Developing Methods Using ToxCast Data for the Classification and Prioritization of Antimicrobials and Inerts

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Abstract

Improved chemical risk management and increased efficiency of chemical prioritization, classification and assessment are major goals within EPA. Towards achieving these goals, EPA's ToxCast™ research program has been designed to rapidly screen hundreds to thousands of chemicals' potential toxicity. In ToxCast, both antimicrobials and inert ingredients are being tested in high-throughput screening systems, 22 in Phase I. Antimicrobial pesticides are chemicals designed to kill or suppress the growth of harmful microorganisms in a variety of use settings, including inanimate objects and surfaces. In total, there are over 300 antimicrobial pesticide active ingredients. Roughly 100 antimicrobial pesticides have undergone re-registration via 41 REDs (re-registration eligibility decision documents), leaving over 200 antimicrobial pesticides requiring some form of hazard evaluation that could be provided by ToxCast. Inert ingredients are substances that are not active ingredients, but which are intentionally included in pesticide products. Limited toxicity data exists for thousands of inert (other) ingredients, creating a need to efficiently determine the potential toxicity of these chemicals. Through the use of ToxCast, toxicity potential has been modeled based on biological activity, pathway-based effects, and estimated dosimetry with a special focus on systemic, cancer, reproductive, and developmental effects. These predictive toxicity scores can then be considered and integrated into the decision process, based on the specific needs of the chemical programs, for classifying antimicrobials and inerts and prioritizing further toxicity testing. *This work does not necessarily reflect official Agency policy.*

Objectives

- *Generate master list from chemical sets of interest*
- *Identify & evaluate toxicity data coverage*
- *Generate physical chemical properties & limited chemical descriptor set for chemical sets*
- *Generate Cramer Classifications for chemical sets*
- *Using ToxCast_309, develop classification model for an endpoint of interest (example Toxicity Signature)*
- *Identify subset of chemicals in chemical sets with Tox21 data (bioactivity profiling data previously generated on 1000's of chemicals)*
- *Integrate data from Tox21 with ToxCast bioactivity profiling, based on assay-gene pairs important to the classification model*
- *Prospectively apply classification model, solely based on data from Tox21, to chemical sets of interest*

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Chemical Lists

Antimicrobials

Roughly 300 Total Chemicals

41 Reregistration Eligibility Documents (REDs) spanning roughly 100 antimicrobials

Master List Developed in Collaboration with ACC Biocides Panel

Toxicity Data Coverage:
- Limited Registration Studies
- Limited Open Literature Coverage
- Dependency on Data Bridging

Current Prioritization/Testing Strategies:
- Separating Food-use and Non-Food-Use
- Group Chemicals by Structural Similarity

Potential ToxCast Applications:
- Re-registration prioritization
- Biologically driven chemical groupings
- Application of Bioactivity Profiling Toxicity Signatures to Targeted Testing

Other Pesticidal Ingredients (Inerts)

Roughly 4500 Inerts

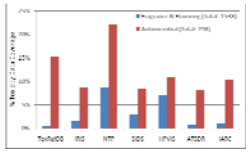
1538 identified as Flavorings or Fragrances

Toxicity Data Coverage:
- Limited to No Registration Studies
- Limited to No Open Literature Coverage

Current Prioritization/Testing Strategies:
- Limited use of QSAR models
- Use limited available information in categorical assessment
- Tackle recognizably safe chemicals 1st (e.g., GRAS)

Potential ToxCast Applications:
- Prioritization & Classification of Ingredients w/ particular emphasis on endocrine disrupting screening
- Biologically driven chemical groupings
- Application of Bioactivity Profiling Toxicity Signatures to Targeted Testing

Toxicity Data Coverage

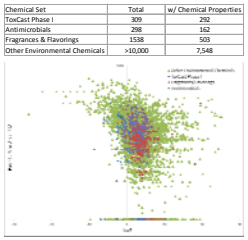


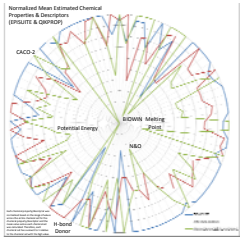
Physical/Chemical Properties

- Defining Chemical Space & Understand chemical difference between various chemical sets
- Use generated descriptors for QSAR approaches and optimizing & interrogating bioactivity profiling signatures
- Clear differences between ToxCast (primarily conventional active pesticides), antimicrobial pesticides, and inert (fragrances & flavorings) ingredients

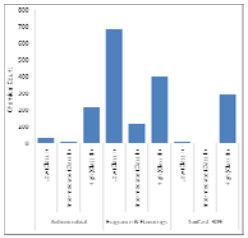
Challenges
- *EPISUITE and QIKPROP are not the only ways of describing chemicals or chemical space*
- *Difficult to globally assess chemical space in meaningful way*
- *Diverse numerical representations of chemical feature space (Estimated experimental values, counts, Boolean, probabilities, etc.)*

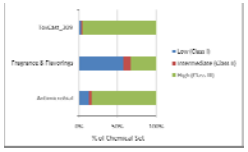
Chemical Set	Total	w/ Chemical Properties
ToxCast Phase I	309	292
Antimicrobials	298	162
Fragrances & Flavorings	1538	503
Other Environmental Chemicals	>10,000	7,548





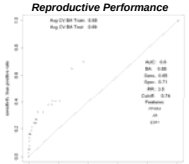
Cramer Class Distribution





- Cramer Class: Expert-derived Tree-based Method
- Compounds are grouped into 3 classes
- Class I –Substances with structures and related data which suggest a low order of oral toxicity
- Class II –Substances which are intermediate. Less innocuous than class I. Lack positive identification of toxicity
- Class III –Substances that permit no initial presumptions on safety or may suggest significant toxicity
- Cramer et al. 1978. Estimation of toxic hazard—a decision tree approach. Fd Cosmet Tox 16: 255.
- ToxCast 309: Generally Class III
- Fragrances & Flavorings: Balanced between Class I & Class III
- Antimicrobials: Generally Class III
- Difficult to use for prioritization due to the large number of Class III chemicals
- Additional research is needed to further refine Class III

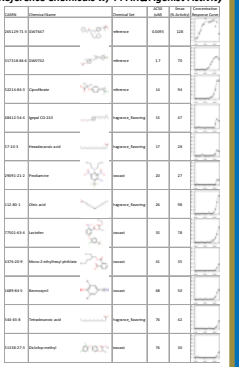
ToxCast Signature Based Classification

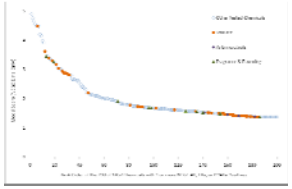


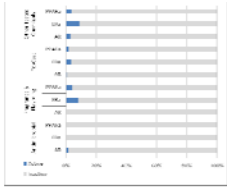
- Simple linear model of AR, ERa, and PPARa activity across 5, 6, and 3 assays, respectively (See Poster #113; Abstract #96)
- Each target gene has at least 1 NCGC (Tox21) assay, which was run on 1462 chemicals, including 98 fragrances & flavorings and 67 antimicrobials
- *Note: Significant loss of information in using single assay source and technology*
- *Note: Majority of antagonist data yet to be analyzed for full chemical set (challenges in distinguishing between cytotoxicity and antagonist activity)*
- Applied Model (NCGC Agonists Assays Only) to 1462 chemical set *Disclaimer: data presented is from a preliminary screening effort, analysis is ongoing and an associated manuscript is in preparation*

GENE	ASSAY COMPONENT
AR	ATG_AR_TRANS
	NCGC_AR_Agonist
	NCGC_AR_Antagonist
	NVS_NR_hAR
	NVS_NR_rAR
ESR1	ATG_ERa_TRANS
	ATG_ERE_CIS
	NCGC_ERalpha_Agonist
	NCGC_ERalpha_Antagonist
	NVS_NR_bER
	NVS_NR_hER
PPARA	ATG_PPARa_TRANS
	NCGC_PPARa_Agonist
	NVS_NR_hPPARa

ToxCast, Fragrance & Flavoring, and Select Reference Chemicals w/ PPARα Agonist Activity

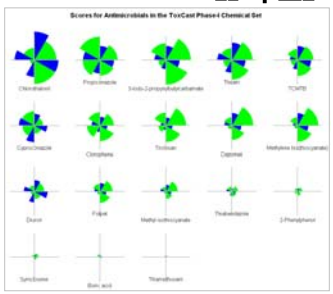




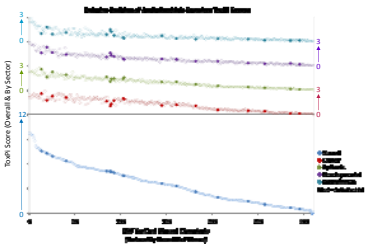


- Little to No Significant AR agonist activity
- Identified set of partial PPARα agonists
- Confirmation of ERα active compounds in progress
- Preliminary results indicate a small fraction of these chemicals are active across AR,ERα, PPARα
- Further Tox21 efforts will include replicate chemicals, additional assays, and targeted testing confirmation

ToxPi (Toxicological Prioritization Index)



- ToxPi: Toxicological Prioritization Index (See Poster #414; Abstract #198)
- 18 antimicrobial pesticides in ToxCast Phase I Chemical Set
- Flexibly incorporates additional information from multiple domains, including QSAR predictions and exposure
- Flexibility to customize components, slices, sectors, and domains for diverse prioritization tasks specific to each regulatory chemical program



Conclusions & Future Directions

- *Need for quality chemoinformatics, including chemical structure annotation (e.g., DSSTox)*
- *Need for capturing in vivo toxicity data on the limited number of chemicals with such data (e.g., ToxRefDB)*
- *Identified generic differences across the various chemical libraries of interest*
- *Cramer Classifications can be useful for high-level categorization, but require additional refinement to inform prioritization decisions*
- *Simple, linear models predictive of specific classes of endpoints enables interrogation and translation of the model leading to more informed prioritization and targeted testing decisions (i.e., not a black box)*
- *Preliminary screening data on 1462 chemical supports need for multiple assays for critical targets (e.g., ERa, AR, PPAR)*
- *ToxPi is a flexible tool for displaying and quantifying diverse information*
- *ToxPi is a tool empowering specific chemical programs to use new data in current prioritization decisions*
- *With ToxCast Phase II and Tox21, large quantities of data will be generated on these and other chemical sets*

Building a scientific foundation for sound environmental decisions