

Using ToxCast high-throughput *in vitro* screening to evaluate food-use chemicals

Agnes Karmaus, PhD

Integrated Laboratory Systems, Inc.

akarmaus@ils-inc.com

Goals

Compile a comprehensive inventory of food-use chemicals in the USA

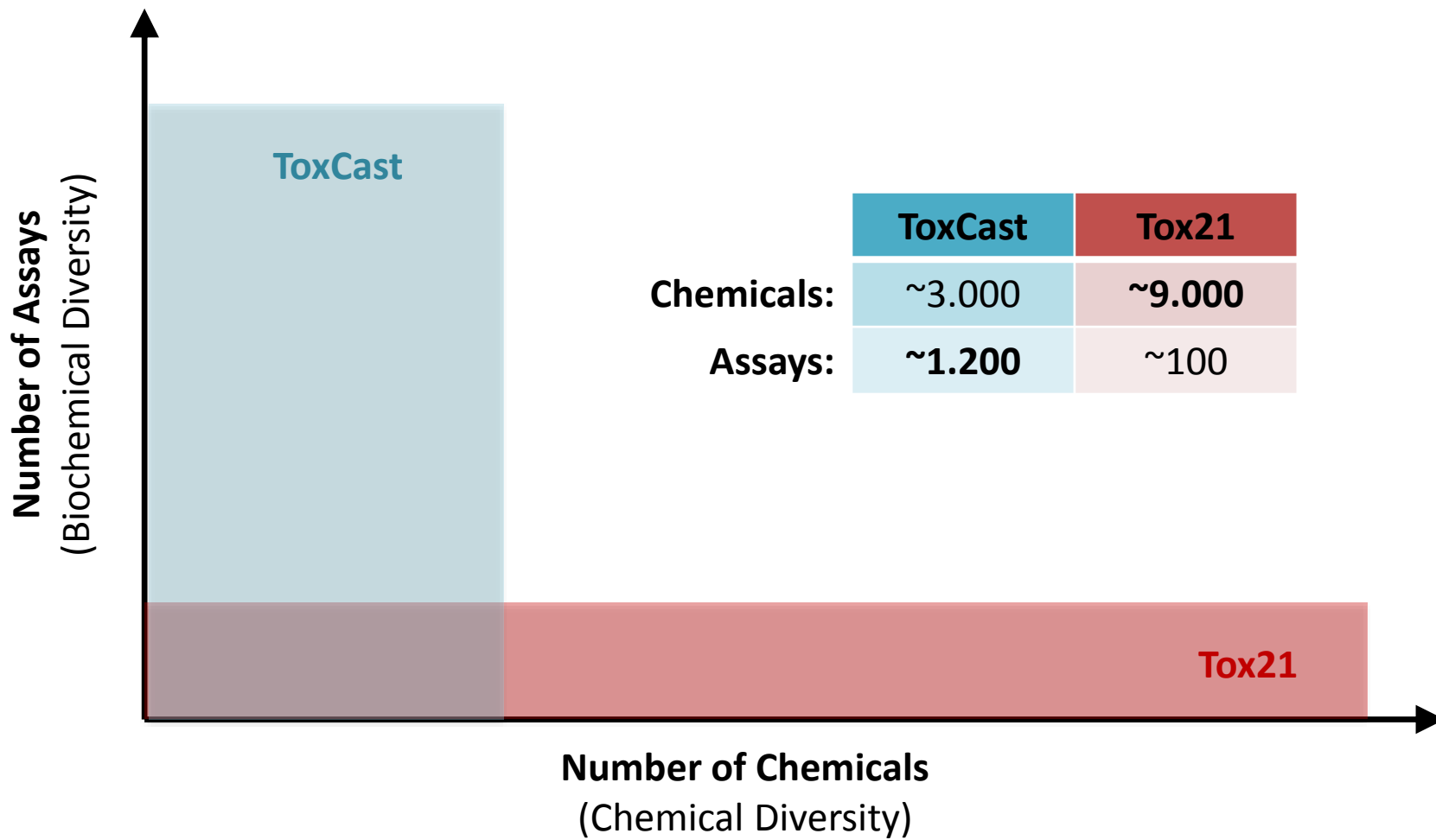
- Apply cheminformatics to evaluate the chemical landscape
- Integrate across ToxCast *in vitro* assays to evaluate bioactivity

Tox21 & ToxCast Programs

- US federal partnership (EPA, NIH, FDA)
- ~1.200 *in vitro* high-throughput assays
- ~9.000 chemicals evaluated in concentration-response
- ALL DATA ARE PUBLIC AND FREELY AVAILABLE

<http://actor.epa.gov/dashboard>

High-Throughput Screening Programs



Overview

1. Identification of food-relevant chemicals
 - Compilation of a comprehensive inventory
 - Cheminformatics evaluation of chemical diversity
 - Categorization of chemicals based on use
2. Food-relevant chemicals in ToxCast
 - Coverage of food-use chemicals in ToxCast
 - Activity of food-use chemicals across ToxCast
 - Cytotoxicity elicited by food-use chemicals

Chemical Inventories

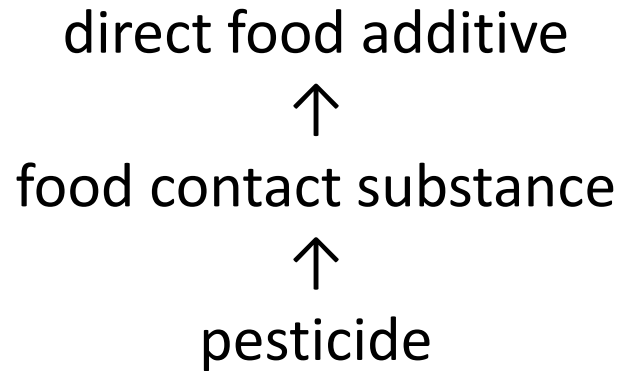
Inventory Source	Entries in Inventory	CASRN in Inventory	Use Category
FDA EAFUS	3968	3277	Direct Food Additives
FDA SCOGS	378	320	
FDA GRAS Notices	603	349	
FEMA GRAS	2796	2659	
FDA Effective FCS	1205	715	Food Contact Substances
FDA Indirect in FCS	3229	2555	
FDA TOR	50	56	
Alan Wood Pesticides	1813	1808	Pesticides
TOTAL	14,042	11,733	

Chemicals were grouped into "Use Categories" based on the database of origin

FDA: United States Food and Drug Administration
 FEMA: Flavour and Extract Manufacturer's Association

Chemical Inventories

Chemicals were restricted to only one use category, with the hierarchy:



Use Category	CASRN in Use Category
Direct Food Additives	3888
Food Contact Substances	3039
Pesticides	1732
	8,659

Cheminformatics: Fingerprinting

Two sources for substructure and physchem property descriptors (fingerprint bits) used:

- 881 PubChem fingerprints
- 166 MACCS fingerprints

Example of PubChem Fingerprints:

<u>Bit Position</u>	<u>Bit Substructure</u>
134	>= 1 unsaturated non-aromatic nitrogen-containing ring size 4
135	>= 1 unsaturated non-aromatic heteroatom-containing ring size 4
136	>= 2 any ring size 4
137	>= 2 saturated or aromatic carbon-only ring size 4
138	>= 2 saturated or aromatic nitrogen-containing ring size 4
139	>= 2 saturated or aromatic heteroatom-containing ring size 4
140	>= 2 unsaturated non-aromatic carbon-only ring size 4
141	>= 2 unsaturated non-aromatic nitrogen-containing ring size 4
142	>= 2 unsaturated non-aromatic heteroatom-containing ring size 4

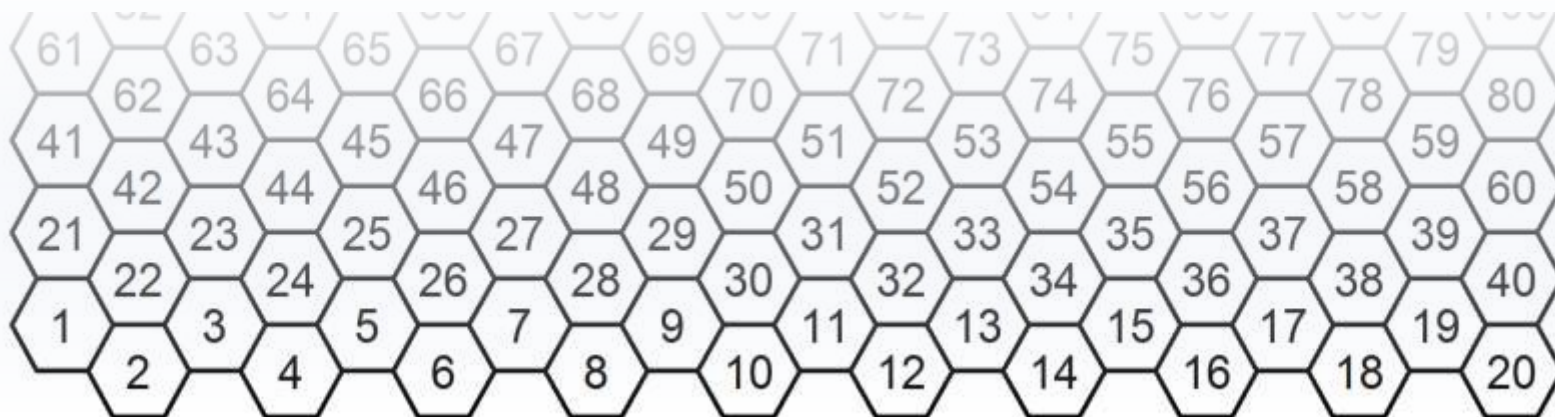
Self Organizing Map (SOM)

Algorithm trained using unsupervised learning where **samples are grouped based on similarity** into bins.

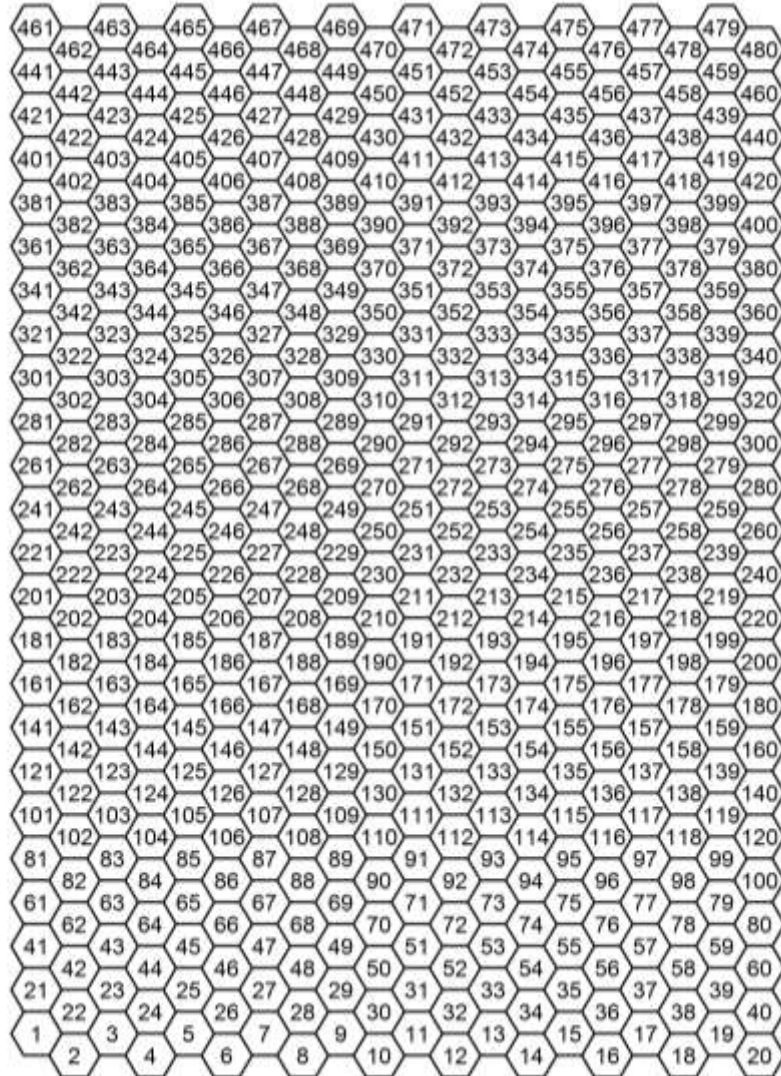
Bins are then laid out so that those most alike are nearest one another.

Number of bins is defined by the user

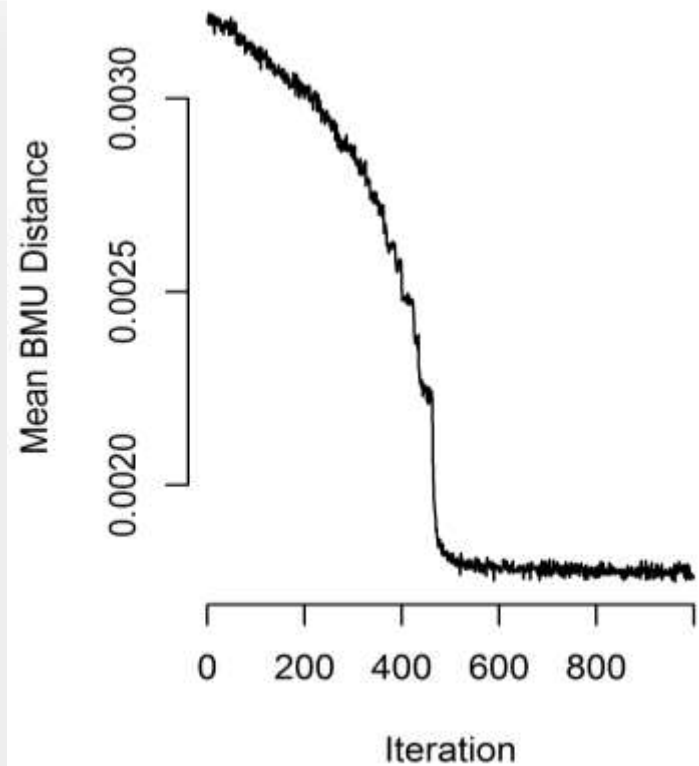
Similarity was based on chemical fingerprints



SOM Results for Food-Use Chemicals

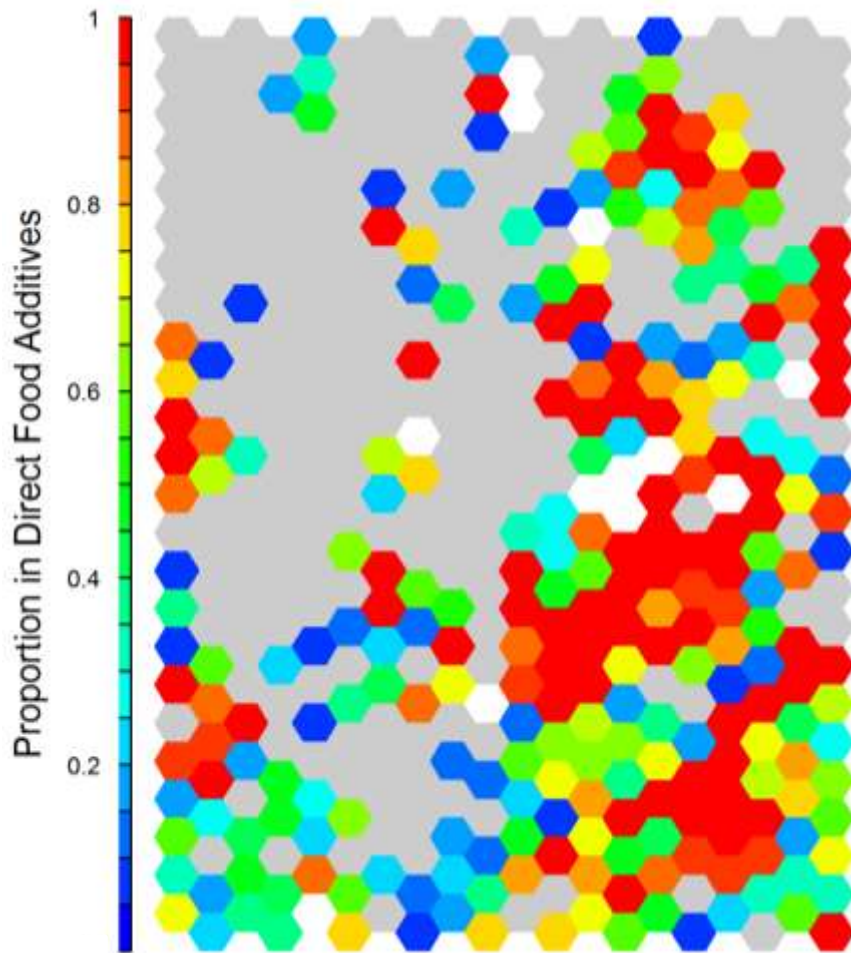


20 x 24 bin layout used, aiming for an *average* of 10 chemicals per bin



SOM Results for Food-Use Chemicals

Direct Food Additives



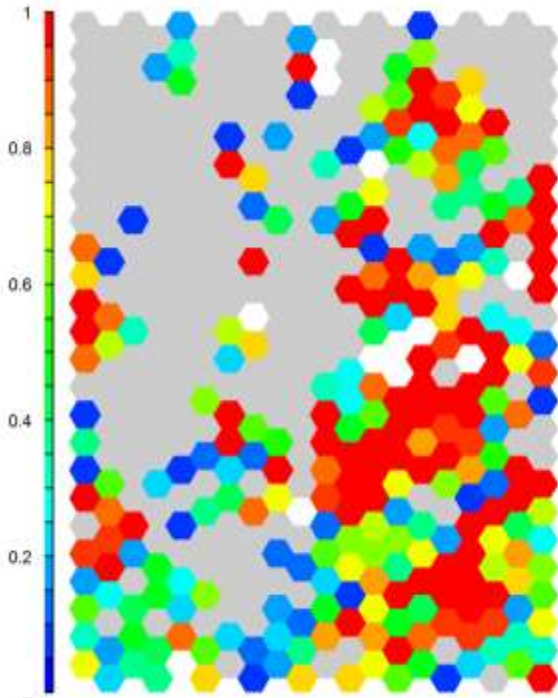
12 bins had zero chemicals (white)

Highlighted to visualize the proportion of chemicals in the bin that are direct food additives

SOM Results for Food-Use Chemicals

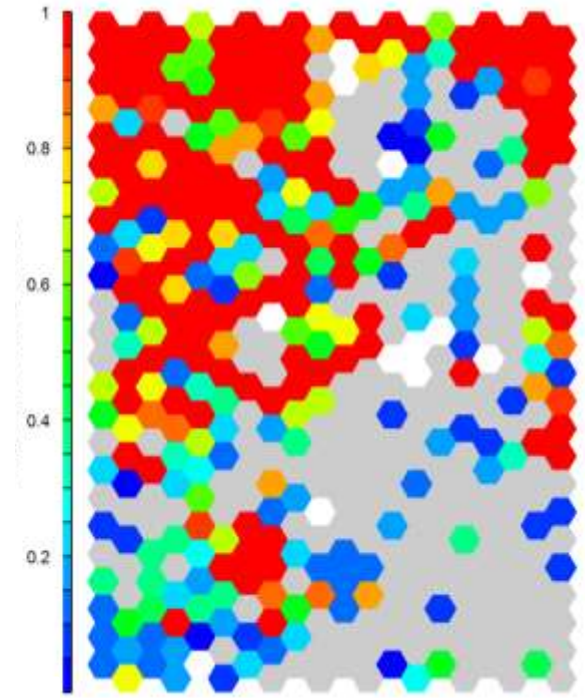
Proportion of chemicals per bin
obtained from direct additive vs. pesticide resource

Direct Food Additives



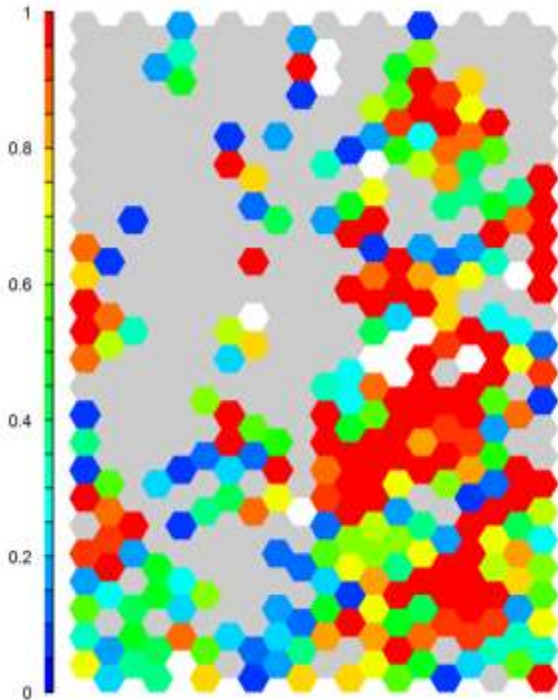
Clear separation
between bins
comprised solely of
direct food additives
versus
pesticides

Pesticides

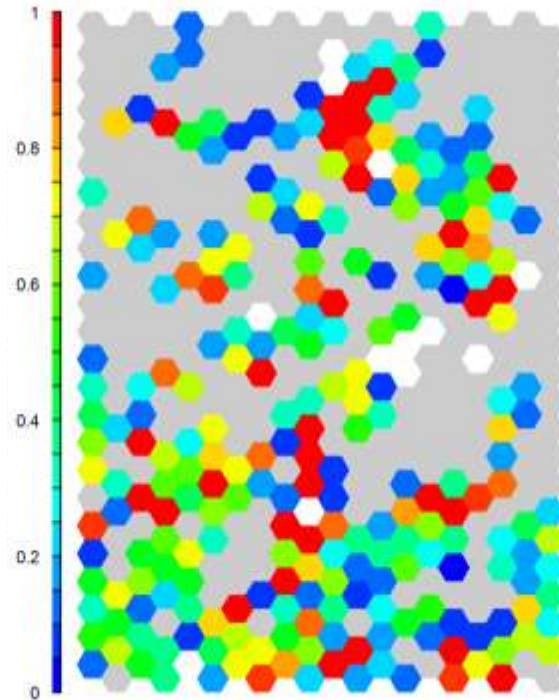


SOM Results for Food-Use Chemicals

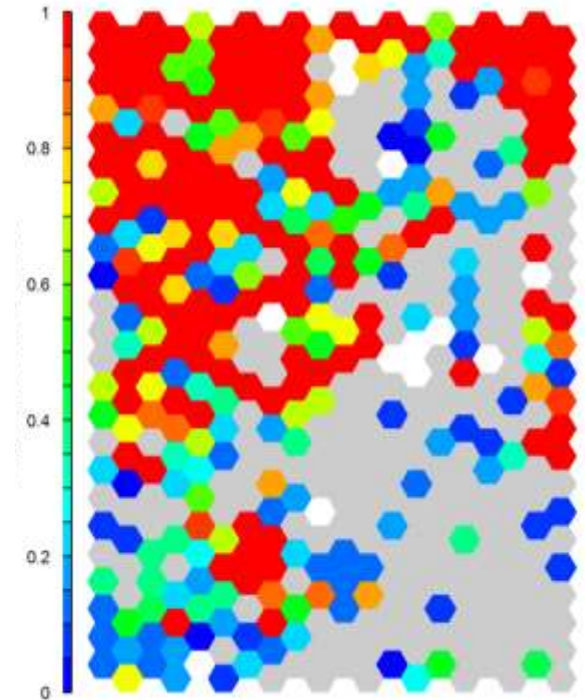
Direct Food Additives



Food Contact Substances

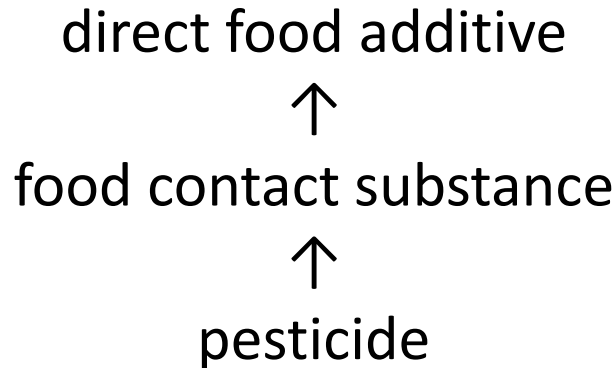


Pesticides



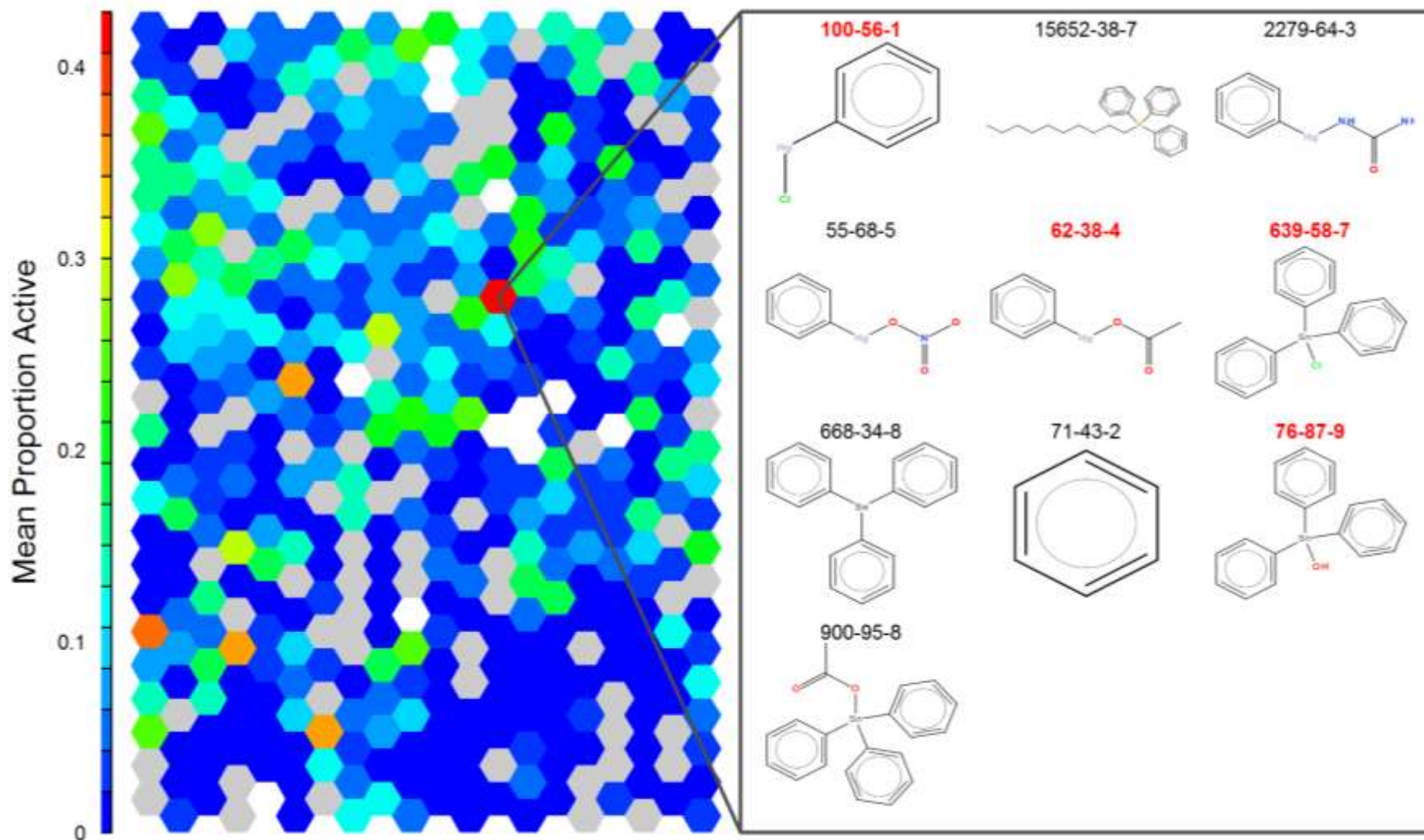
Chemical Inventories

Chemicals hierarchy:

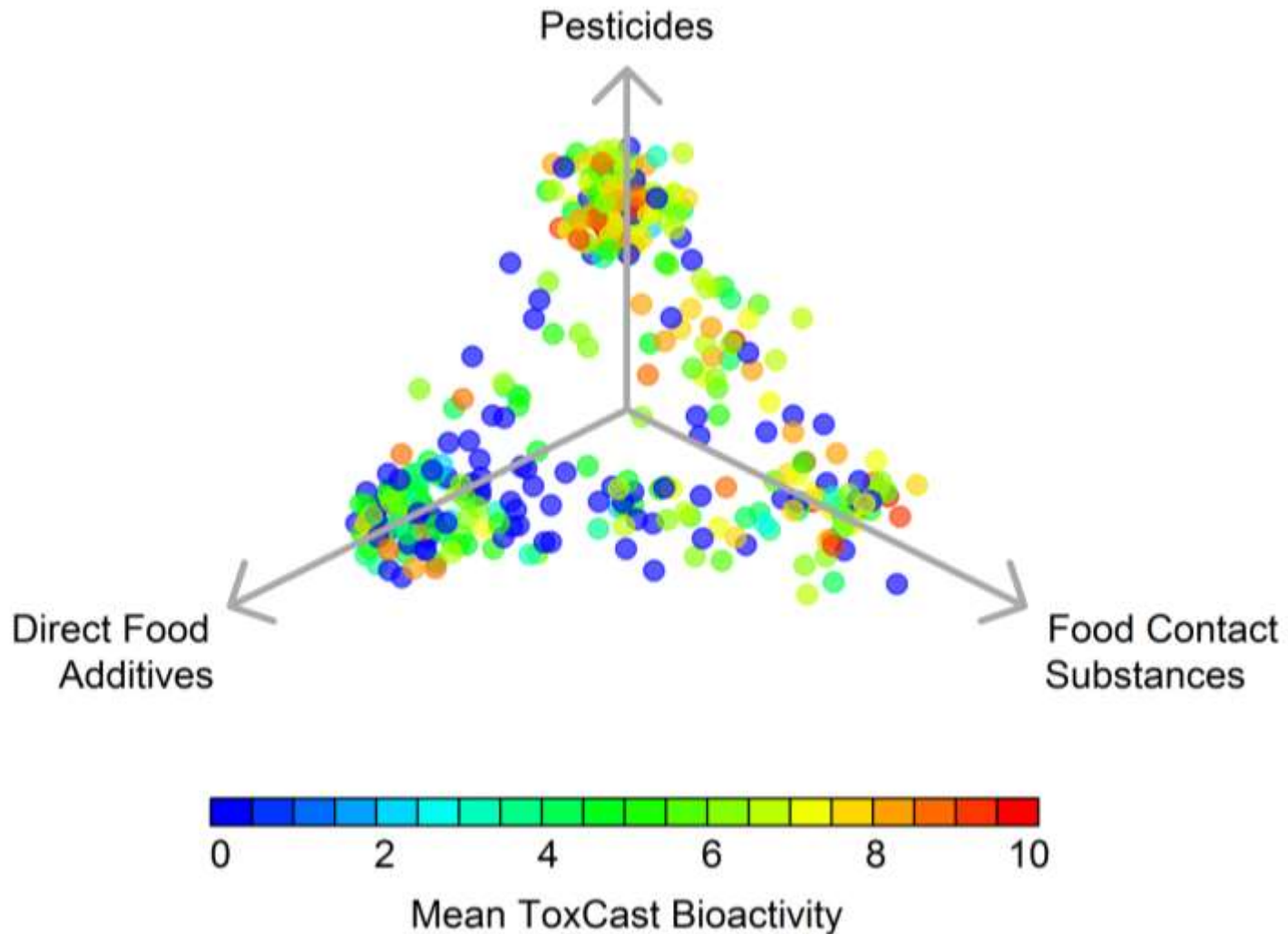


Use Category	CASRN in Use Category	CASRN in ToxCast
Direct Food Additives	3888	616
Food Contact Substances	3039	371
Pesticides	1732	543
	8,659	1,530

ToxCast Activity for Food-Use Chemicals

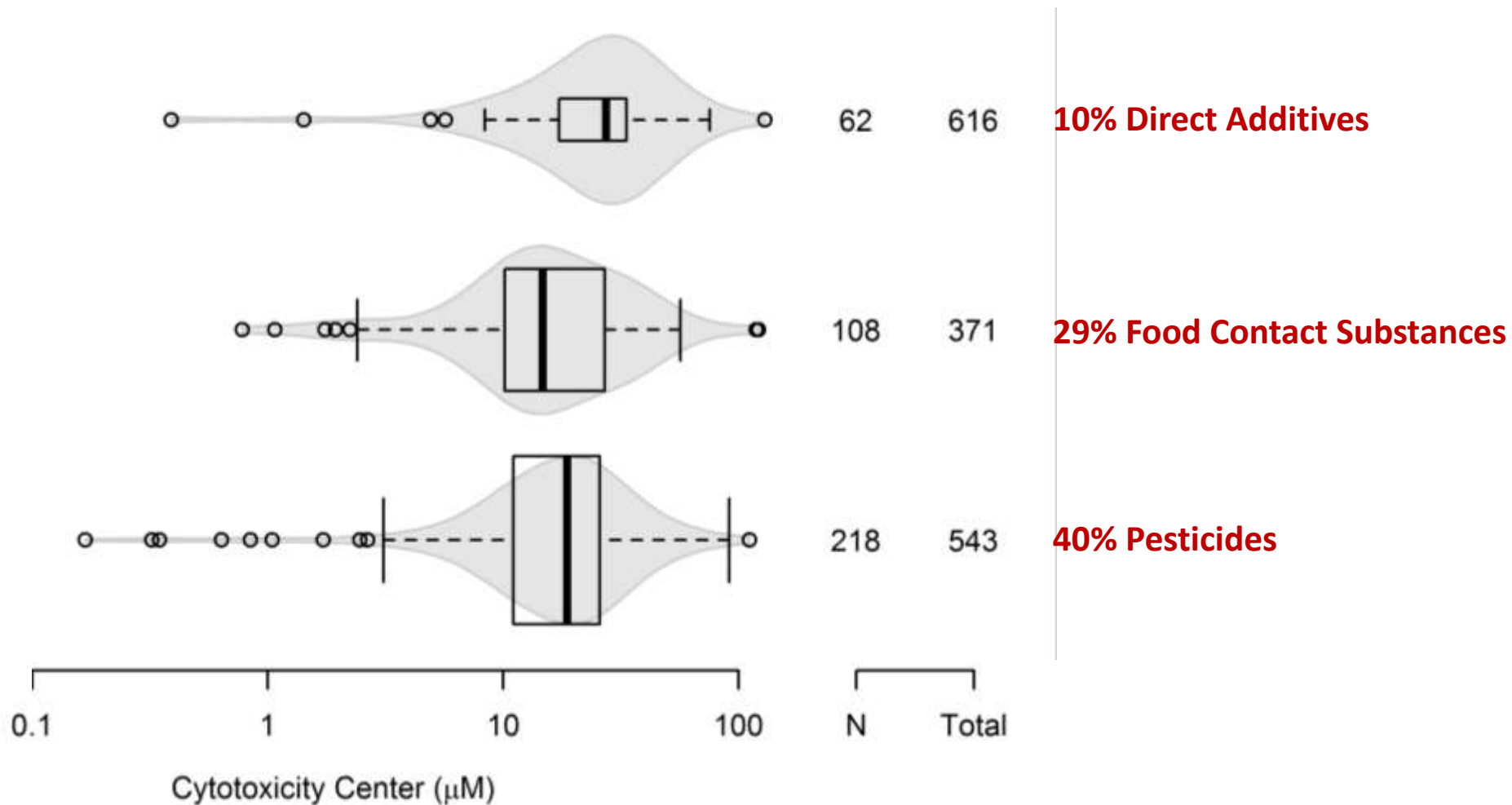


Food-Use Chemicals Bioactivity in ToxCast

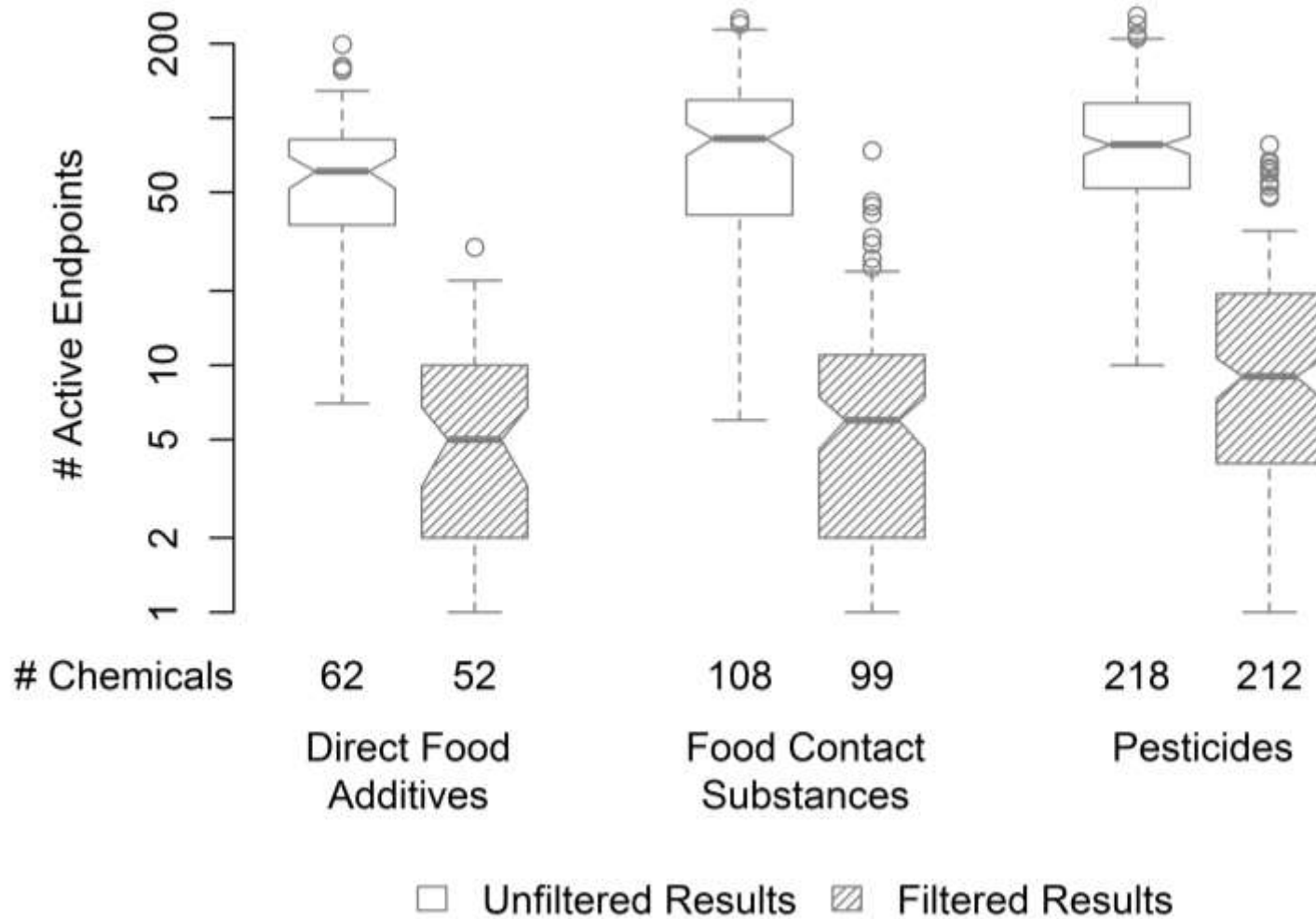


Evaluating Cytotoxicity Across ToxCast

All chemicals were evaluated in ≥ 14 of 35 cytotoxicity assays,
Chemicals had to elicit cytotoxicity in ≥ 3 assays for calculation



Evaluating Activity Across ToxCast



Summary of Chemical Inventory so far...

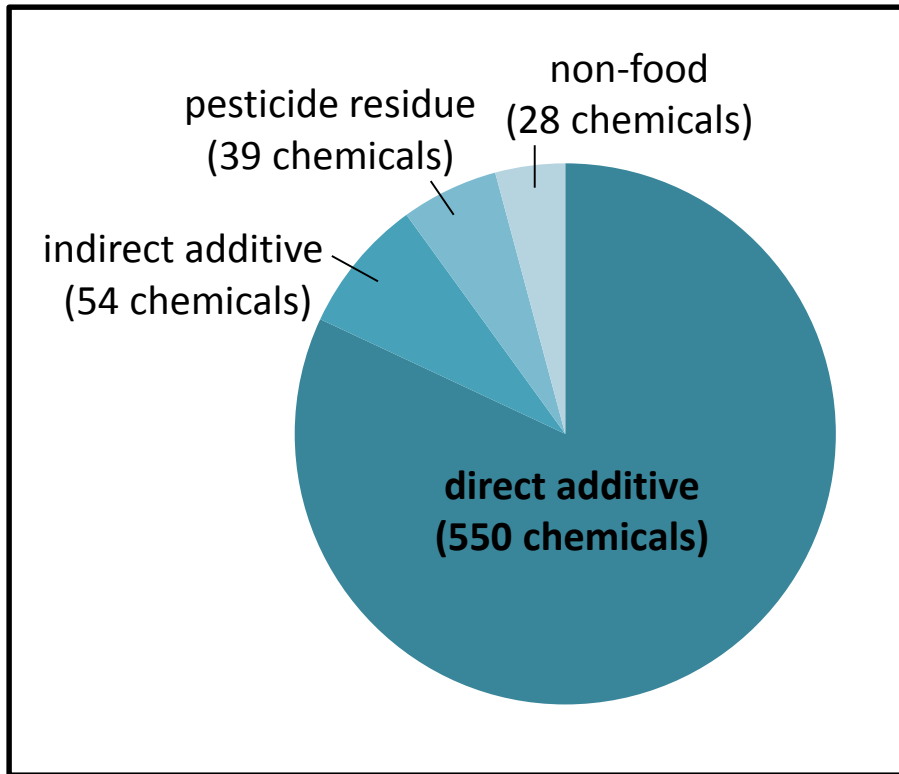
- Identified food-relevant chemicals by mining publicly available databases
- Chemicals were categorized based on database, assuming comparable usage
- Observations:
 - Some chemicals from databases are no longer allowed for use in food
 - Some chemicals have multiple uses or were misclassified (ie. GRAS chemicals)

Manual Curation of Food-Use Chemicals in ToxCast

- Focus on chemicals that are of relevance for food in current-day US
 - Eliminate chemicals that are no longer approved for food-use in the US
 - Eliminate chemicals that are only foreign use and have no importation tolerance
 - Confirm categorization based on use and exposure likelihood

Refining ToxCast Food-Relevant Chemical Categorization

former 616 direct additives category

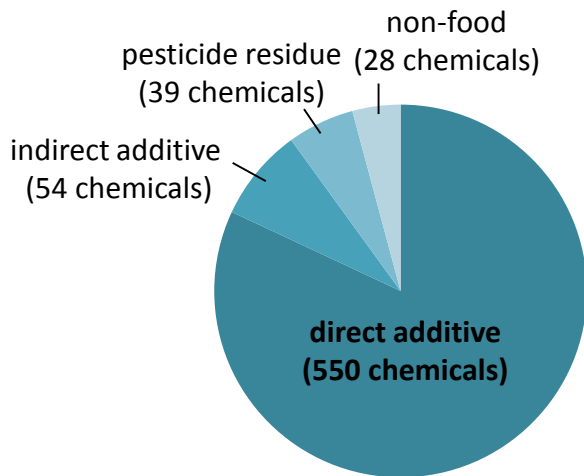


Manual curation evaluated:

- Current registration or tolerance status in the USA
- Exposure likelihood from food in the USA
- Allowed chemicals in >1 category

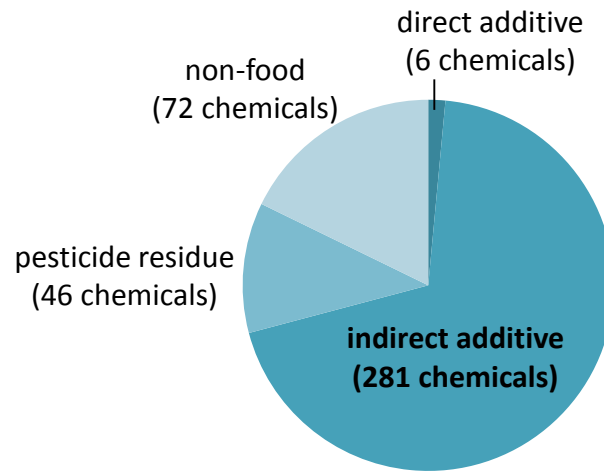
Manual Curation Results

formerly 616
direct additives



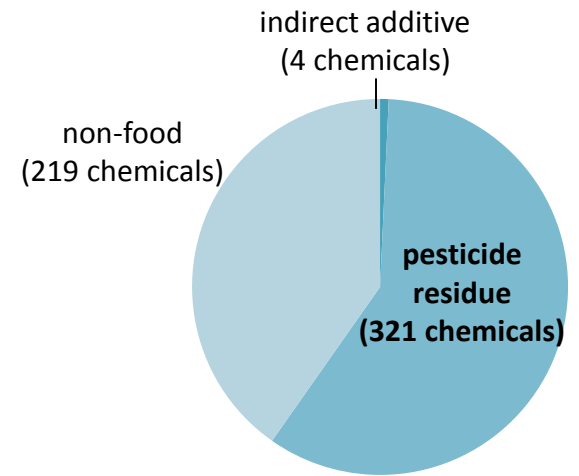
Refined: 556
direct additives

formerly 371
food contact substances



Refined: 339
indirect additives

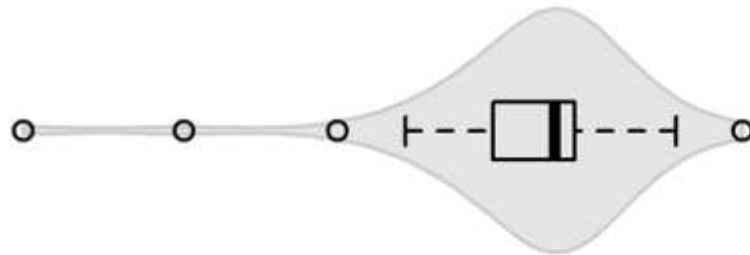
formerly 543
pesticides



Refined: 406
pesticides & residues

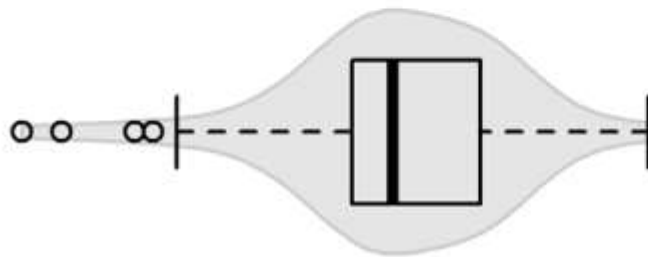
1,211 Manually Curated Food-Use Chemicals in ToxCast

Curated Chemical Categories: Cytotoxicity Across ToxCast



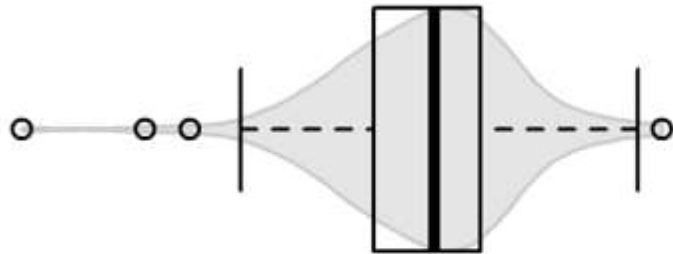
9.7%
(54/556)

Direct Additives



24.2%
(82/339)

Indirect Additive



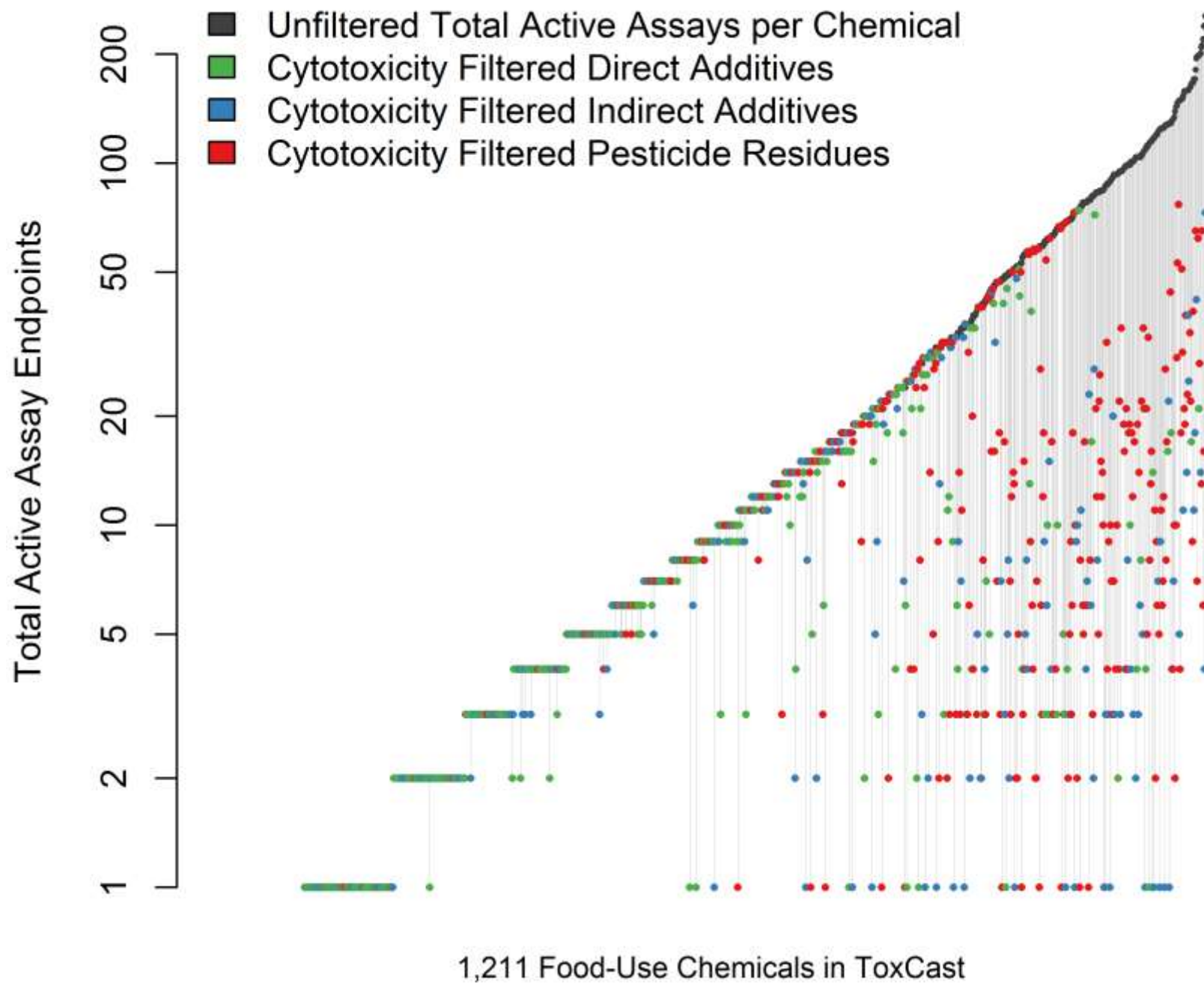
40.9%
(166/406)

Pesticide Residue



% of category with
cytotoxicity center

Cytotoxicity Center (μM)

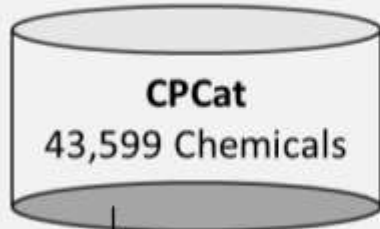


Summary

- Identified 8,659 unique food-use chemicals
- Chemical fingerprints confirm grouping based on “use”
- ToxCast includes 1,211 food-use chemicals after manual curation to confirm current-day use in the USA
 - 556 Direct food additives
 - 339 Indirect food additives
 - 406 Pesticides/residues
- Direct food additives are overall less cytotoxic compared to indirect food additives and pesticides/residues

12 use informative resource databases

Dionisio *et al.* compile/annotate



Isolate chemicals with
the word "food" in
description fields

10,972 "Food" Chemicals

Isolate chemicals in the
ToxCast inventory

**1,749 "Food" Chemicals
in ToxCast**

Next Steps...

- Compare food-use chemical inventory to chemicals identified from the Chemical and Product Categories (CPCat) Database

**1,530 "Food" Chemicals
in ToxCast**

Isolate chemicals in the
ToxCast inventory

8,965 Food-Relevant Chemicals

Karmaus *et al.* compile/categorize
8 food-relevant resource databases

Acknowledgements

- ILSI North America
 - Technical Committee for Food and Chemical Safety
- Dr. Tom Trautman (retired, General Mills)
- Dr. Lori Fix (Unilever)
- US EPA National Center for Computational Toxicology
 - Dr. Matt Martin, Dr. Keith Houck, Dayne Filer

Karmaus et al. 2016. *Food Chem Tox.* 92: 188-196.

Karmaus et al. 2017. *Food Chem Tox.* 103: 174-182.

Email: akarmaus@ils-inc.com