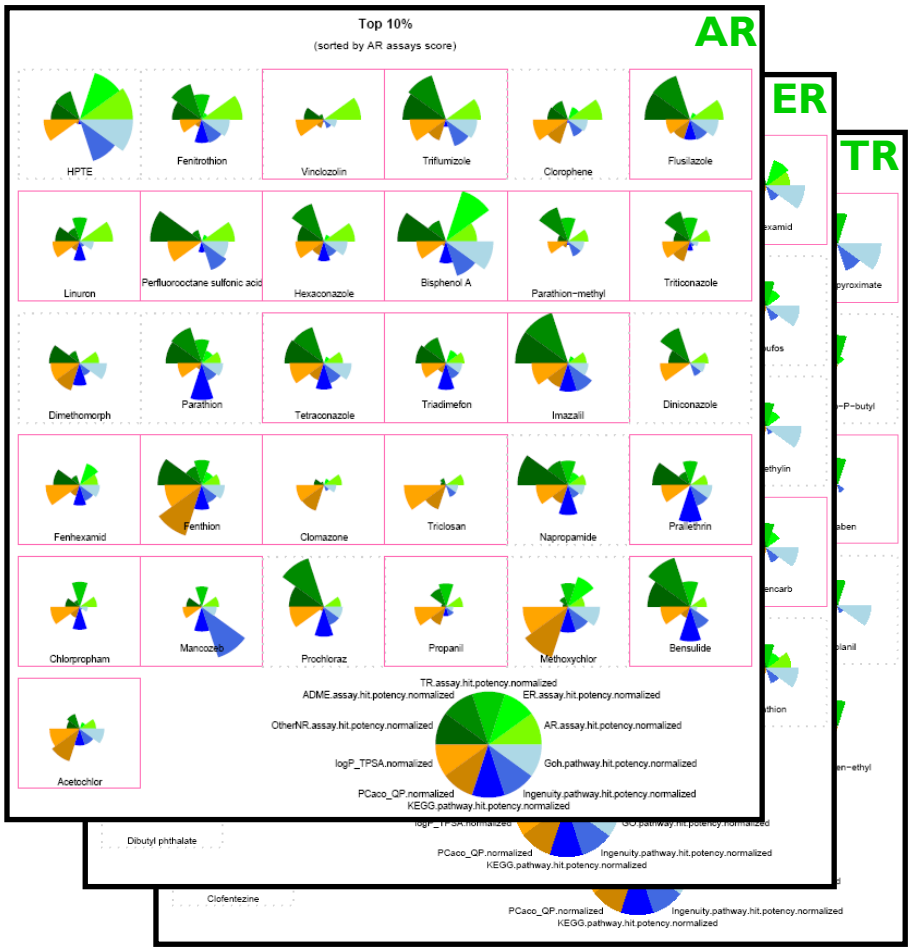
Triadimenol

[**not tested** in-vivo]

Parathion

Here, the in vivo aggregated endpoints include multigenerational study effects in endocrine organs, the reproductive tract, offspring survival, reproductive outcome and performance, and sexual developmental landmarks (e.g. PPS, VO, AGD)

Rank by specific slices (top 10% AR, ER, or TR) in the context of in vivo results



Any Endo/Repro in vivo toxicity

No in vivo test results for this endpoint

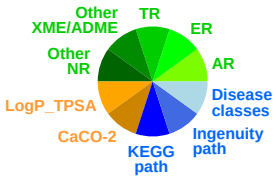
While the in vivo endpoints from multi-gen studies do not necessarily reflect endocrine disruption, potent in vitro assay hits warrant further inspection of the chemicals involved.

Different in vitro assays have differing levels of association with in vivo endpoints.

The in vitro assays may provide new information on biological mechanisms that are not targeted by current in vivo studies.

There are many chemicals for which there are no in vivo test results for particular endpoints

Chemical classes: ToxScore plots for all Triazoles

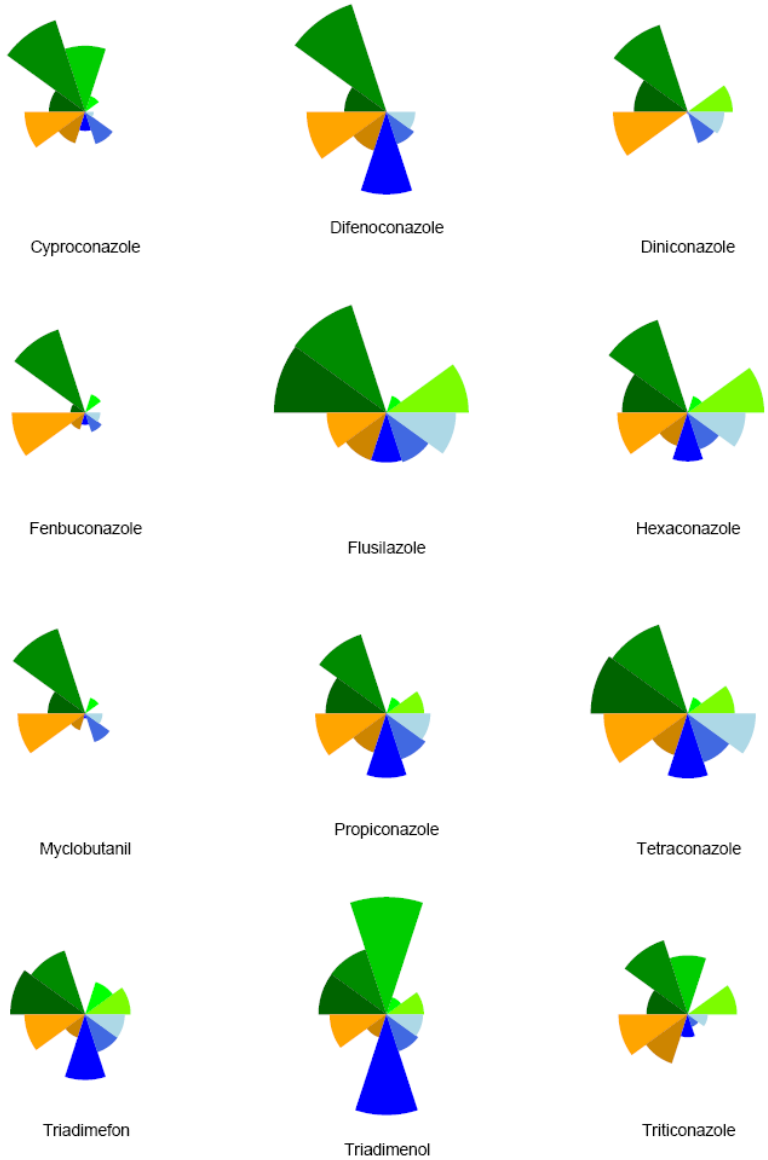


For any (sub)class of chemicals, the profiles are informative for comparing and contrasting class members.

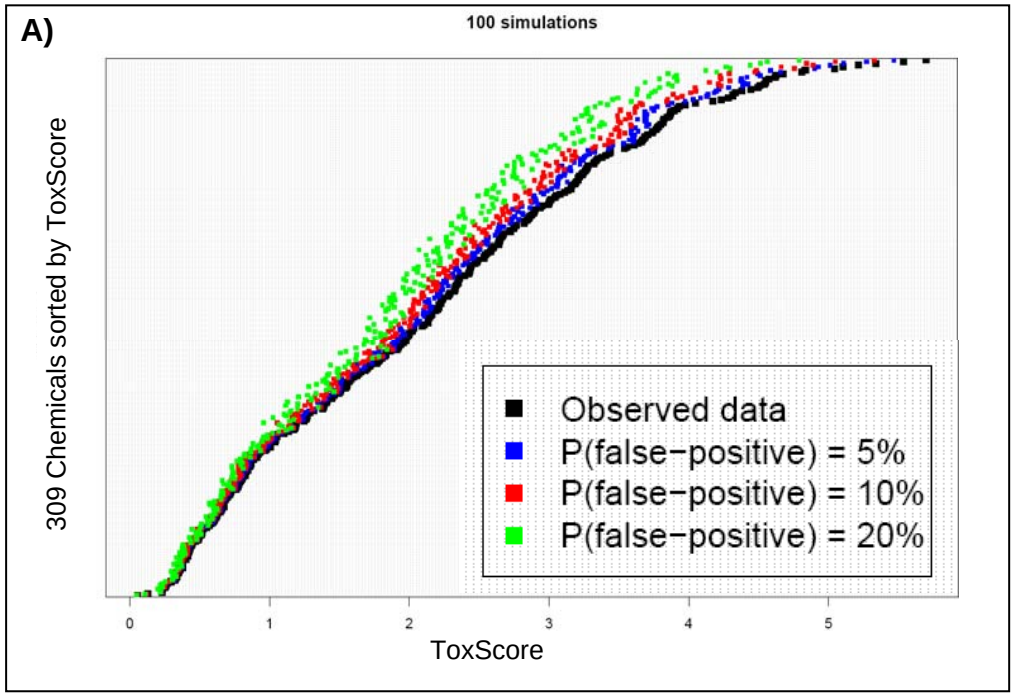
For example, the Triazoles show:

Similar *LogP_TPSA* scores
Similar *Ingenuity pathway* scores
Similar *Other XME/ADME* scores

Different *AR*, *ER*, and *TR* scores



Assessing the stability of ToxScore rankings in the presence of spurious assay results



Methods: The simulated probability of a spurious result on a given component assay varies from 5% - 20% (NOTE: results for *chemProp* slices were held constant because they do not represent stochastic assays). Each colored data point in the figure shows the mean simulated ToxScore under each condition.

Results: While the absolute *value* of ToxScores may change, the relative ranks of chemicals are generally preserved. In situations where a chemical's absolute rank changes, it tends to swap positions with a neighbor. This is in contrast to the large shifts in rank that would occur in a prioritization scheme reliant on singular pieces of information, wherein individual errors would shift chemicals between entire priority regions (e.g. a chemical assigned a top quartile priority rank is shifted to the bottom quartile).

Presentation outline

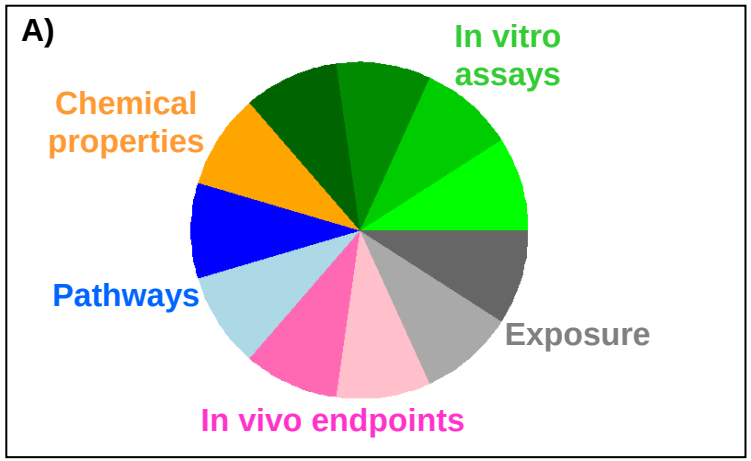
I. Prioritization framework
   i. Rationale for integrated prioritization scheme
   ii. Definitions and notation
   iii. Interpreting ToxScores for individual chemicals

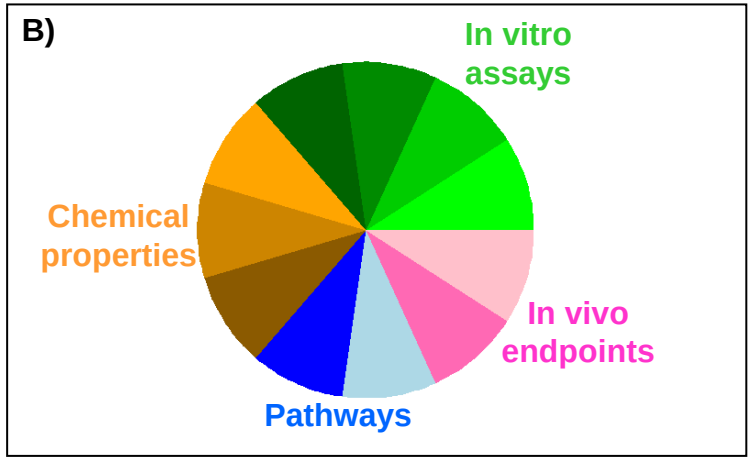
II. Implementation for the task of endocrine prioritization
   i. EDSP prioritization
   ii. Developing a prioritization scheme for EDCs
   iii. Data sources
   iv. Results: EDSP chemicals of interest in ToxCast Phase-I
   v. Results: empirical distribution of EDSP chemicals of interest
   vi. Results: guidepost ("spike-in") chemicals
   vii. Results: exploring ToxScores in the context of in vivo results
   viii. Results: rank by specific slices (e.g. AR) in the context of in vivo results
   ix. Results: chemical classes
   x. Results: simulation studies assess sensitivity to spurious assay results

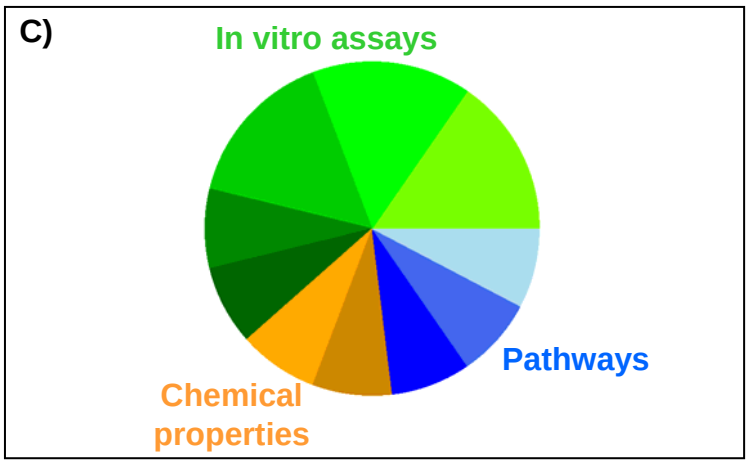
III. Future directions
   i. Alternative implementations
   ii. Incorporation of new/other data (e.g. QSAR models and other extant tools)

IV. Conclusions

Alternative implementations







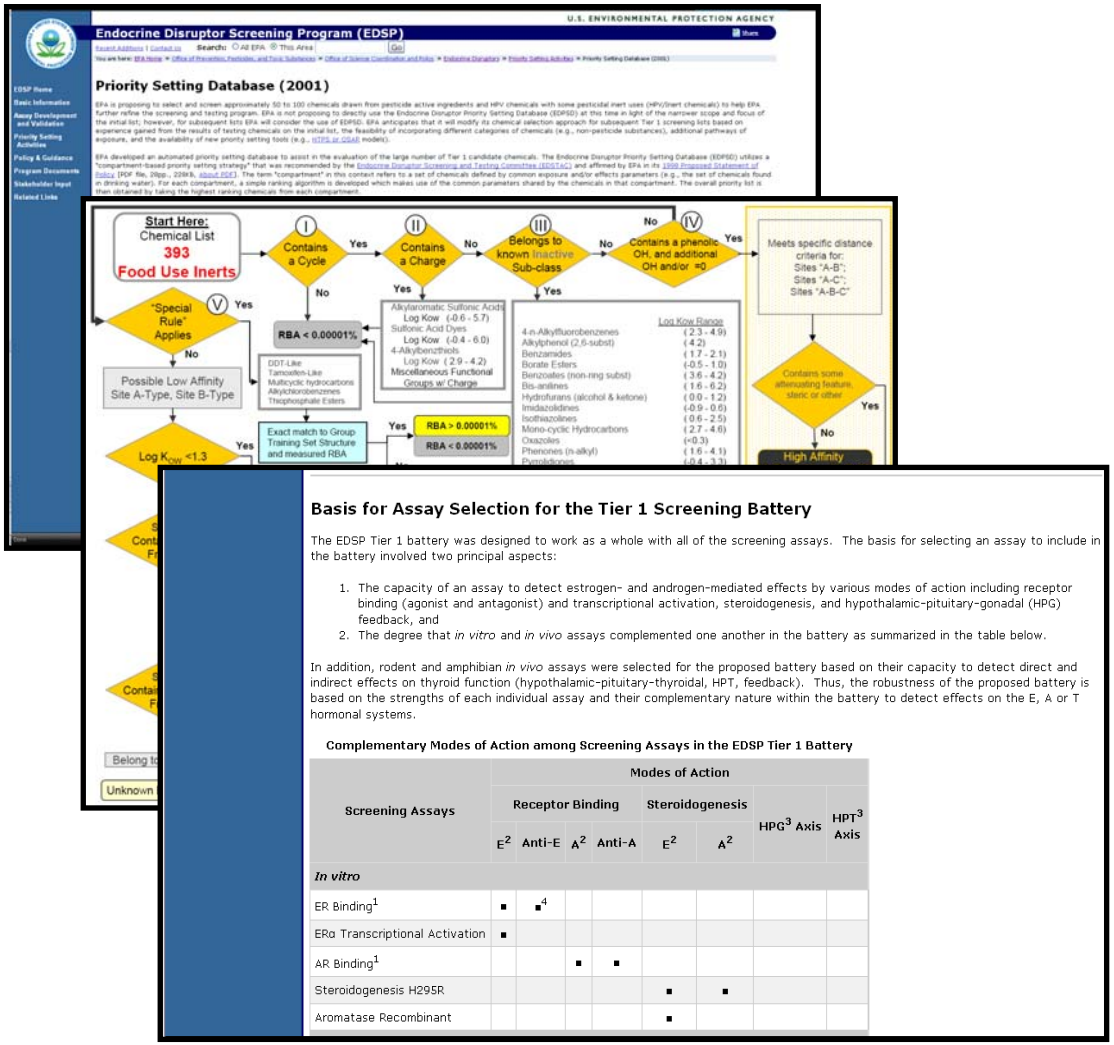
Prioritization tasks might:

A) Incorporate additional components (slices) that may be from other domains (e.g. Consideration of exposure potential);

B) Customize individual domains (e.g. Add a targeted set of chemical descriptors);

C) Adjust weighting schemes according to specific prioritization tasks or component (slice) meaning (e.g. The weights ($w_{i=1,2,3}$) of In vitro assay slices 1, 2, and 3 (representing AR, ER, and TR, respectively) have been increased).

Incorporation of new/other data



The framework is amenable to incorporation of new data from the EDSP Tier-1 battery, subsequent phases of ToxCast, and other data from any number of sources.

For example:

QSAR models, such as the Mid-Continent Ecology Division's system for ER binding potential, could be incorporated into the *chemProp* domain.

Toxicological knowledgbases, such as the Endocrine Disruptor Priority Setting Database, could be used to assign priors to particular chemicals.

Exposure tools, such as ExpoCast, could provide data for the *exposure* domain.

Predictive signatures, such as those developed as part of ToxCast, could be added as components.

. . . and many more . . . .

United States Environmental Protection Agency

Conclusions

This work was reviewed by EPA and approved for presentation but does not necessarily reflect Agency policy

This implementation indicates that an integrated approach, wherein multiple domains of toxicological knowledge are simultaneously incorporated into chemical prioritization, gives a reasonably stable priority rank across the ToxCast Phase-I chemicals.

The inclusion of benchmark chemicals (akin to a "spike-in" set) as internal controls reduces the probability that potentially hazardous chemicals will be improperly assigned low priority for further testing and makes this a promising approach for diverse chemical prioritization tasks.

The ToxRefDB in vivo results may be useful for evaluation of other, specific prioritization tasks.

The framework developed here provides graphical insight into the multiple domains considered in chemical profiling and prioritization.

It is amenable to incorporating extant prioritization schemes and relevant data from diverse sources, thereby facilitating meta-analysis across Agency resources.

Because ToxScores are intended for relative ranking, particular implementations of this framework can be continually updated with new chemicals and future data.