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

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Traditional toxicology testing paradigm needs to change because . . .

Too Many Chemicals

![Graph showing data collection for various categories such as IRIS, TRI, Pesticide Actives, CCL 1&2, Pesticide Inerts, HPV, MPV Current, MPV Historical, and TSCA Inventory.](image)

Too High a Cost

![Mouse data collection and millions of dollars spent on different types of toxicology, including Cancer, DevTox, NeuroTox, ReproTox, ImmunoTox, and PulmonaryTox.](image)

...and not enough data.

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

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.

ToxPi (Toxicological Priority Index)

- In vitro assays (ToxCast)
- Chemical properties (descriptors)
- Pathways (endocrine)

Putative endocrine profiles

Reif et al., 2010, submitted
Rationale for an integrated chemical prioritization scheme

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ToxPi (Toxicological Priority Index)

Chemical properties

Pathways

In vitro assays

Putative endocrine profiles

Reif et al., 2010, submitted
Definitions & notation

Slice: “Pie” slices representing individual components or aggregations of multiple related components.

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

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

ToxPi = \( f(\text{In vitro assays} + \text{Chemical properties} + \text{Pathways}) \)

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

Reif et al., 2010, submitted
Interpreting ToxPis for individual chemicals

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

36 assays
1 technology

38 assays
4 technologies

Calculated using LeadScope software

Calculated using QikProp software

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Prioritization of ToxCast™ chemicals
(sorted by overall ToxPi endocrine score)
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)
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.
  - Environment Canada: Identifies existing substances from Domestic Substances List that are persistent or bioaccumulative to non-human species.
    - Canadian Environmental Protection Act (CEPA), 1999
      [Link](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

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![Graph of BCF (L/kg wet-wt) vs. # of Chemicals for ToxCast Phase I](image1)

![Graph of log BCF vs. # of Chemicals for ToxCast Phase I](image2)
Source for preliminary exposure data of environmental fate

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  - **Persistence** (half life air, half life water, persistence time) from Level III fugacity model, BIOWIN, AOPWIN programs

- 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

Bisphenol A

Perfluorooctane sulfonic acid (PFOS)

Log P
ER
TR
Other NR
AR
Other XME/ADME
Persistence
BCF/BAF
CaCO-2
KEGG path
Ingenuity path
Disease classes

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• 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)
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)
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.

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

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

• 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

- Analyzed 89 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

<table>
<thead>
<tr>
<th>CHEMICAL CLASS</th>
<th>SOURCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalates</td>
<td>Plastics, adhesives, personal care products, and other sources</td>
</tr>
<tr>
<td>Alkylphenols</td>
<td>Surfactants in cleaners, inerts in pesticides, personal care products, plastics, and other sources</td>
</tr>
<tr>
<td>Pesticides, pesticide metabolites</td>
<td>Pesticides</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons (PAHs)</td>
<td>Products of combustion</td>
</tr>
<tr>
<td>Parabens</td>
<td>Personal care products and other sources</td>
</tr>
<tr>
<td>Phenolics (e.g., bisphenol A)</td>
<td>Plastics, personal care products, and other sources</td>
</tr>
<tr>
<td>Miscellaneous (e.g., dichlorophenol, nitrophenol)</td>
<td>Miscellaneous household products</td>
</tr>
<tr>
<td>Polychlorinated biphenyls (PCBs)</td>
<td>Electrical equipment</td>
</tr>
</tbody>
</table>

EDC compounds detected in indoor air and dust

- Of 89 compounds analyzed in 120 homes:

<table>
<thead>
<tr>
<th>Indoor Air</th>
<th>Household dust</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 52 compounds detected</td>
<td>• 66 compounds detected</td>
</tr>
<tr>
<td>• 23 pesticides</td>
<td>• 27 pesticides</td>
</tr>
<tr>
<td>• 13 to 28 compounds per home</td>
<td>• 6 to 42 compounds per home</td>
</tr>
<tr>
<td>• Most abundant chemicals include:</td>
<td>• Penta- and tetrabrominated diphenyl ethers (flame retardants) frequently</td>
</tr>
<tr>
<td>phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant),</td>
<td>detected in dust; Most abundant pesticides include permethrins and</td>
</tr>
<tr>
<td>4-nonylphenol (detergent metabolite) and 4-tert-butylphenol (adhesive)</td>
<td>synergist piperonyl butoxide</td>
</tr>
<tr>
<td>• Typical concentrations in range of 50-1500 ng/m³</td>
<td></td>
</tr>
<tr>
<td>• 2,3-dibromo-1-propanol (carcinogenic intermediate of a flame retardant</td>
<td></td>
</tr>
<tr>
<td>banned in 1977) detected in both air and dust</td>
<td></td>
</tr>
</tbody>
</table>

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

PCB 153  PCB 105  4,4’-DDT  PBDE 100  PBDE 99  PCB 52  PBDE 47  4,4’-DDD  gamma-chlordane  alpha-chlordane

... 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

- HPET
- Bisphenol A
- Parathion
- Methoxychlor
- Piperonyl butoxide
- Diazinon
- Lindane
- Cypermethrin
- Alachlor
- Chlorpyrifos

- Parathion-methyl
- Malathion
- Metolachlor
- Carbaryl
- Dicofol
- Trifuralin
- 2-Phenethylphenol
- Dibutyl phthalate
- Diethylhexyl phthalate

- Cyanazine
- Atrazine
- Dichlorvos
- Simazine
- Propoxur

- Bendiocarb
- XME/ADME
- Other

- Log P
- Disease classes

- TROther
- NR

- Other

- KEGG path

- Ingenuity path

- Indoor Air Conc.

- Dust Conc.
Indoor air and dust chemicals—Overlap with ToxCast Phase I chemicals

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

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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

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