United States Environmental Protection Agency

Source and Use Information for Aiding Compound Identification in Non-Targeted Analysis (NTA) Studies

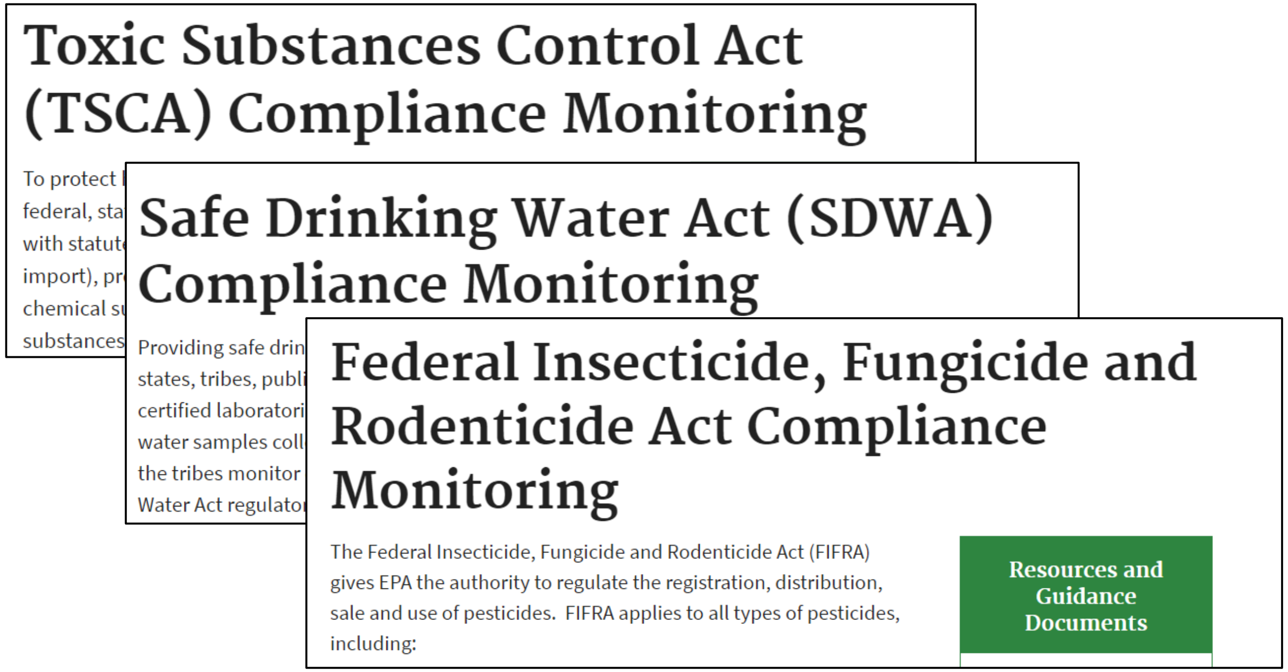
Jon R. Sobus

QSUR Summit

Nov 3, 2022

Why Does EPA Need Measurement Data?

- **Measurement data needed to ensure chemical safety**
  - Characterize risk
  - Regulate use & disposal
  - Manage human & ecological exposures
  - Ensure compliance under federal statutes


Toxic Substances Control Act (TSCA) Compliance Monitoring

To protect
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Safe Drinking Water Act (SDWA) Compliance Monitoring

Providing safe drin
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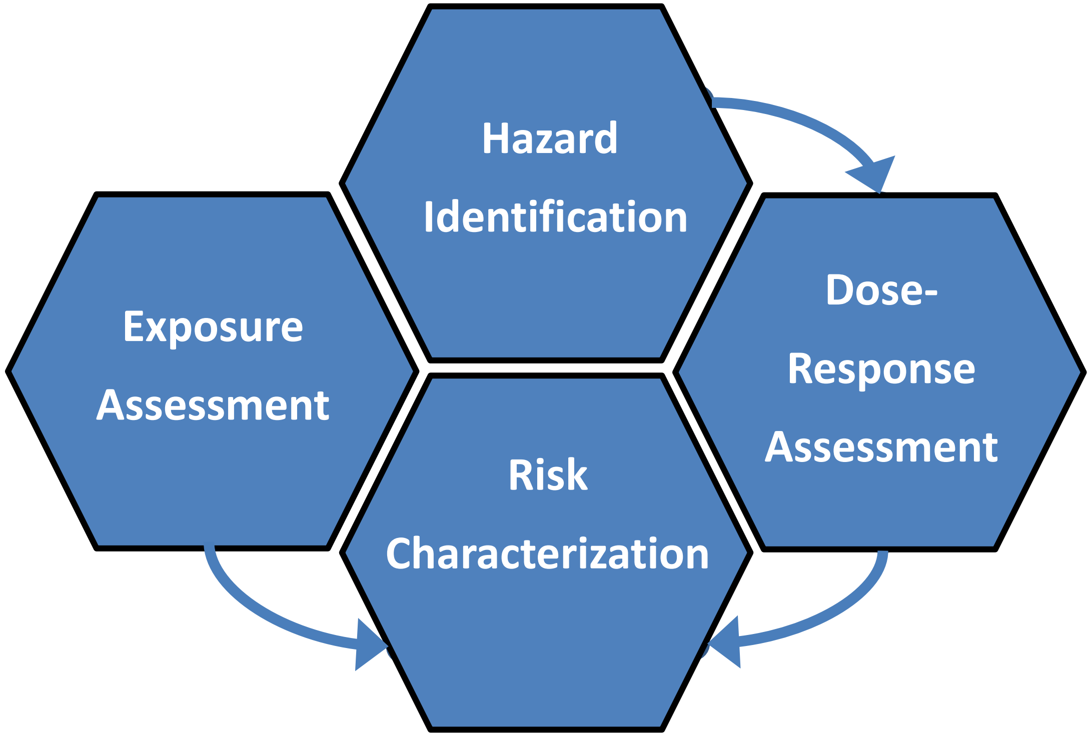
the tribes monitor
Water Act regulator

Federal Insecticide, Fungicide and Rodenticide Act Compliance Monitoring

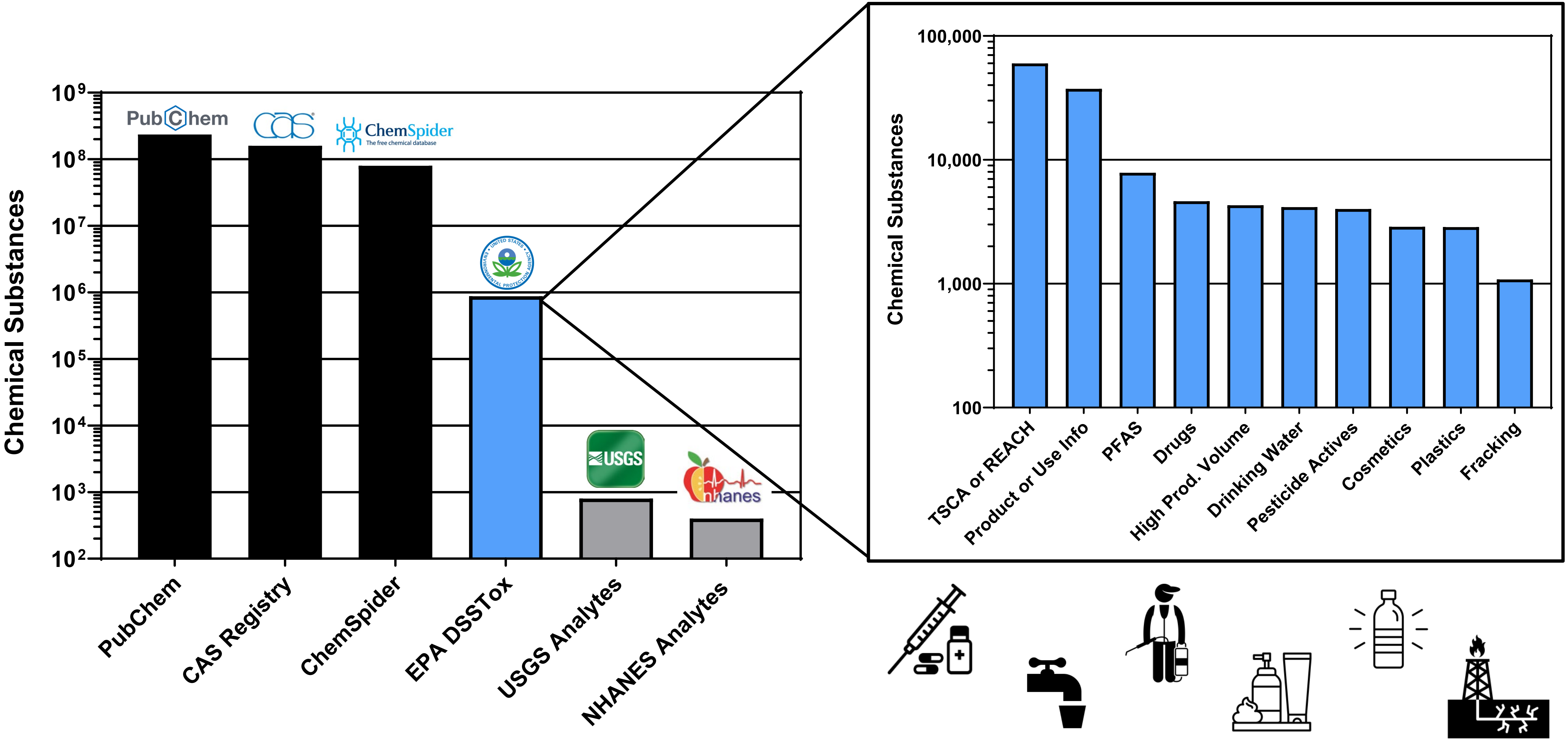
The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) gives EPA the authority to regulate the registration, distribution, sale and use of pesticides. FIFRA applies to all types of pesticides, including:

Resources and Guidance Documents

Chemical Monitoring Needs



Data Disparity: Have vs. Need



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Challenges

- High-quality monitoring data are unavailable for most chemicals
- Measurement data traditionally generated using “targeted” methods
- Targeted analytical methods:
  - Require *a priori* knowledge of chemicals of interest
  - Produce data for few selected analytes (10s-100s)
  - Require standards for method development & compound quantitation
  - Are blind to emerging contaminants
  - Can’t keep pace with the needs of 21st century risk characterizations
- Data gaps being filled with exposure models and “NTA” methods

Exposure Models for Gap Filling & Aggregation

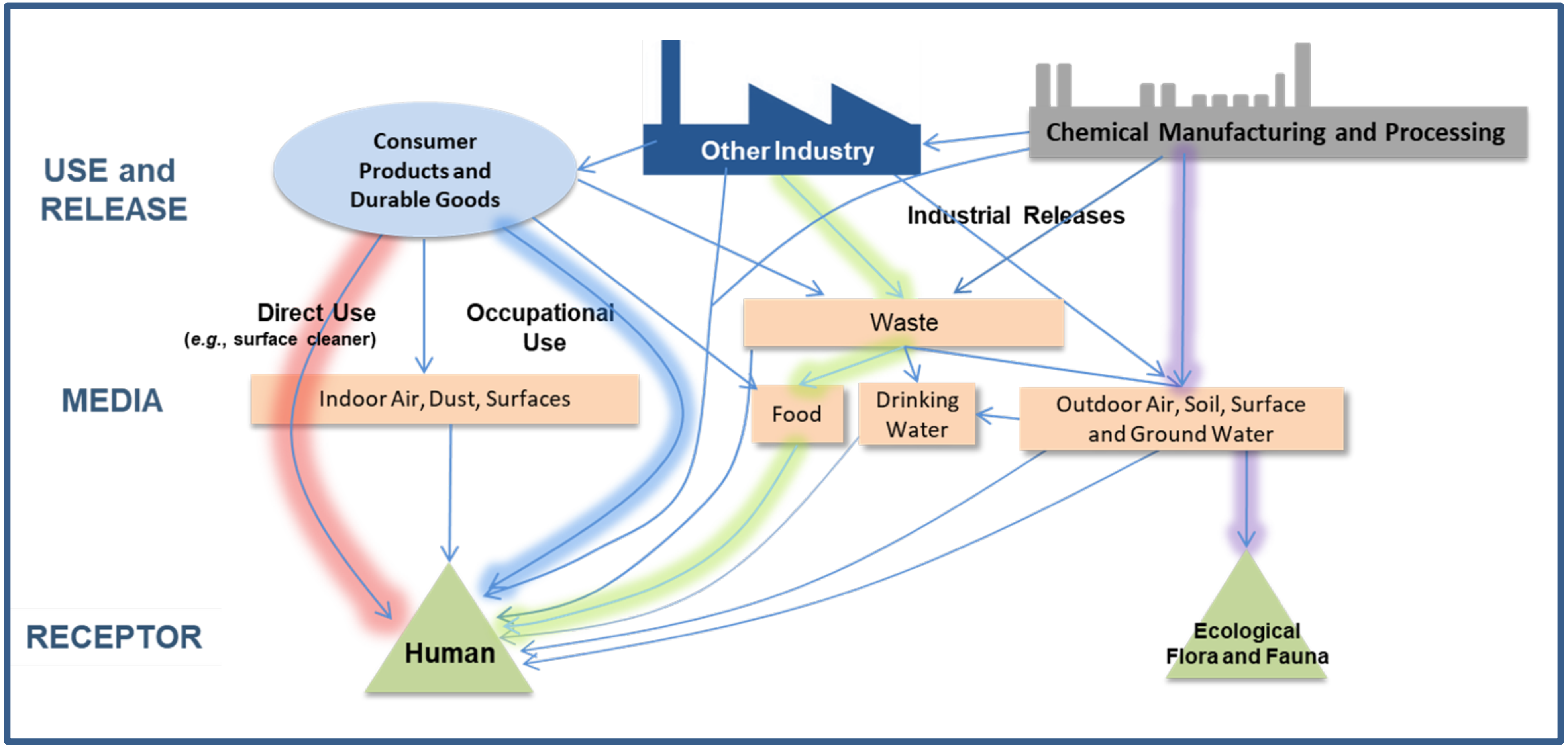
EPA Considers 4 Pathway Types:

Consumer

Occupational

Ambient

Ecological



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Benefits and Challenges of Exposure Modeling

- Benefits:
  - Able to predict:
    - Pathway-specific exposures
    - Scenario-specific exposures
    - Aggregate exposures
    - Cumulative exposures
  - Efficient (compared to analytical monitoring)

- Challenges:
  - Requires use, property, and pathway info for prediction
  - Requires monitoring data for evaluation & refinement

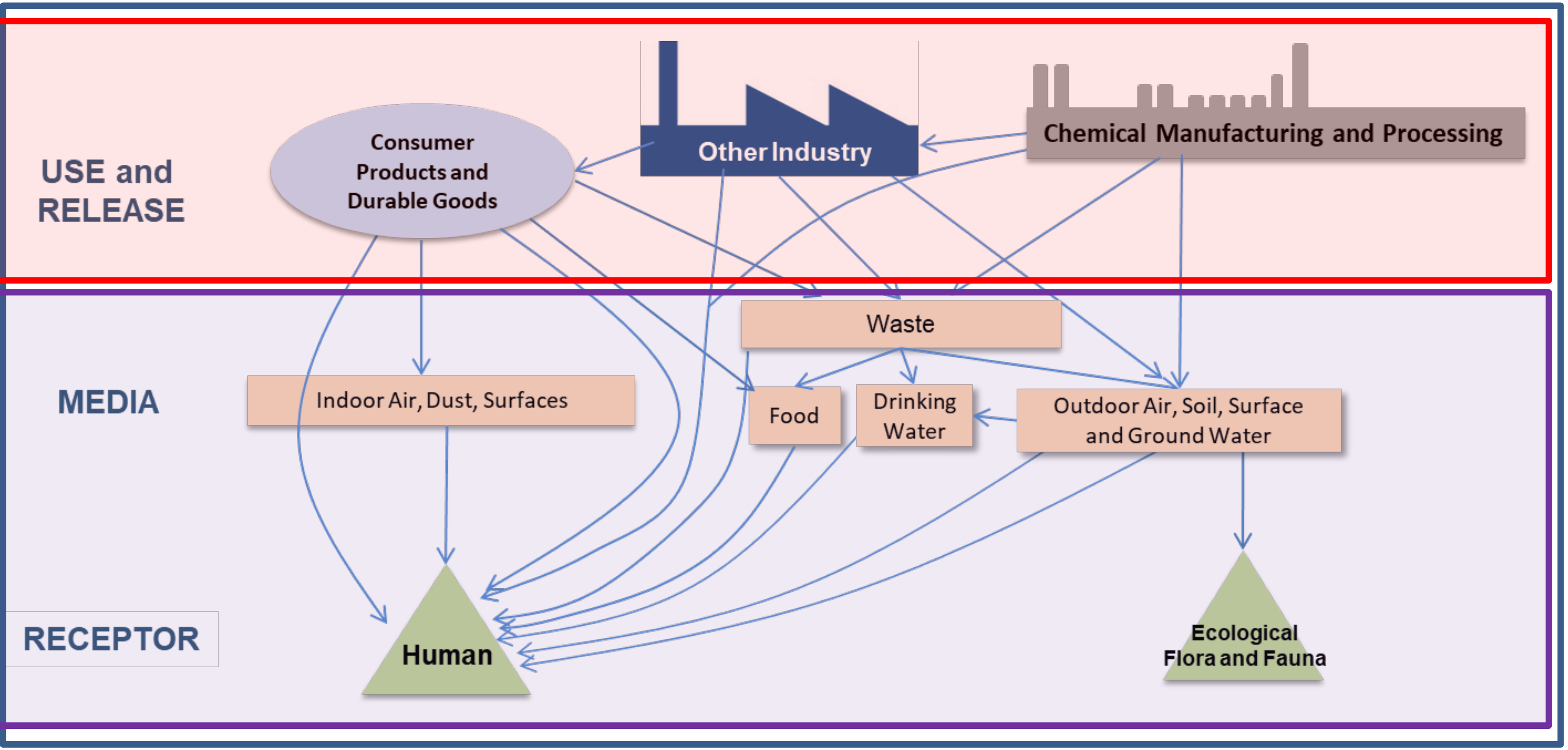
Integration of Measurements and Models

QSUR Domain:

How & why is it used?

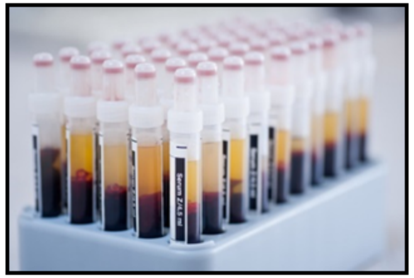
Measurement Domain:

Where and in what quantities is it found?

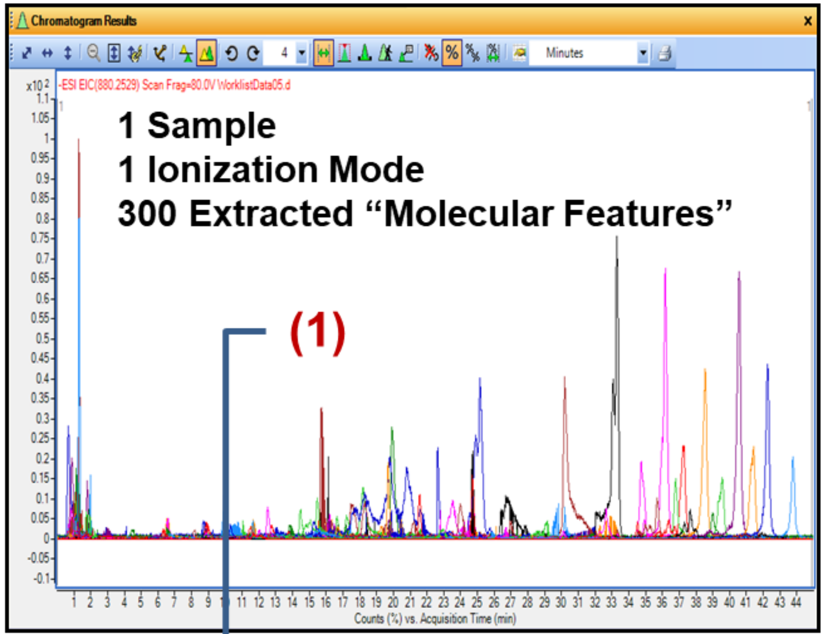


Rudimentary NTA Workflow

Samples



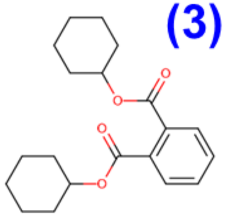
High-Resolution MS



(2)

$C_{20}H_{26}O_4$

(3)



(4)

12 µg/mL

(5)

1) Prioritize "molecular features"
2) Correctly assign formulas
3) Correctly assign structures
4) Predict chemical concentrations
5) Determine chemical sources

Relevant Questions of NTA Studies?

- Which chemicals are where?
- Do we see any “new” chemicals?
- Do observed co-occurrences highlight:
  - Important exposure sources?
  - Stressor-response relationships?
- What is the concentration of each chemical?
- Do estimated concentrations suggest unacceptable risk?

Answers supported via QSURs and other QSxRs

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Example 1: Consumer Product Analysis

Environmental Science & Technology

Article

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Suspect Screening Analysis of Chemicals in Consumer Products

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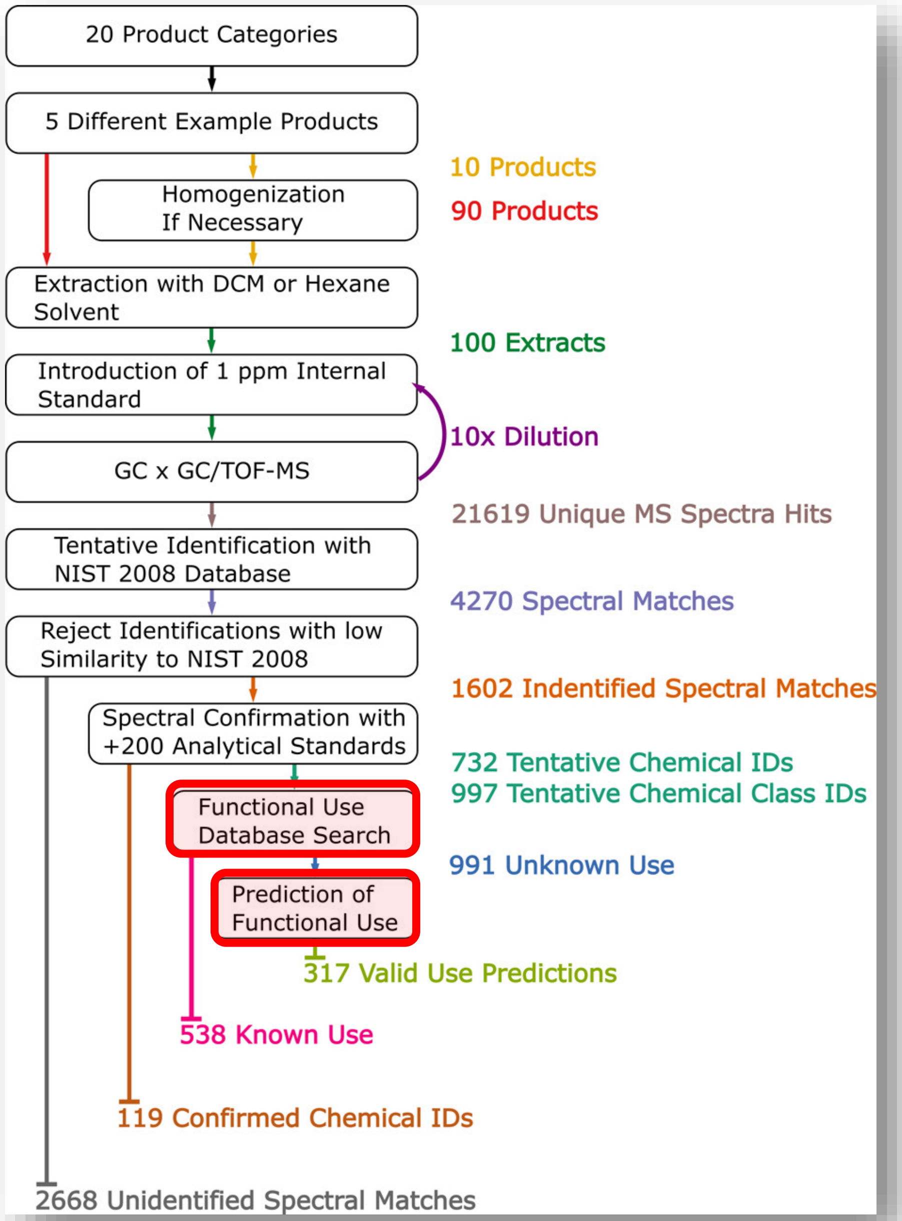
Supporting Information

ABSTRACT: A two-dimensional gas chromatography-time-of-flight/mass spectrometry (GC×GC-TOF/MS) suspect screening analysis method was used to rapidly characterize chemicals in 100 consumer products—which included formulations (e.g., shampoos, paints), articles (e.g., upholsteries, shower curtains), and foods (cereals)—and therefore supports broader efforts to prioritize chemicals based on potential human health risks. Analyses yielded 4270 unique chemical signatures across the products, with 1602 signatures tentatively identified using the National Institute of Standards and Technology 2008 spectral database. Chemical standards confirmed the presence of 119 compounds. Of the 1602 tentatively identified chemicals, 1404 were not present in a public database of known consumer product chemicals. Reported data and model predictions of chemical functional use were applied to evaluate the tentative chemical identifications. Estimated chemical concentrations were compared to manufacturer-reported values and other measured data. Chemical presence and concentration data can now be used to improve estimates of chemical exposure, and refine estimates of risk posed to human health and the environment.



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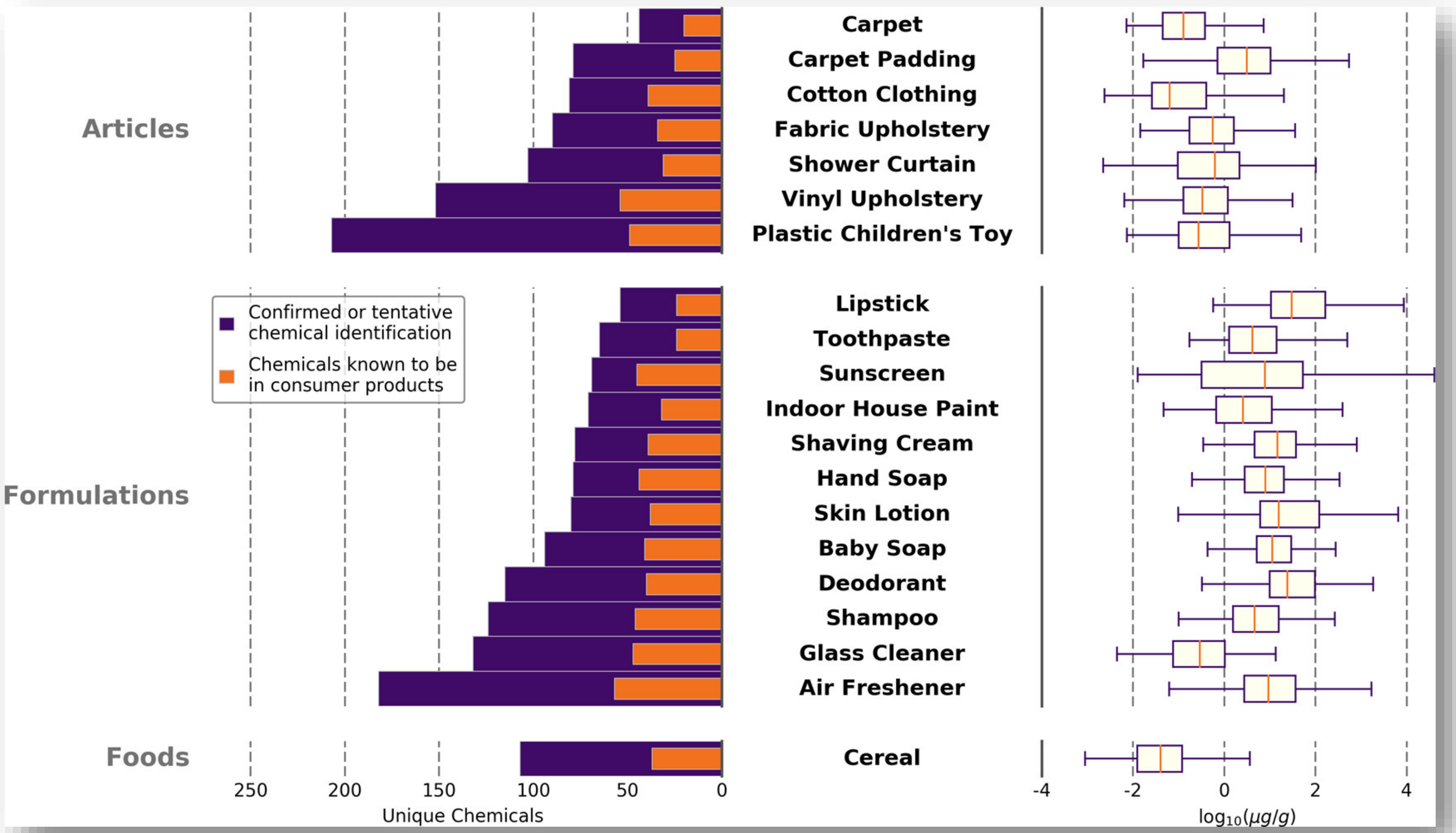
Example 1: Consumer Product Analysis



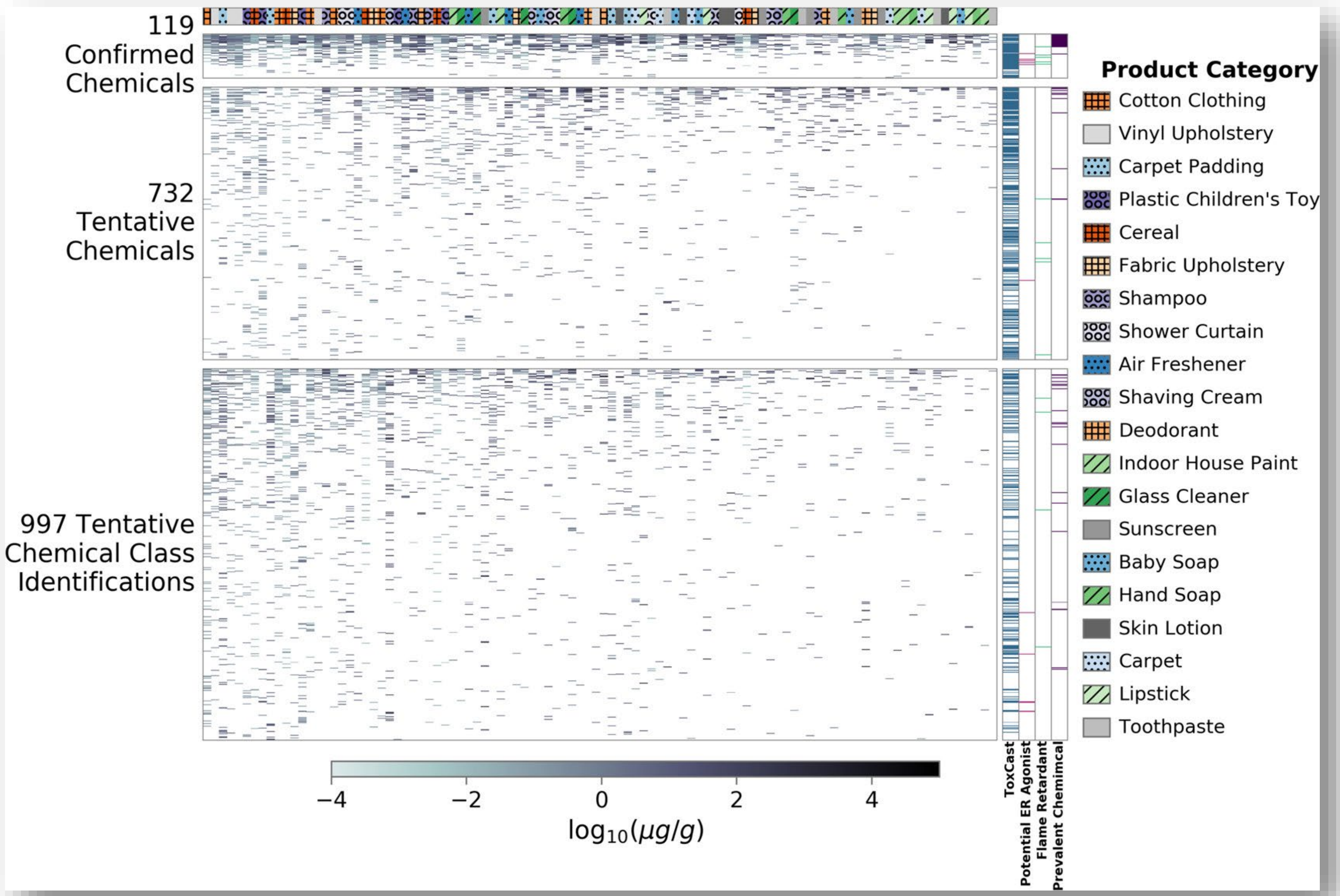
Many chemicals observed in consumer product extracts

Many observed chemicals known to be in consumer products

More observed chemicals not known to be in consumer products

Why might the 'other' chemicals be in the products?

Example 1: Consumer Product Analysis



Few chemicals confirmed due to limited availability of standards

Many chemicals only tentatively identified

Even more chemicals only identified at the "class" level (e.g., isomers)

How do we provide further evidence for correct structures?

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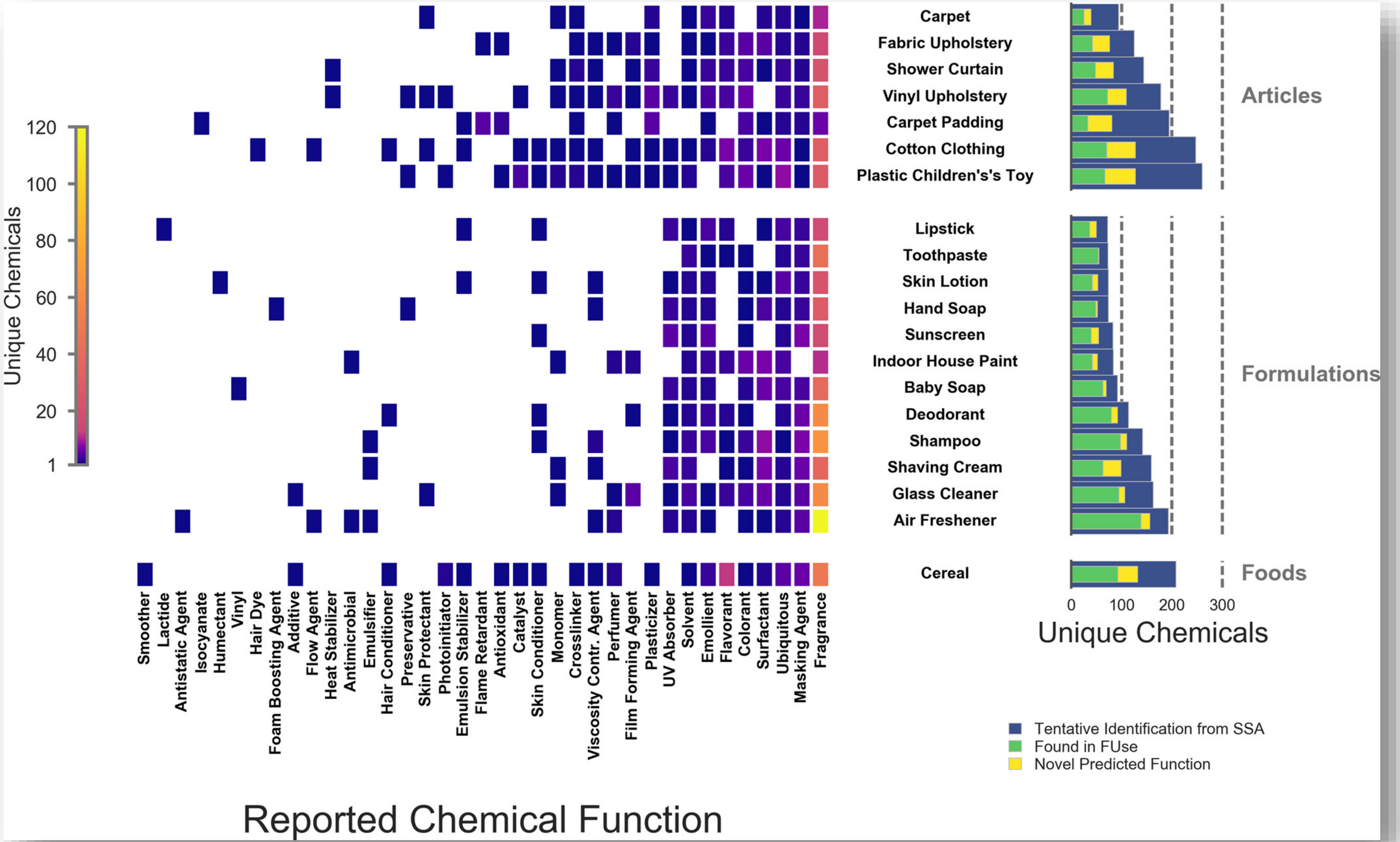
Example 1: Consumer Product Analysis



Known functional uses support presence in specific products

Certain functional uses are represented across many products

Other functional uses are more product-specific

Predicted functional uses can support tentative chemical identifications

Example 2: Recycled Product Analysis

Environmental Science & Technology

pubs.acs.org/est Article

Chemical Characterization of Recycled Consumer Products Using Suspect Screening Analysis

Charles N. Lowe, Katherine A. Phillips, Kristin A. Favela, Alice Y. Yau, John F. Wambaugh, Jon R. Sobus, Antony J. Williams, Ashley J. Pfirrman, and Kristin K. Isaacs*

Cite This: *Environ. Sci. Technol.* 2021, 55, 11375−11387 Read Online

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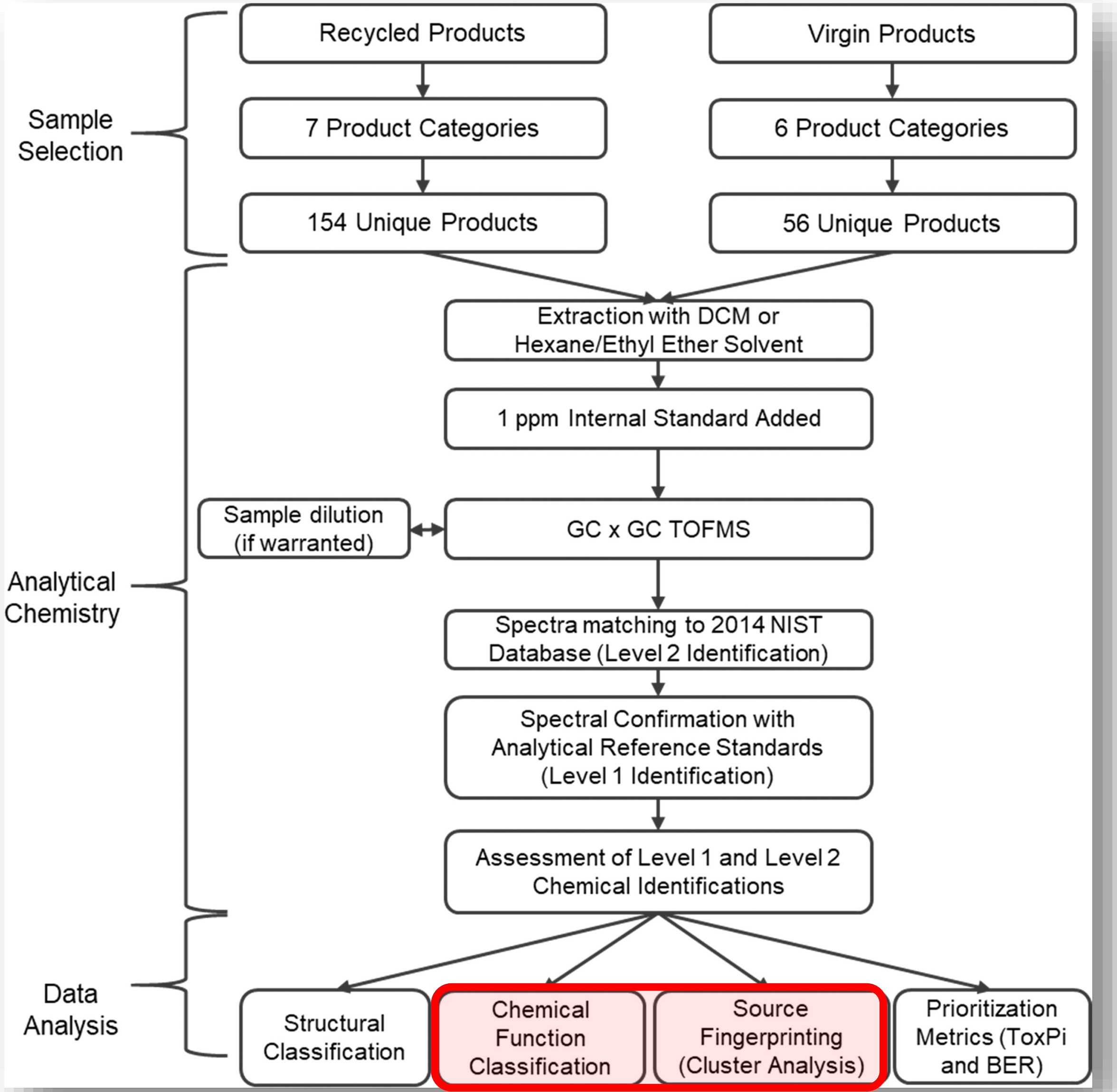
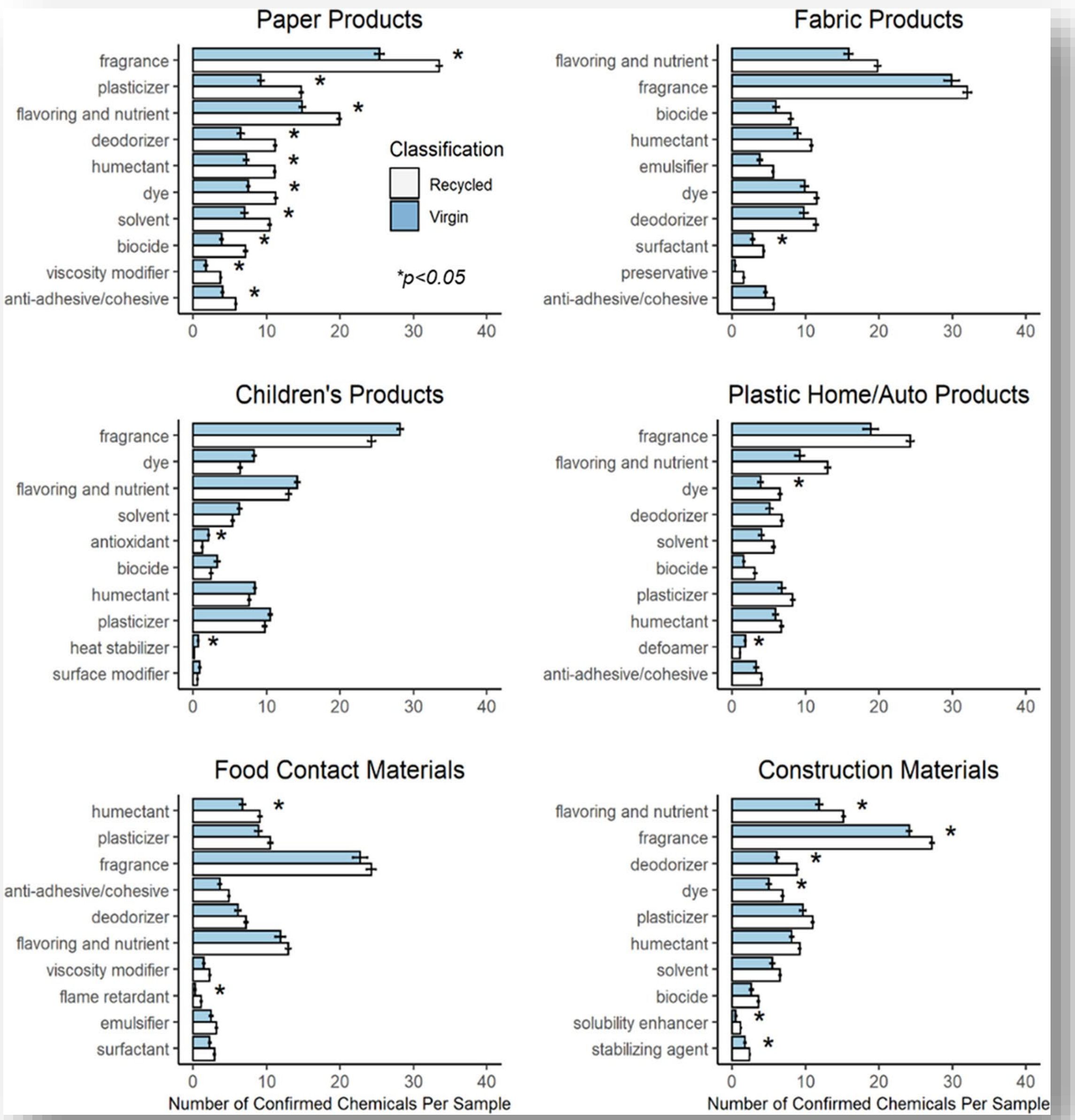
ABSTRACT: Recycled materials are found in many consumer products as part of a circular economy; however, the chemical content of recycled products is generally uncharacterized. A suspect screening analysis using two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) was applied to 210 products (154 recycled, 56 virgin) across seven categories. Chemicals in products were tentatively identified using a standard spectral library or confirmed using chemical standards. A total of 918 probable chemical structures identified (112 of which were confirmed) in recycled materials versus 587 (110 confirmed) in virgin materials. Identified chemicals were characterized in terms of their functional use and structural class. Recycled paper products and construction materials contained greater numbers of chemicals than virgin products; 733 identified chemicals had greater occurrence in recycled compared to virgin materials. Products made from recycled materials contained greater numbers of fragrances, flame retardants, solvents, biocides, and dyes. The results were clustered to identify groups of chemicals potentially associated with unique chemical sources, and identified chemicals were prioritized for further study using high-throughput hazard and exposure information. While occurrence is not necessarily indicative of risk, these results can be used to inform the expansion of existing models or identify exposure pathways currently neglected in exposure assessments.

KEYWORDS: *recycling, consumer products, human exposure modeling, consumer exposure, ExpoCast, nontargeted analysis, suspect screening*



Example 2: Recycled Product Analysis

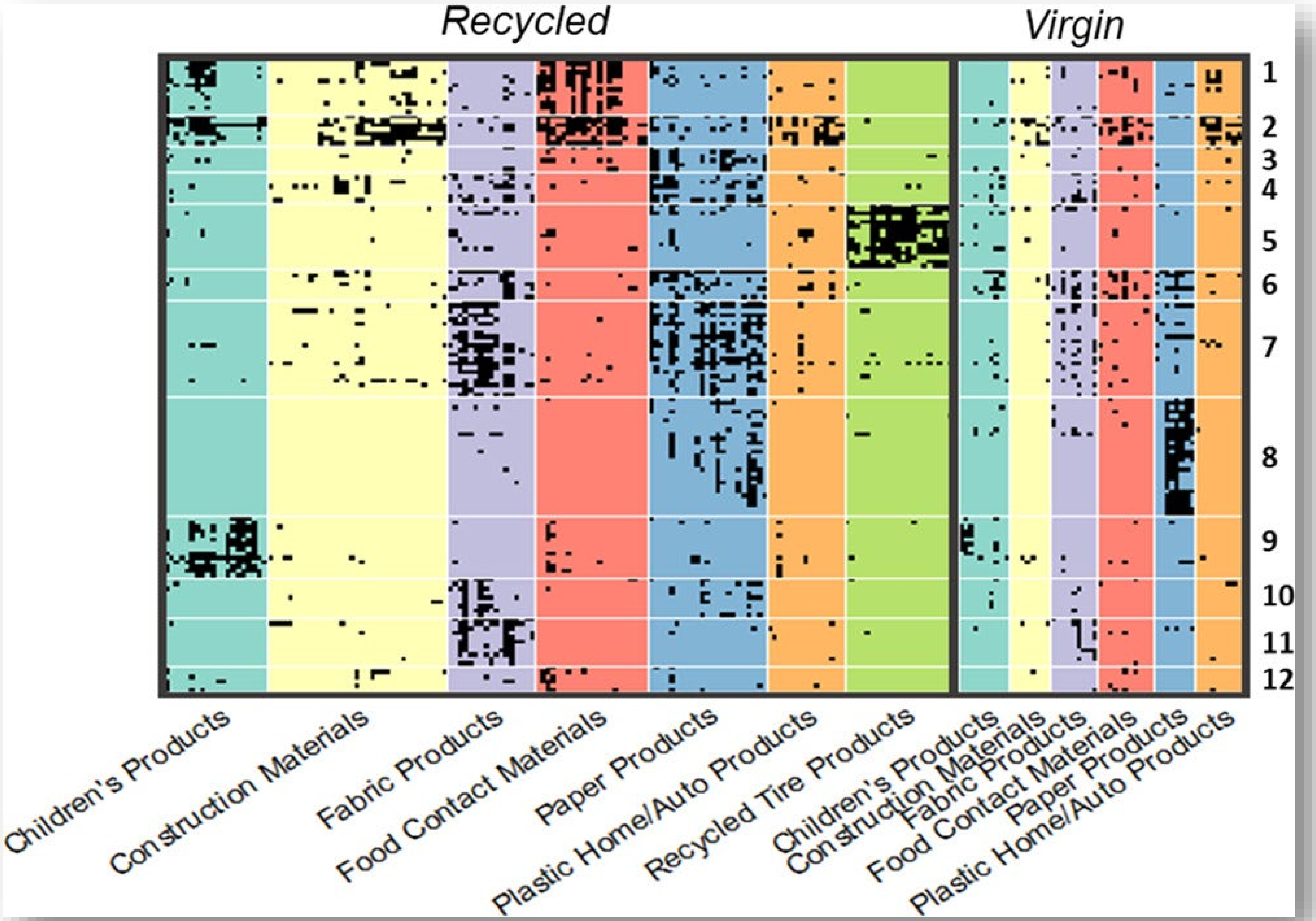


Significant differences between chemicals in recycled vs. virgin products for certain product & use categories

Most differences observed in paper products and construction materials

Some uses (e.g., fragrances) highly represented across all product/use categories

Example 2: Recycled Product Analysis



Some feature clusters (e.g., #2) show broad presence across product types & categories

Some feature clusters (e.g., #5) show specificity to a particular recycled product

Some feature clusters (e.g., #9) show specificity to a product type across both categories

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Example 2: Recycled Product Analysis

Table 2. Summary of Use Information for of Chemicals Co-occurring in Multiple Products[a]

cluster ID	number of chemicals	primary classification	primary categories of occurrence[b]	frequently occurring uses, sectors, or functions[c]	example chemicals
1	13	recycled	children's products, construction products, food contact materials	pesticide actives and inerts	permethrin, bifenthrin, chlorpyriphos
2	7	both	children's products, construction materials, food contact materials, plastic home/auto products	plastics and plastics manufacturing (including intermediates), polymer additives (UV stabilizer, antioxidant, odor agent)	tris(2,4-di-*tert*-butylphenyl) phosphite, octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate, 2-(phenylmethylene) octanal
3	6	recycled	paper products	manufacture of ink, paints/coatings, or paper surface treatments; pesticides	2,2-dimethoxy-1,2-diphenylethanone, propylbenzene, DEET, *p,p'*-methoxychlor olefin
4	7	both	construction materials, fabric products, and paper products, fabric products	manufacture of ink, paints, or dyes; use in ink, toner, and colorant products	2-(2-butoxyethoxy)ethanol, (1-hydroxycyclohexyl)(phenyl) methanone, phthalic anhydride
5	15	recycled	recycled tire products	intermediates, rubber components, and processing aids used in the manufacture of rubber products or rubber tires, or in rubber recycling	aniline, diphenylamine, dicyclohexylamine, phthalimide
6	7	both	fabric and paper products, children's products, food contact materials	manufacture of plastics, including plasticizers or plasticizer precursors and other polymer additives.	triethyl citrate, dimethyl phthalate, benzaldehyde
7	22	both	paper products and fabric products	cleaning product, ink, and apparel manufacturing; solvents, fragrances, biocides, dyes, flame retardants	1-phenoxy-2-propanol, *p*-cresol, tris(2-chloroisopropyl) phosphate
8	27	both	paper products	dyes and dye manufacturing, fragrances, pigments and pigment manufacturing	leucomalachite green, Michler's ketone, dehydroabietic acid
9	14	both	children's products	an alternative plasticizer used in children's products due to its low toxicity; adhesives, colorants, and chemicals used in their production	bis(2-ethylhexyl) terephthalate, tetradecanoic acid, 1,4-bis(2-hydroxy-2-propyl)benzene
10	9	recycled	fabric and paper products	fragrances, flavorants, manufacturing of chemicals, cleaning and washing	methyl benzoate, triclosan, dimethyl succinate
11	11	both	fabric products	flame retardants, fragrances, apparel manufacturing	2-butyl-1*H*-isoindole-1,3(2*H*)-dione, octrizole, biphenyl phosphate
12	6	both	food contact materials	polymer additives (e.g., odor agent, stabilizers); intermediates	2-hydroxy-4-methoxybenzophenone, hexyl salicylate, 3,5-di-*tert*-butyl-4-hydroxyhydocinnamic acid

Chemical use information is often consistent with desired product characteristics

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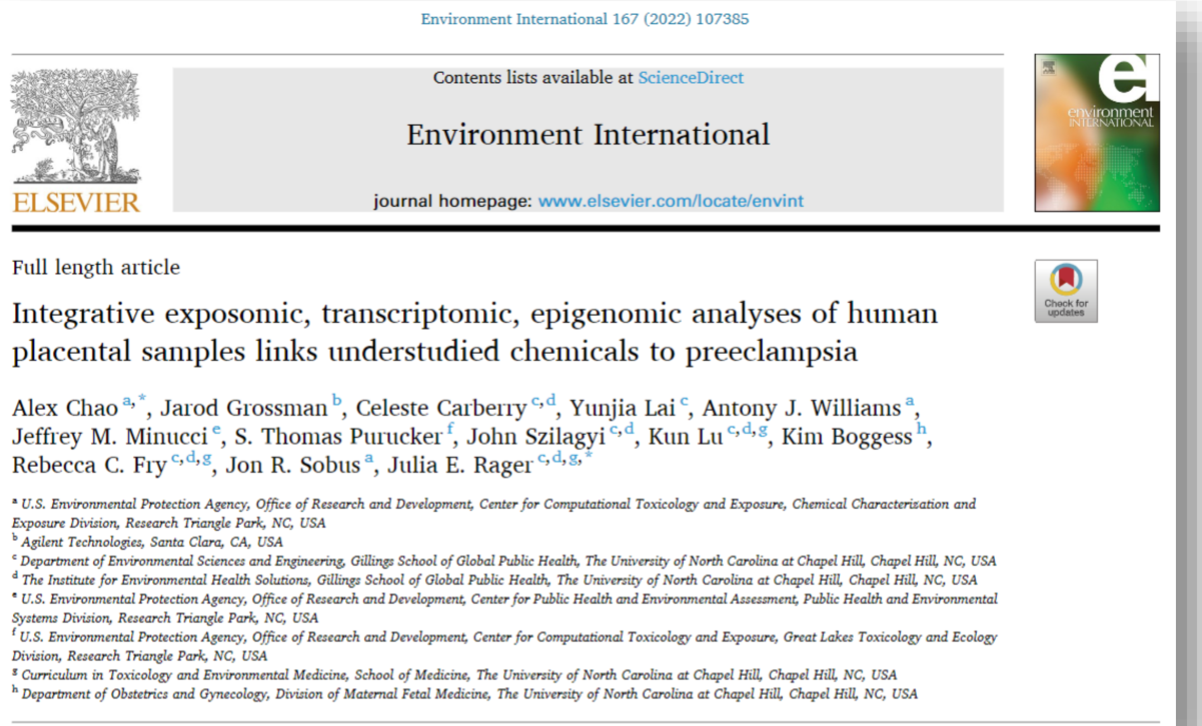
Example 3: Placental Tissue Analysis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Full length article

Integrative exposomic, transcriptomic, epigenomic analyses of human placental samples links understudied chemicals to preeclampsia

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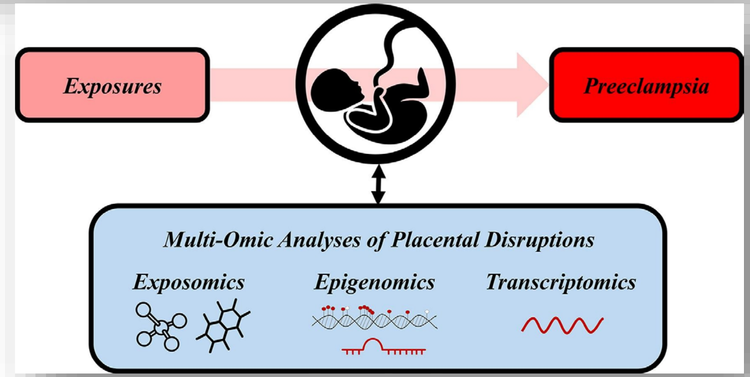
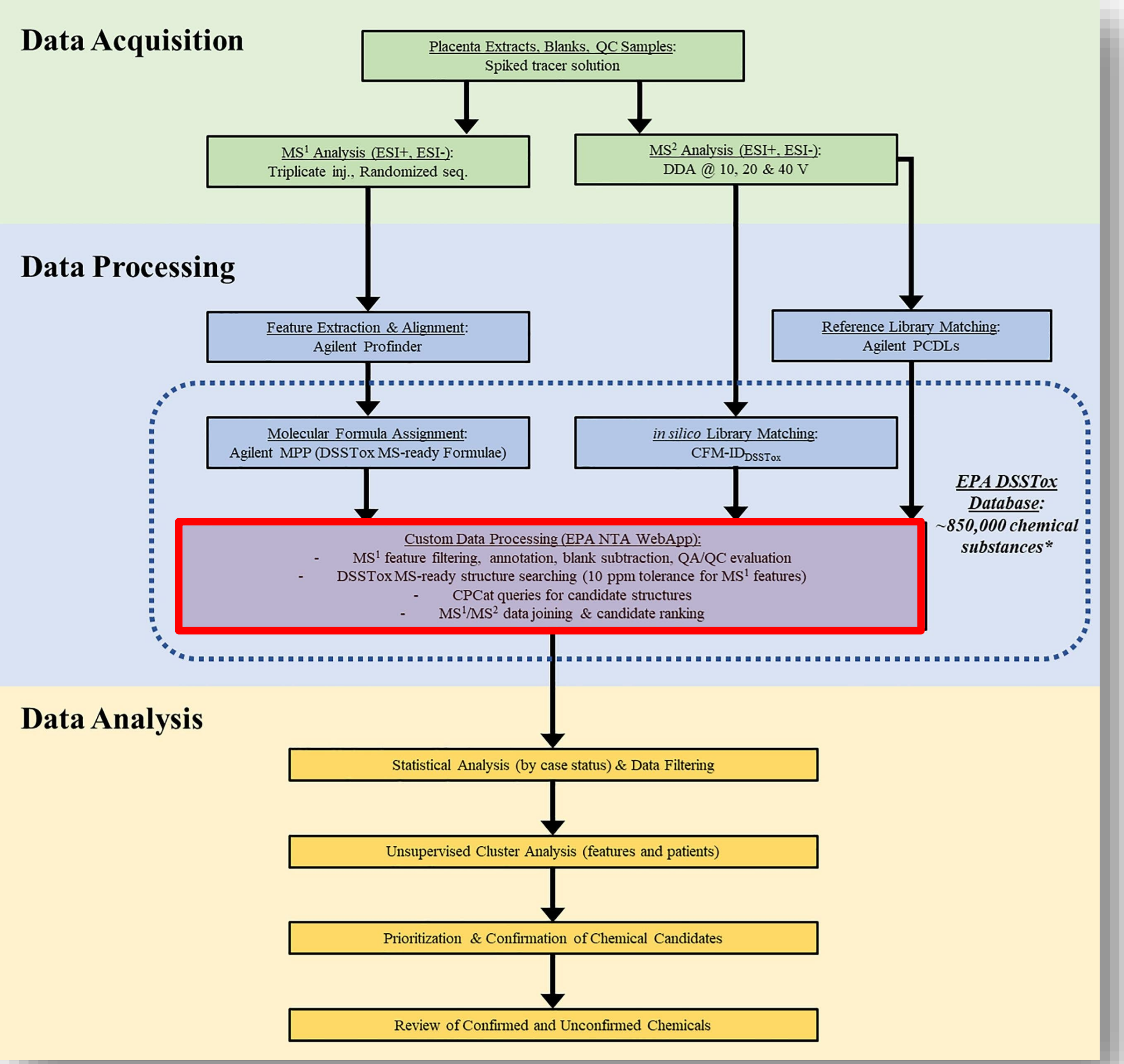
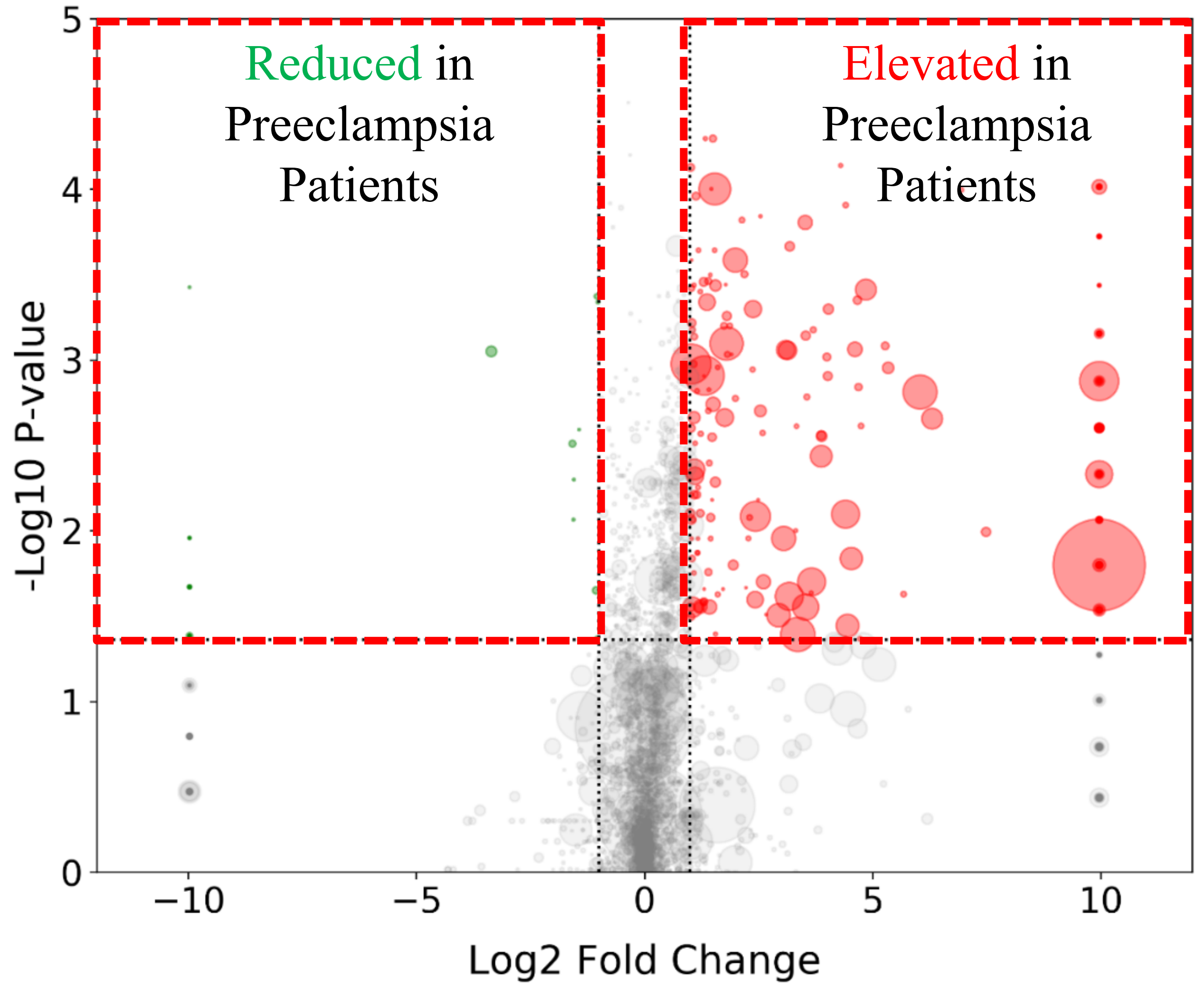




Example 3: Placental Tissue Analysis



NTA on placenta samples:

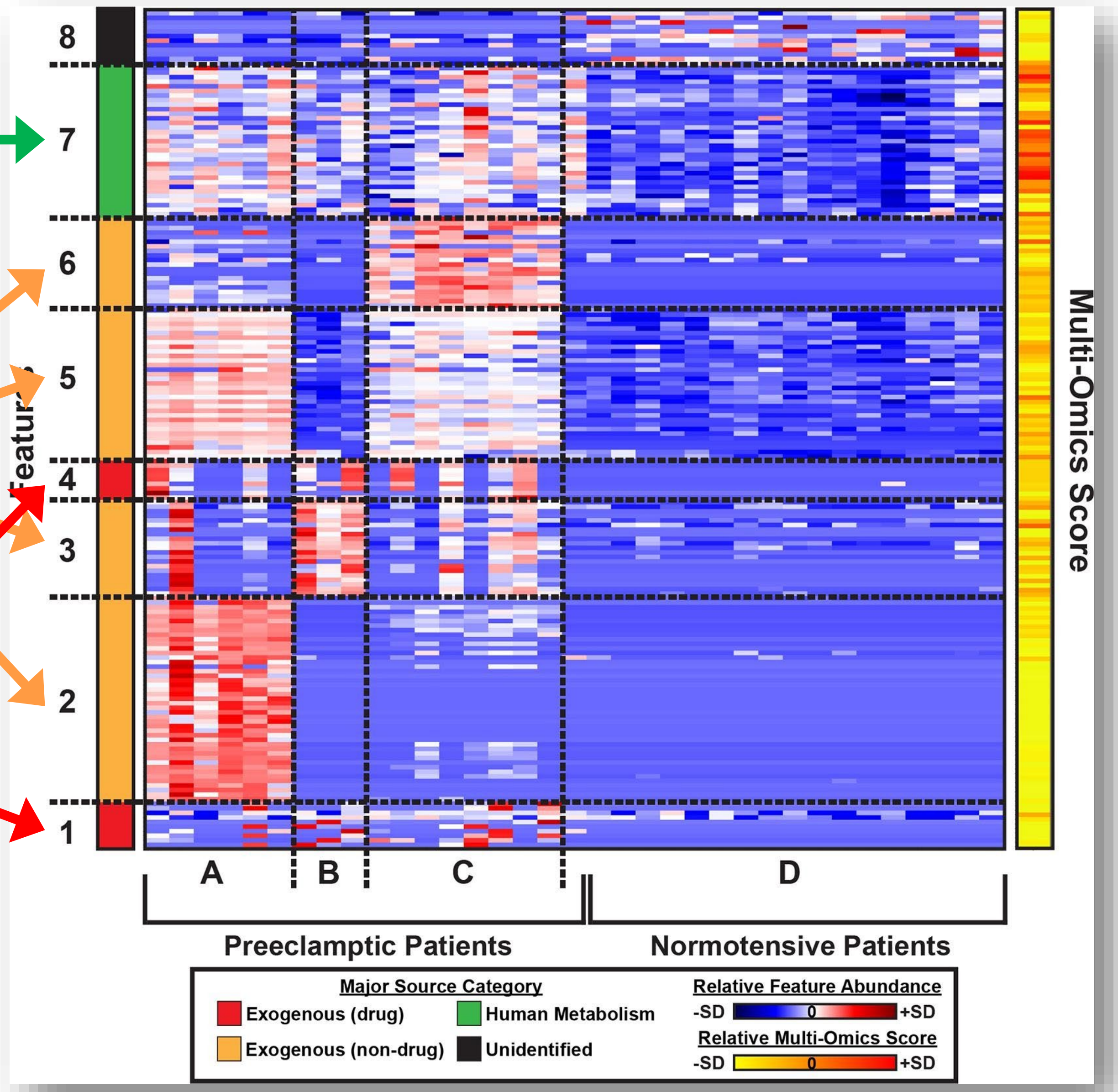
- Normotensive (n = 17) and preeclamptic (n = 18)
- **183 molecular features** found significantly different (~6000 potential candidates)
- Feature chemicals prioritized for targeted confirmatory work via:
  - Reference MS2 spectrum match
  - *In silico* MS2 spectrum match
  - Data source counts
  - CPCat database presence
  - **46 chemicals** prioritized / acquired
- **25 chemicals** confirmed via targeted analyses

Example 3: Placental Tissue Analysis

Human Metabolism Chemicals

Exogenous Chemicals (not drugs)

Exogenous Chemicals (drugs)

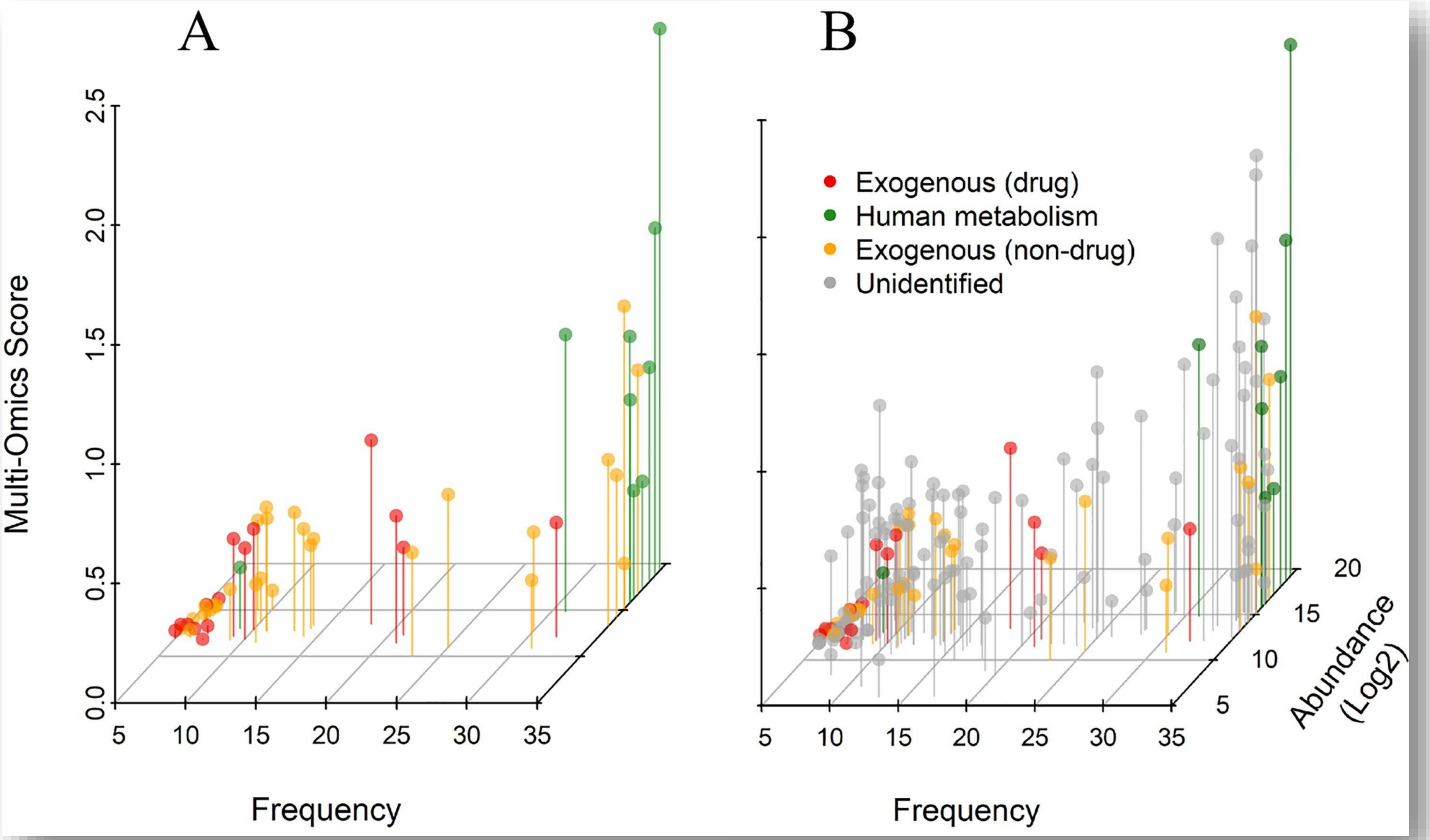


Stronger association with biological changes

Modest association with biological changes

Weaker association with biological changes

Example 3: Placental Tissue Analysis



More work is needed to identify all compounds elevated in preeclamptic patients

Source and use information, along with clustering patterns, provide clues to chemical origins

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Take-Home Points

- NTA methods can detect many analytes in virtually any sample matrix
- Tentative IDs in NTA studies far outweigh confirmed IDs
- Methods and tools are needed to prioritize tentative IDs for confirmation
- Prioritization should be based on:
  - Likelihood of presence (informed by source and use information)
  - Likelihood of importance (informed by provisional risk metrics)
- Future work will move towards:
  - Efficient prediction of source & use for tentative IDs
  - Network approaches to identify sources of feature clusters
  - Approaches to quantify tentatively identified chemicals without standards

Research Contributors

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