MS2Tox: Discovering Endocrine Disruptors via Molecular Networking

Yvonne Kreutzer[a], Ida Rahu[b], Ulf Norinder[c,d], Anneli Kruve[a,b]

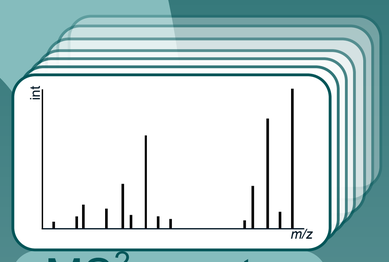
[a]Department of Chemistry, Stockholm University, Svante Arrhenius väg 16, 106 91 Stockholm, Sweden, [b]Department of Environmental Science, Stockholm University, Svante Arrhenius väg 8, 106 91 Stockholm, Sweden, [c]Department of Computer and Systems Sciences, Stockholm University, P.O.Box 1073, SE-164 25 Kista, Sweden, [d]MTM Research Centre, School of Science and Technology, Örebro University, 701 82 Örebro, Sweden

1 Background and Aim

Discovering endocrine disruptors (EDs) in complex mixtures is challenging as <10% of the chemicals are structurally annotated[1].

Machine learning models, from the MS2Tox toolbox, can predict toxicity from MS^2 spectra[2,3] and Molecular networks aid in annotating unknown transformation products in wastewater[4].

This study assesses molecular networks for pinpointing EDs from mass spectrometric data. Furthermore, it is compared with existing MS2Tox and newly trained conformal predictions approach.



Potential
aryl hydrocarbon receptor (AhR) agonist?
Labelling of MS^2 spectra with 3 different approaches prior to identification

2 Materials and Methods

Training Set

ESI(+)/HRMS spectra from MassBank, MoNA, GNPS, NIST23 of 4274 unique chemicals with available endpoint information from Tox21 Data Challenge.

Test Set

Comparison of approaches based on common test set, 861 compounds. Approaches are compared on false positive rate (FPR) at 50% and 90% recall.

Wastewater samples

Influent and effluent samples measured with LC/ESI(+)/HRMS Orbitrap.

Molecular Networks (MS_2 similarity based)

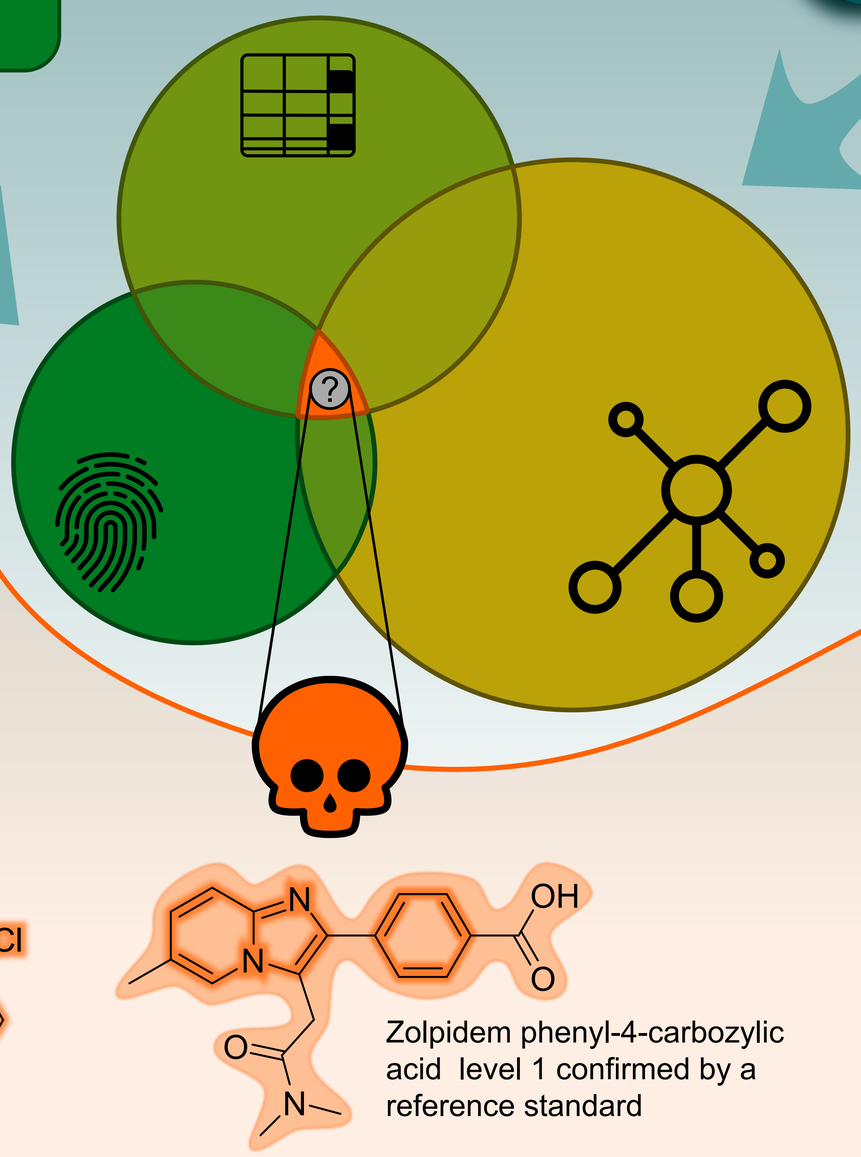
Nodes are connected based on MS2DeepScore, indicating MS^2 spectral similarity. The propability of an LC/ESI(+)/HRMS feature being active is calculated through its active neighboring nodes. A feature was classified as active if its probability exceeded a threshold that guaranteed recall of 50% or 90%.

Conformal Predictions (MS_2 similarity based)

Random forest classifier with subsequent error rate estimation.

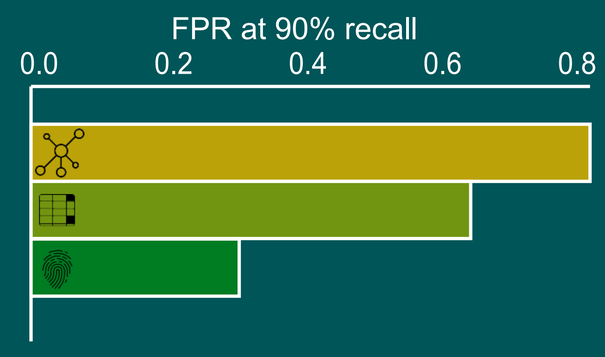
MS2Tox (Fingerprint based)

SIRIUS+CSI:FingerID (v5.8.6) fingerprints used to train a MS2Tox Extreme Gradient Boosting (XGBoost) Classifier.



3 Results and Discussion

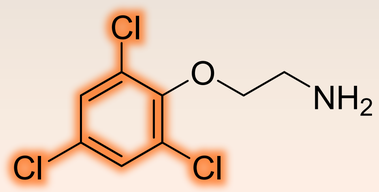
Test set results on AhR endpoint

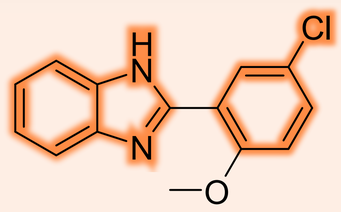


Fingerprint models have the lowest FPR.

Unknown LC/ESI(+)HRMS features labelled as potentially posing endocrine-disrupting activity by all approaches are further investigated.

Structural candidates are obtained through library matching and SIRIUS+CSI:FingerID.





Zolpidem phenyl-4-carbozylic acid level 1 confirmed by a reference standard

highlighed scaffolds have been shown to be associated with AhR interaction.

Sample application

Structural candidates of labelled features contain scaffolds related to AhR interaction.

We recommend selecting methods such as MS2Tox models, trained to associate specific spectral or structural features with the bioactivity, over approaches that rely on general spectral/structural similarity for feature prioritization in non-target screening workflows.

[1] Papazian, S.; et al. Commun. Earth Environ. 2022, 3 (1), 1–14. 7
[2] Rahu, I.; et.al. J. Chem. Inf. Model. 2024, 64 (8), 3093–3104.
[3] Peets, P.; et al. Environ. Sci. Technol. 2022, 56 (22), 15508–15517.
[4] Oberleitner, D.; et al. Anal. Bioanal. Chem. 2021, 413 (21), 5291–5300.

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Kruve lab

Contact:
Yvonne Kreutzer
yvonne.kreutzer@su.se