EPA United States Environmental Protection Agency

Incorporating exposure information into Toxicological Priority Index (ToxPI) for Chemical Prioritization

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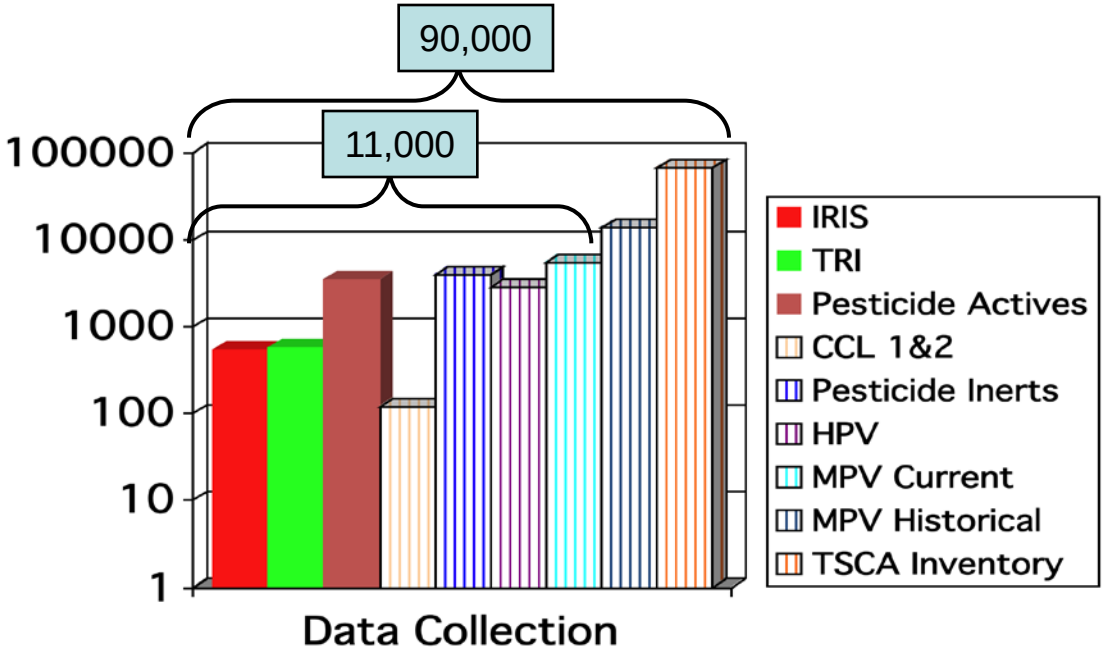
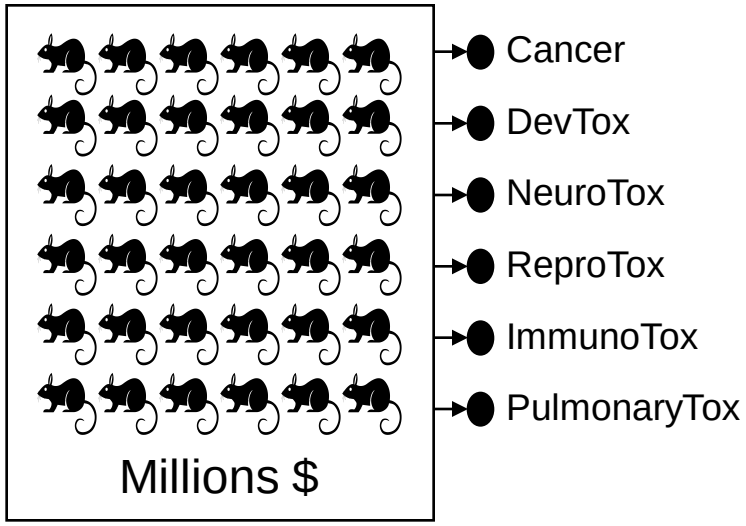
National Center for Computational Toxicology

Traditional toxicology testing paradigm needs to change because . . .

Too Many Chemicals



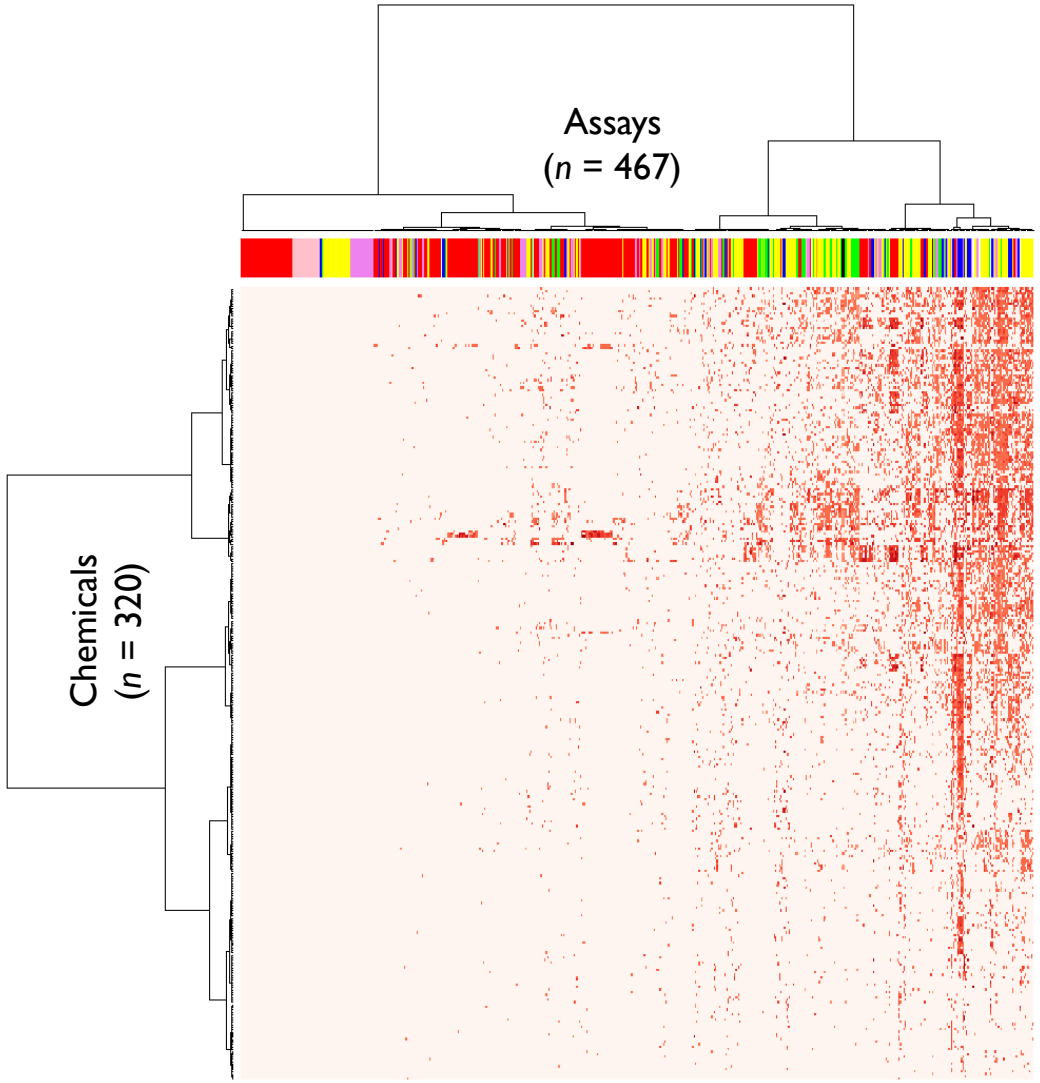
Too High a Cost



…and not enough data.

Judson et al., 2009, *Environ. Health Perspect.*

Diversity of data from 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

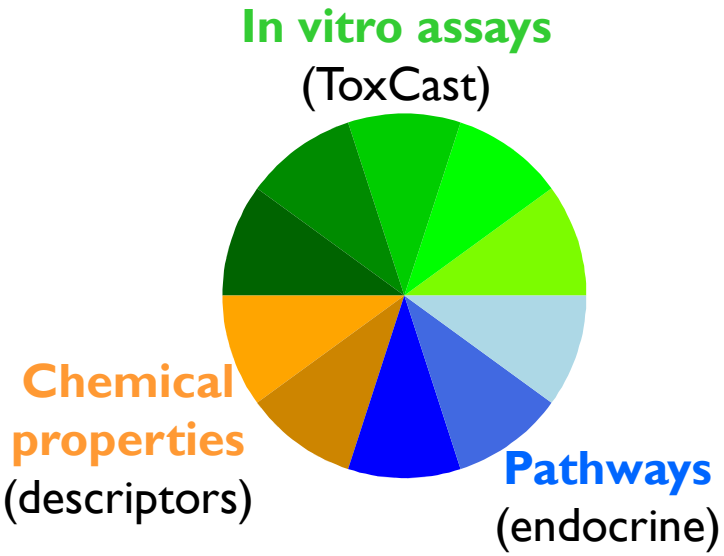
http://www.epa.gov/ncct/toxcast/

Judson et al., 2010, *Environ. Health Perspect*. (doi: 10.1289/ehp.0901392)

Rationale for an integrated chemical prioritization scheme

- Integration over multiple domains of information
- Extensibility to incorporate additional types of data
- Transparency in score derivation and visualization
- Flexibility to customize components for diverse prioritization tasks

ToxPi (Toxicological Priority Index)



Putative endocrine profiles

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

Reif et al., 2010, *submitted*

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ToxPi (Toxicological Priority Index)

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Definitions & notation

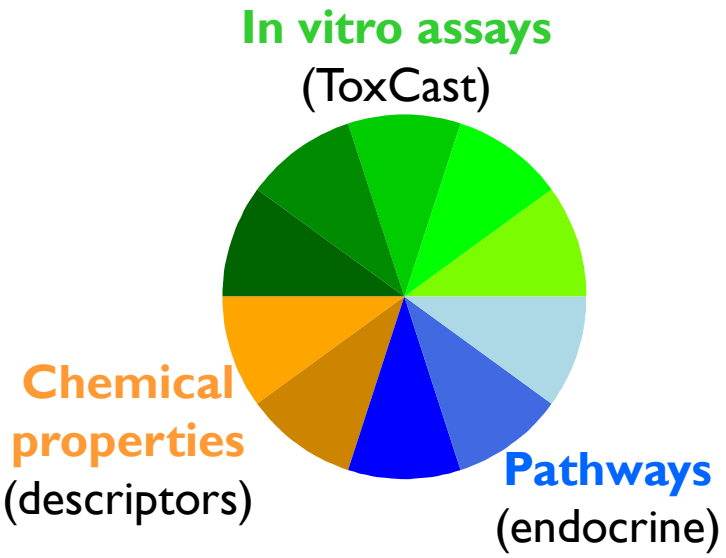
Each chemical profile/ signature/ fingerprint gives a score index (ToxPi) used for ranking chemicals

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

Domain: Domain/field of knowledge; represented by the slice(s) of a given color family

Slice: "Pie" slices representing individual *components* or aggregrations of multiple related *components*

Component: Individual in-vitro assays, chemical properties/descriptors, etc.

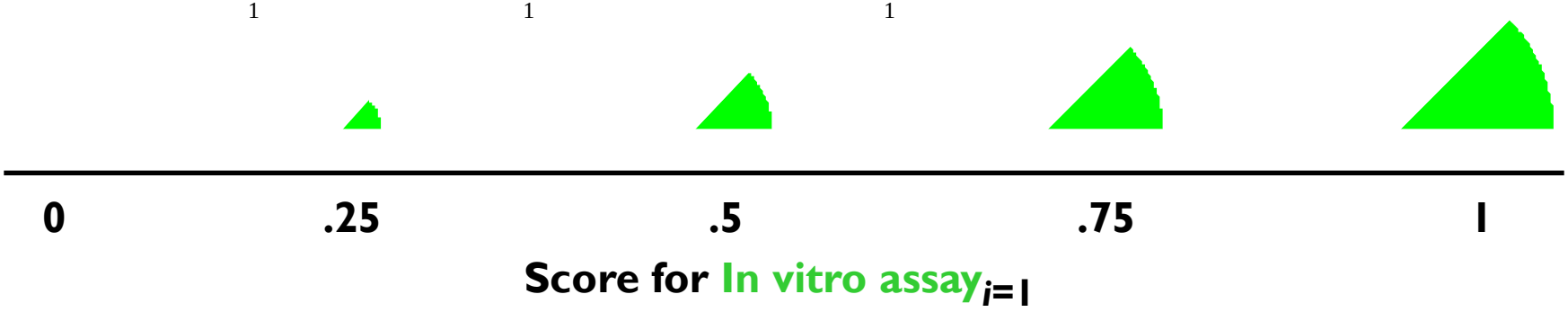


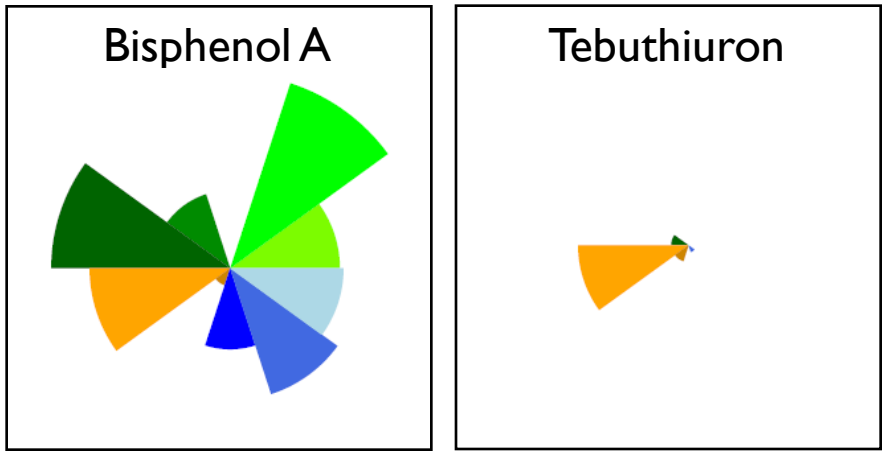
Reif et al., 2010, *submitted*

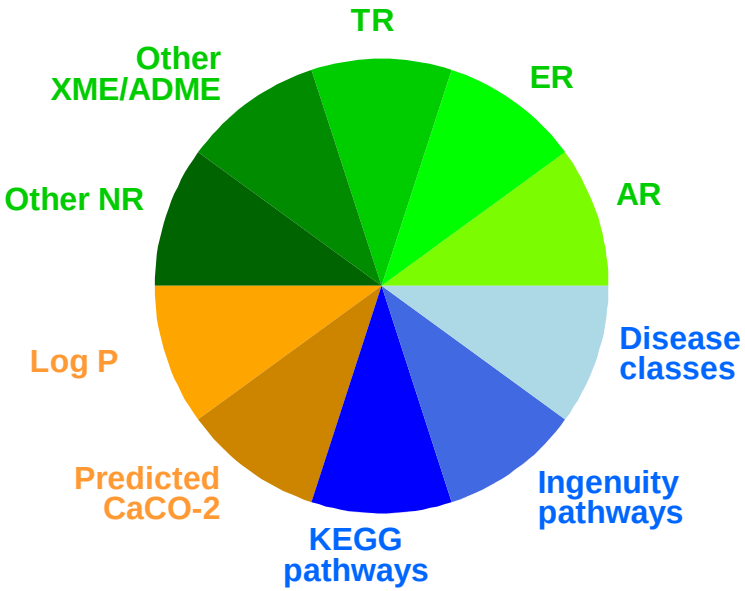
Interpreting ToxPis for individual chemicals

Example: Endocrine profiling and prioritization of environmental chemicals using ToxCast™

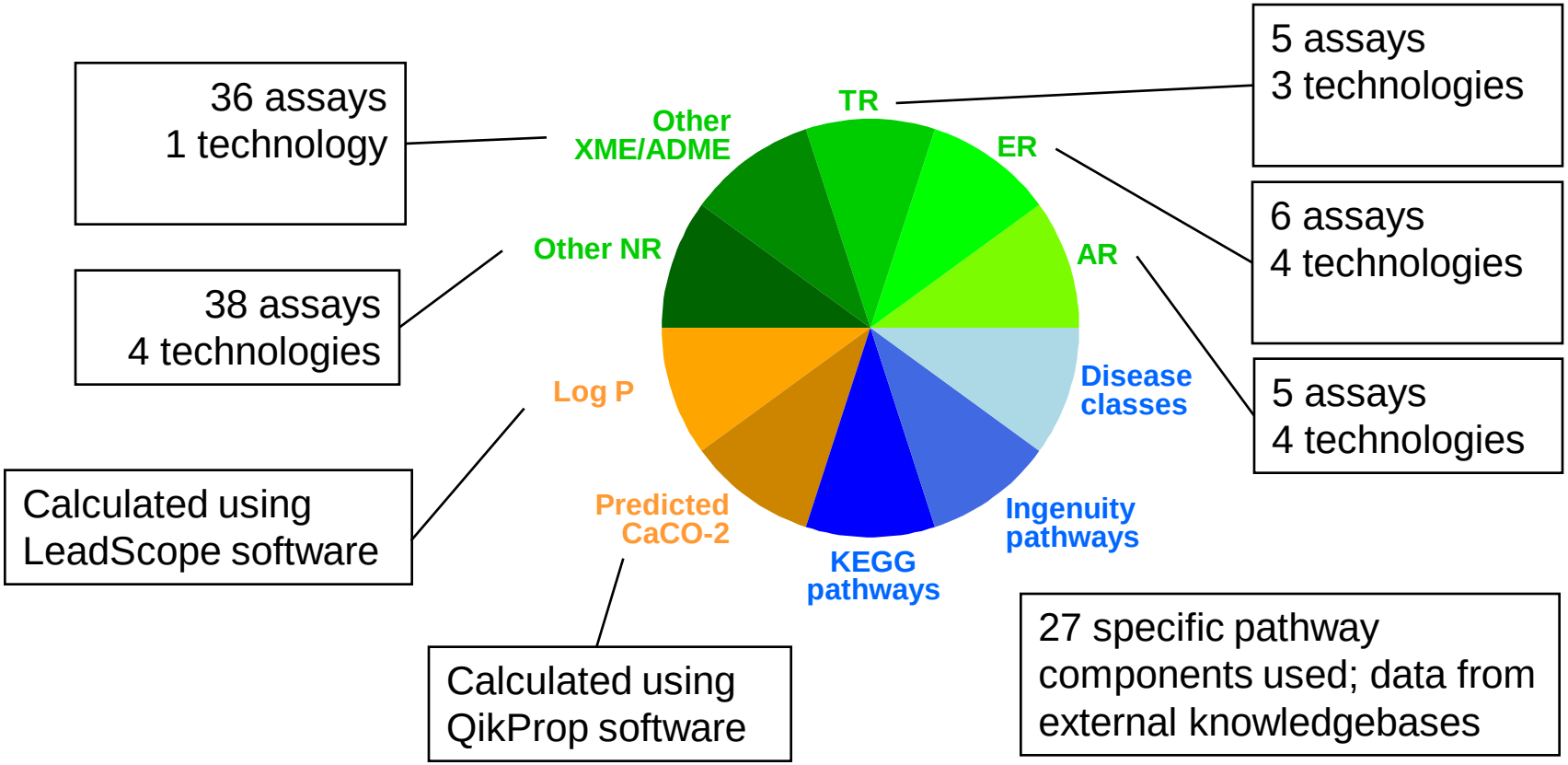
$$\text{ToxPi} = \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{P} w_p * \text{pathway}_p$$





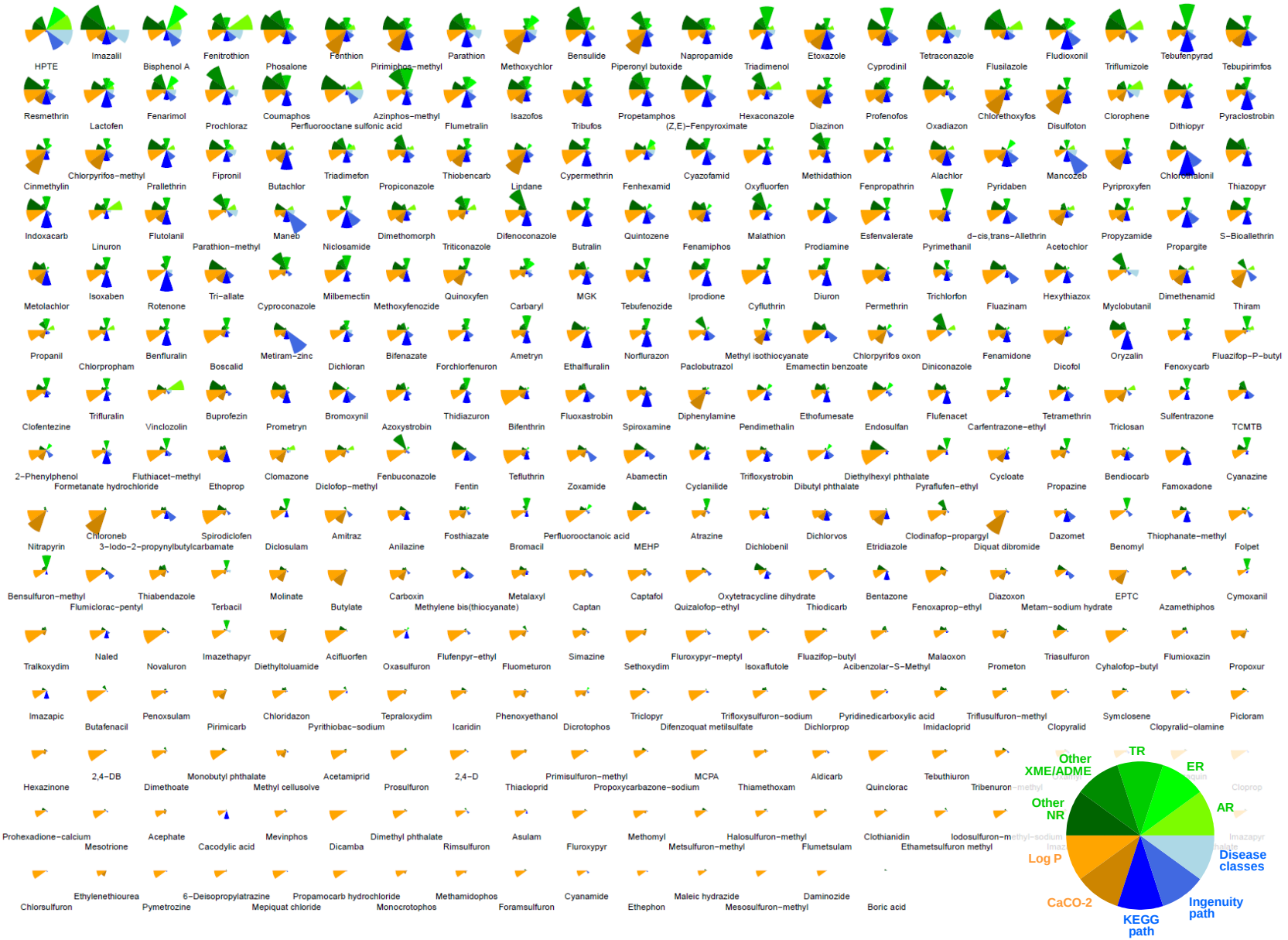


Example of data sources



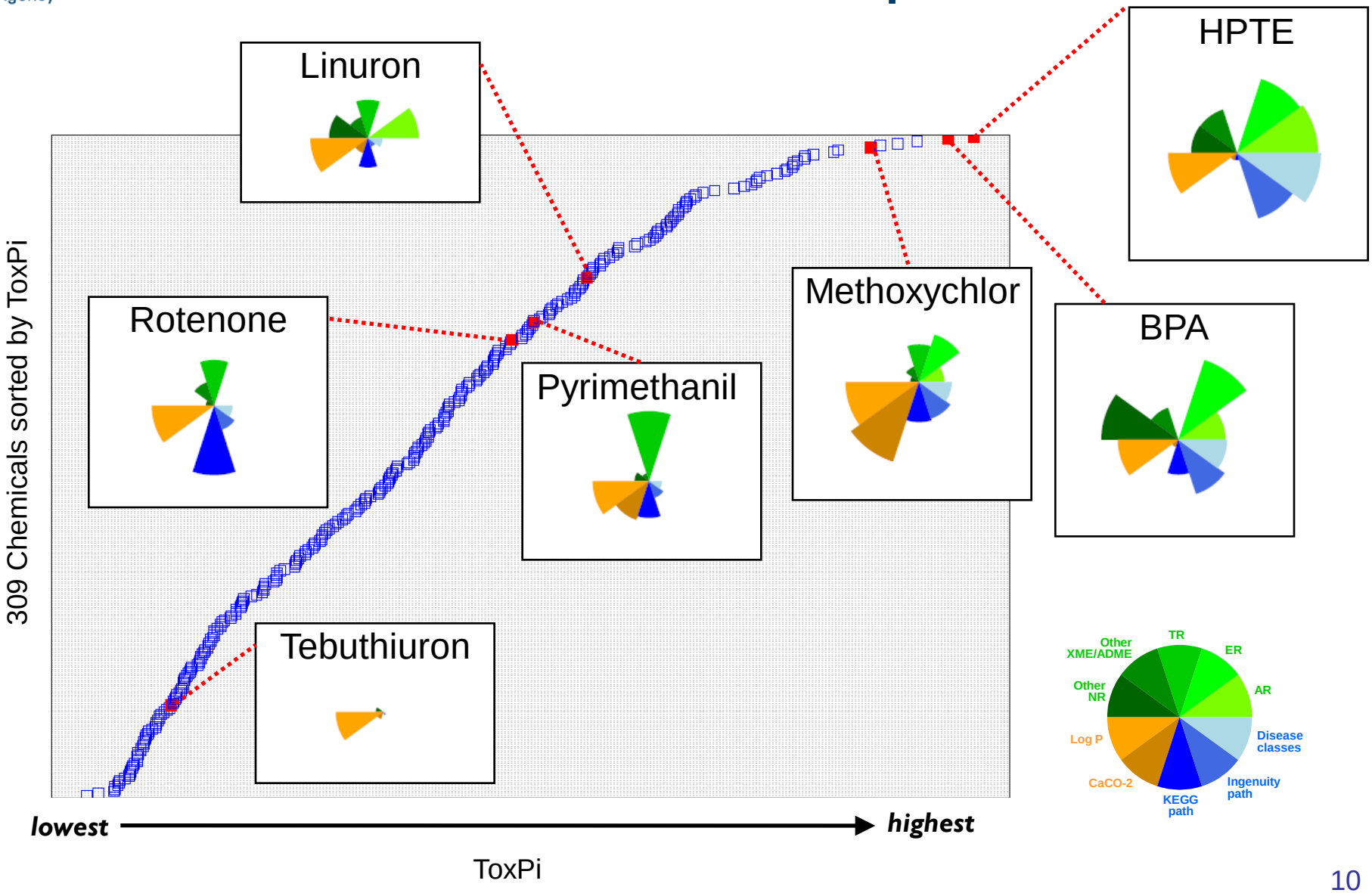
Prioritization of ToxCast™ chemicals

(sorted by overall ToxPi endocrine score)



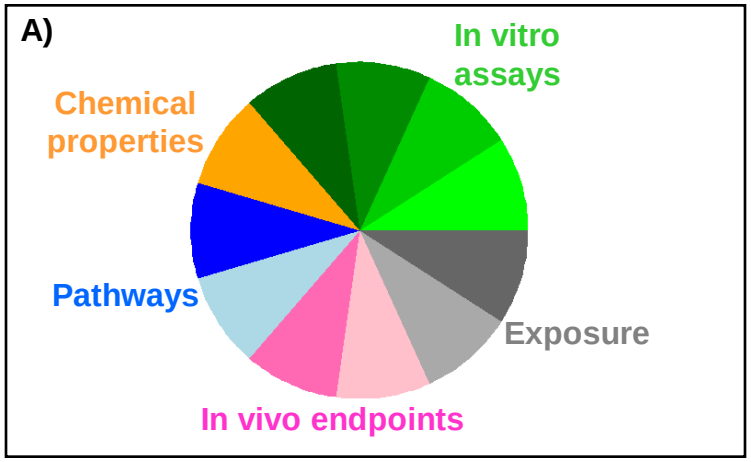
Reif et al., 2010, *submitted*

Example ToxPi scores for reference chemicals from ToxCast™ phase I

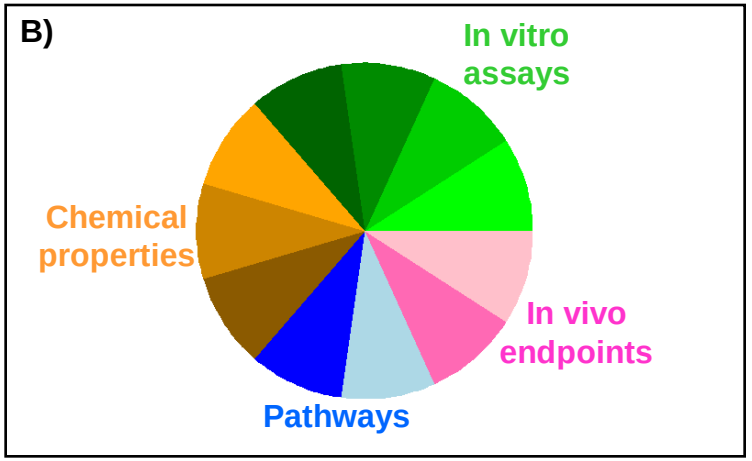


Ranks and scores consistent with published bioactivity

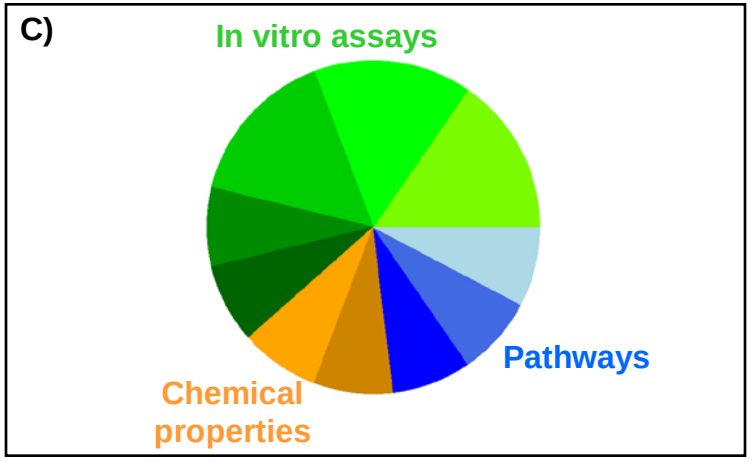
Alternative ToxPi implementations for different applications



A) Incorporate additional components (slices) from other domains



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



C) Adjust weighting schemes (e.g. Weights of In vitro assay slices AR, ER, and TR have been increased)

Reif et al., 2010, *submitted*

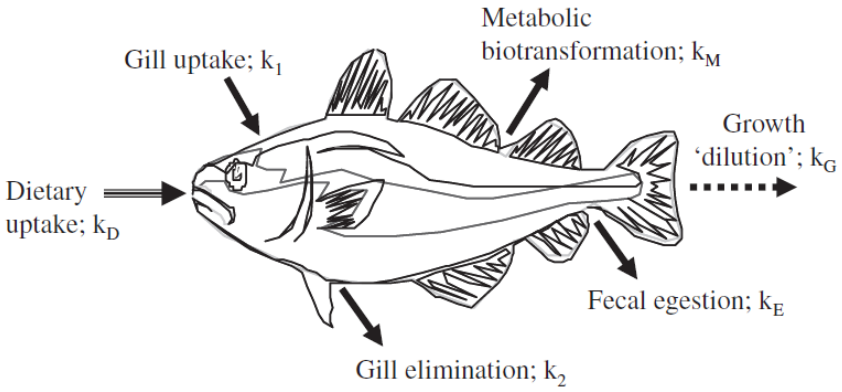
Does typical info used to prioritize based on potential for exposure change ToxPi ranking?

- Environmental fate parameters (bioaccumulation, persistence)
  - U.S. EPA: Identifies new/existing chemicals as persistent or bioaccumulative → Completing screening level risk assessment.
    - U.S. EPA. Category for Persistent, Bioaccumulative, and Toxic (PBT) New Chemical Substances. Federal Register, 1999, Vol. 64, pp 60194-60204.
  - Environment Canada: Identifies existing substances from Domestic Substances List that are persistent or bioaccumulative to non-human species.
    - Canadian Environmental Protection Act (CEPA), 1999 http://www.ec.gc.ca/substances/ese/eng/dsl/cat_criteria_process.cfm

- Manufacturing production / use information
  - U.S. EPA's New Chemicals Program: Requires production volume info and use category for premanufacturing notice submission.
  - Health Canada ranked chemicals by quantity in commerce, # of submitters, and sum of expert ranked use codes → Greatest potential for exposure.
    - "Exposure-based Prioritization – Health Canada Experience under the Canadian Environmental Protection Act", Christine Norman, March 2010

Source for preliminary exposure data of environmental fate

- Exposure data obtained from EPI Suite™ v4.00 (http://www.epa.gov/oppt/exposure/pubs/episuite.htm):
  - **Bioaccumulation/bioconcentration factor** (Log BCF, Log BAF) from BCFBAF program
  - **Persistence** (half life air, half life water, persistence time) from Level III fugacity model, BIOWIN, AOPWIN programs



Arnot and Gobas, 2006, *Environ. Rev*.

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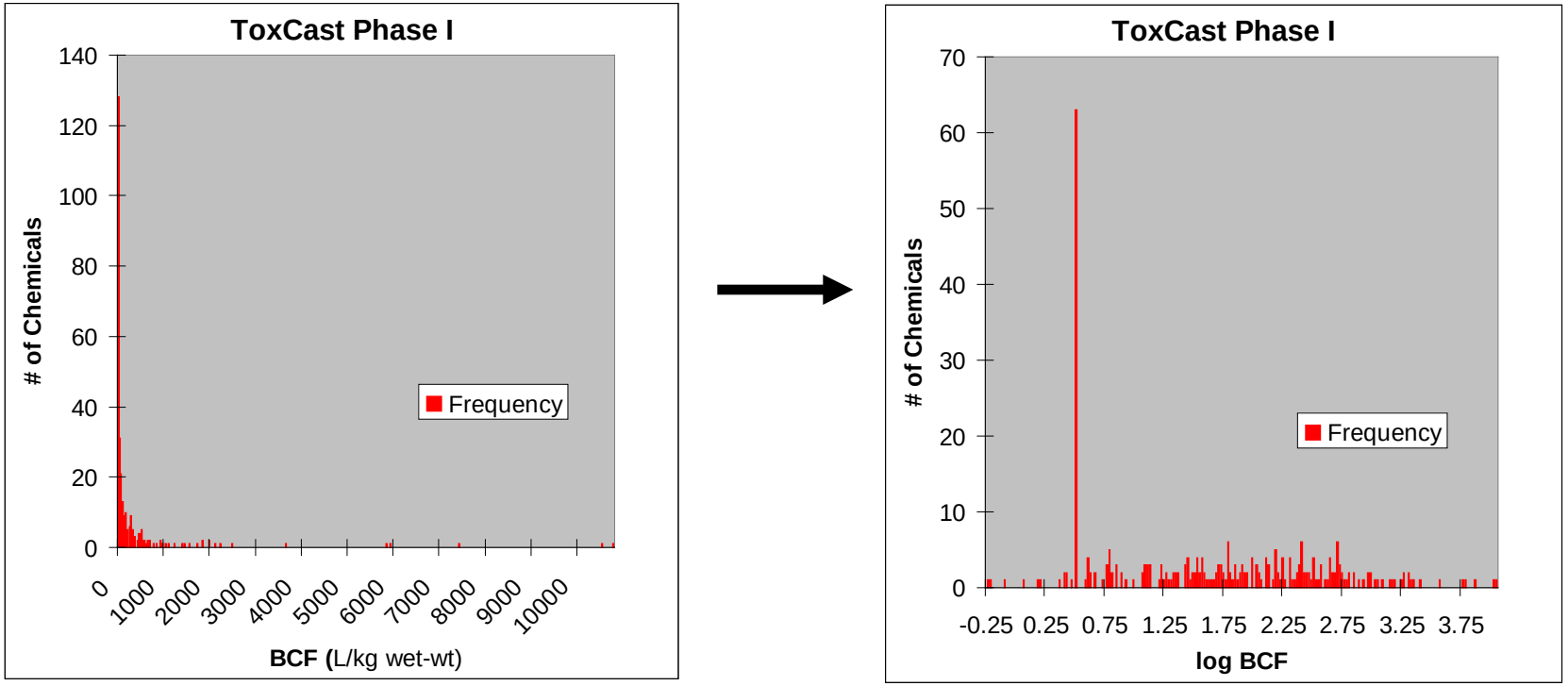

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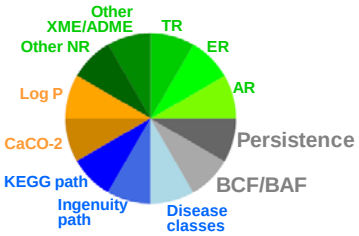
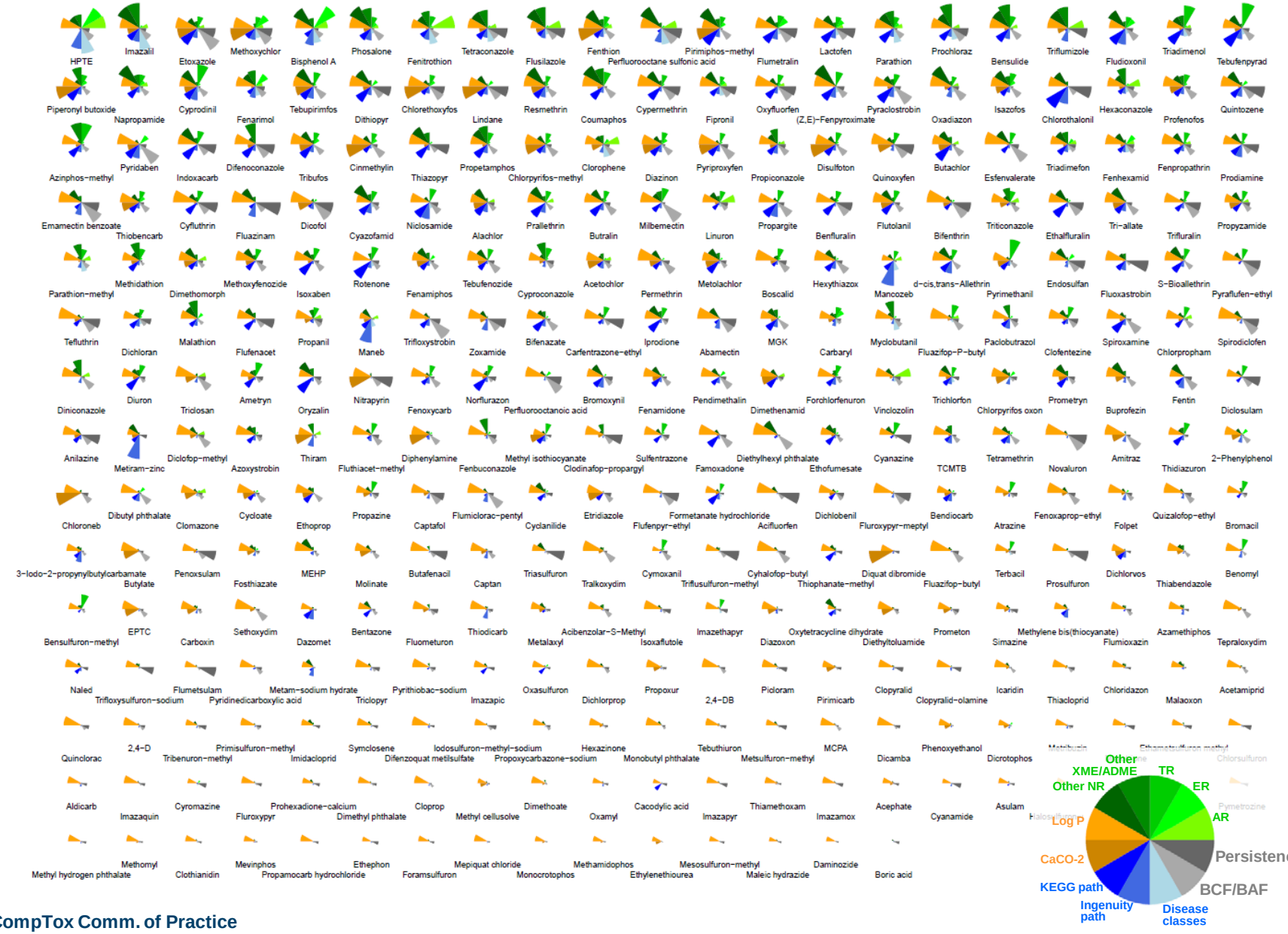
- Ran EPI Suite™ in batch mode passing chemicals smiles/CAS. From summary results, extracted data for 309 ToxCast Phase I chemicals

- Adjusted data range for negative values

- Normalized data to incorporate exposure domain into ToxPi framework with other data domains



Incorporating exposure information: Preliminary ToxPi endocrine scores (sorted by overall ToxPi score)



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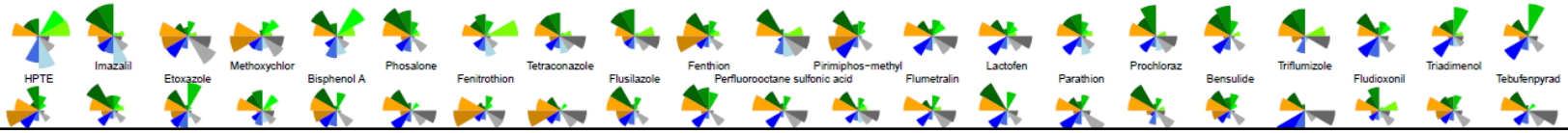
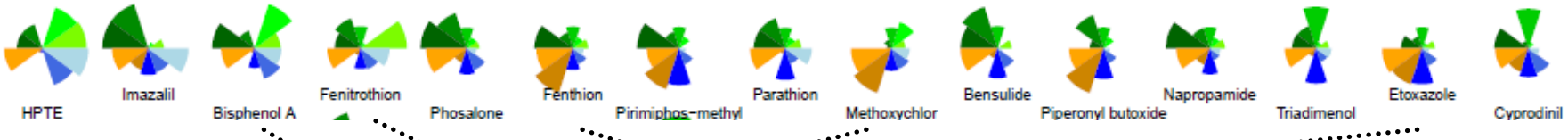
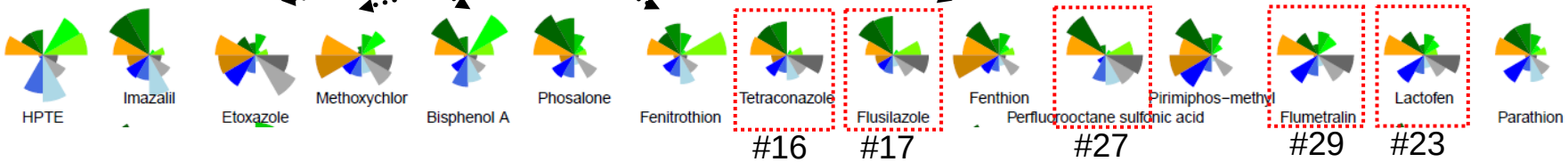
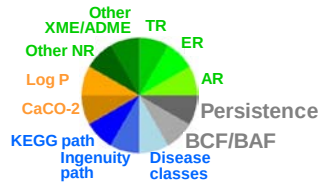


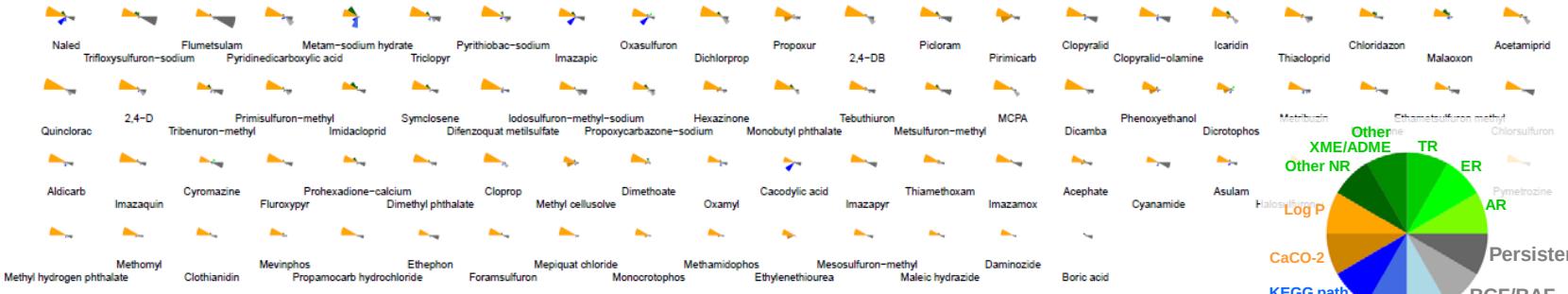
Previous top 15 prioritized chemicals by overall ToxPi score



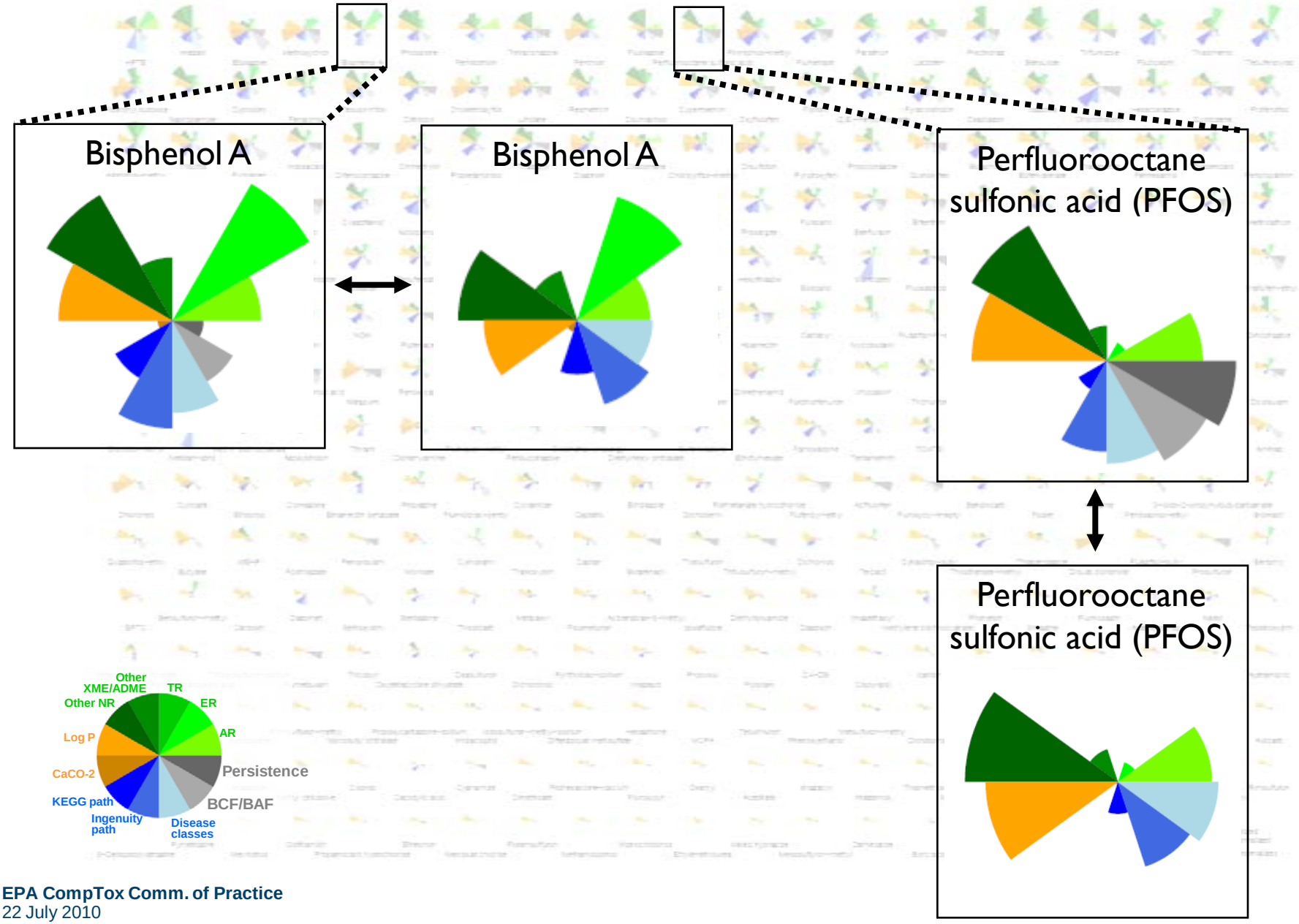
New top 15 prioritized chemicals with exposure domain







Interpreting ToxPis with exposure domain for individual chemicals



Pesticide agricultural usage data

- Data provider is GfK Kynetec, a private market research firm.
  Proprietary database is called Agro Trak.
  Based on surveys of pesticide use on over 50 agricultural crops.

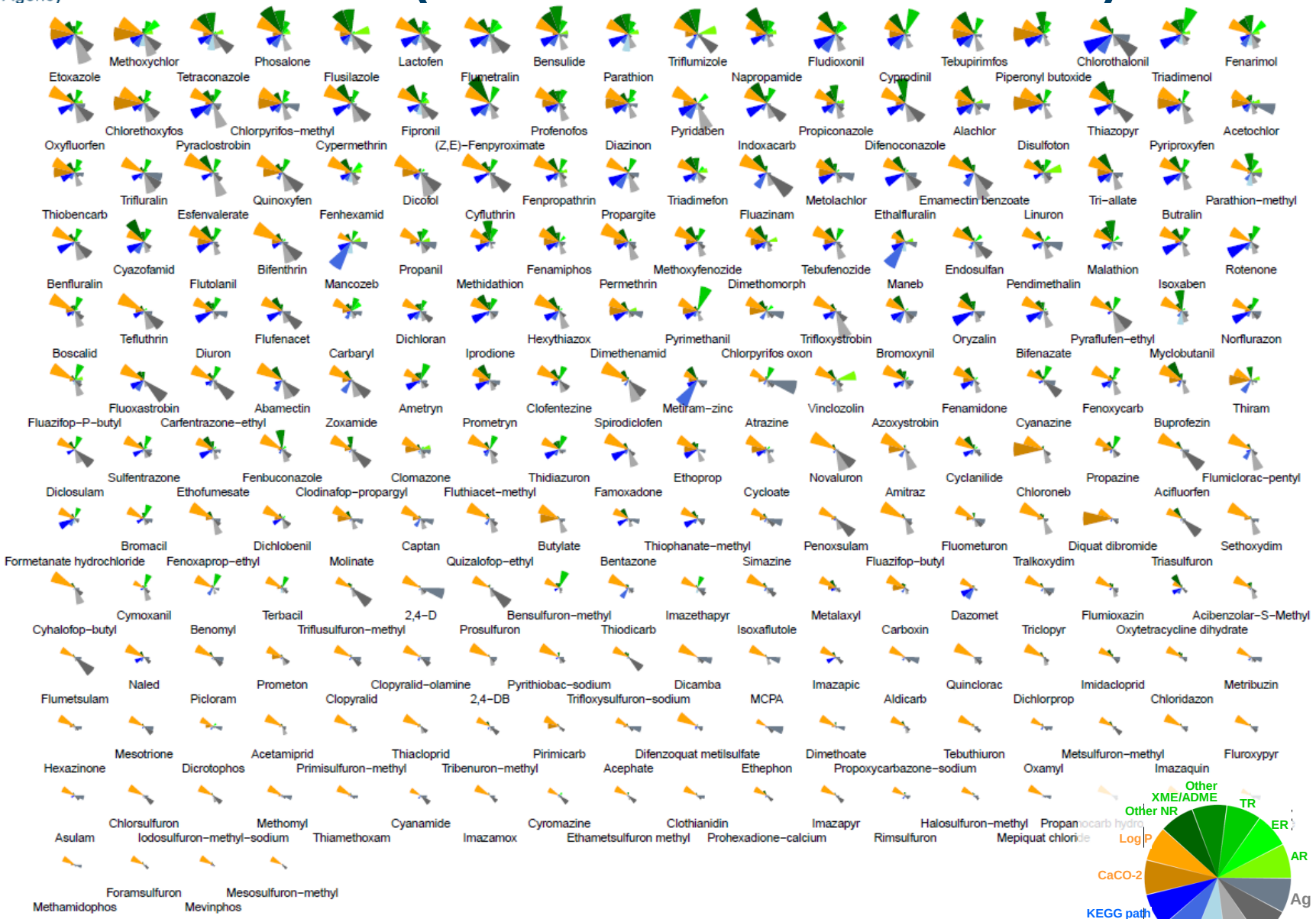
↳ **Data provides an estimate of the pounds of each active ingredient (AI) applied to agricultural crops on a national level.** Does not include Non-Ag. data (home and garden use, turf and ornamentals, etc.).

↳ Data available for 220 of 309 ToxCast Phase 1 chemicals for which agricultural pesticide usage was reported.

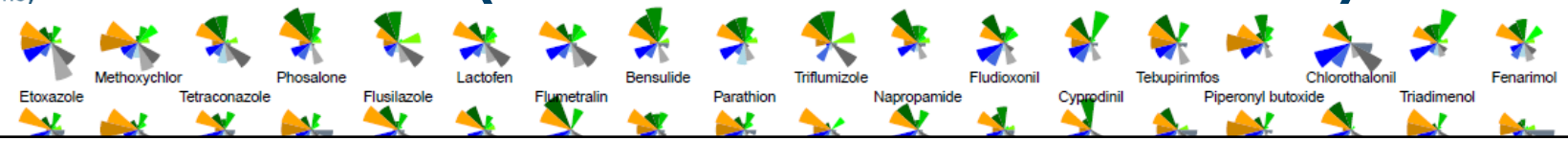
↳ Reported pounds of AI applied per year (from 1998 to 2008) was summed over the 11 years and then normalized.

Rankings after adding agricultural usage data (220 of 309 Phase I chemicals)

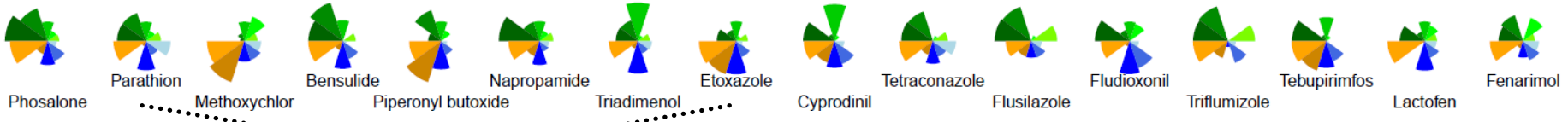
United States Environmental Protection Agency



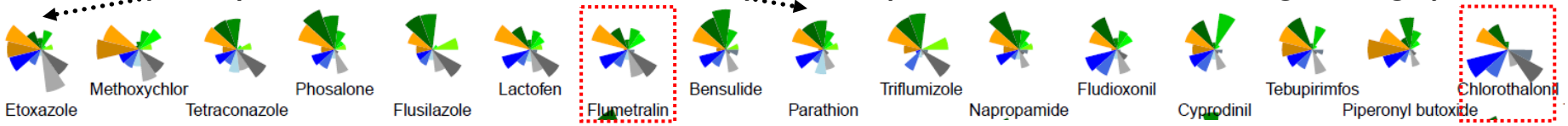
Rankings after adding agricultural usage data (220 of 309 Phase I chemicals)



Previous top 16 prioritized chemicals by overall ToxPi score

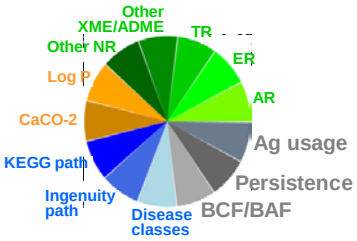


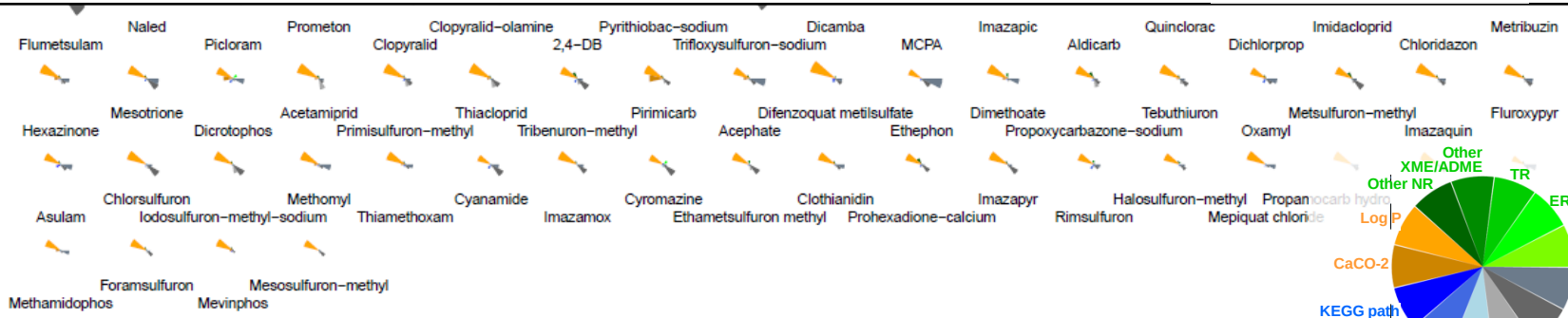
New top 16 prioritized chemicals with exposure (environmental fate, ag. usage)



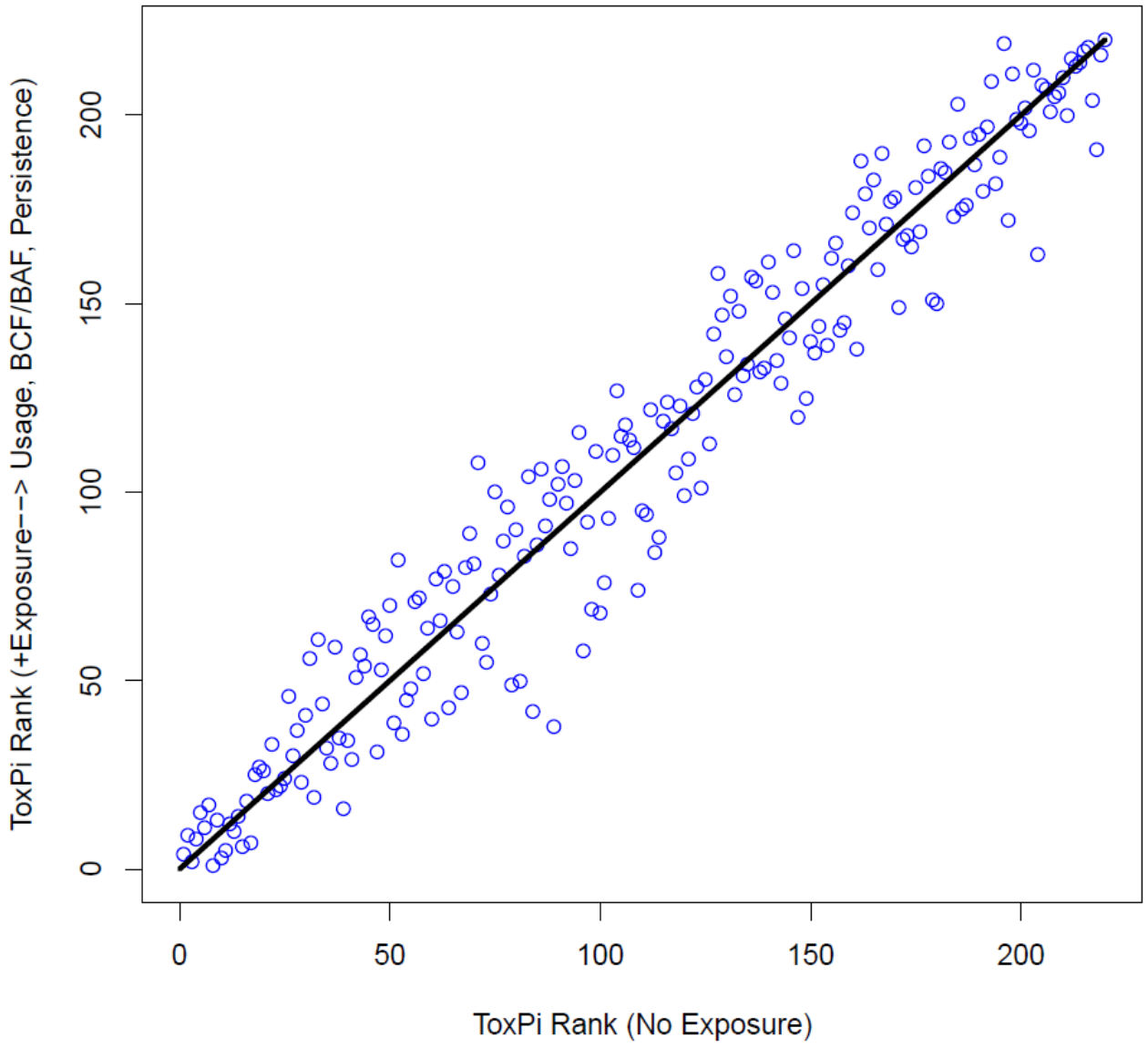
#17

#39





Change in ToxPI ranking after adding agricultural usage data (220 chemicals)



Environmental fate and usage data change ToxPI rankings

Limitations of environmental fate parameters

- Bioaccumulation in humans → NOT studied
  - Humans at top of both aquatic and terrestrial food chains→ Evidence suggests bioaccumulation based on aquatic food chains only may not be appropriate for humans.

    Czub and McLachlan, 2004, *Environ. Sci. Technol*.

- Uncertainty in BCF and BAF values can be high (up to a factor of ~3 for BAF).

  Arnot and Gobas, 2006, *Environ. Rev*.

- Decisions for screening assessments by setting cutoffs for BCF and persistence can be flawed.
  - Proposed holistic method integrating persistence, bioaccumulation, toxicity (PBT) and quantity.

    Arnot and Mackay, 2008, *Environ. Sci. Technol.*

Do fate parameters prioritize chemicals detected during residential exposure?

- Humans spend much of their time indoors.
  - Exposure to semivolatile organic compounds (SVOCs) indoors contribute to detectable body burdens (CDC's National Report on Human Exposure to Environmental Chemicals)

    Weschler and Nazaroff, 2008, *Atm. Environ*. ; Weschler and Nazaroff, 2010, submitted

- For example, phthalates are detected not only in consumer products → Also in food and in indoor environment (air and household dust).
  - But, exposure to phthalates does not result in bioaccumulation (based on chemical properties).

    Heudorf et al., 2007, Int. *J. Hyg. Environ. Health*

Endocrine disrupting compounds in indoor air and dust

CHEMICAL CLASS (IN ORDER OF ABUNDANCE IN DUST)	SOURCES
Phthalates	Plastics, adhesives, personal care products, and other sources
Alkylphenols	Surfactants in cleaners, inerts in pesticides, personal care products, plastics, and other sources
Pesticides, pesticide metabolites	Pesticides
Polycyclic aromatic hydrocarbons (PAHs)	Products of combustion
Parabens	Personal care products and other sources
Phenolics (e.g., bisphenol A)	Plastics, personal care products, and other sources
Miscellaneous (e.g., dichlorophenol, nitrophenol)	Miscellaneous household products
Polychlorinated biphenyls (PCBs)	Electrical equipment

- Analyzed 89 compounds compounds found in indoor air and house dust samples from 120 homes in Cape Cod, MA.
  - Eligible women either breast cancer cases or age-matched controls. Lived in their homes at least 10 years.
- Criteria for compounds selection
  - Evidence that they were EDCs
  - Reported to be in commercial products or building materials
  - Compatible with one of two GC/MS analytical methods for detection

Rudel et al., 2003, *Environ. Sci. & Tech*.

EDC compounds detected in indoor air and dust

• Of 89 compounds analyzed in 120 homes:

Indoor Air	Household dust

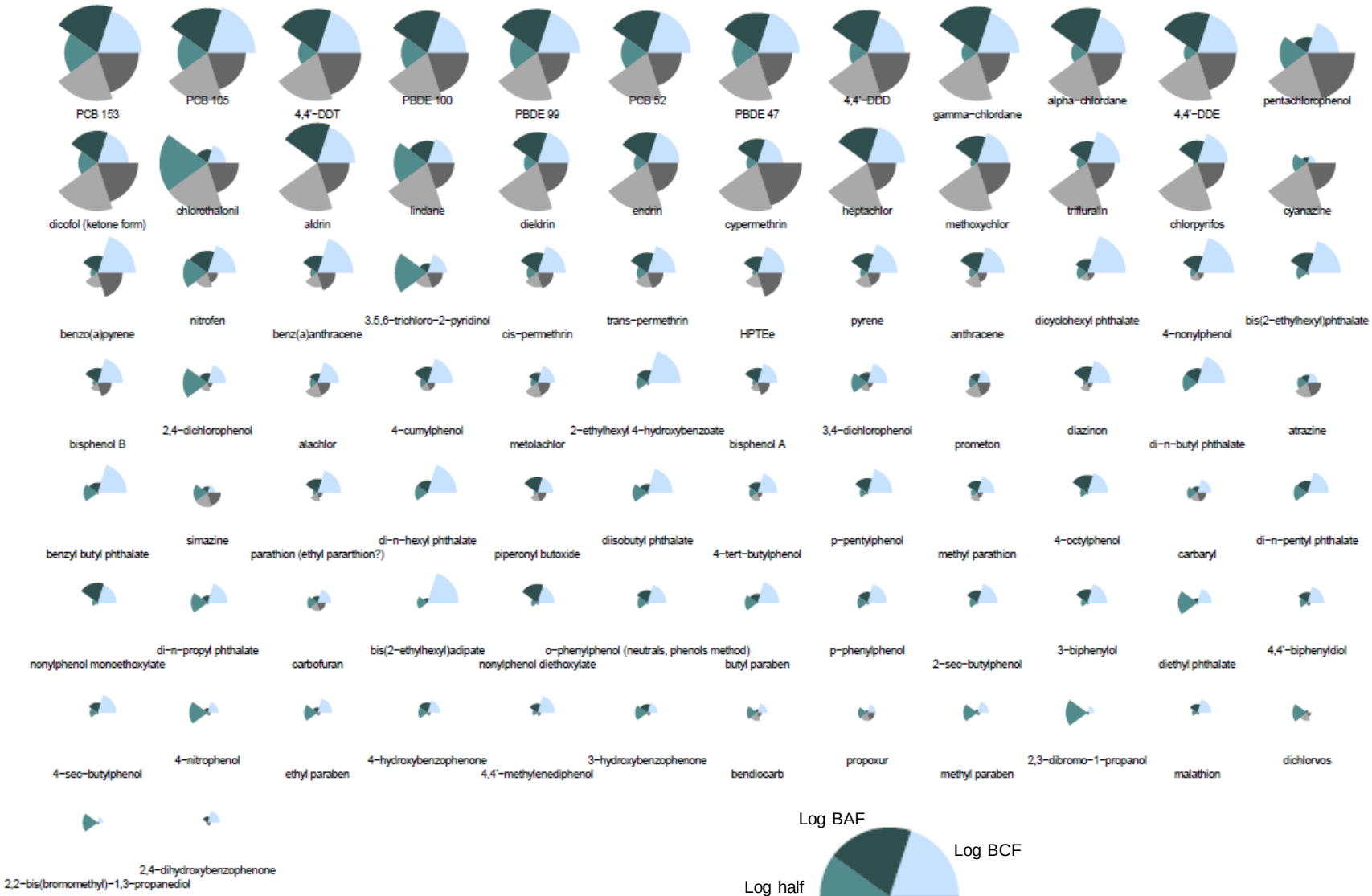
• 52 compounds detected
  • 23 pesticides
	• 66 compounds detected
	  • 27 pesticides

• 13 to 28 compounds per home	• 6 to 42 compounds per home
• Most abundant chemicals include: phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant), 4-nonylphenol (detergent metabolite) and 4-tert-butylphenol (adhesive) • Typical concentrations in range of 50-1500 ng/m^3	• Penta- and tetrabrominated diphenyl ethers (flame retardants) frequently detected in dust; Most abundant pesticides include permethrins and synergist piperonyl butoxide

• 2,3-dibromo-1-propanol (carcinogenic intermediate of a flame retardant banned in 1977) detected in both air and dust

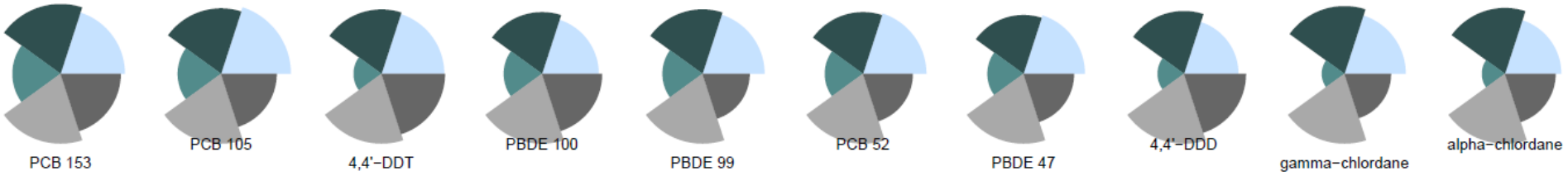
Rudel et al., 2003, *Environ. Sci. & Tech.*

Chemicals detected in indoor air and dust – ranking by BCF / BAF / Persistence

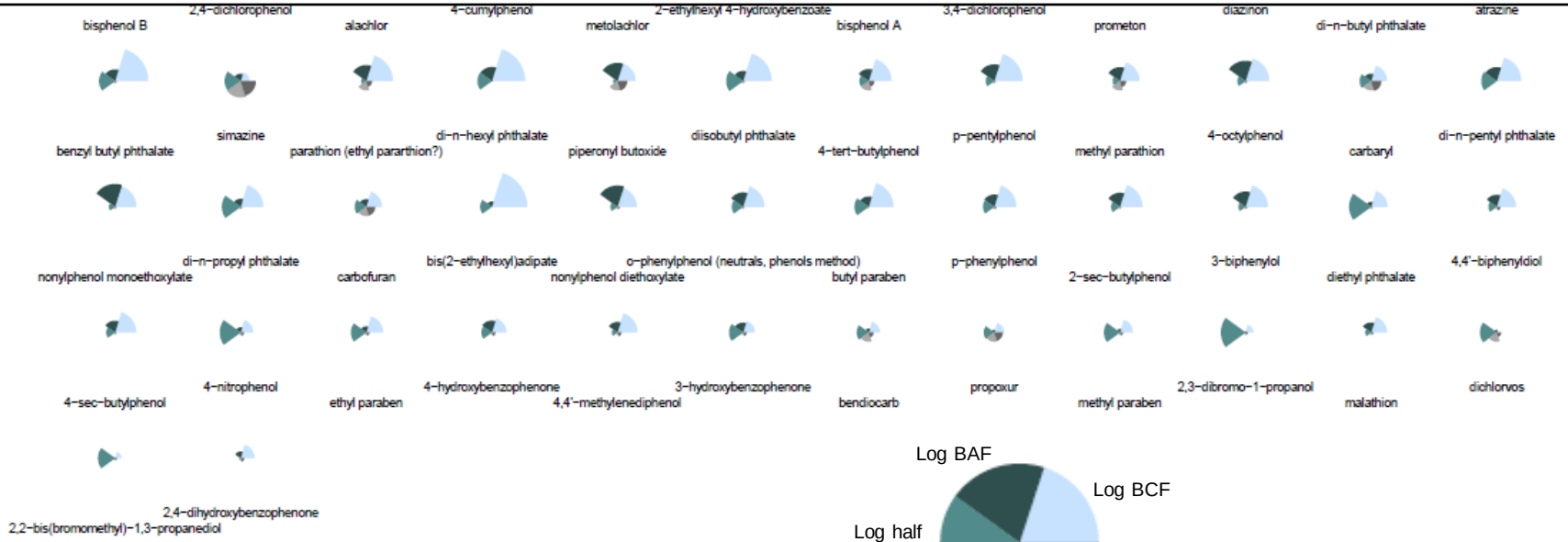


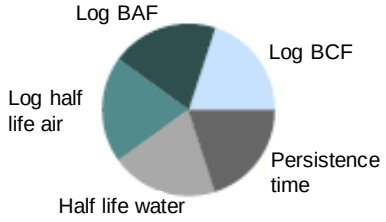
Chemicals detected in indoor air and dust – ranking by BCF / BAF / Persistence

Top 10 chemicals ranked by environmental fate parameters



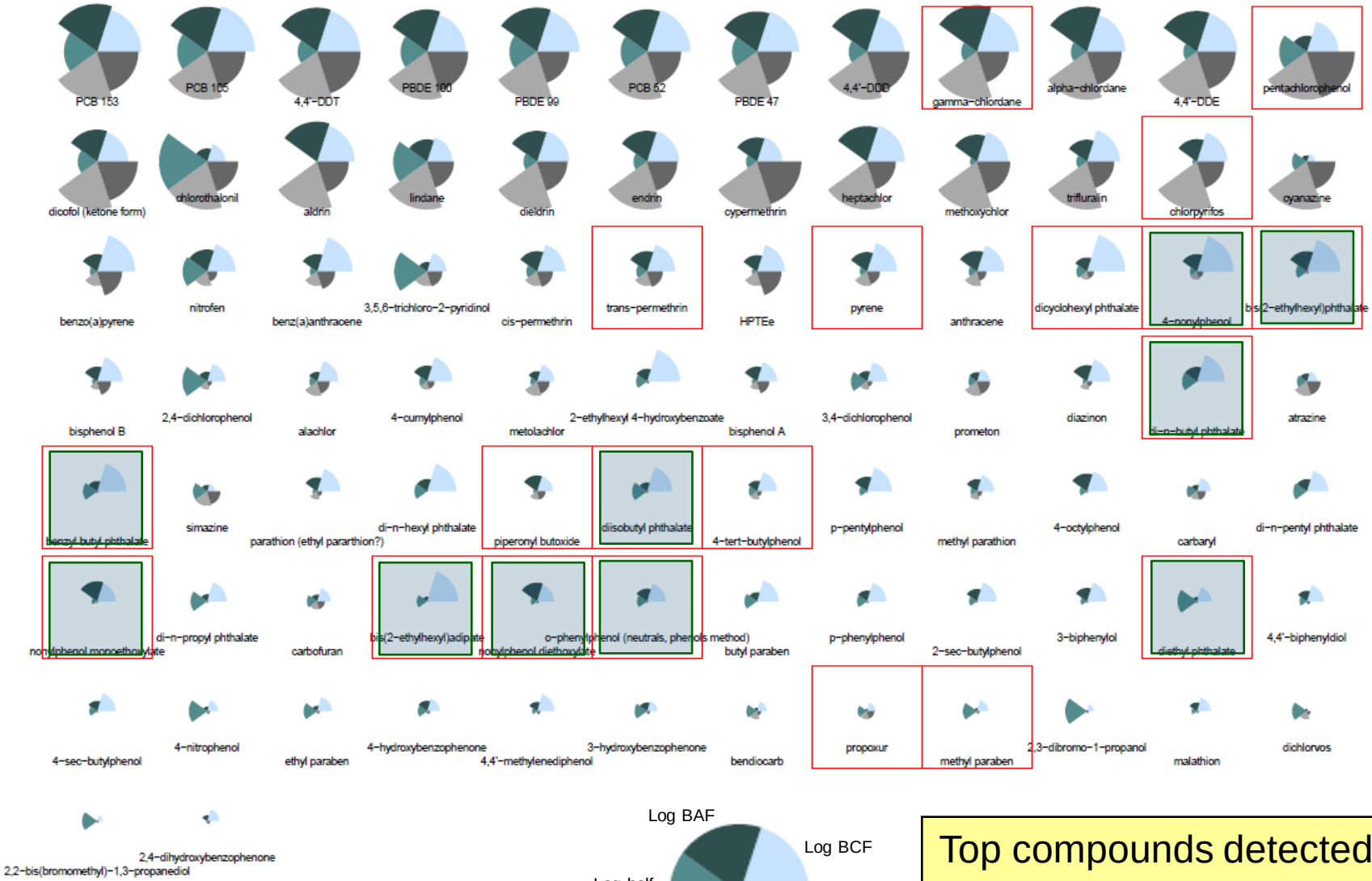
. . . How does this ranking compare to what is detected in air and dust?

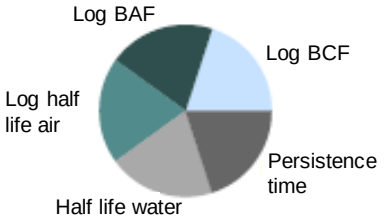




Chemicals detected in indoor air and dust

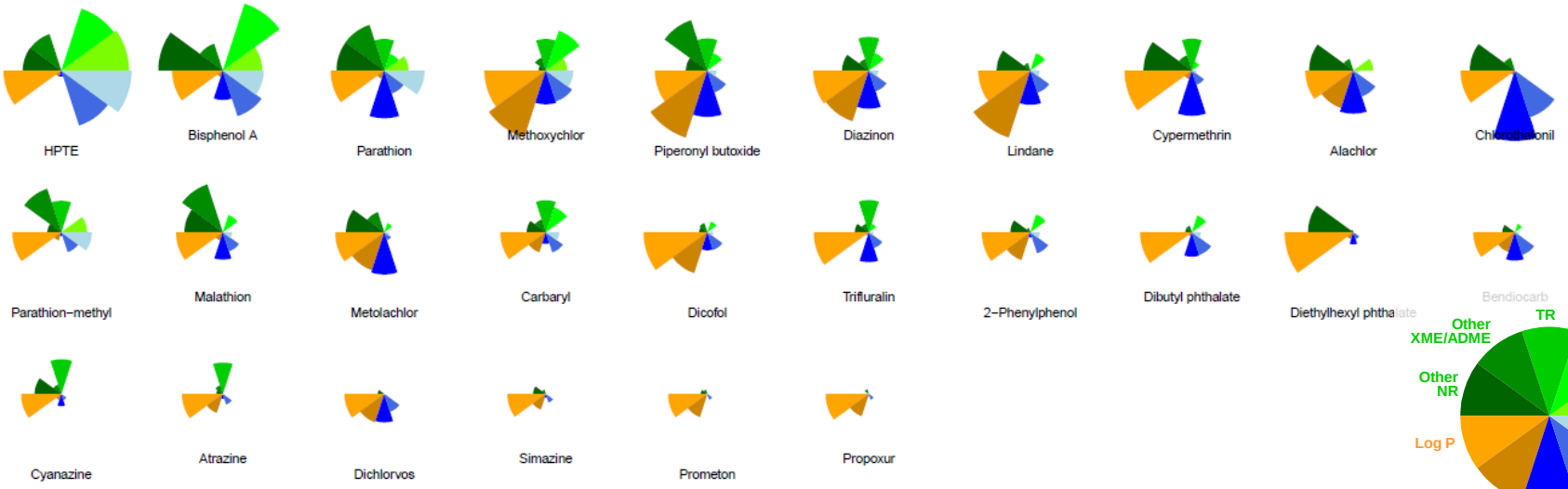
(top 20 concentrations highlighted by red boxes; top 10 conc. shaded)

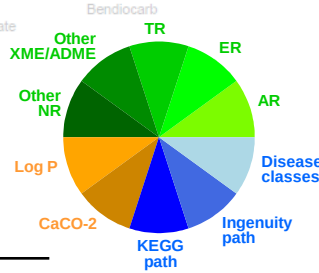




Top compounds detected in air/dust may not be prioritized by env. fate parameters

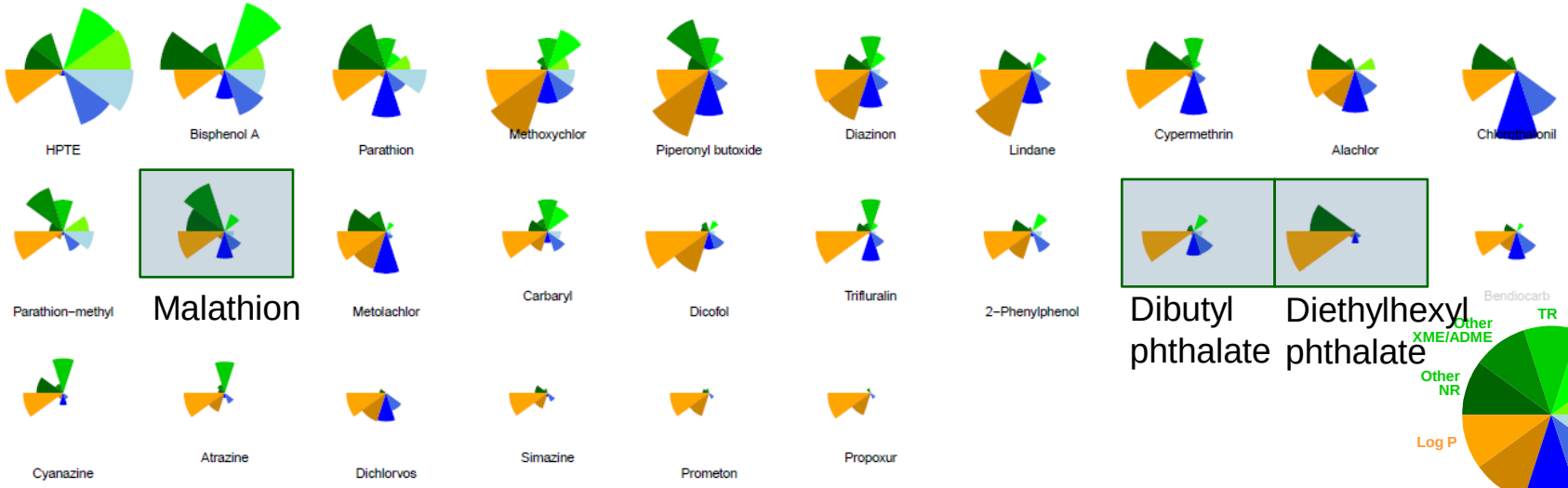
Indoor air and dust chemicals—Overlap with ToxCast Phase I chemicals

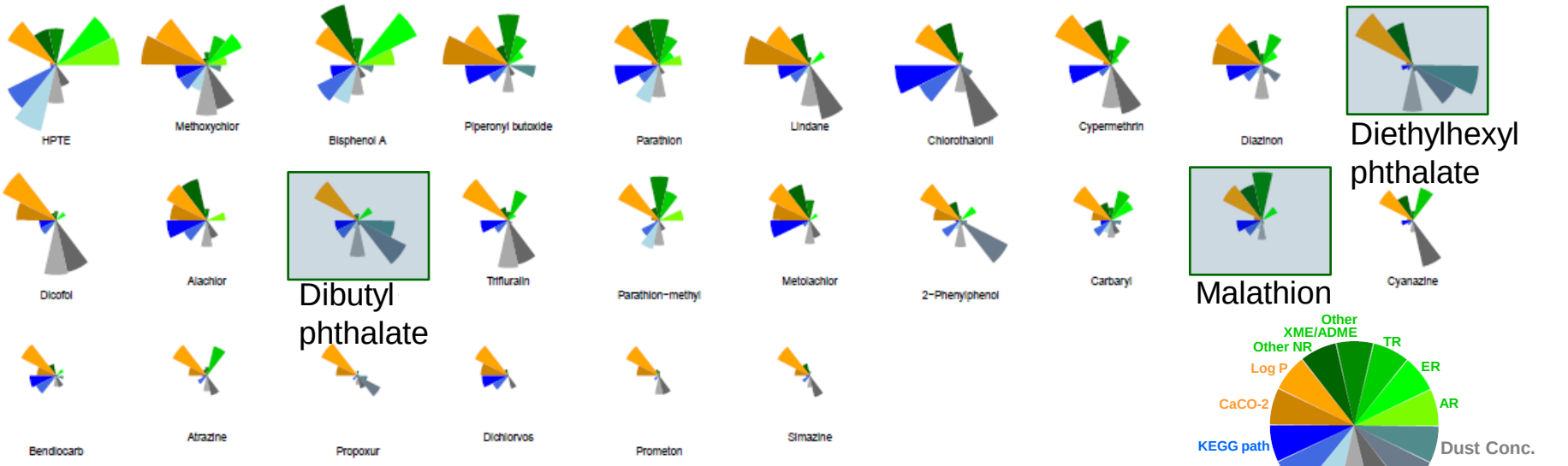




Indoor air and dust chemicals—Overlap with ToxCast Phase I chemicals

(shaded chemicals have moved 5 or more positions in ranking)





Conclusions

- ToxPi profiles provide transparent visualization of relative contribution of all info sources to an overall priority ranking.

- Toxicological data components selected based on putative endocrine relevance.
  → Method developed readily adaptable to diverse chemical prioritization tasks.

- Adding exposure domain changes ToxPi scores for ToxCast compounds.

- Environmental fate parameters may not prioritize key chemicals to which humans are exposed to indoors

- Future plans:
  - Incorporate other exposure metrics (manufacturing volumes, non-agricultural usage, measured data in food, biomonitoring data, etc.) into ToxPi analysis.

  - Perform value of information analysis. Weight slices differently or remove slices.

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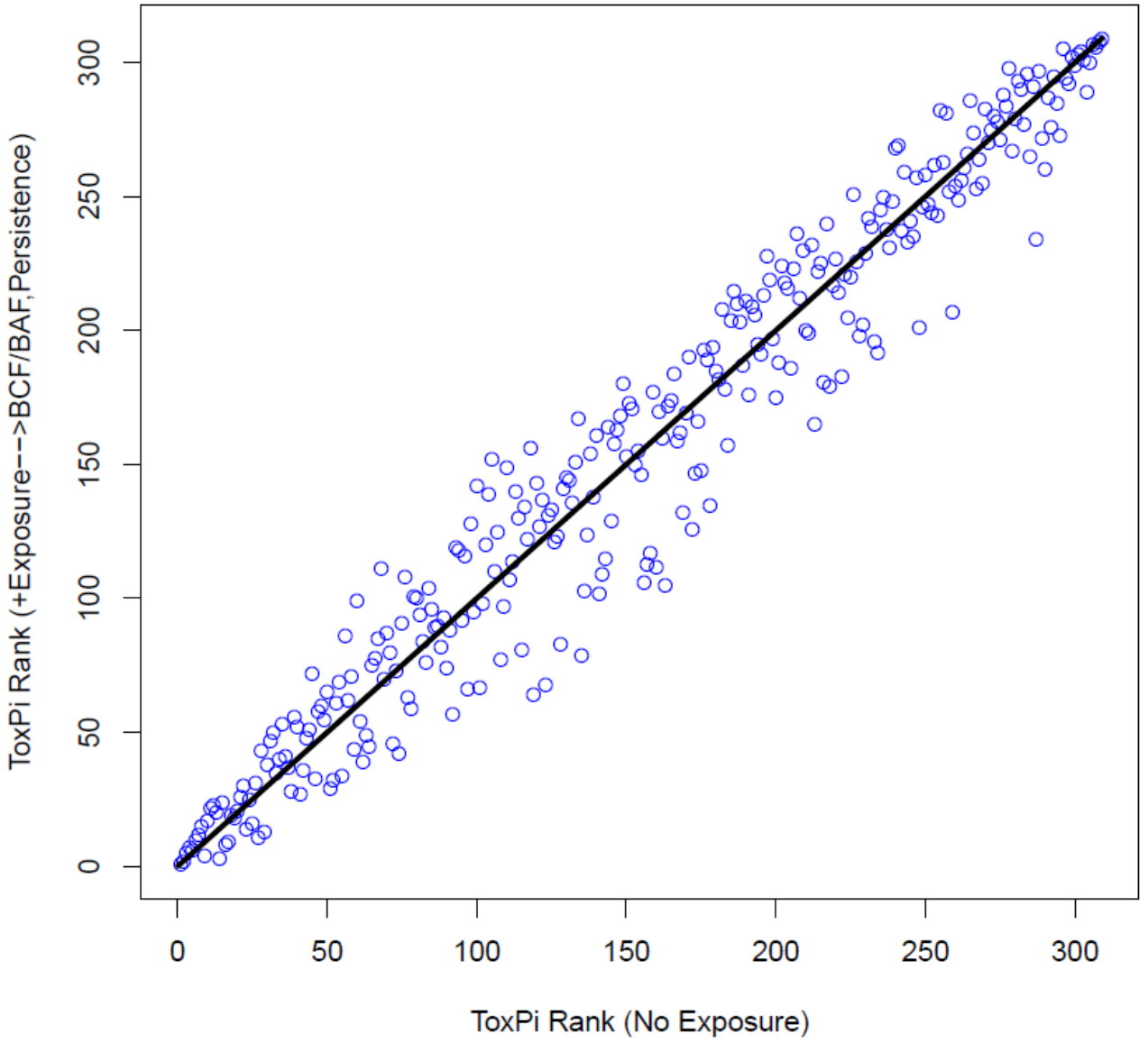
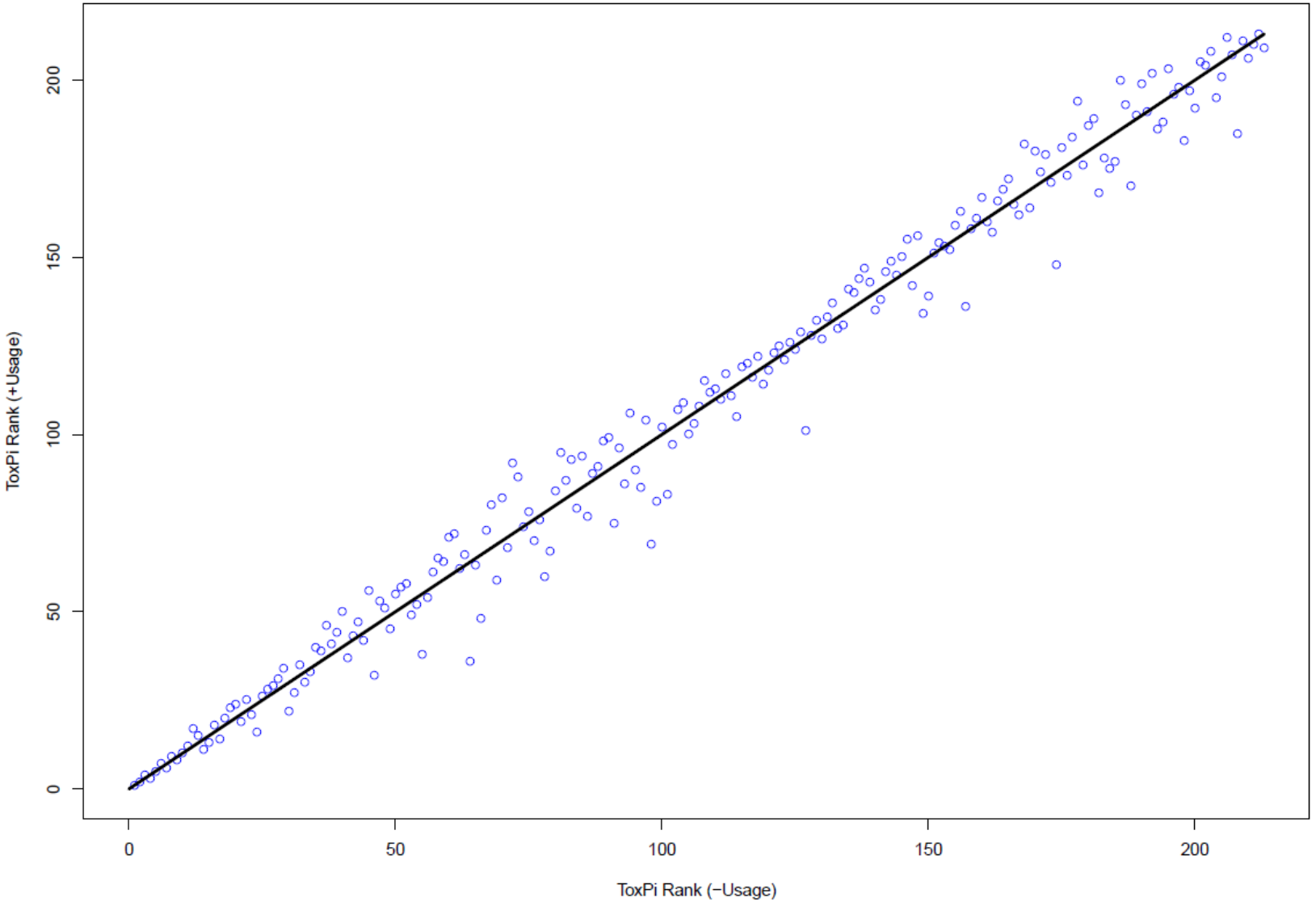
…plus many more EPA colleagues that are participating in the continued development of this project

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

Change in ToxPI ranking after adding environmental fate metrics

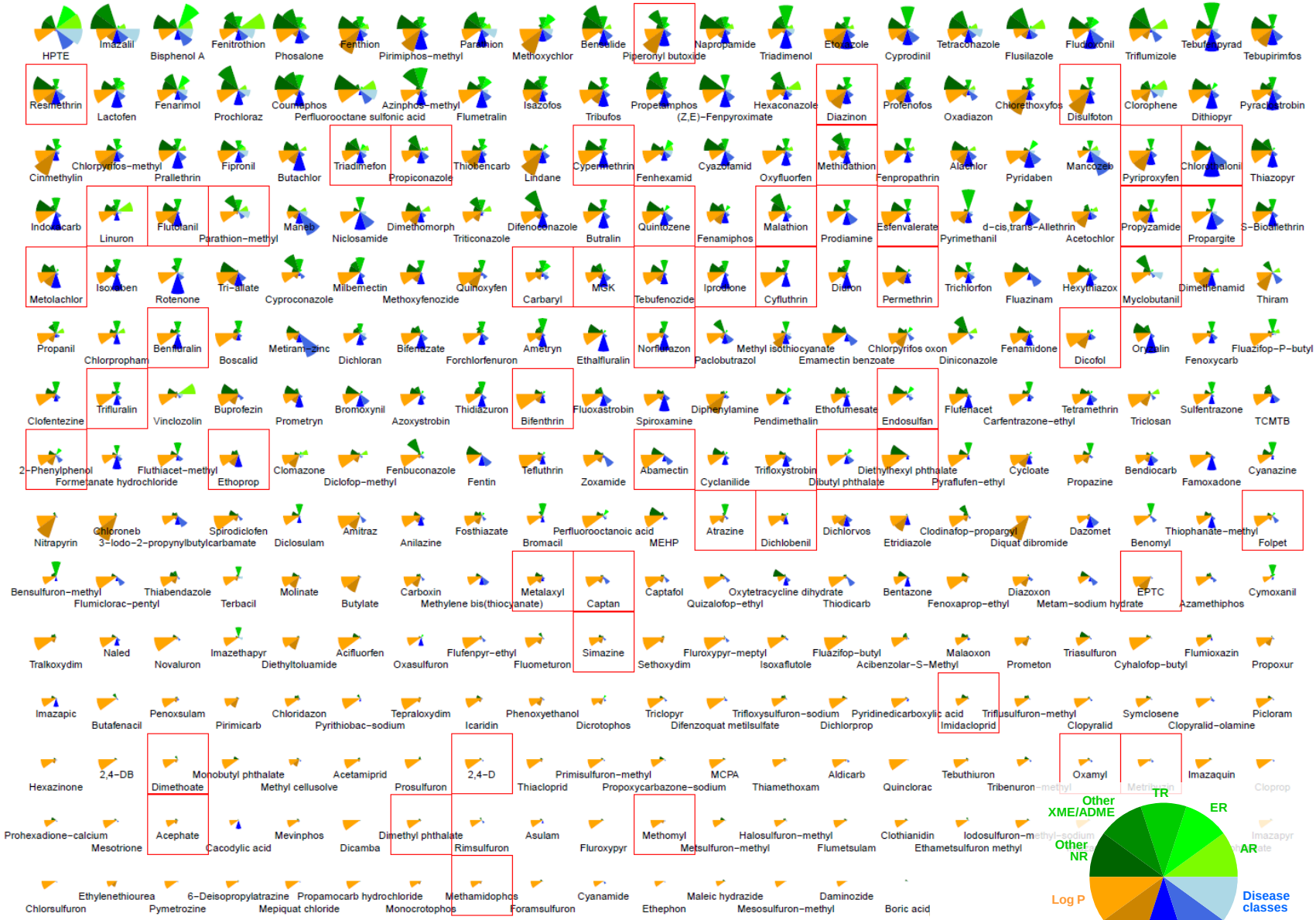


Change in ToxPI ranking after adding agricultural usage data (subset of 309 chemicals)



Prioritization of ToxCast™ chemicals

(sorted by overall ToxPi endocrine score, highlight EDSP chemicals)



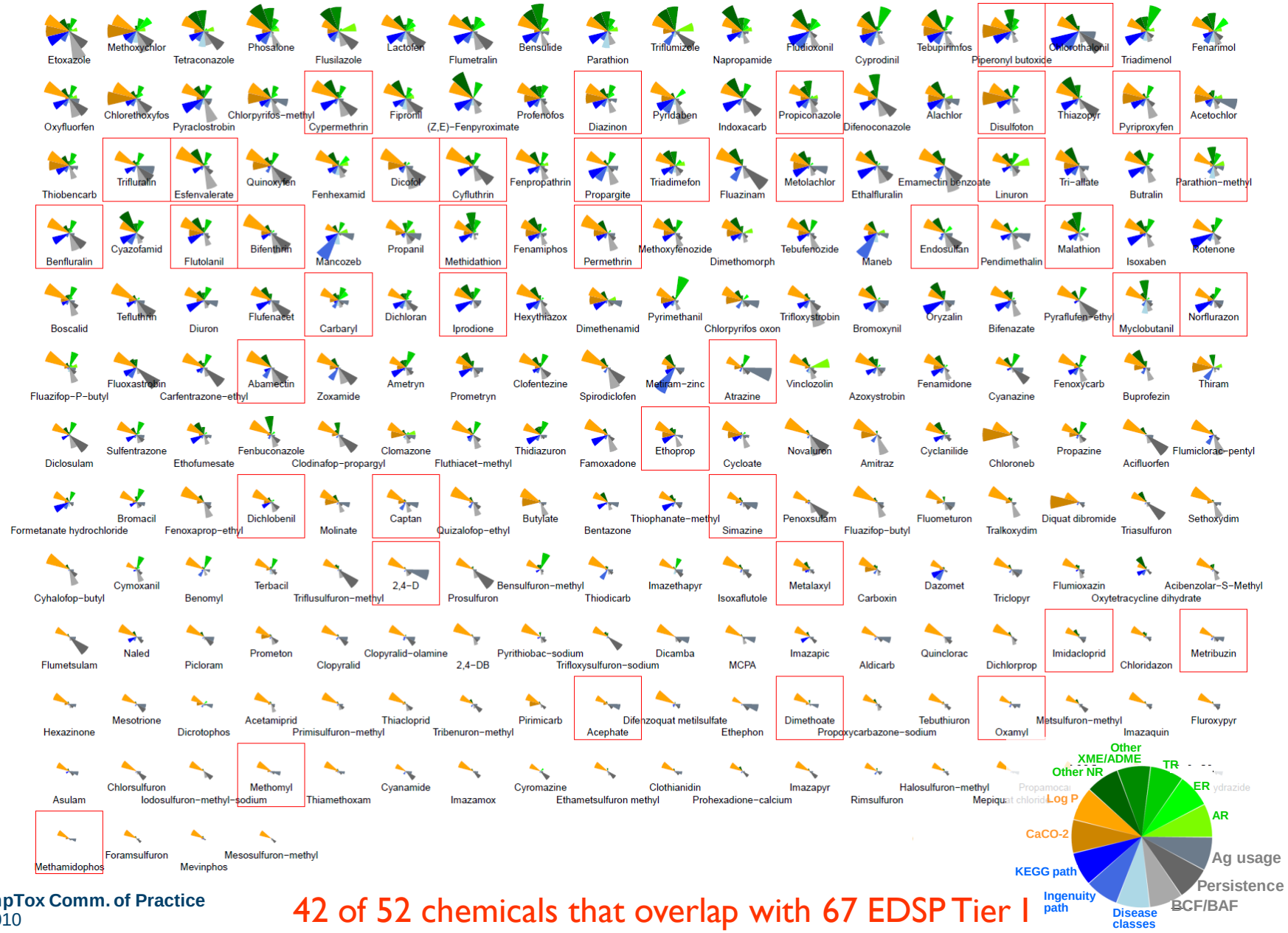
Overlap of 52 of 67 Tier I EPA's EDSP chemicals

Incorporating exposure information: Preliminary ToxPi endocrine scores (sorted by overall ToxPi score, highlight EDSP chemicals)

Overlap of 52 of 67 Tier I EPA's EDSP chemicals

Rankings after adding usage slice

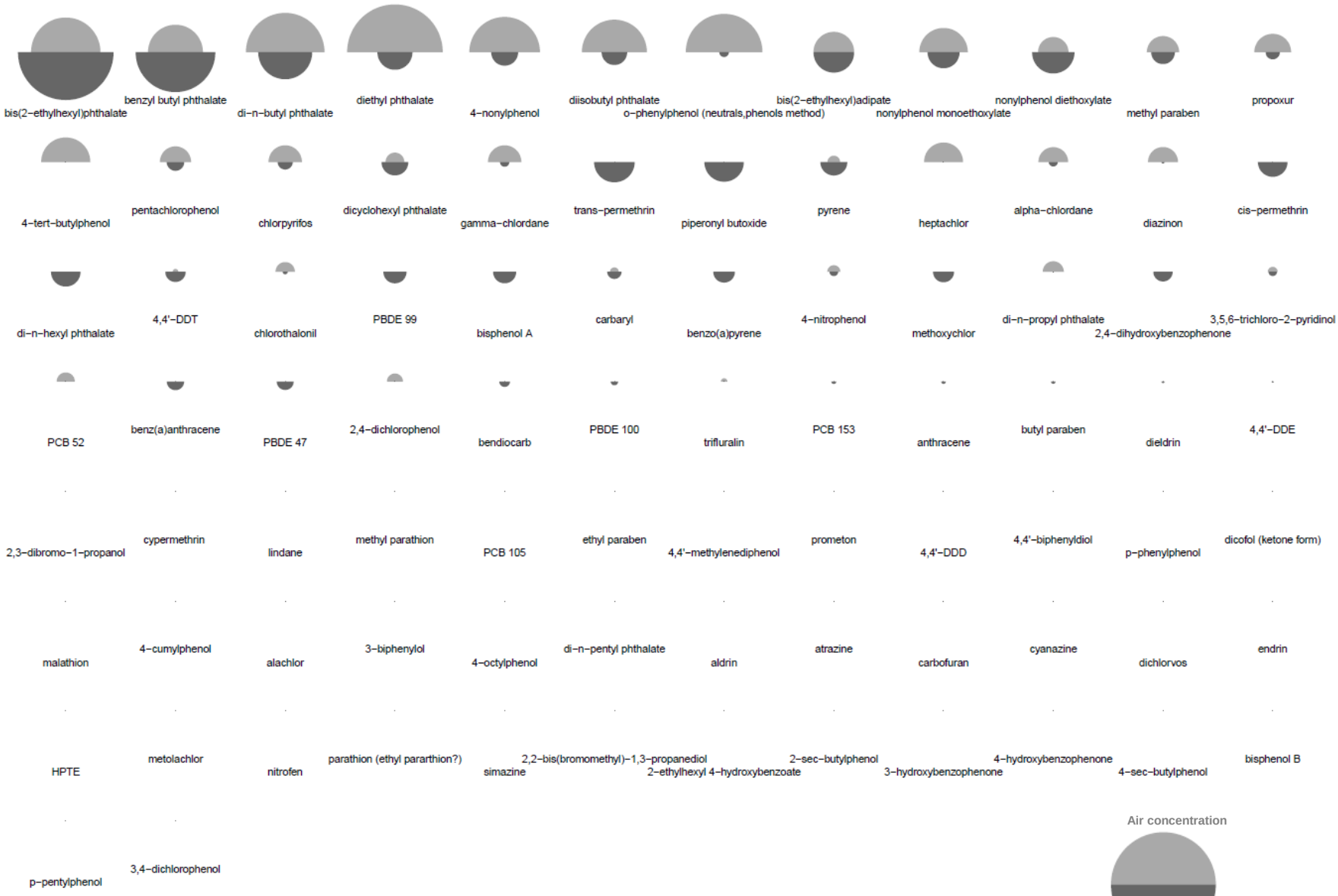
(highlight EDSP chemicals)



42 of 52 chemicals that overlap with 67 EDSP Tier I

Endocrine disrupting compounds in indoor air and dust

United States Environmental Protection Agency



Based on data from Rudel et al., 2003, Environ. Sci. & Tech.