Kruve lab

Assessing the removal of tetracycline and toxicity of its transformation products with non-target LC-HRMS analysis and MS2Tox

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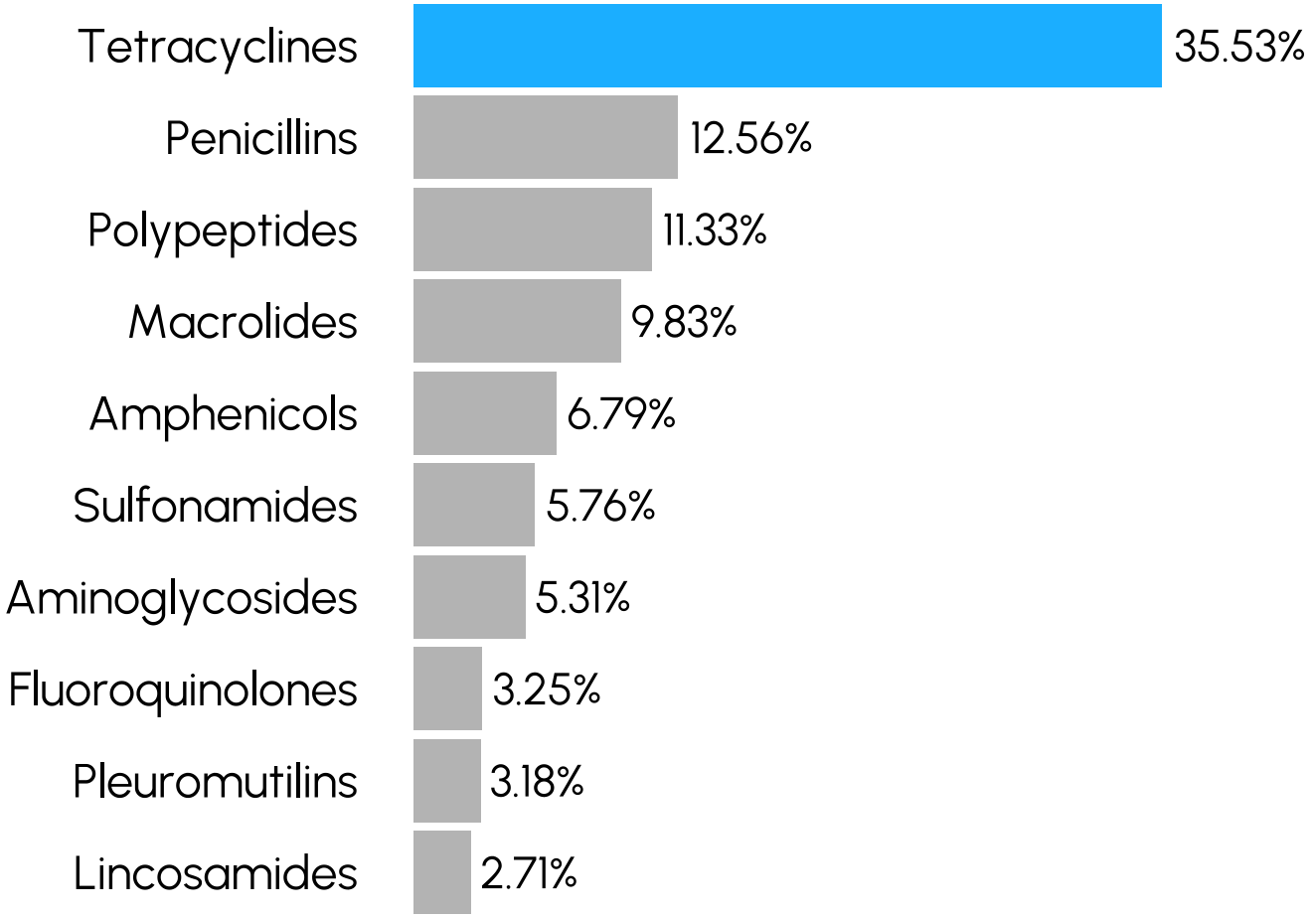
Tetracyclines – most abundant antibiotics

Proportion of antimicrobial classes reported for use in animals in 2021



Adapted from: World Organisation for Animal Health. (2024). *Annual report on antimicrobial agents intended for use in animals (8th ed.)*.
Wang et al., S, Science of The Total Environment, 949 (2024), 175126,

Tetracyclines – most abundant antibiotics

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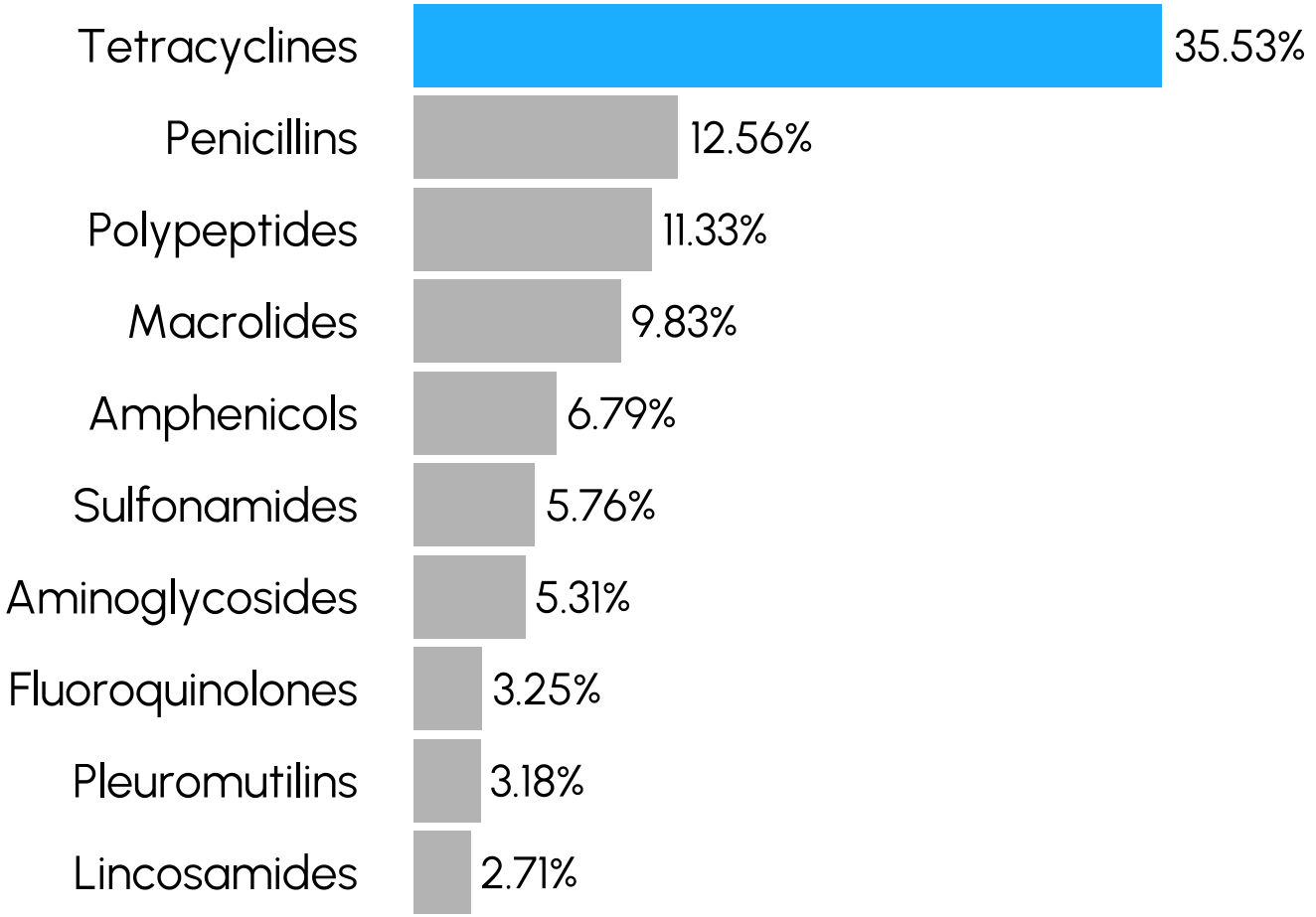
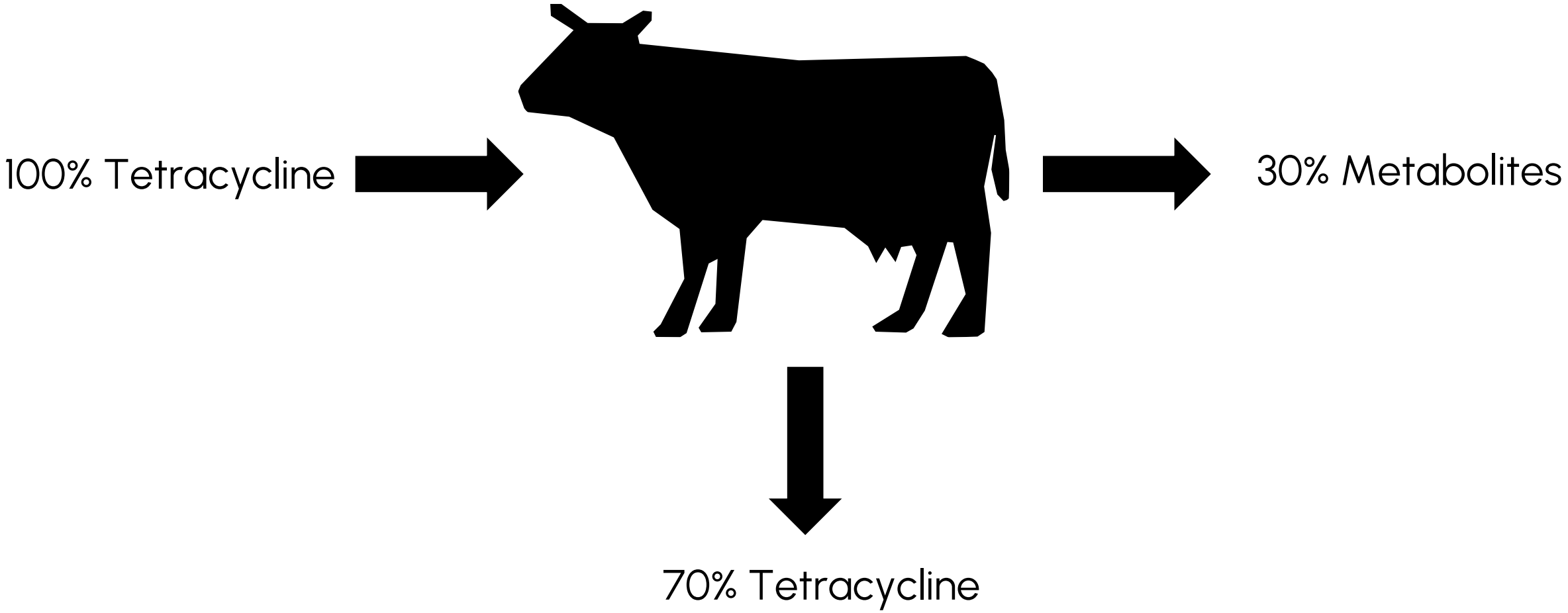


2024

33 305 tons
Tetracyclines

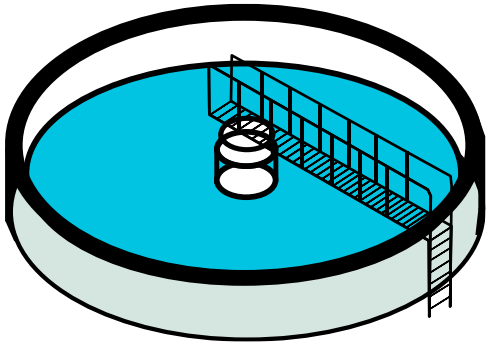
Adapted from: World Organisation for Animal Health. (2024). *Annual report on antimicrobial agents intended for use in animals (8th ed.).*
Wang et al., S, Science of The Total Environment, 949 (2024), 175126,

Tetracycline is insufficiently metabolized



Daghrir et al., Environmental Chemistry Letters, 11 (2013), 209–227.

Setup: wastewater treatment plant



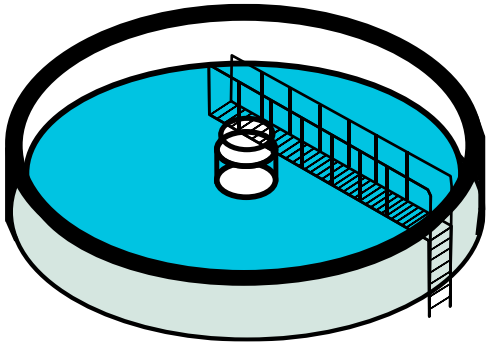
1st Sedimentation

2nd Biological reactor

3rd Cl_2 Chlorination

UV

Setup: wastewater treatment plant



1st Sedimentation

2nd Biological reactor

3rd Cl_2 Chlorination

UV

4th

C Granular activated carbon

O_3 Ozonation

HO• UV/ H_2O_2

Fenton reactions

Advanced Oxydation Processes (AOPs)

Photo-electro-Fenton reaction

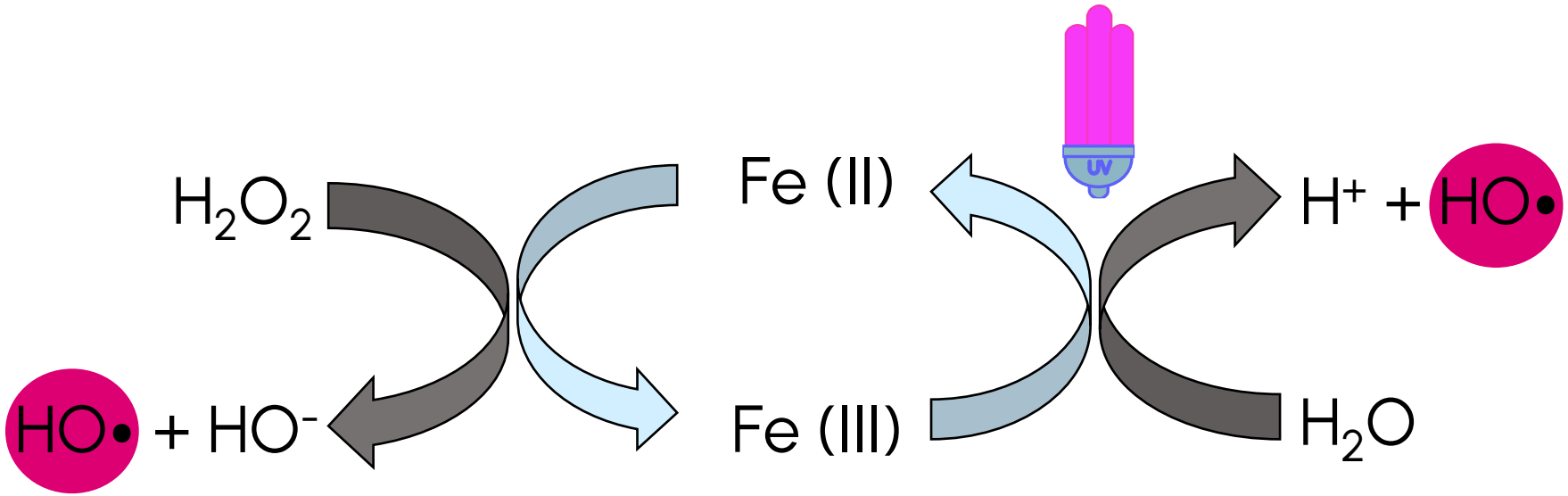
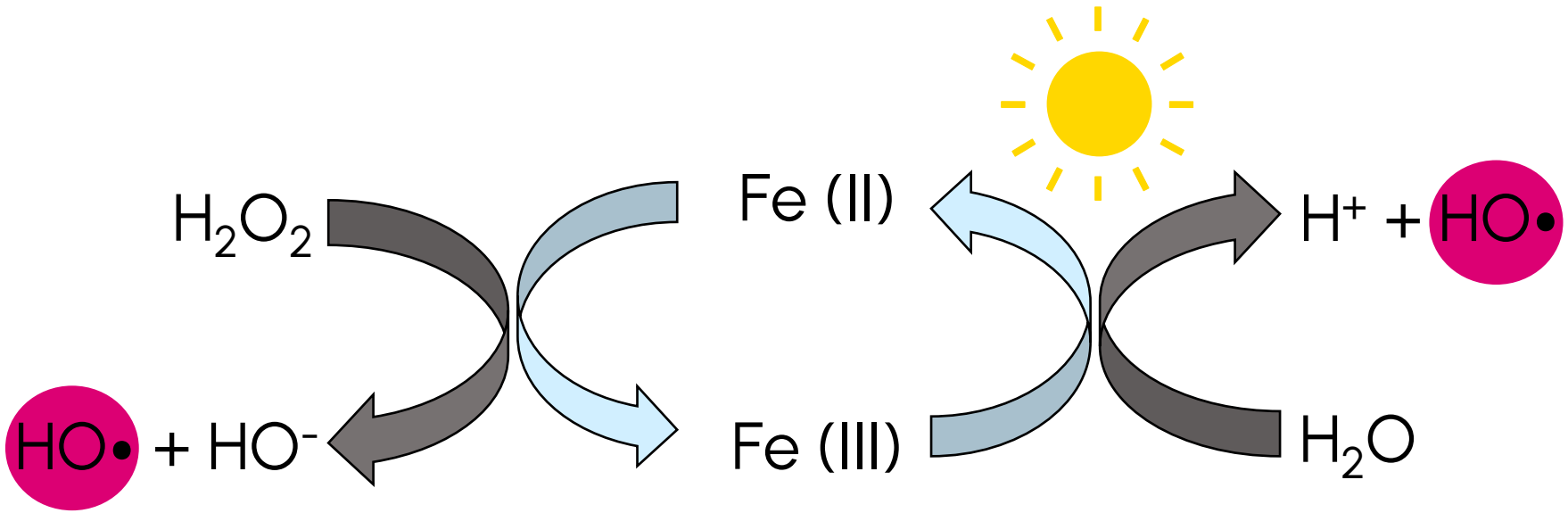


Photo-electro-Fenton reaction



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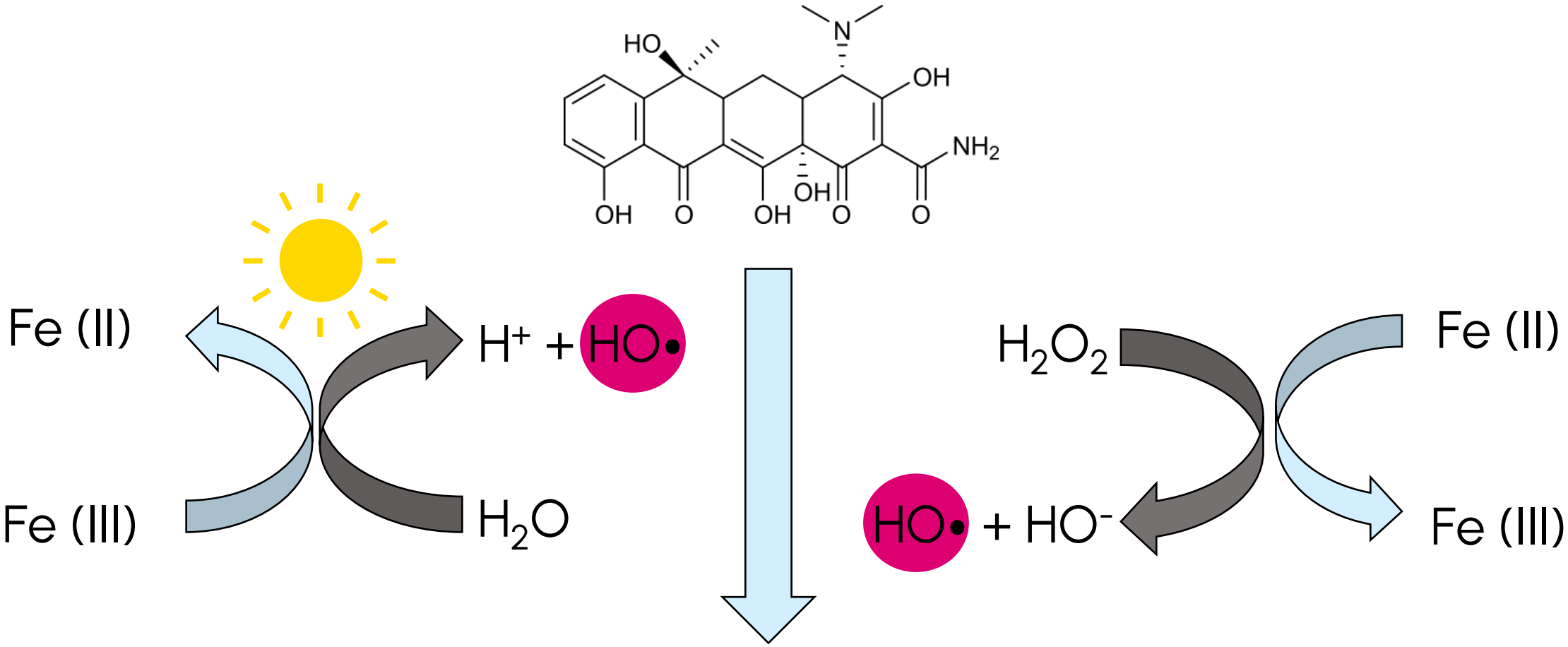
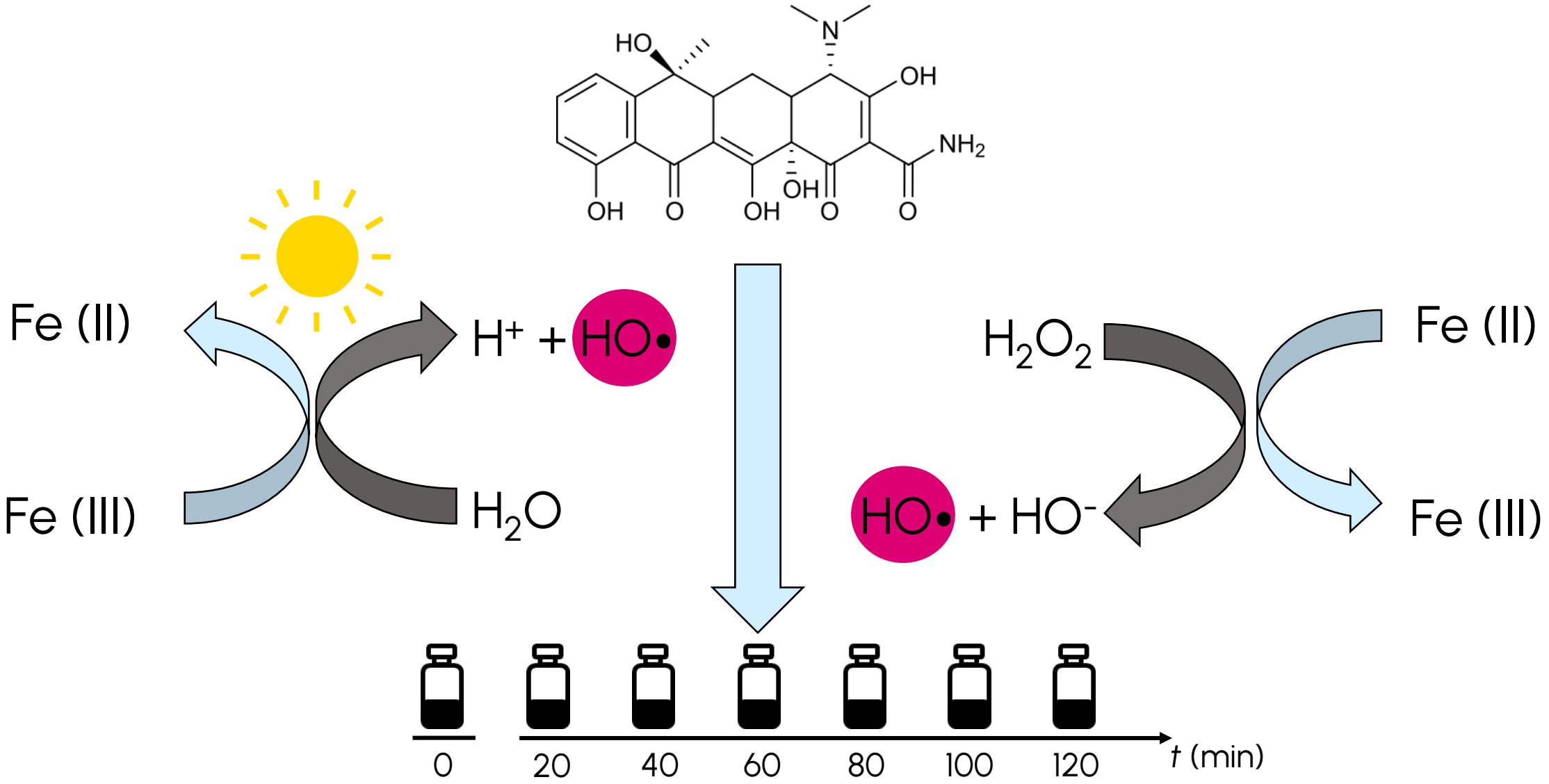


Transformation products (TPs)

Photo-electro-Fenton reaction



Aim

1. Validate degradation: tetracycline

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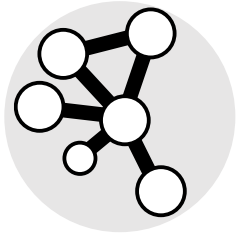
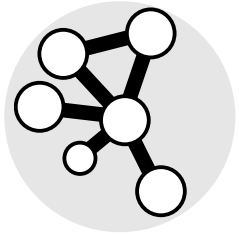


2. Find TPs of tetracycline via molecular networking

Aim

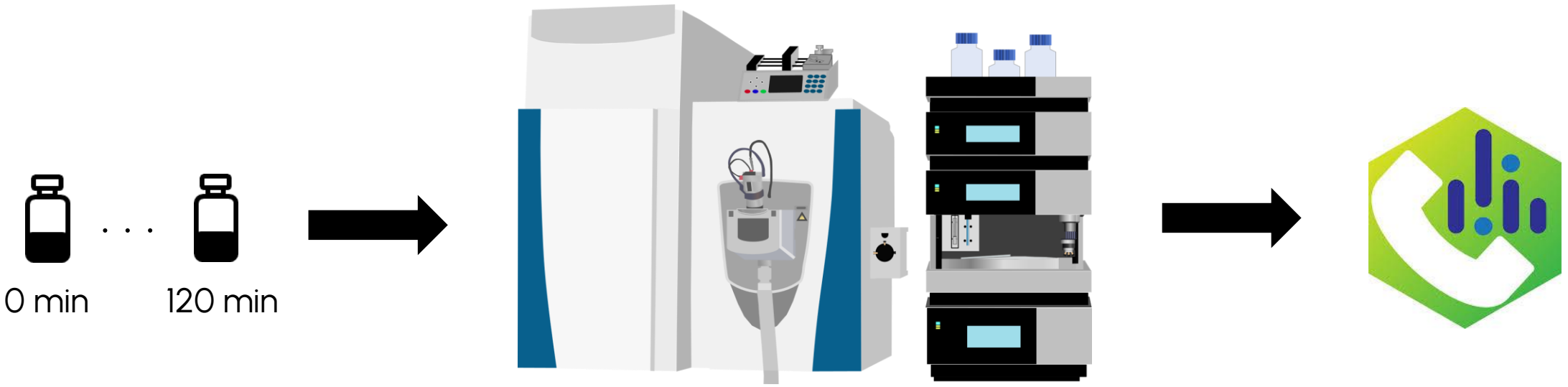
1. Validate degradation: tetracycline



2. Find TPs of tetracycline via molecular networking

3. Evaluate TPs: Predict adult fish toxicity with MS2Tox

Workflow of analysis



Samples

RPLC-HRMS

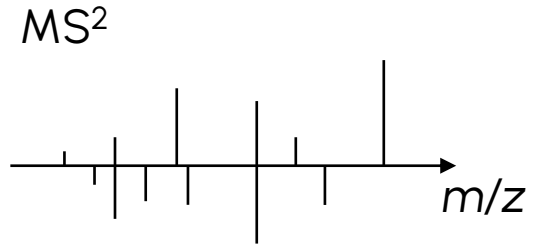
Kinetex C18/ Q Exactive Orbitrap

Data processing

MS-DIAL 5.3

Molecular networking: parameters

Modified dot product ≥ 50%



Data processing

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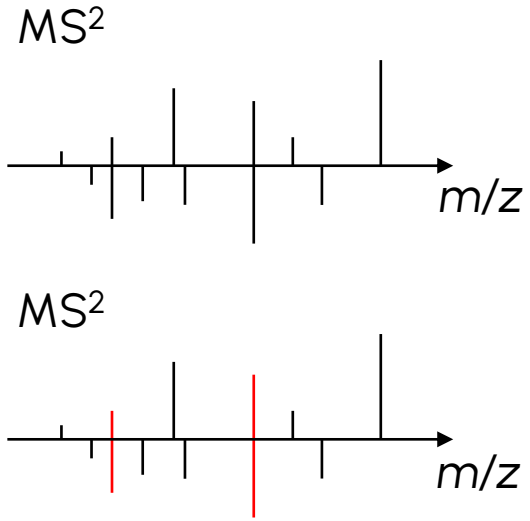
Molecular networking: parameters

Data processing

MS-DIAL 5.3

Modified dot product ≥ 50%

Matching peaks ≥ 2
with $\Delta m/z \leq 0.025$



Molecular networking: parameters

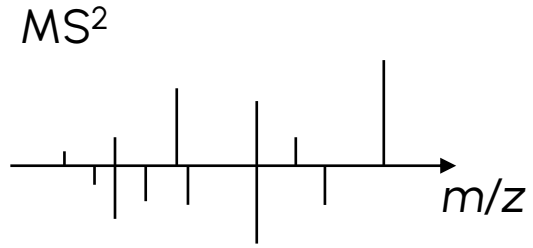
Data processing

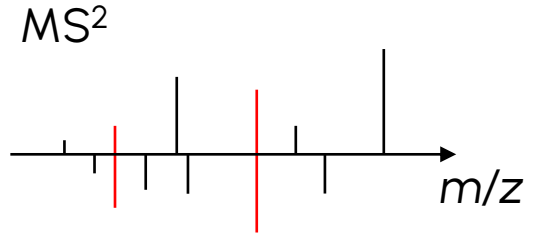
MS-DIAL 5.3

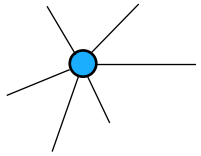
Modified dot product ≥ 50%

Matching peaks ≥ 2
with Δ*m/z* ≤ 0.025

Edge number ≤ 6







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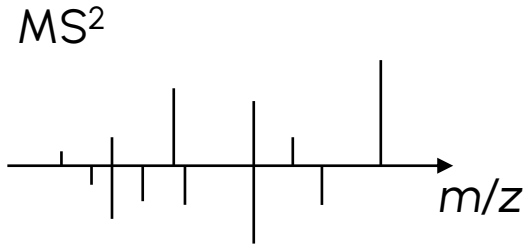

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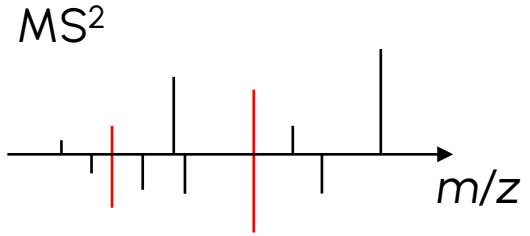
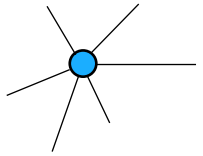
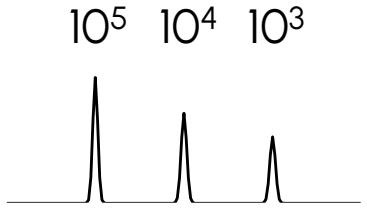


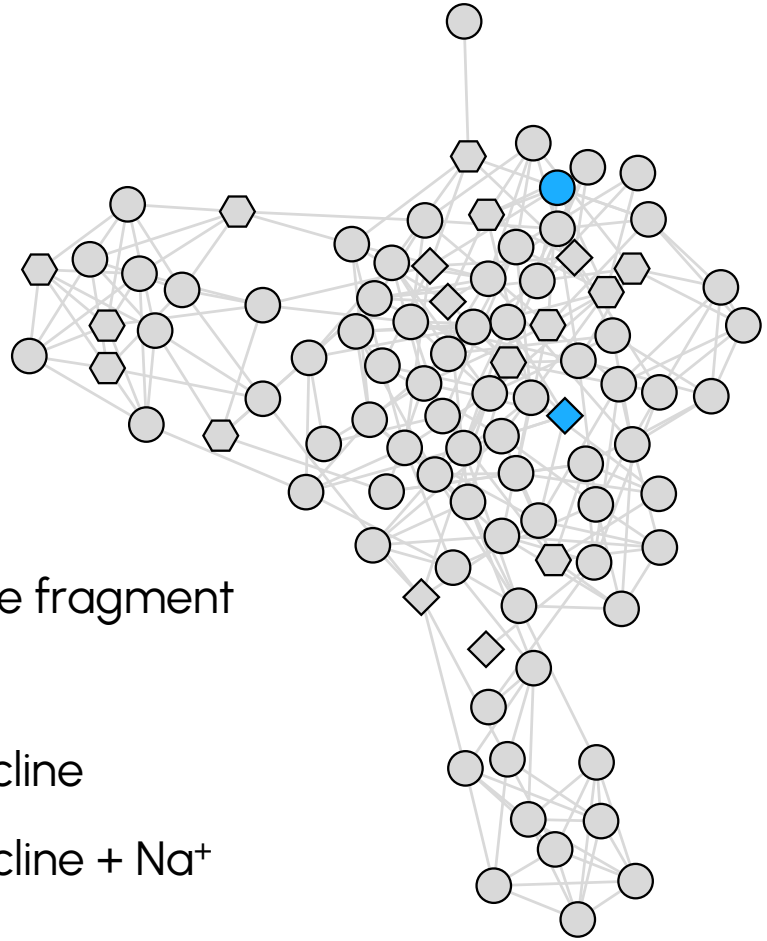
Edge number ≤ 6



Absolute intensity ≥ 10^5, 10^4, 10^3

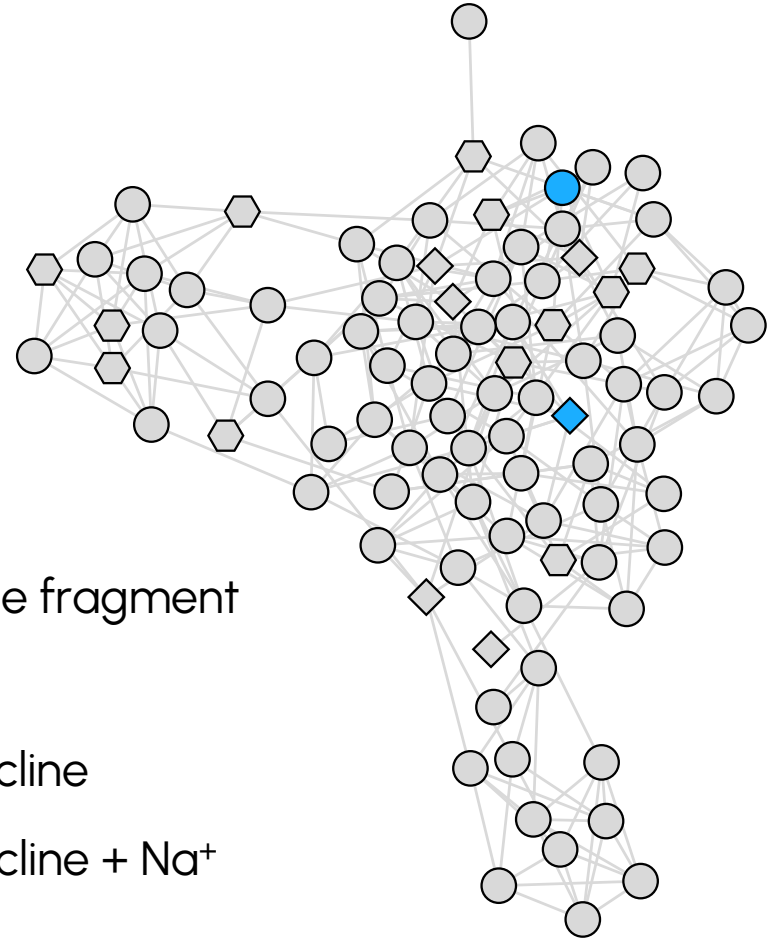


Molecular networking: tetracycline



- In-source fragment
- Adduct
- Tetracycline
- Tetracycline + Na^+

Molecular networking: tetracycline



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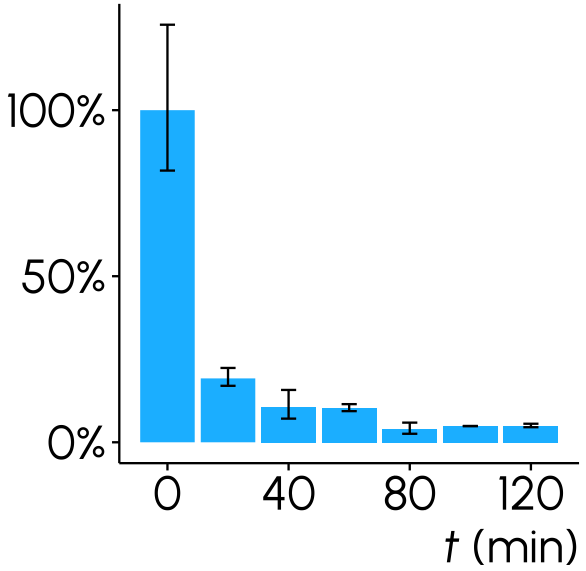

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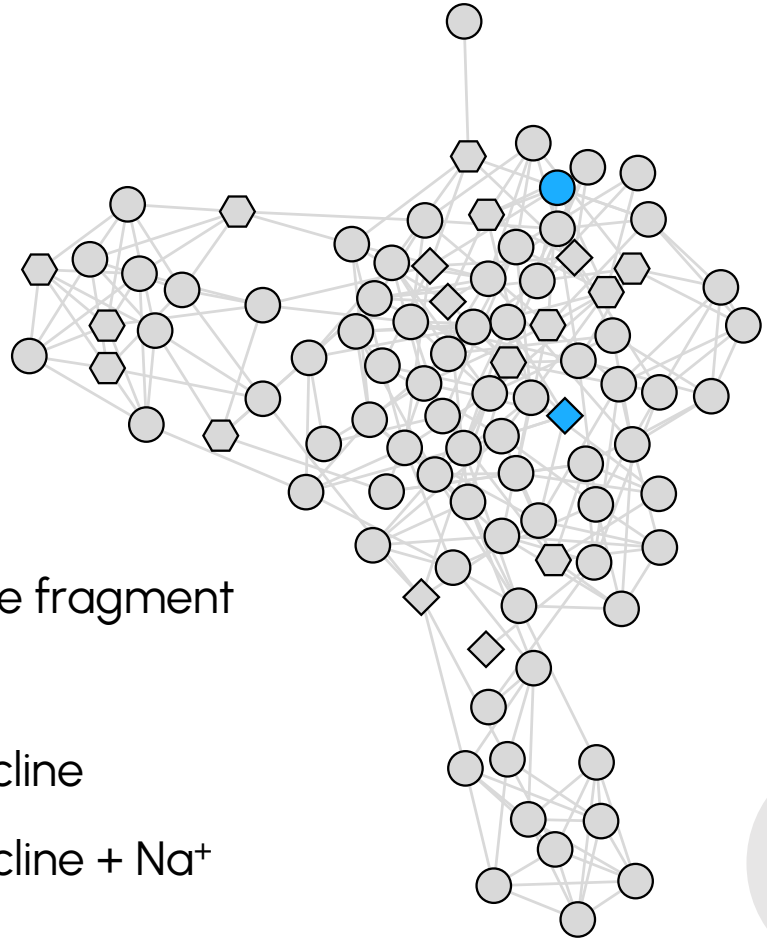

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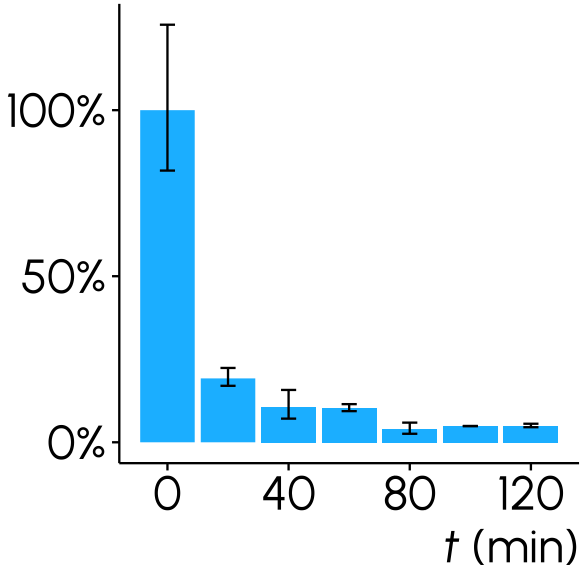
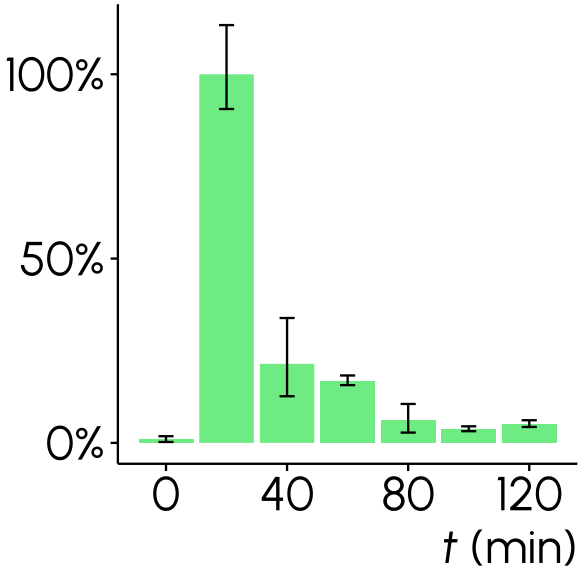


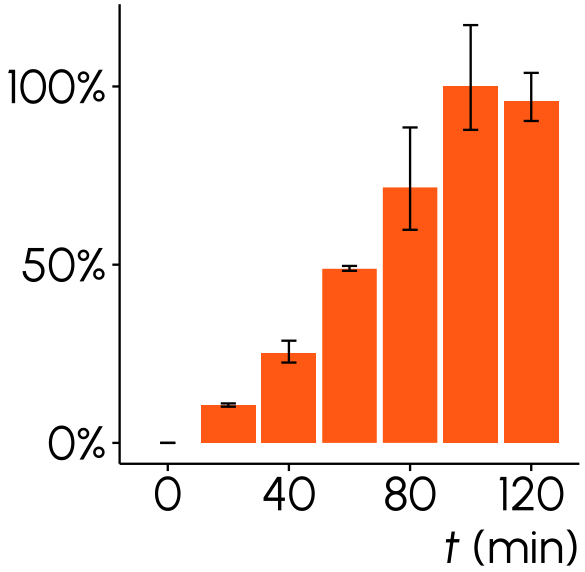
Degradation of Tetracycline by 81% with 20 min

Hierarchical clustering

TP_{early}

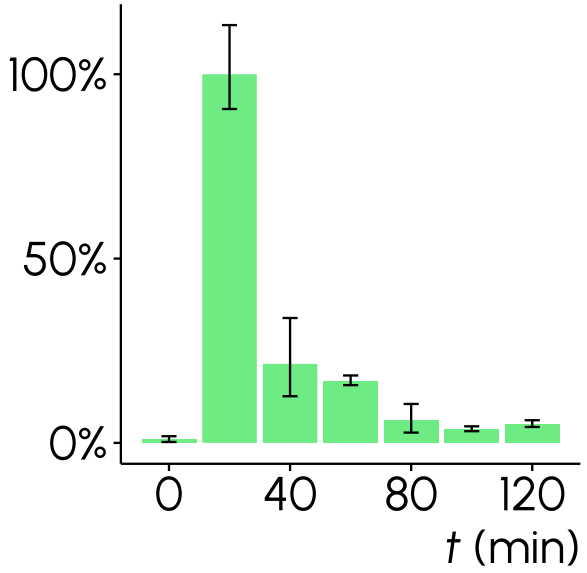


TP_{late}

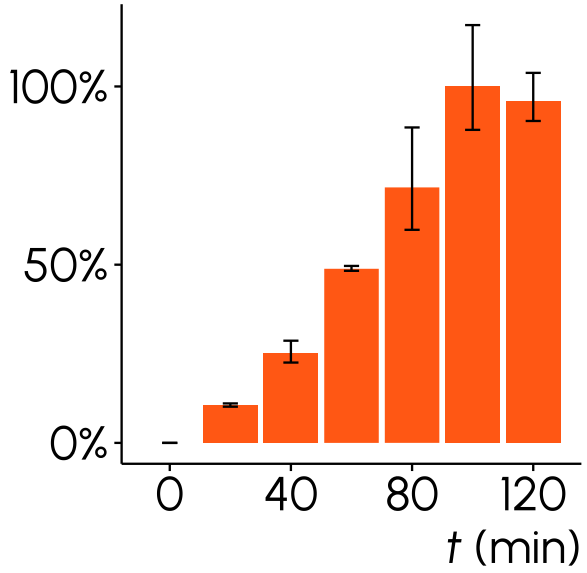


Hierarchical clustering

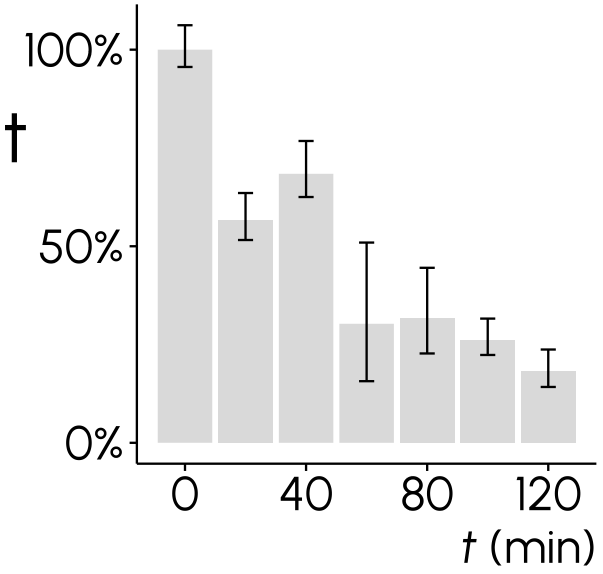
TP_{early}



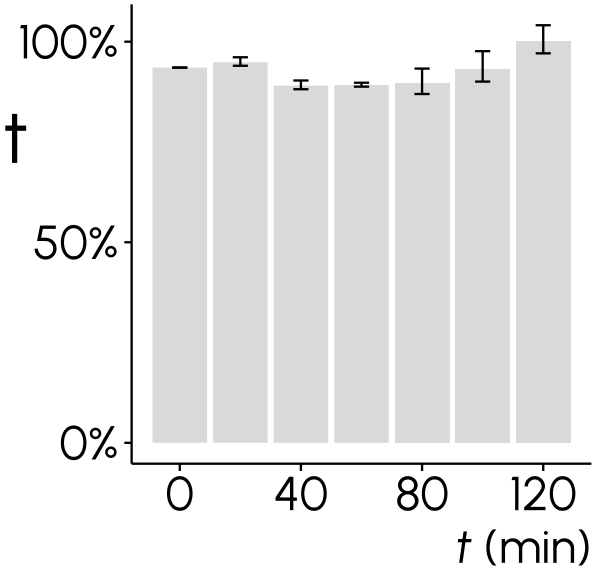
TP_{late}



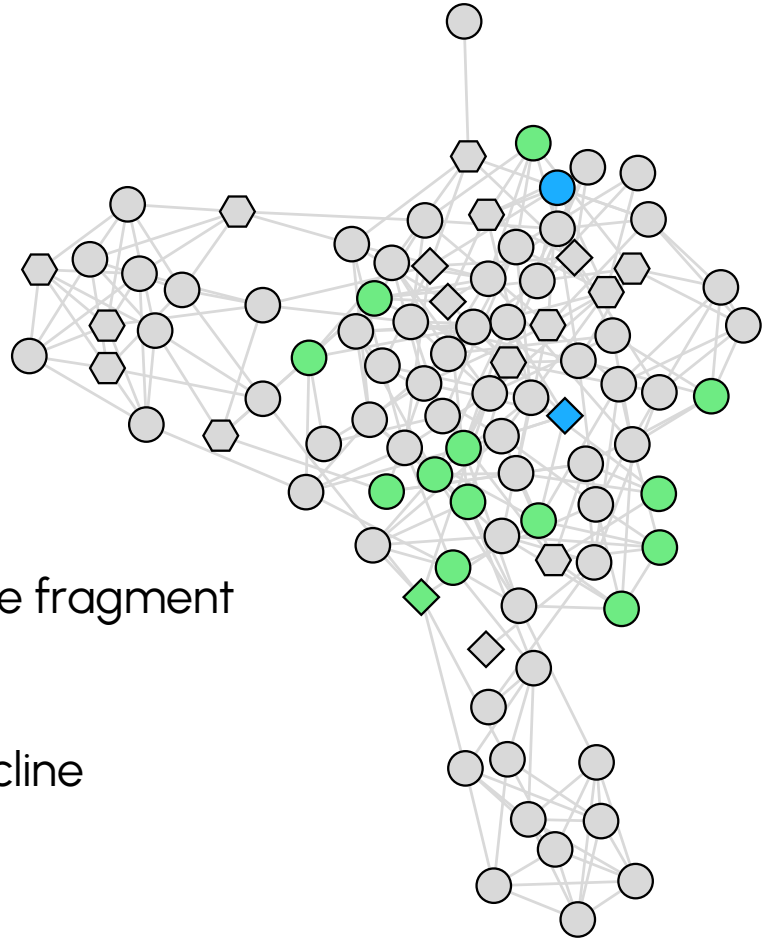
degrading contaminant



persistent contaminant



Molecular networking



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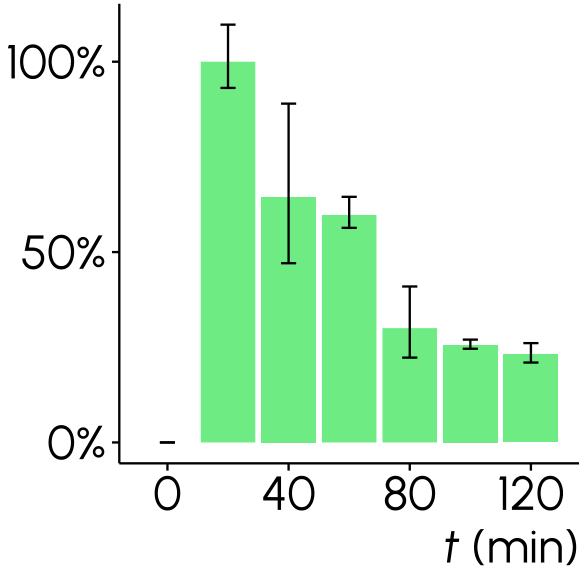
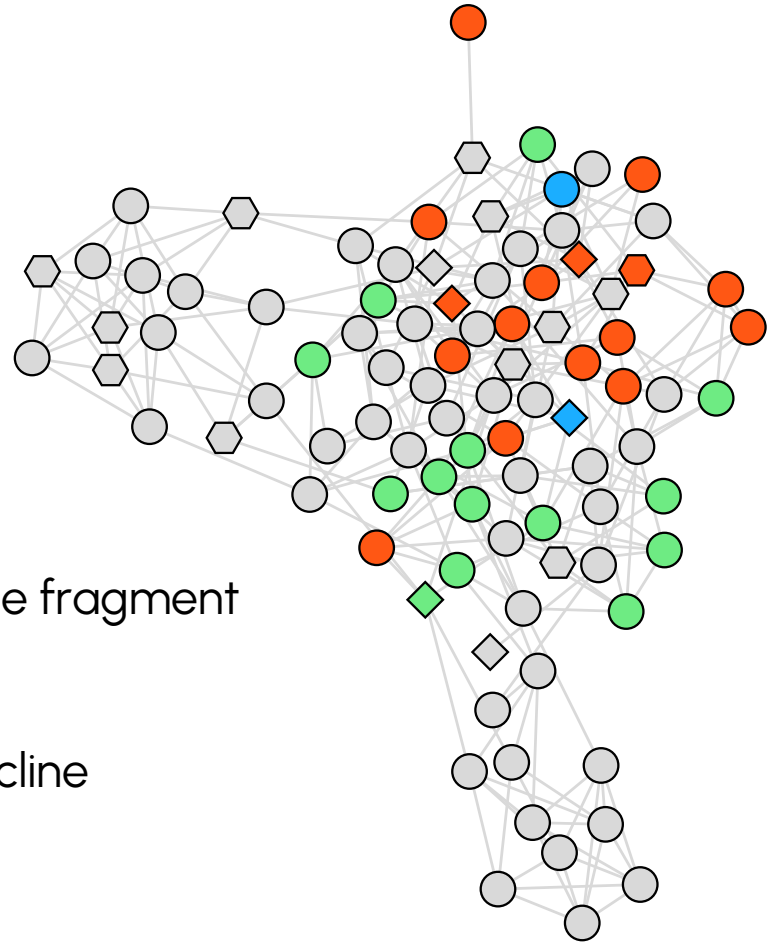
- Adduct
- Tetracycline
- TP_{early}



Molecular networking



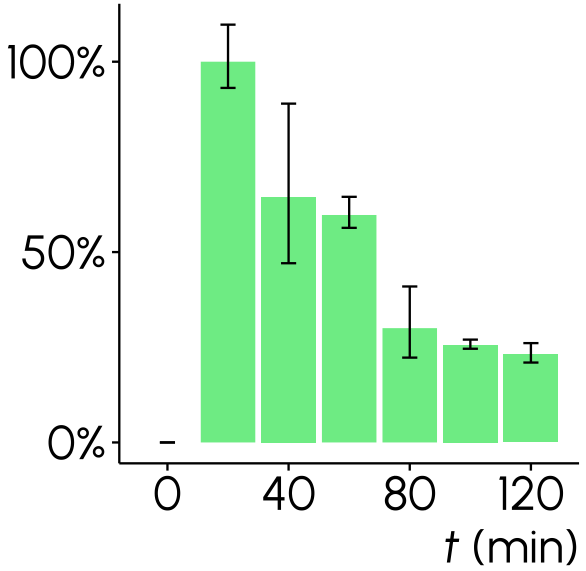
In-source fragment

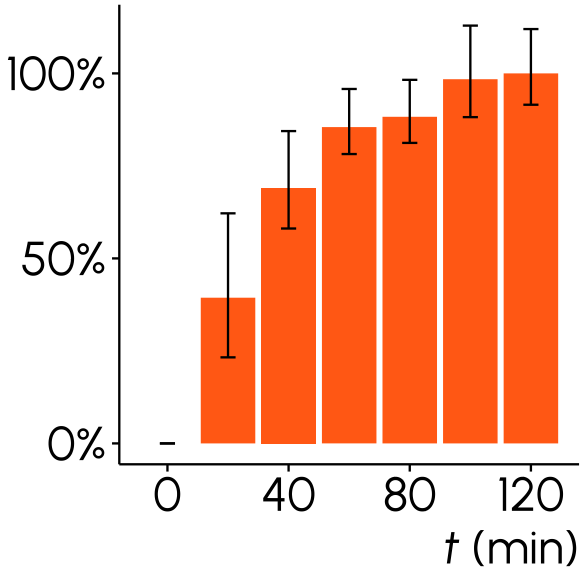
Adduct

Tetracycline

TP_{early}

TP_{late}





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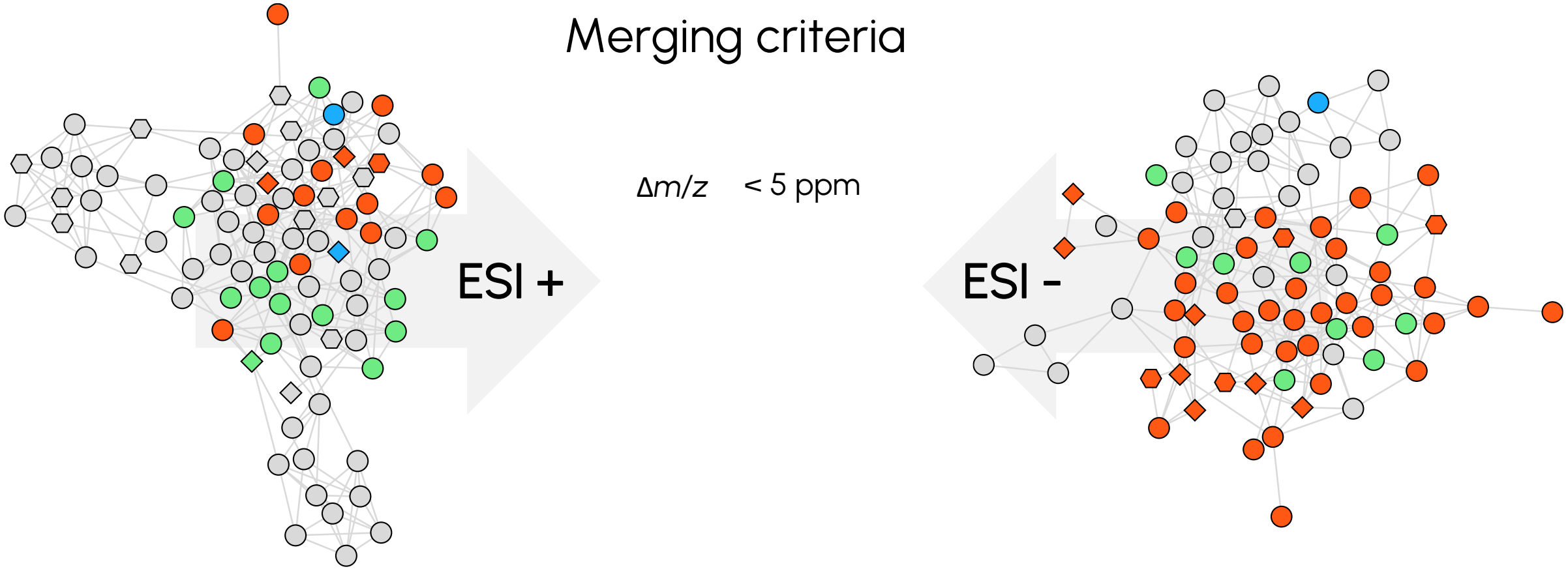

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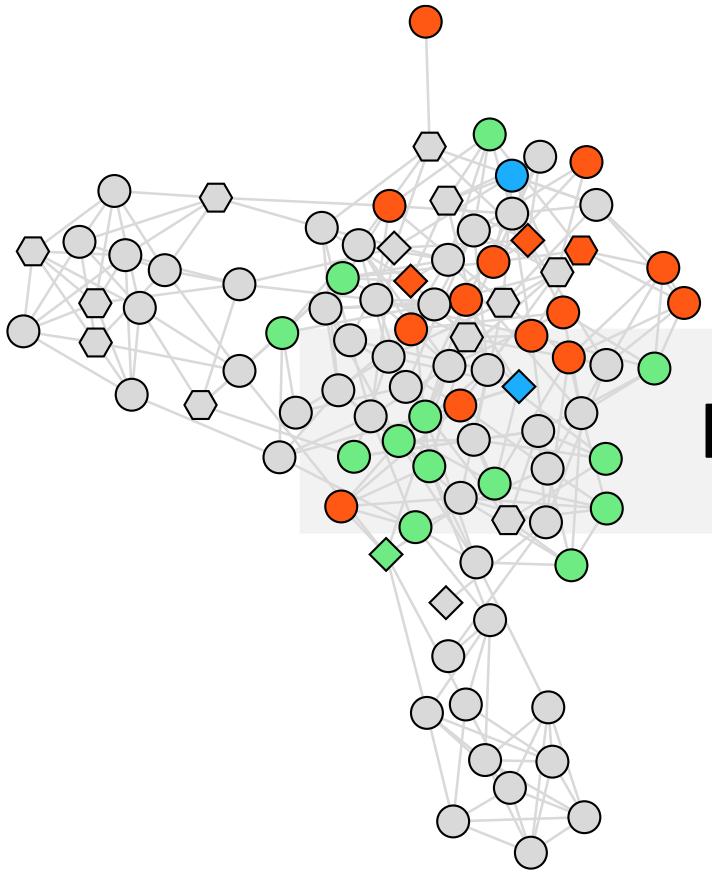


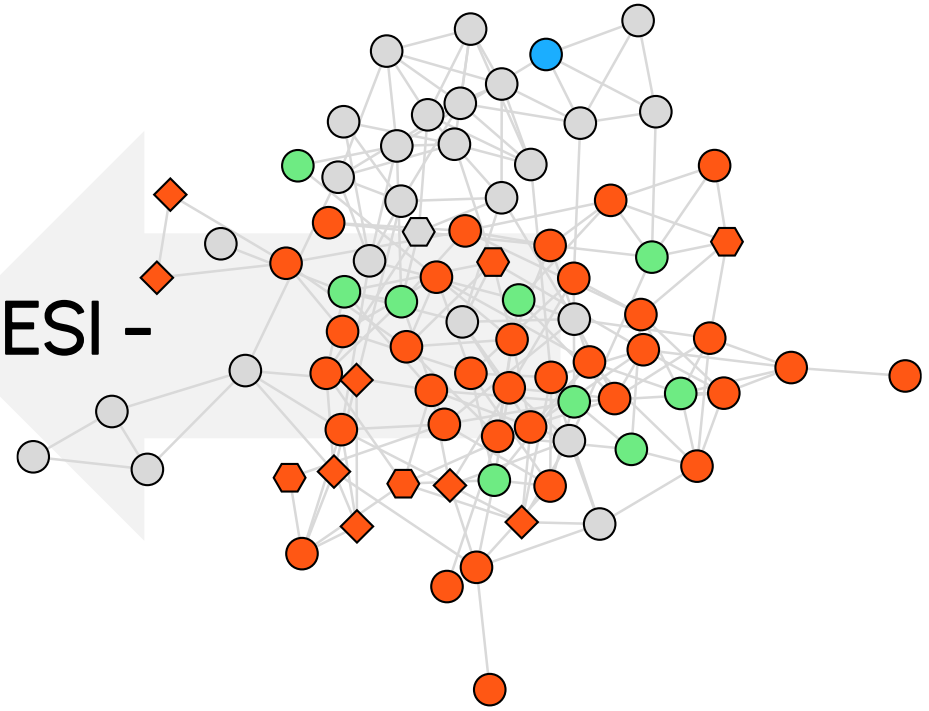
ESI +

Merging criteria

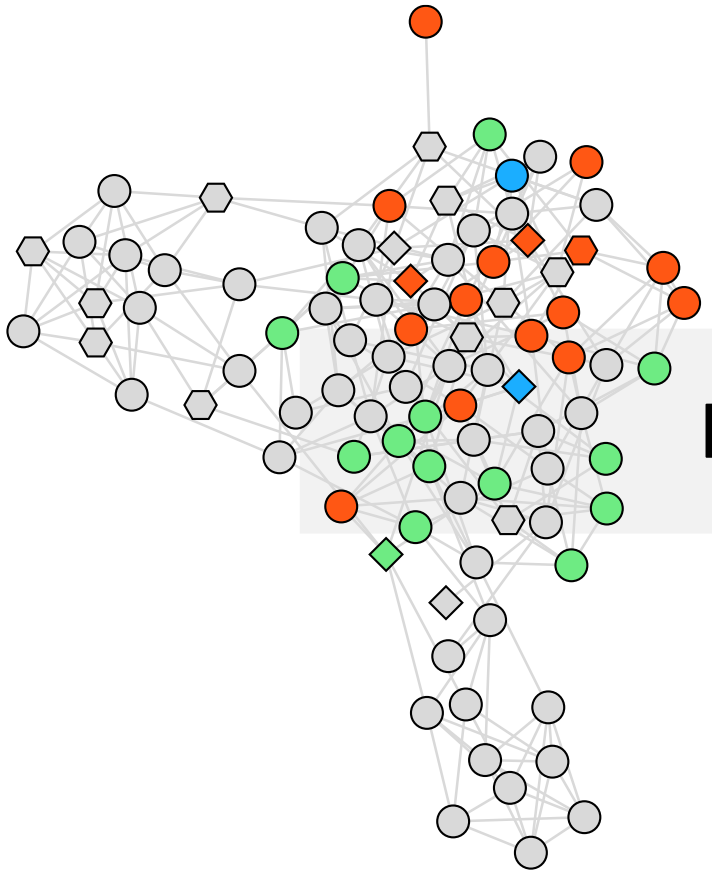
$\Delta m/z$ < 5 ppm

Δt_R < 0.2 min

ESI -



Molecular networking



ESI +

Merging criteria

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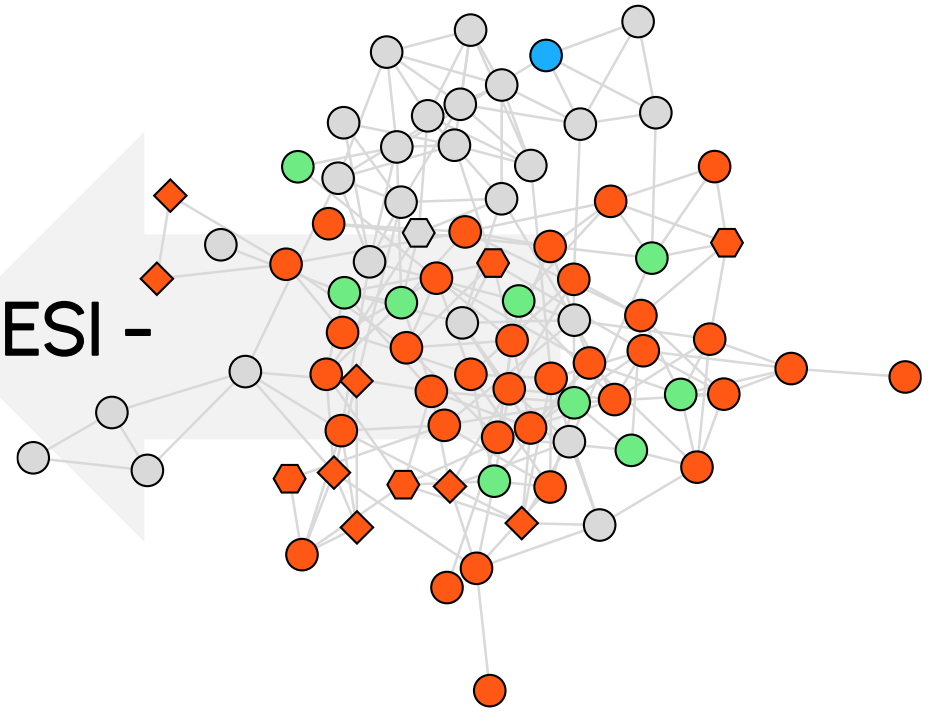
Δt_R < 0.2 min

$R^2_{Pearson}$ > 0.7

ESI -



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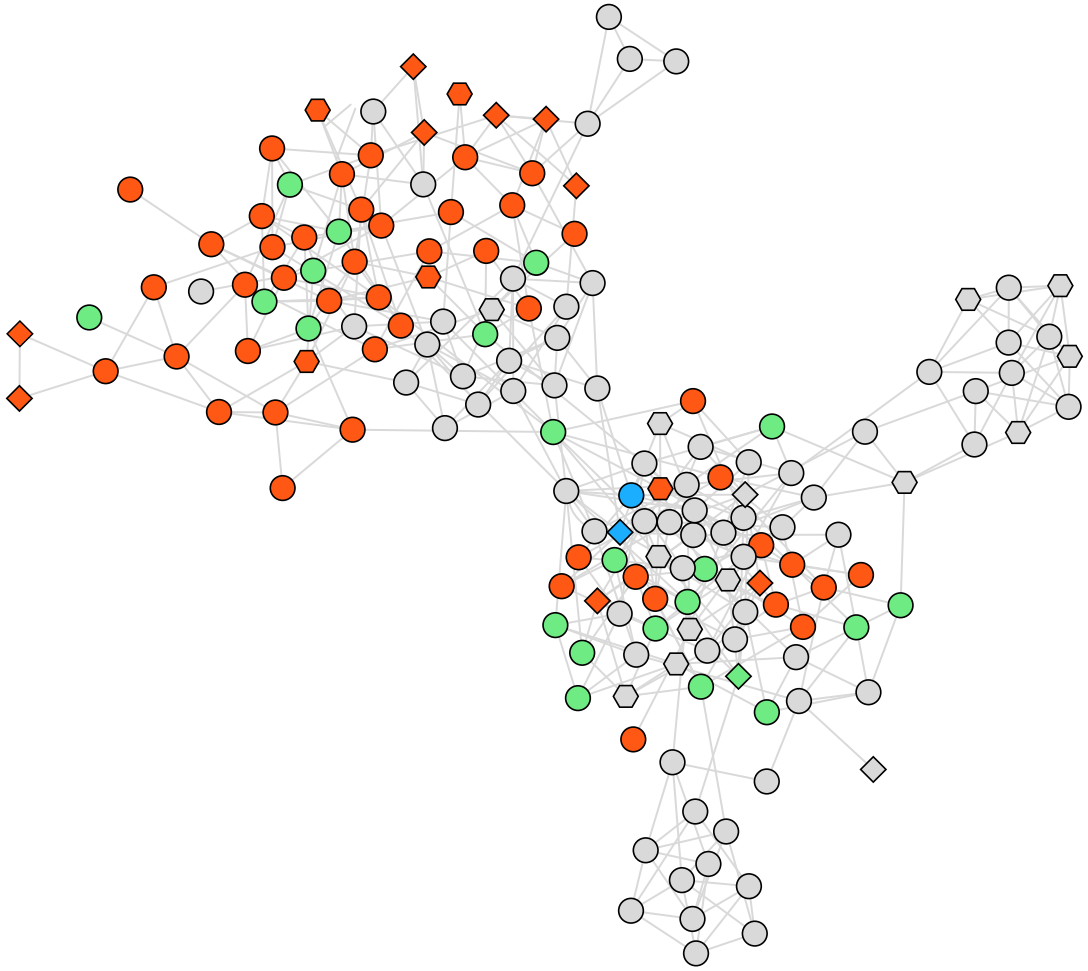

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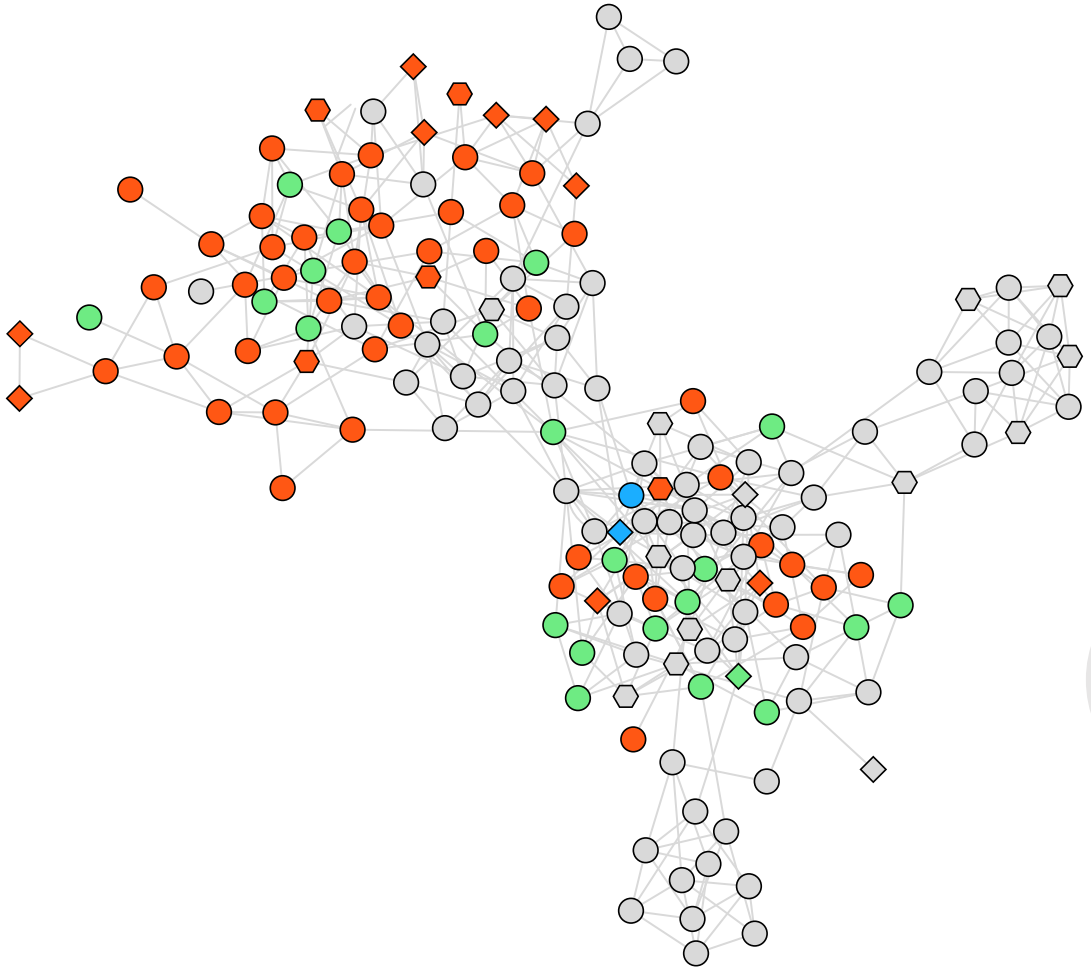
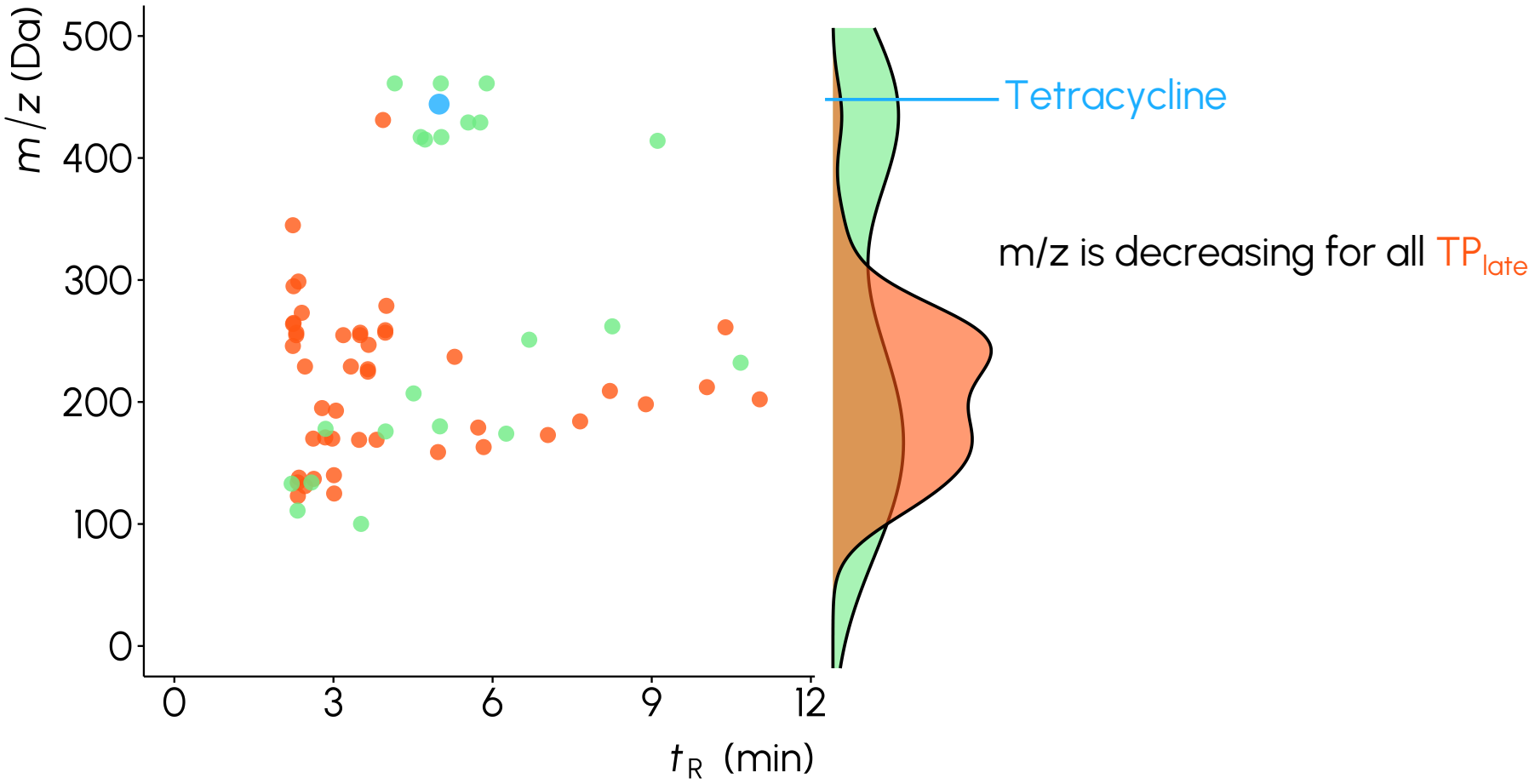
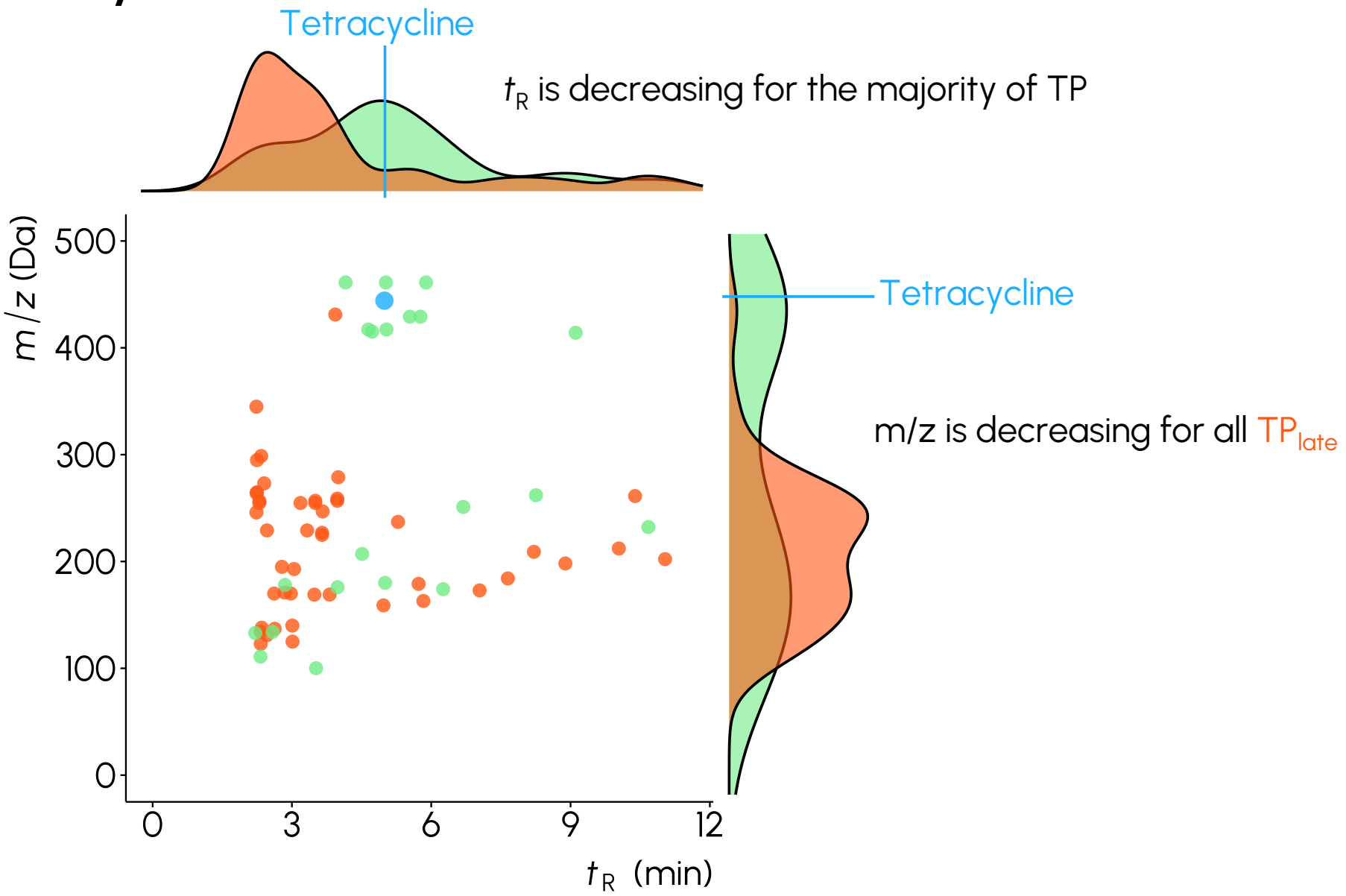


TP_{early} (21) and TP_{late} (46) pinpointed

Oxydative Breakdown



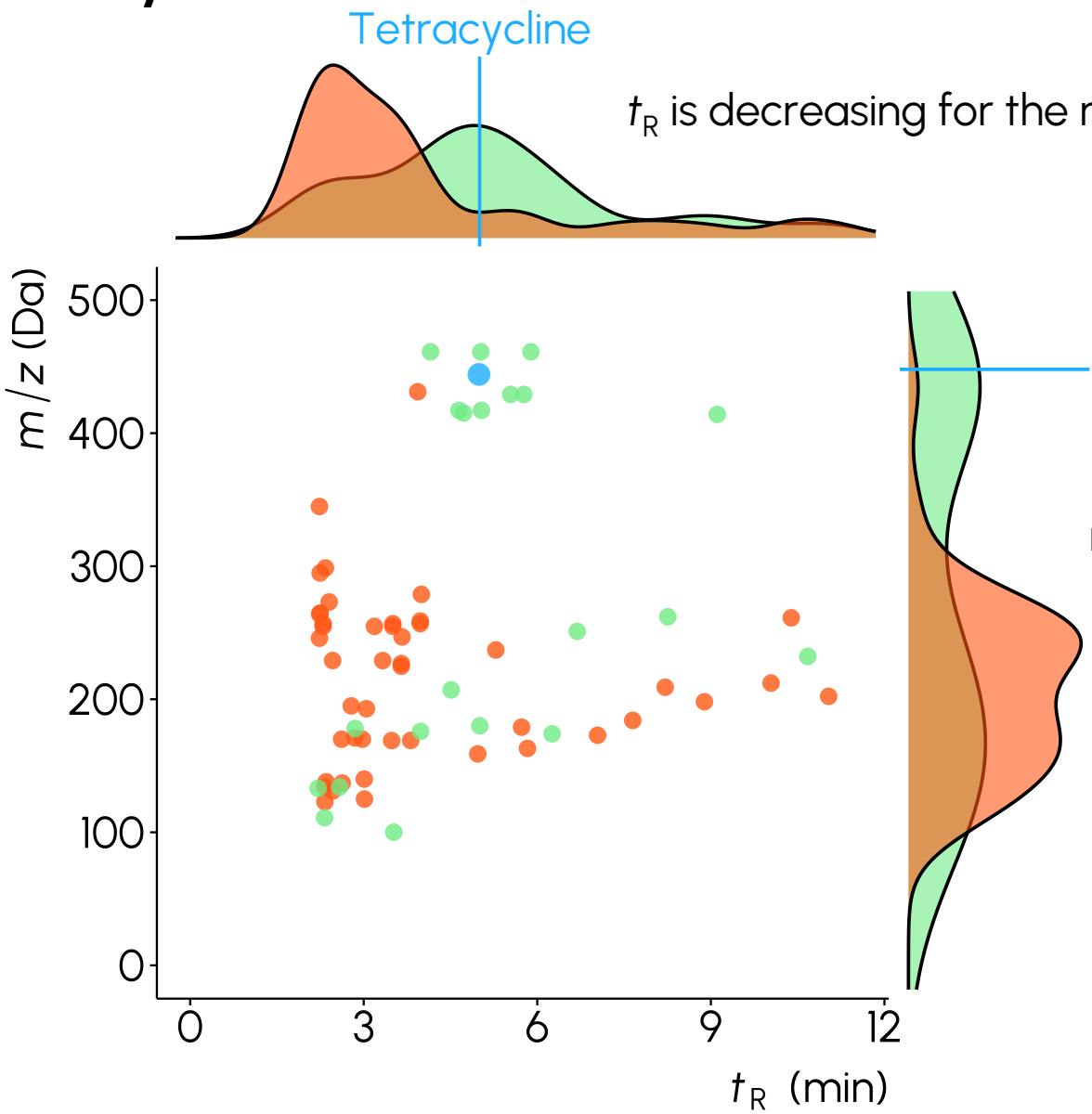
Oxydative Breakdown



t_R is decreasing for the majority of TP

m/z is decreasing for all TP_{late}

Oxydative Breakdown



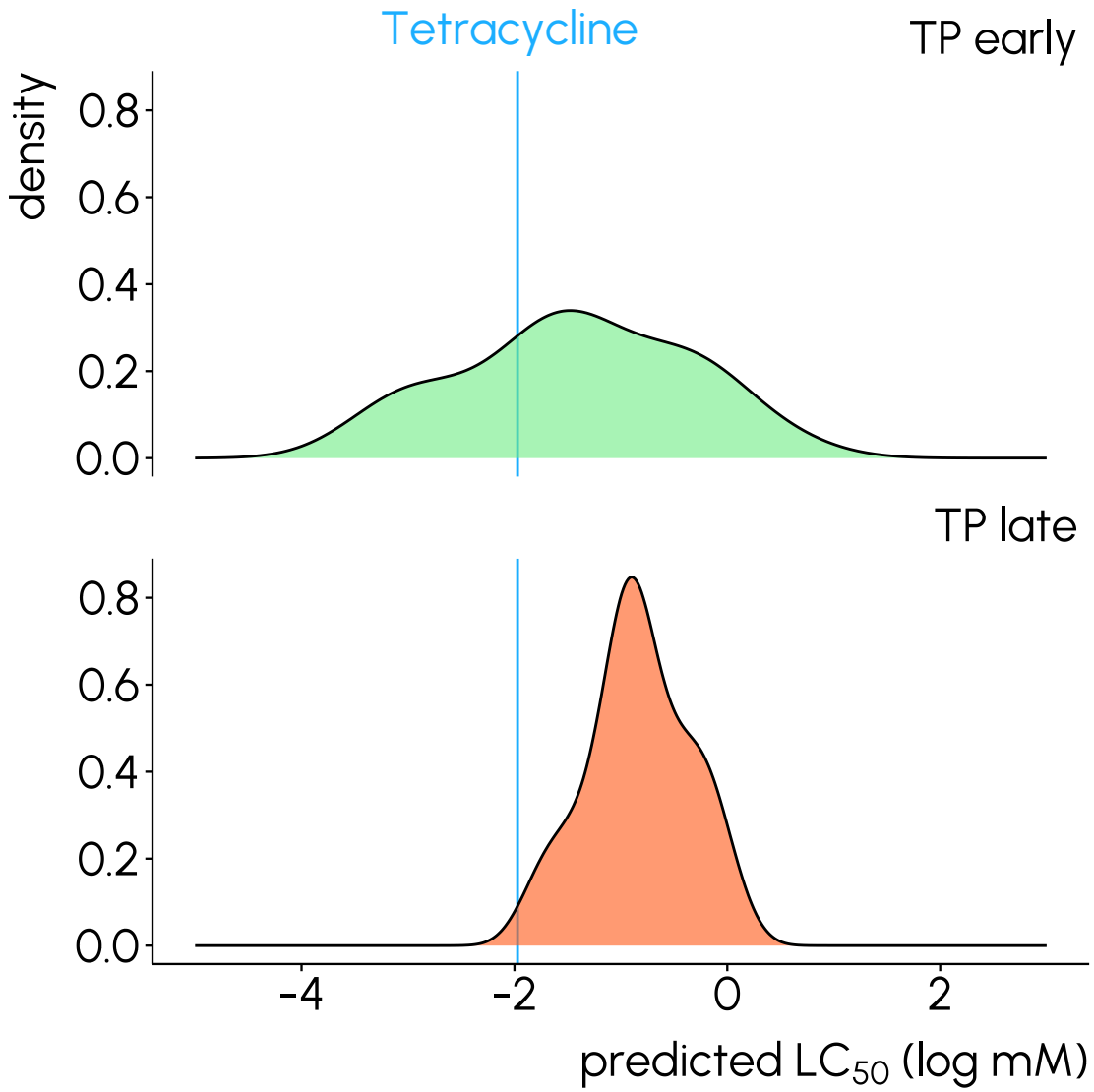
t_R is decreasing for the majority of TP

Tetracycline

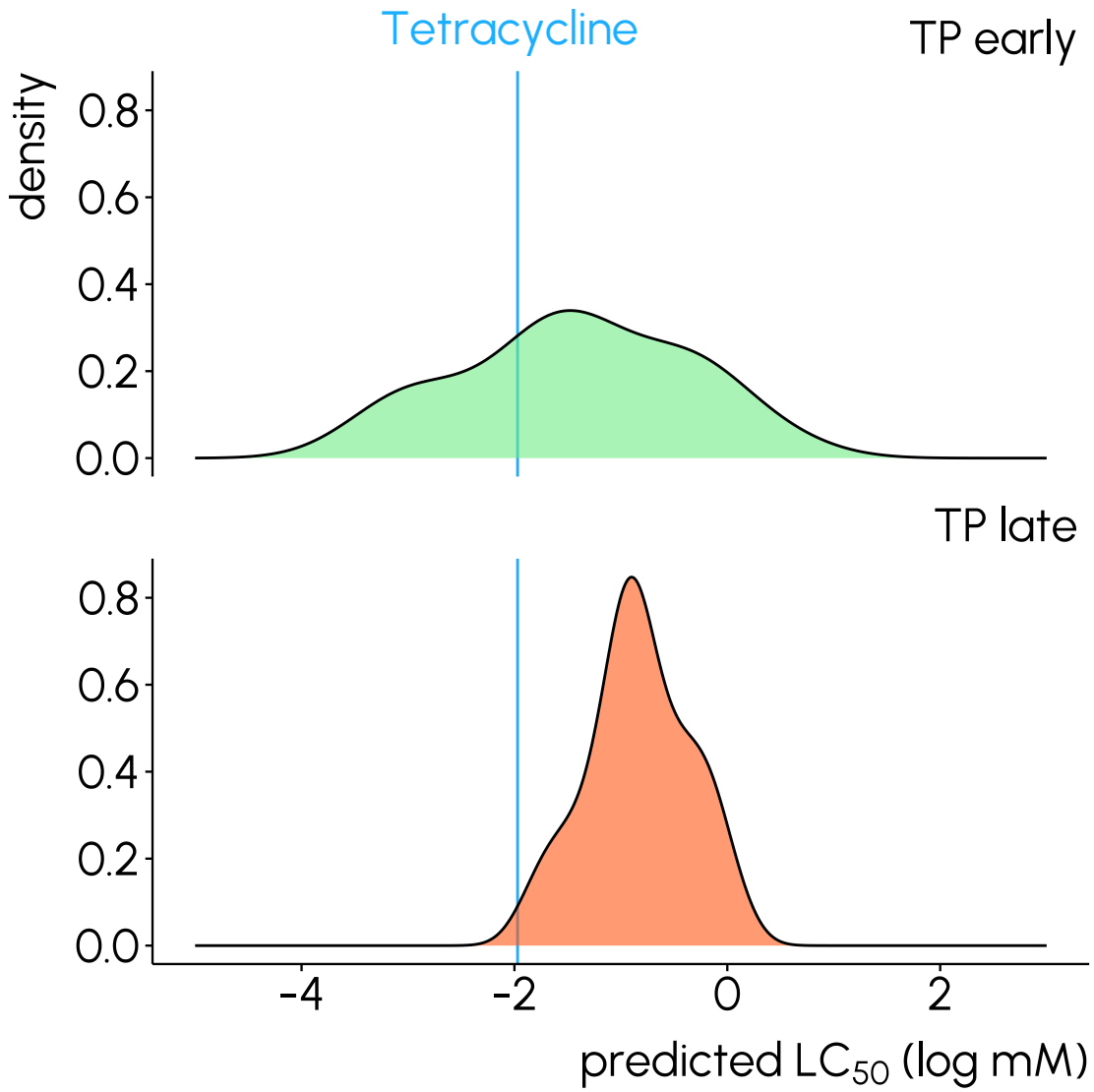
m/z is decreasing for all TP_{late}

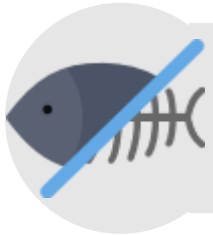
TP_{late} increase in polarity

Predicted LC_{50} in comparison



Predicted LC_{50} in comparison





Ecotoxicity decreased for all TP_{late}

Conclusion

1. Tetracycline is degrading rapidly: 81% in 20 min.

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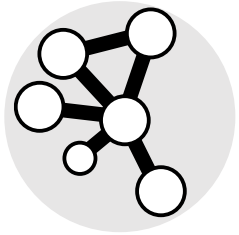
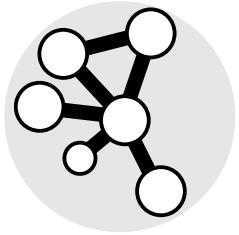


2. TPs can be pinpointed and categorized: 21 TP_{early}, 46 TP_{late}.

Conclusion

1. Tetracycline is degrading rapidly: 81% in 20 min.



2. TPs can be pinpointed and categorized: 21 TP_{early}, 46 TP_{late}.

3. Predicted adult fish toxicity of TP_{late} decreases compared to tetracycline.

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