A Reference Library for Suspect Screening of Environmental Toxicants using Nontargeted Ion Mobility Spectrometry-Mass Spectrometry Analyses

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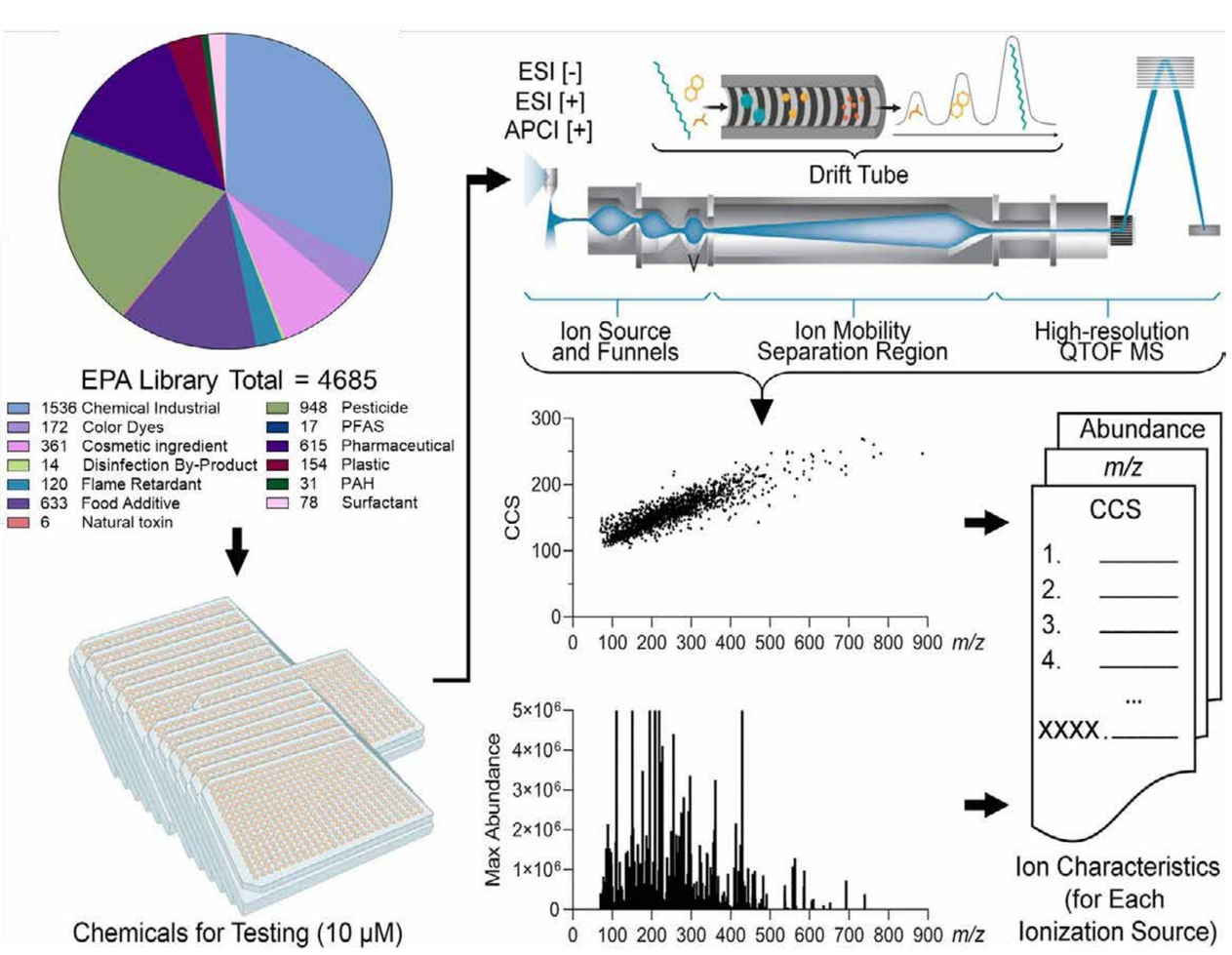
ABSTRACT

Exposure assessment traditionally relies on time-intensive extraction and analytical methods to evaluate <40 chemicals, which is infeasible for mixture analyses. Ion mobility spectrometry-mass spectrometry (IMS-MS) is a rapid post-ionization separation technique, applicable to targeted and non-target analyses of chemicals and mixtures. IMS-MS separates compounds based on mass-to-charge ratio (m/z) and size specific drift time (DT), enabling the calculation of collisional cross section (CCS) values crucial for molecular and isomeric distinctions in complex samples. In this project, we utilized 4,000+ diverse chemicals from the ToxCast Program to establish a comprehensive CCS database for future IMS-MS-enabled exposomic studies. Classified into 13 categories, chemicals were prepared at 10 µM in a 50:50 water/methanol solution and analyzed via IMS-MS using electrospray ionization (ESI, positive and negative modes) and atmospheric pressure chemical ionization (APCI, positive mode). The Agilent IM-MS Browser was then used to calculate CCS values followed by manual verification of each ion envelop across all detected compounds. Approximately 40% of all ToxCast compounds were detected in at least one of the ionization modes, with CCS reproducibility within +/-1% Å². Of the 40% of chemicals detected, ~65% were detected with ESI+, ~45% with ESI-, and ~45% with APCI+. Approximately 25% of the tested compounds were exclusively detected in ESI+, 25% in ESI-, and 8% in APCI+. These numbers showcase the need for diverse ionization modes in suspect screening. In summary, this database will be a pivotal tool for high-throughput suspect screening of environmental contaminants, enabling rapid exposure and risk assessments of complex environmental samples.

OBJECTIVES

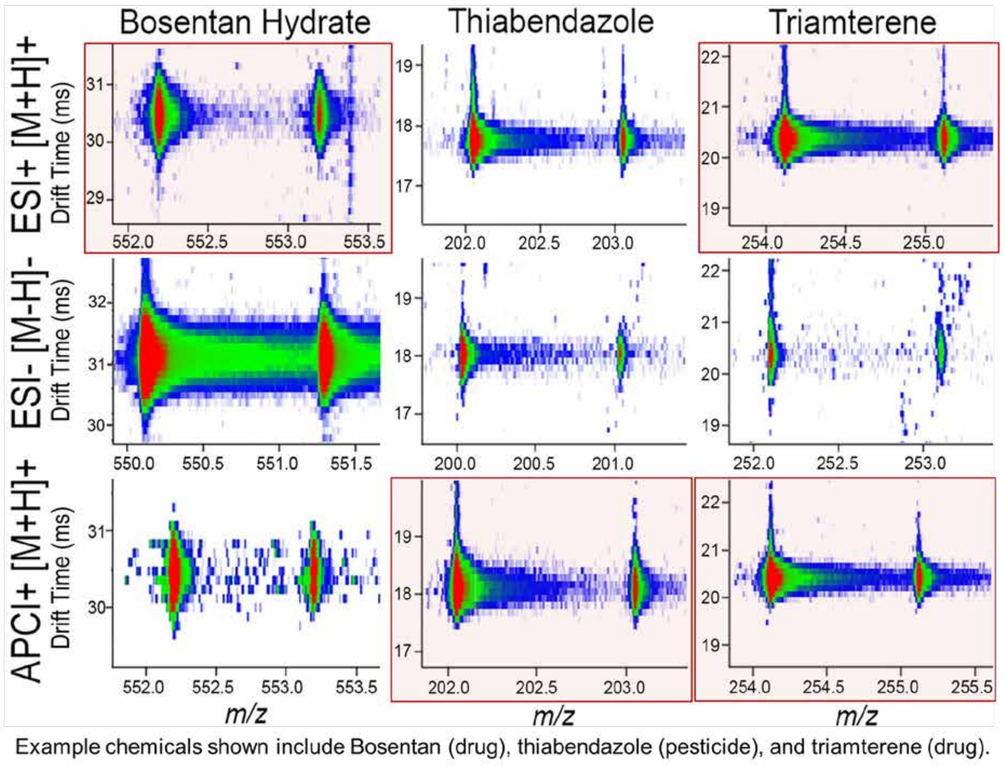
- Out of 4,685 chemicals analyzed via IMS-MS in three different ion modes: ESI(+), ESI(-), and APCI(+):
  1. Determine which ion mode(s) are best ionizers for each chemical and derive corresponding CCS values.
  2. Investigate instrumental reproducibility between CCS replicates to establish confidence in the CCS value.
  3. Compile all validated CCS values into a database available for public use.
- **Overall objective:** Facilitate mixtures exposure and risk assessment by compiling CCS spatial data for a large library of chemicals from EPA ToxCast library. This will enable more confident constituent identification in complex samples.

MATERIALS & METHODS



RESULTS

Ion Detection In Each of Three Ionization Modes: ESI(+), ESI(-), and APCI(+)



Example chemicals shown include Bosentan (drug), thiabendazole (pesticide), and triamterene (drug).

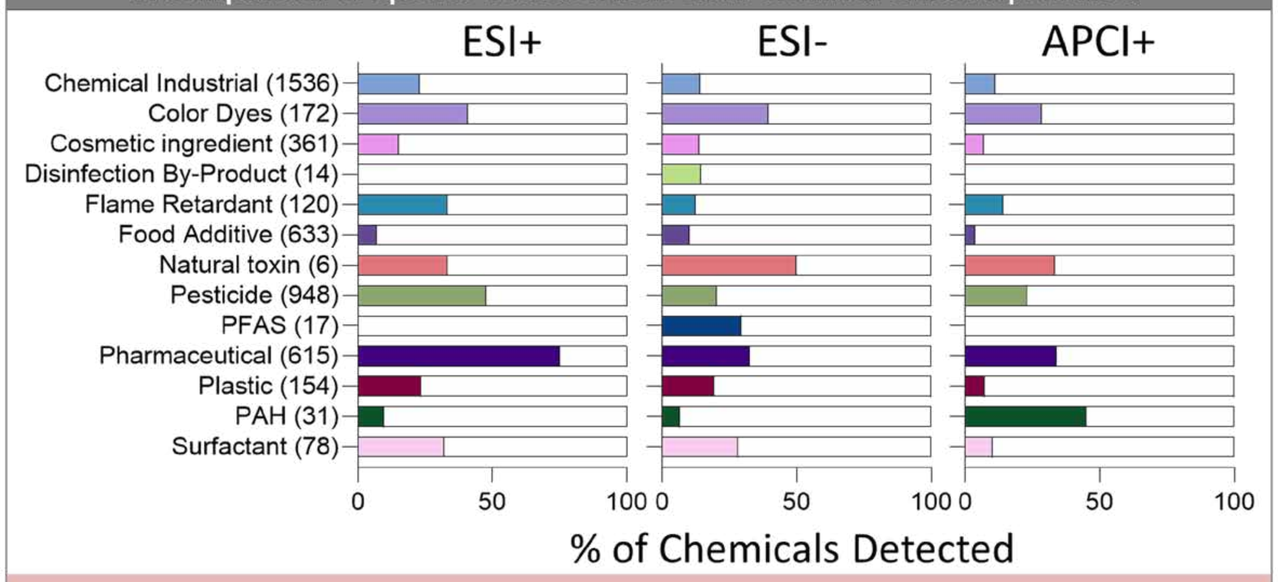
- Chemicals shown were ionizable in all three ion modes.
- Adducts observed include ESI [M+H]+, ESI [M-H]-, and APCI [M+H]+.
- For each feature, the parent ion and the ^{13}C isotope are shown.
- Abundance is shown as feature intensity. Ionization source & mode(s) preferred (red boxes) for each chemical correspond to spectra of highest abundance and ^{13}C isotope presence.

RESULTS

A Comparison of Species Detected with Each Ionization Mode

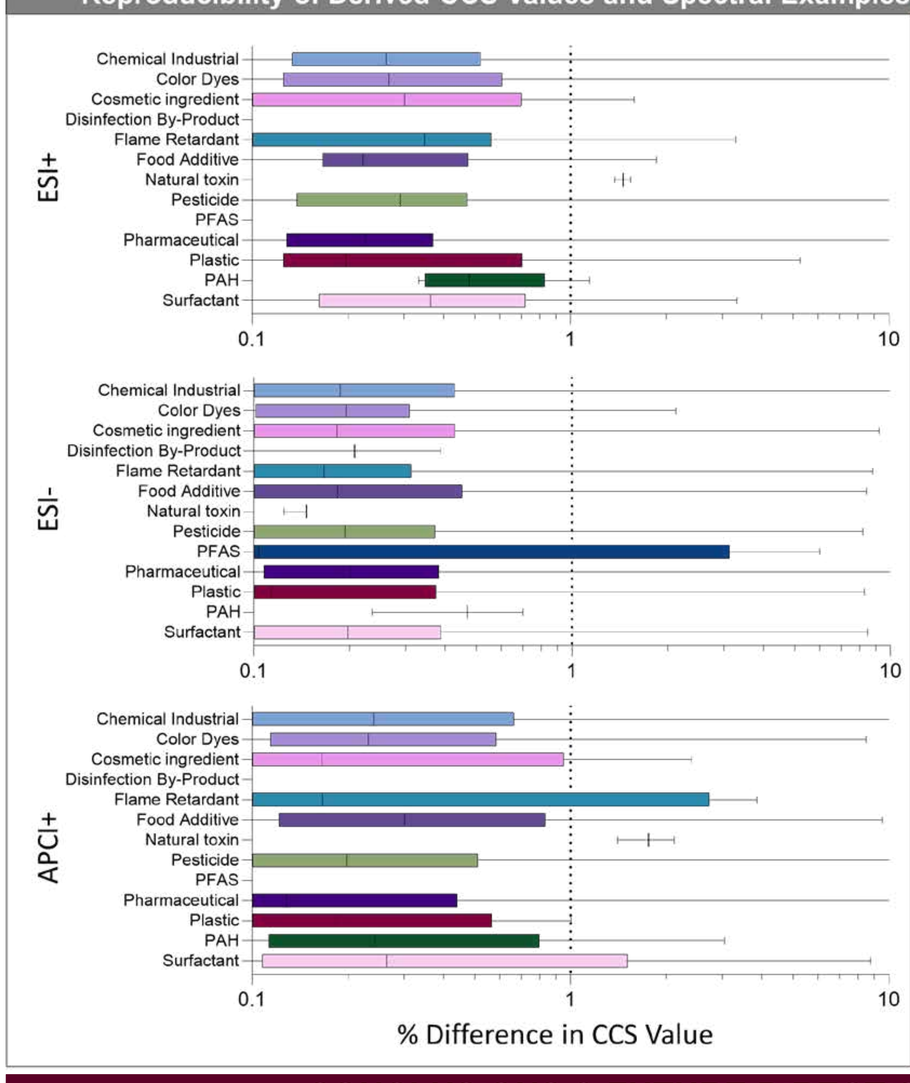
- ~40% of chemicals were ionized for derivation of a CCS value.
- ESI was the most robust source, able to ionize 1,607 out of 1,751 chemicals.
- [M+H]+ and [M+Na]+ were the adducts most frequently observed in positive ion mode.
- 139 chemicals were deemed unsuitable for the instrument and were not analyzed (e.g. metals).

A Comparison of Species Detected from Each Chemical Class Represented



- Overall, ESI(+) and ESI(-) ion modes enable derivation of a varying number of CCS values, depending on chemical classification.
- ESI(-) show detection of Disinfection By-Products and PFAS, neither of which is detected in ESI(+) nor APCI(+).
- Detection by APCI+ is more limited (primarily aromatics), as supported by literature.
- While multiple modes of ionization are needed to detect a wide range of chemicals, certain chemical classification ionize better with a particular mode.

Reproducibility of Derived CCS Values and Spectral Examples of Features Detected



- Two replicates of each chemical were analyzed. Instrumental reproducibility was measured by calculating the % difference in CCS values derived from each replicate.
- ESI(+) had the best reproducibility; a majority of derived CCS values are in +/- 1% of the duplicate runs.
- ESI(-) and APCI(+) exhibited greater variability in derived CCS values.
- CCS variability can be a result of several factors (right panel), including the detection of isomeric features, saturation of the detector, multimer clustering, and low levels of abundance (but not below LOD).
- Future analysis must be conducted to determine the relationship between each ion mode and CCS variability.
- All CCS values that fall outside of the +/- 1% range must be reanalyzed to establish confidence in the value.

CONCLUSIONS & FUTURE DIRECTIONS

Conclusions

- 4,685 EPA ToxCast chemicals were analyzed in three ion modes to determine CCS values: ESI(+), ESI(-), and APCI(+).
- CCS values could be derived for ~40% of the chemicals.
- Most of the compounds analyzed are polar; ESI is therefore a more suitable choice to derive CCS value than APCI. However, choice of ionization source is dependent upon the chemical structure of the compound in question.

Future Directions

- Spectra will be analyzed further to determine why CCS values are less reproducible for specific chemicals, and whether they should be included or excluded from the database. Individual chemical structures will also be investigated to determine a potential link between chemistry and detection.
- Data will be compiled into a database for public use.

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