In silico MS/MS fragmentation spectra for identifying chemical unknowns: applications and performance validation

Abstract #239

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OBJECTIVES

- Demonstrate identification of unknown chemicals using high resolution mass spectrometry (MS) utilizing workflows with relevant data and software analysis tools [1-3]
- Examine whether the comparison of experimental MS fragmentation data with predicted fragmentation data can increase confidence in compound identification [4]
- Demonstrate whether predicted fragmentation data, coupled with relevant metadata, helps identify unknowns

APPROACH

- Use "MS-Ready" forms of structures from US-EPA CompTox Chemicals Dashboard [5] as input files: ~800,000 structures
- Use CFM-ID package (https://cfmid.wishartlab.com/) to generate mass spec. fragmentation spectra for +ve and –ve ion LCMS and EI GCMS spectra. 7 spectra per chemical.
- Combine rich Dashboard metadata with fragmentation matching of experimental spectra to rank candidate hit lists

MAIN RESULTS

- The identification of "known-unknowns" using non-targeted analysis benefits from the use of CFM-ID as an *in silico* fragmentation prediction tool CFM-ID Competitive Fragmentation Modeling for Metabolite Identification
- Combining metadata candidate ranking of hits based on mass or formula searches gives improved results
- CFM-ID predicted spectra are available as FAIR Open Data
- Proof-of-concept web applications are in testing

IMPACT

- The free availability of the CompTox Chemicals Dashboard for the community, coupled with MS-Ready structures to generate *in silico* MS/MS fragmentation data, and metadata for candidate ranking, is a basis for the development of structure identification software tools at EPA
- **For more information, contact:** Antony Williams, williams.antony@epa.gov

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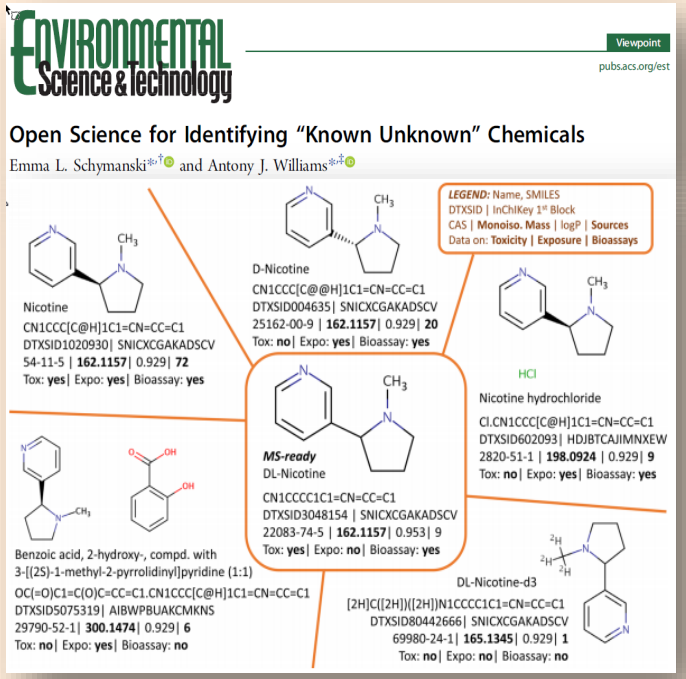
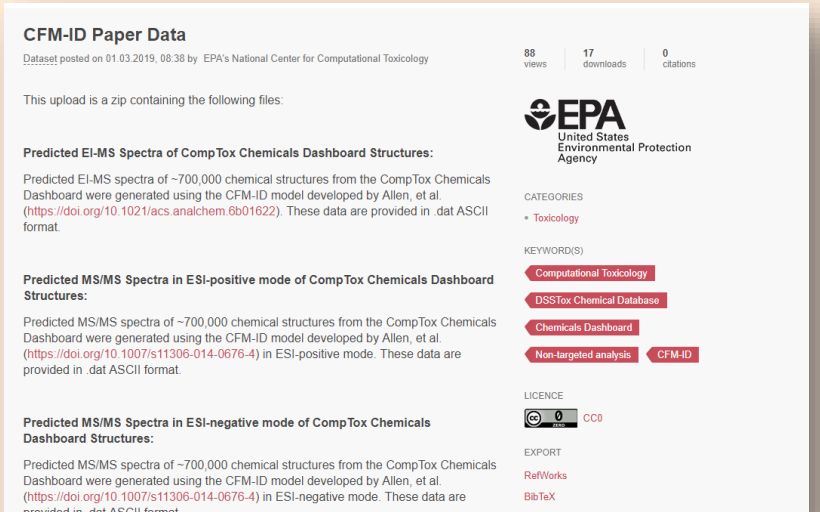
MAIN RESULTS



MS-Ready Structures [6] are the inputs to *in silico* fragmentation. This approach removes stereobonds, desalts and splits multicomponent chemicals but maps back to the original substances in the CompTox Chemicals Dashboard. This mapping provides association with substance

~800,000 MS-Ready structures were used to predict fragmentation [7]. The dataset is available as a FAIR dataset for repurposing: https://doi.org/10.23645/epacomptox.7776212.v1



Validation of performance of combined approach with 5 years of CASMI contest data [8]. Percentage of compounds from each dataset ranked in the top (number 1) position by *in silico* MS/MS match only, Data Source count (DS) only, and the combined score of *in silico* MS/MS data with Data Source counts.

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Summary

In silico MS/MS fragmentation is highly beneficial for the identification of unknowns and supporting non-targeted analysis

- Our multiple studies [1-3,7-9] demonstrate the benefit of *in silico* prediction especially when coupled with metadata for candidate ranking of hits
- MS-Ready structure generation [6] is an essential step to the production of input structures for processing

Future Plans

Following testing and performance validation the software applications described here will be released.

- Public access to the CFM-ID experimental search tool
- A new non-targeted analysis web application (NTA WebApp) reading instrument data and using both *in silico* fragmentation data and metadata for candidate ranking will be made available for community use [9]
- Public access to MS-Ready structure set processing

Ongoing updates to CFM-ID fragmentation predictions will be provided as FAIR data for reuse and repurposing

References

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