

# Food-Use Chemicals in ToxCast: Identification, Curation, and Evaluation

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# Goals

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

- Evaluate the chemical landscape
- Evaluate bioactivity in ToxCast

# 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
<b>TOTAL</b>	<b>14,042</b>	<b>11,733</b>	

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

# Chemical Inventories

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

direct food additive  
↑  
food contact substance  
↑  
pesticide

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

# Chemical 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



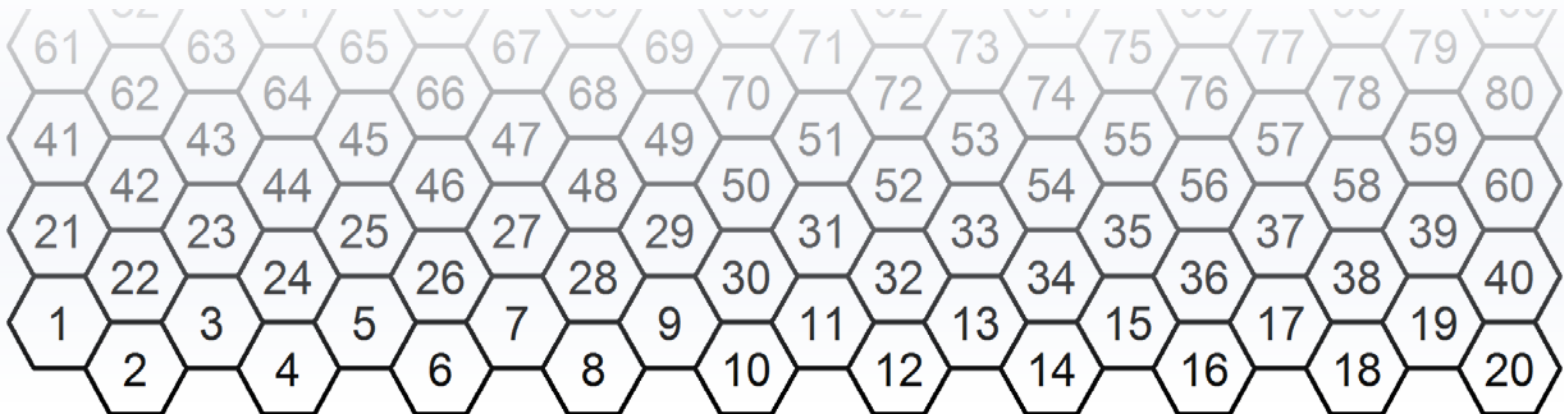
# Self Organizing Map (SOM)

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

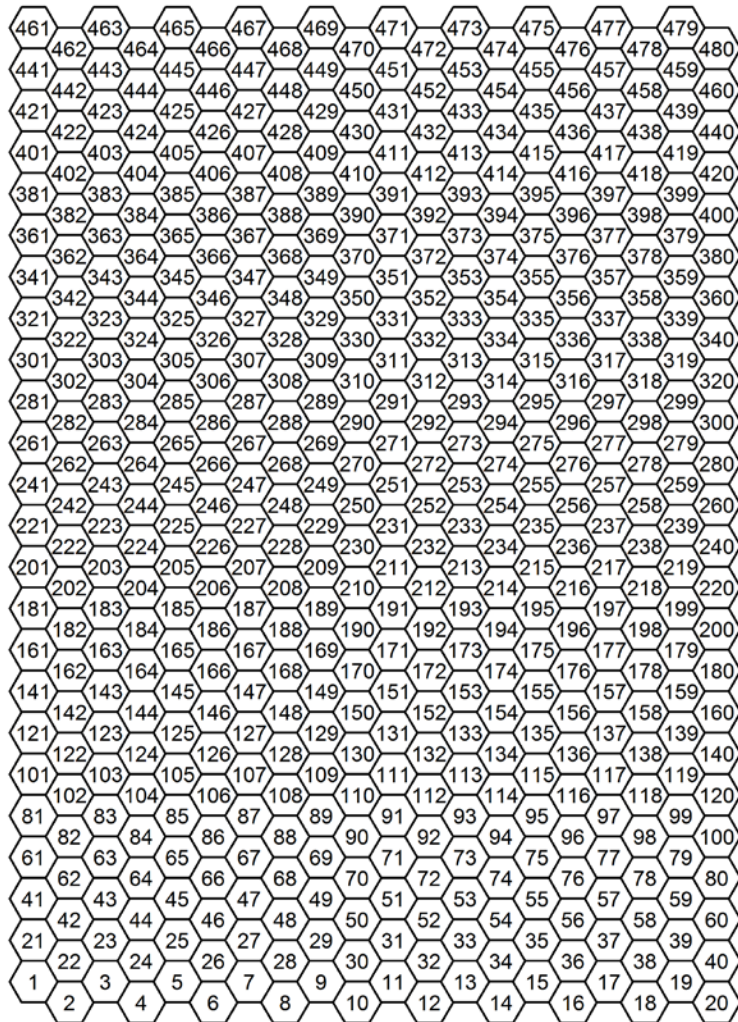
Similarity was  
based on  
chemical  
fingerprints





# 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**

**Pesticides**

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

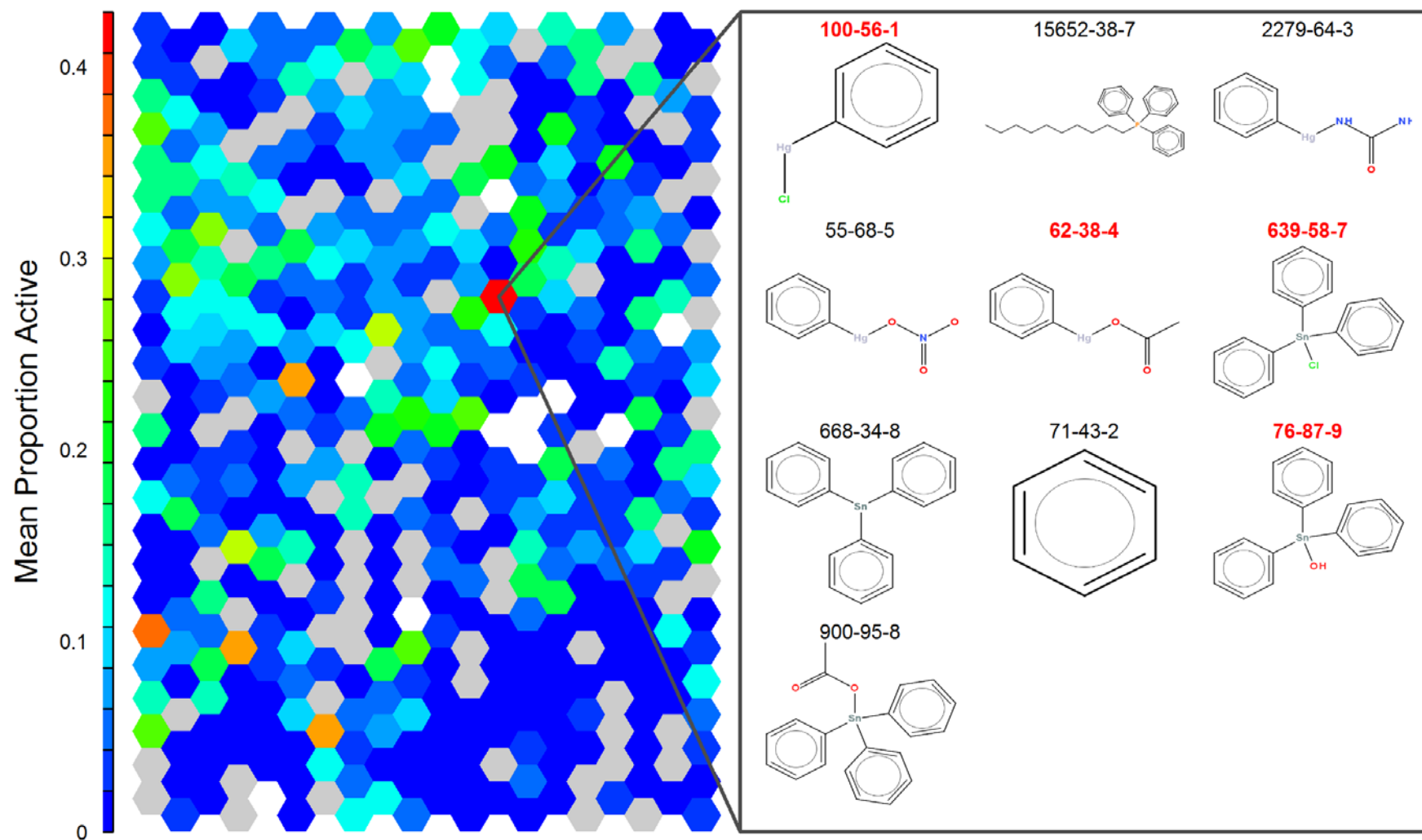
# SOM Results for Food-Use Chemicals

**Direct Food Additives**

**Food Contact Substances**

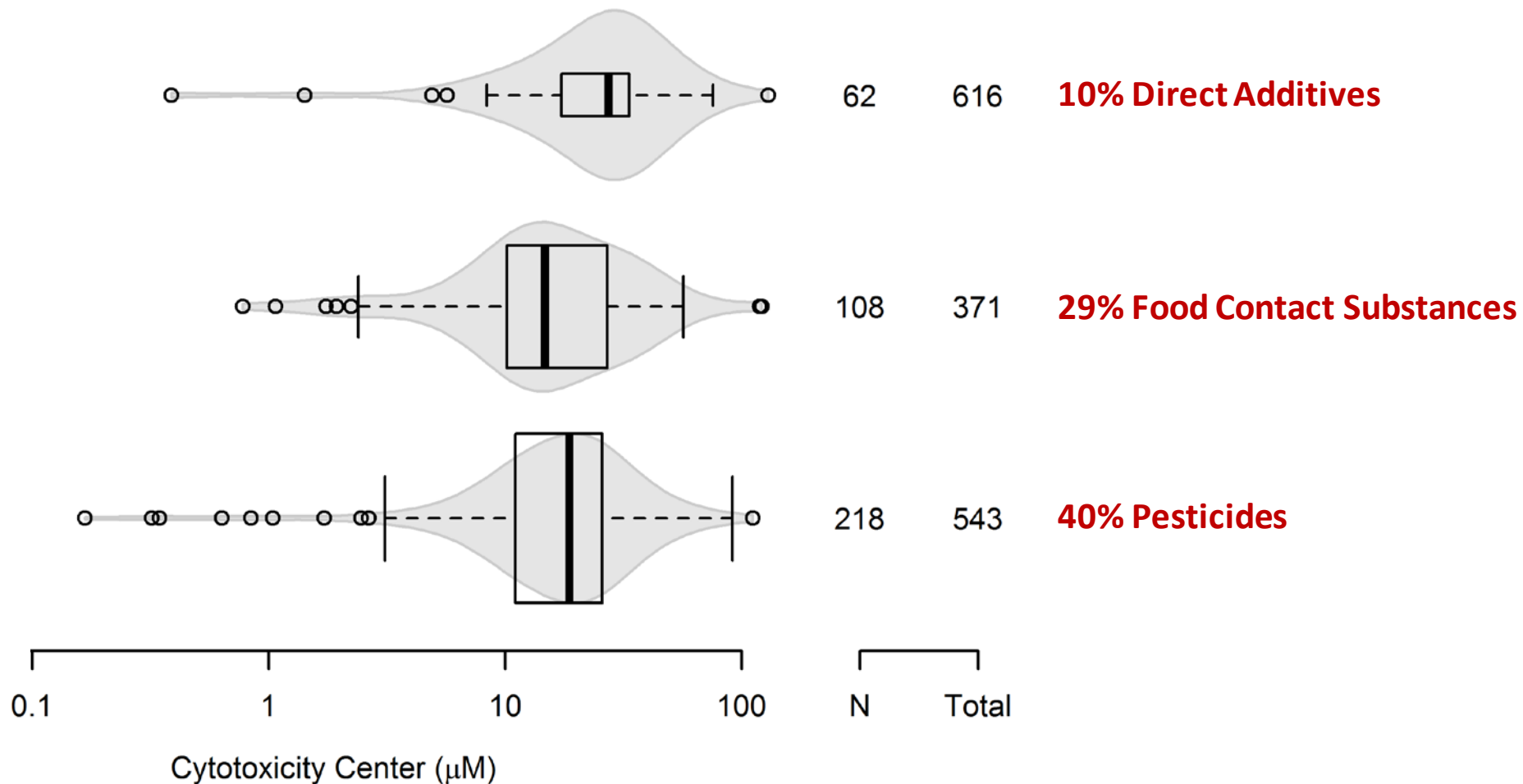
**Pesticides**

# ToxCast Activity for Food-Use Chemicals

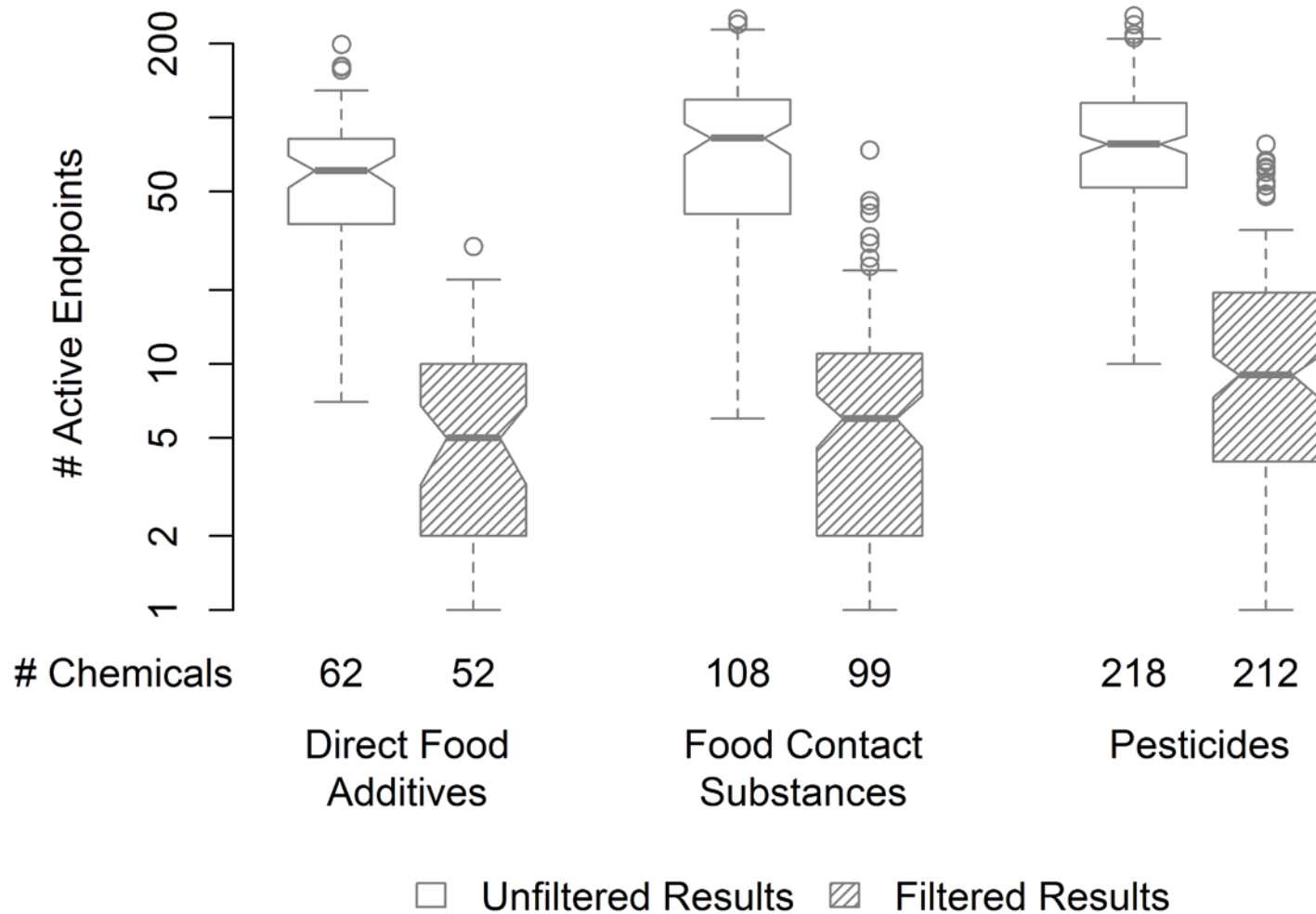


# Evaluating Cytotoxicity Across ToxCast

All chemicals were evaluated in  $\geq 14$  of 35 cytotoxicity assays,  
Chemicals had to elicit cytotoxicity in  $\geq 3$  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)

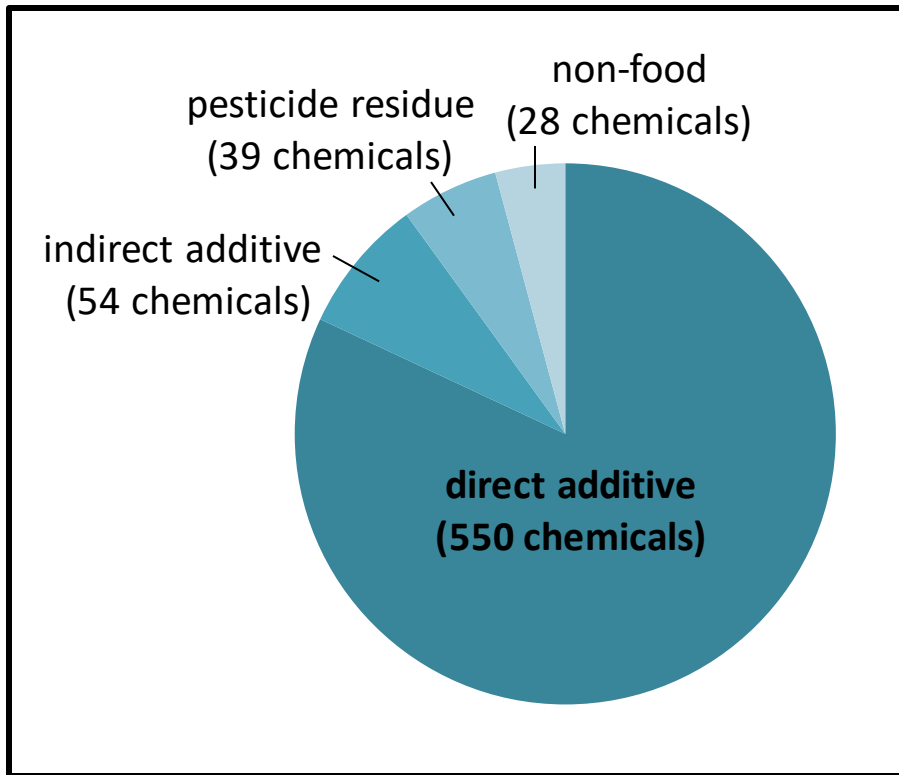


# Manual Curation of Food-Use Chemicals in ToxCast

- 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**

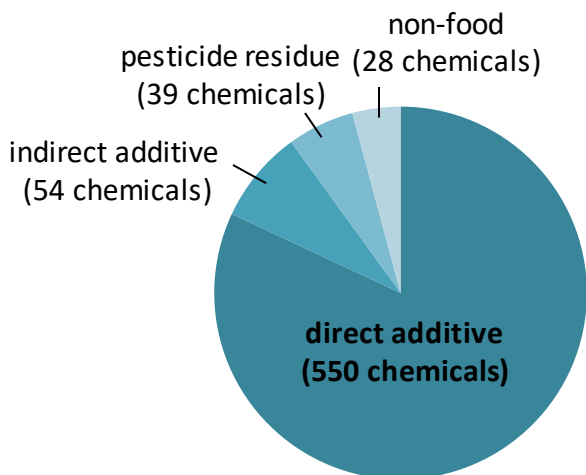


Manual curation evaluated:

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

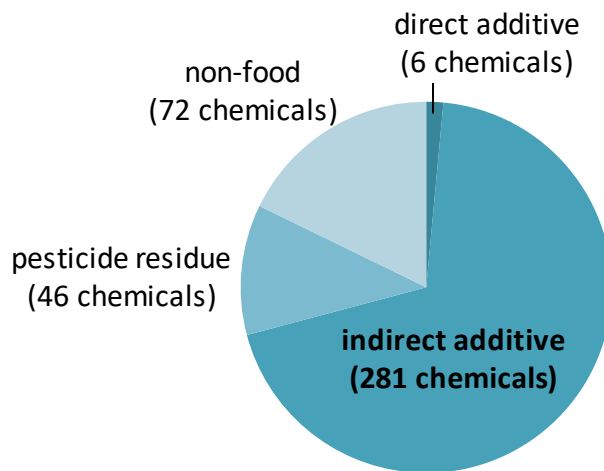
# Manual Curation Results

**formerly 616  
direct additives**



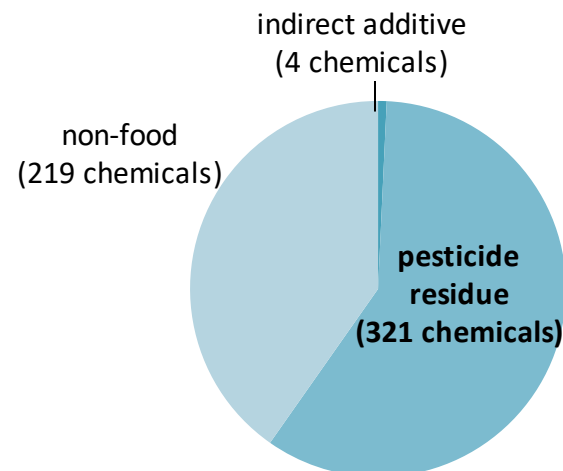
**Refined: 556  
direct additives**

**formerly 371  
food contact substances**



**Refined: 339  
indirect additives**

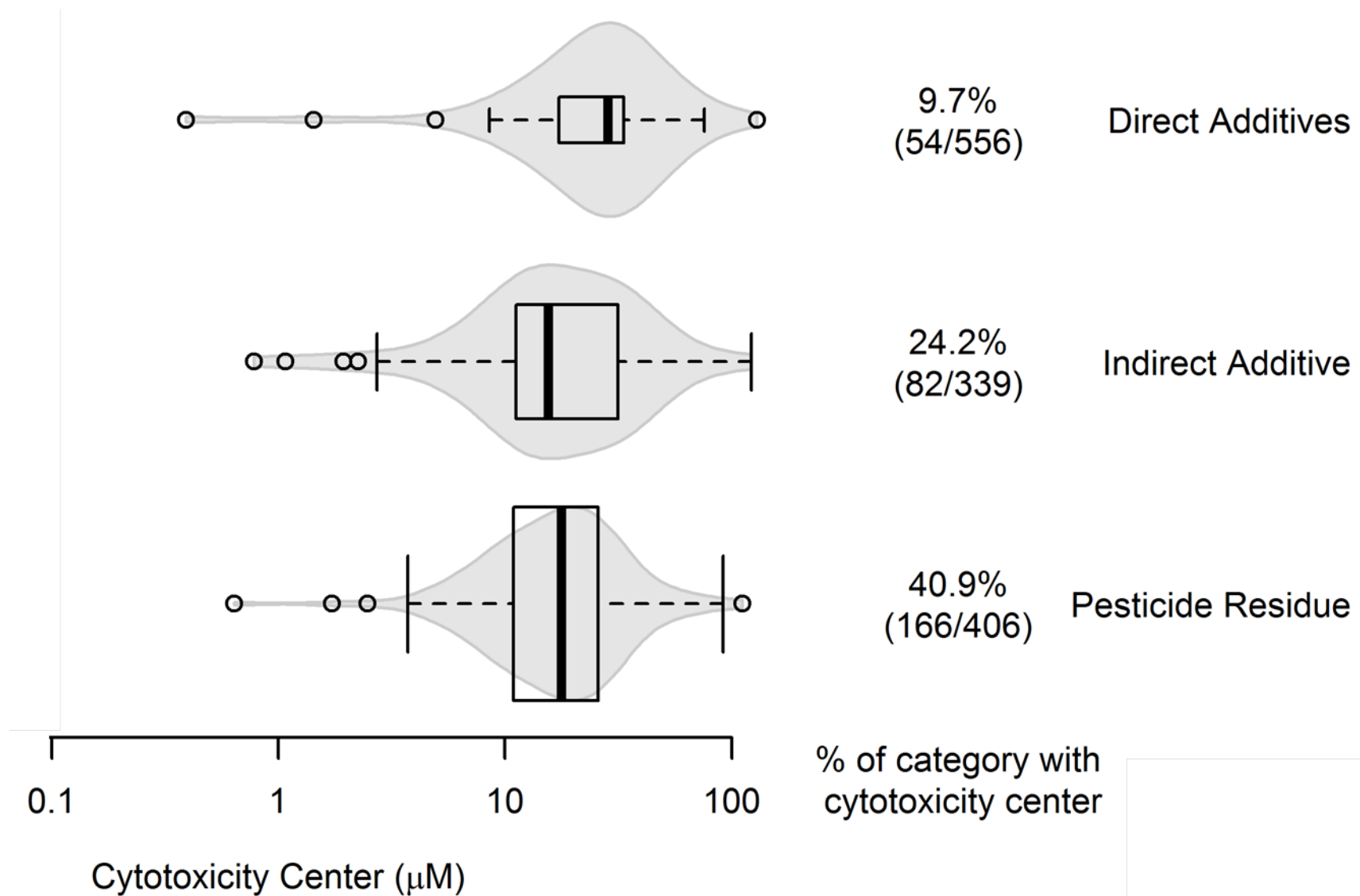
**formerly 543  
pesticides**

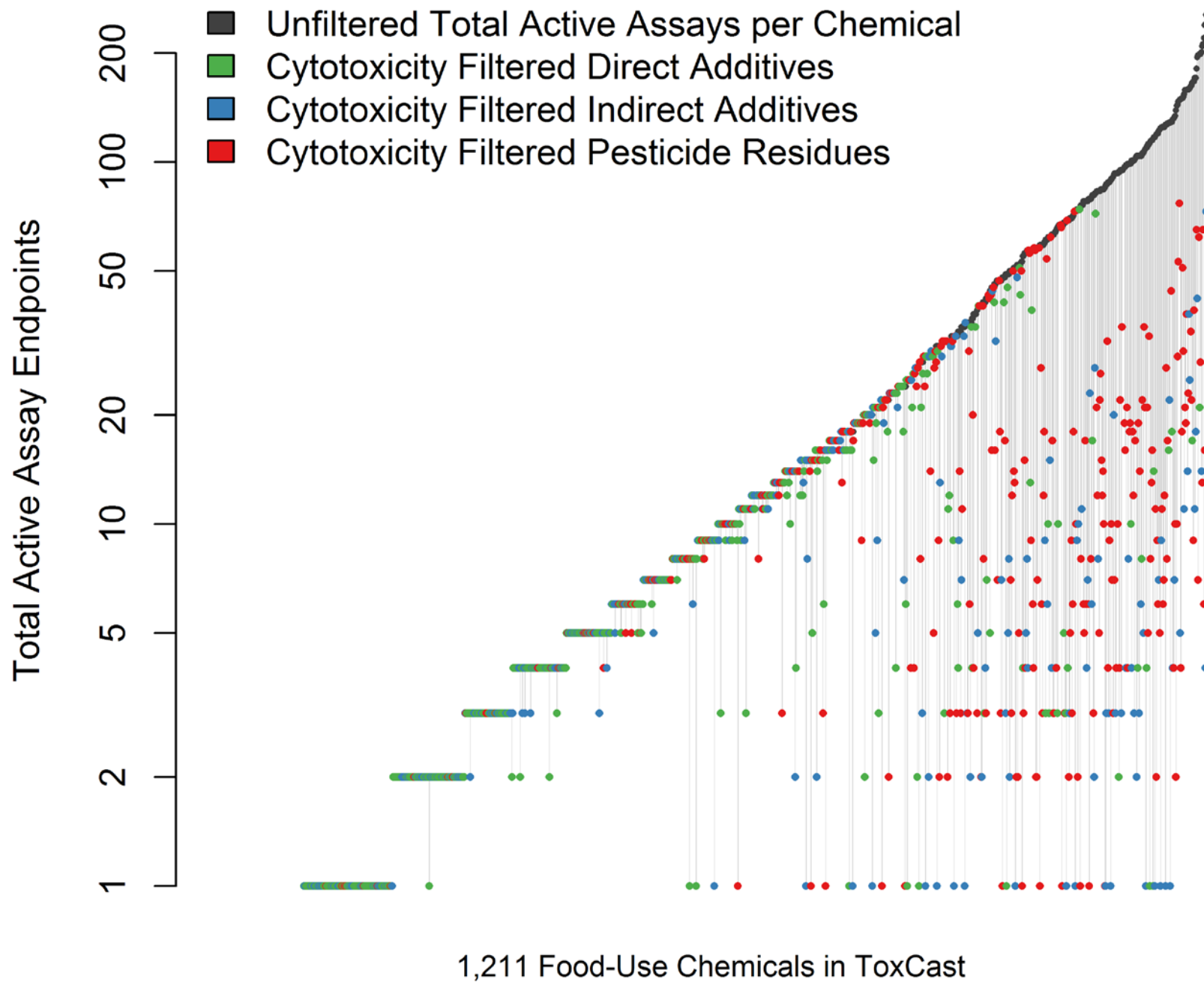


**Refined: 406  
pesticides & residues**

**1,211 Manually Curated Food-Use Chemicals in ToxCast**

# Curated Chemical Categories: Cytotoxicity Across ToxCast



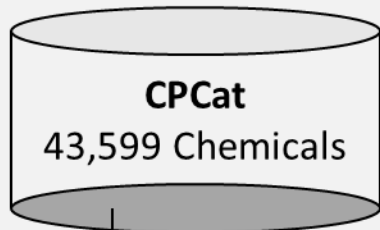


# Summary

- 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

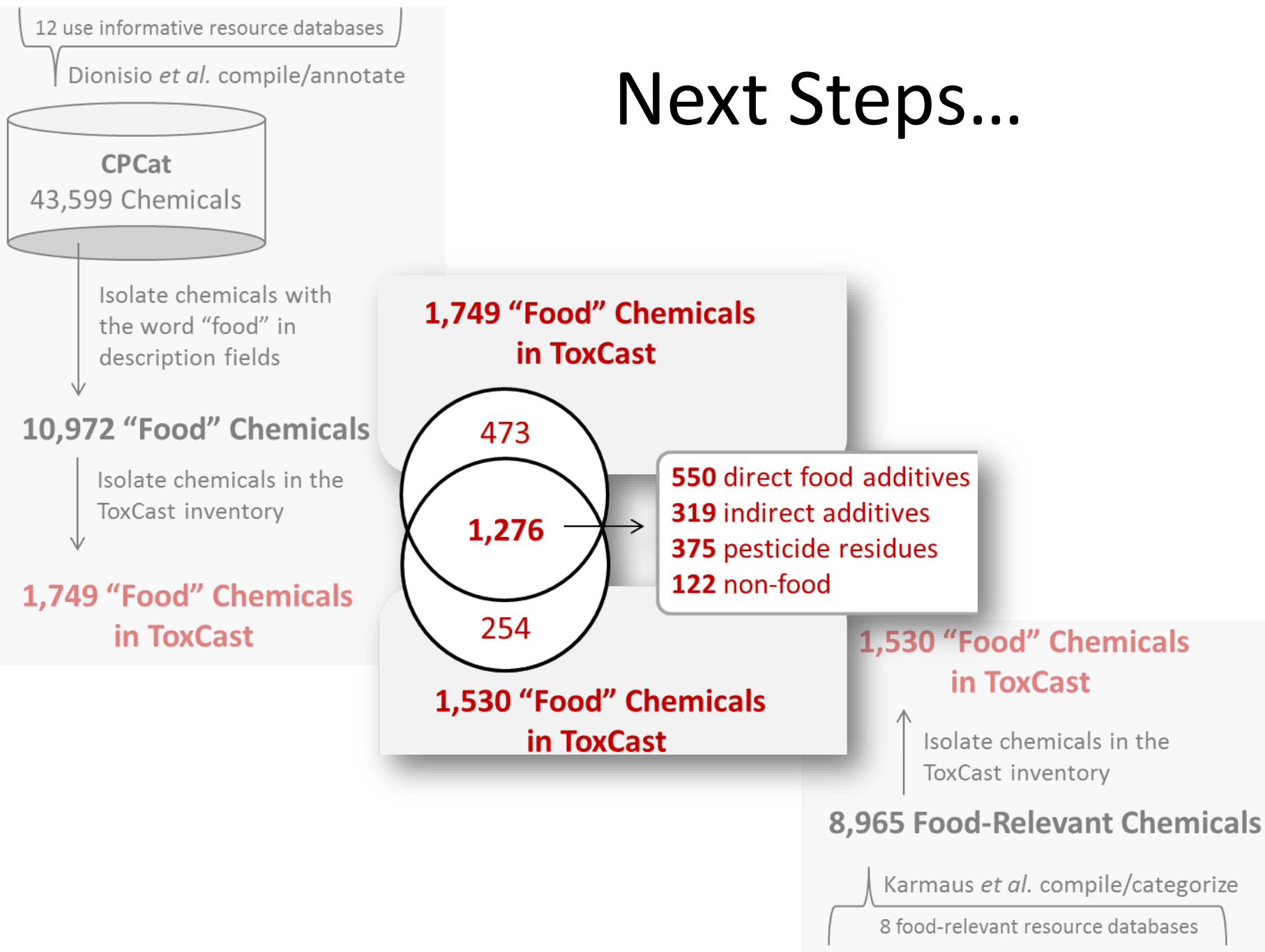
**1,530 "Food" Chemicals  
in ToxCast**

Isolate chemicals in the  
ToxCast inventory

**8,965 Food-Relevant Chemicals**

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

# Next Steps...





# Acknowledgements

- ILSI North America
  - Technical Committee for Food and Chemical Safety
  - Alison Kretser and Dr. Mansi Krishan
- Dr. Tom Trautman (retired, General Mills)
- Dr. Lori Fix (Unilever)
- EPA/NCCT
  - Dr. Matt Martin, Dr. Keith Houck, Dayne Filer

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