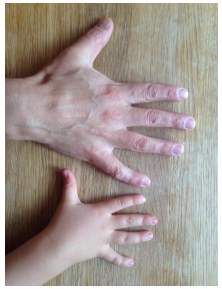
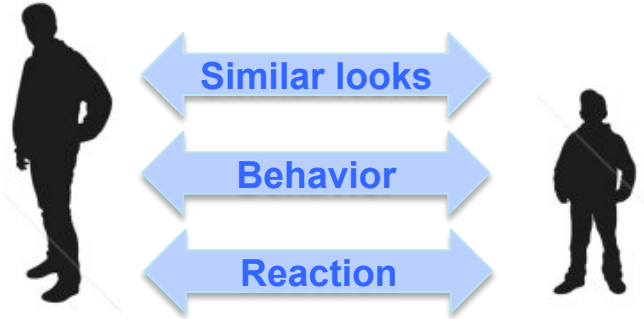
Transformation product analysis: Ready to go beyond suspect screening?

Kathrin Fenner, Rebekka Gulde, Juliane Hollender, Jennifer Schollée, Heinz Singer

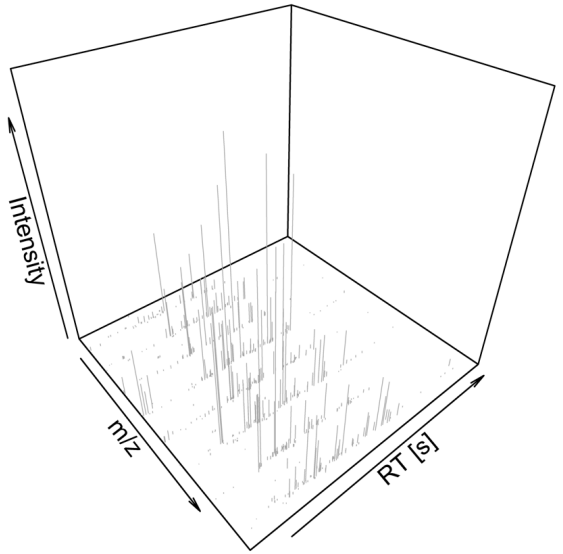
Thoughts of a chemist parent

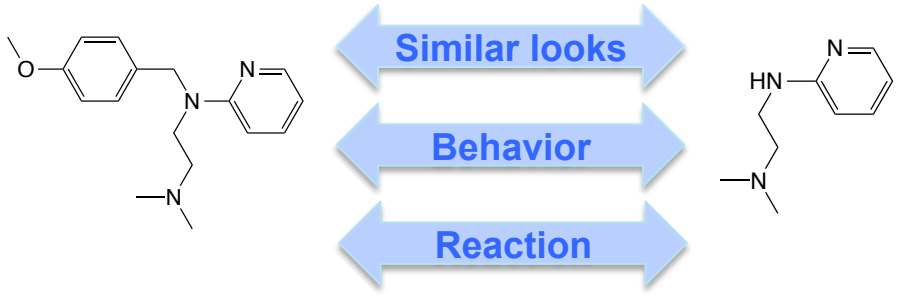
At the playground





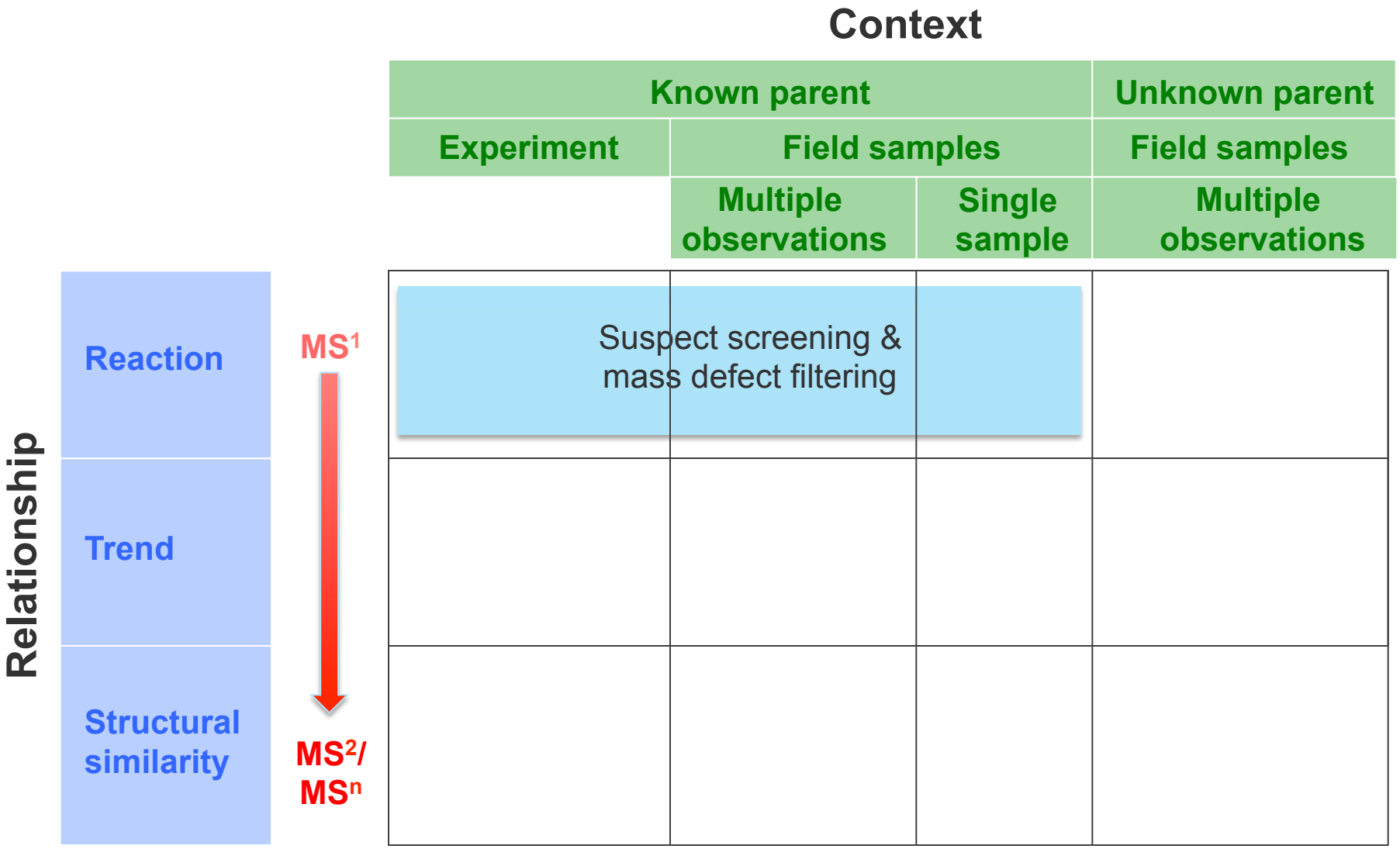
In the lab





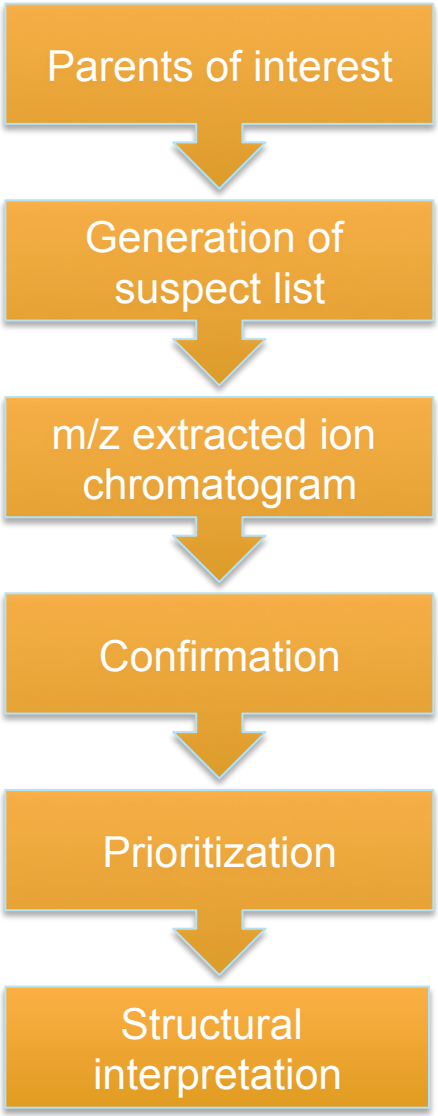
TP search with high-resolution MS

Overview of strategies



Suspect screening

Looking for expected transformation products



Environmental relevance, chemical classes

Batch studies, literature, prediction systems, library of transformations

HR-MS full scan of sample(s)

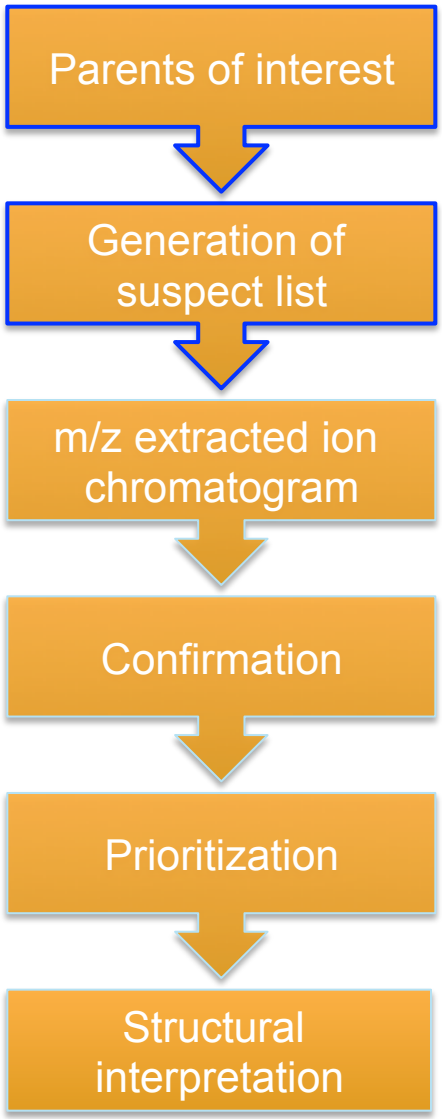
Isotope fit, plausible retention time, absence in blank, ionization behavior

Expected trend, intensity

Data-dependent MS/MS, interpretation of fragmentation patterns

Suspect screening

Transformation products in natural waters



52 highly used biocides, pesticides and pharmaceuticals

1794 possible transformation products from literature and Eawag-PPS

HR-MS full scan of sample(s)

Isotope fit, plausible retention time, absence in blank, ionization behavior

Expected trend, intensity

Data-dependent MS/MS, interpretation of fragmentation patterns

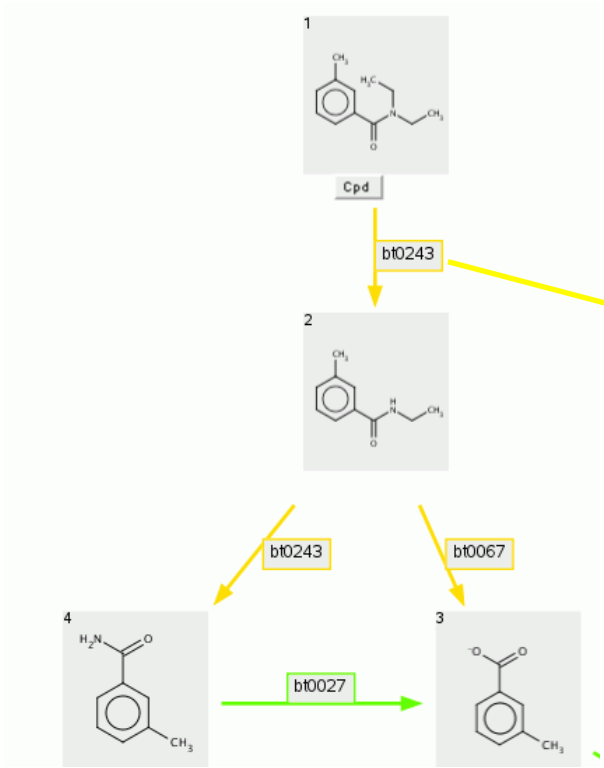
Kern et al., 2008, ES&T

Prediction of microbial biotransformation

Eawag-PPS

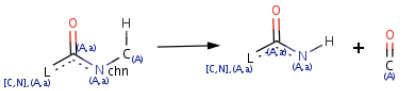
- Artificial intelligence system to predict likely microbial transformation pathways
- About 250 biotransformation rules applied
- Batch mode available upon request

The predicted pathway:



Rule bt0243

[Pathway Prediction Engine] [All Rules List] [BBD Main Menu]



N-substituted Amide -> Amide + Aldehyde or Ketone
N, N-disubstituted Amide -> N-substituted Amide + Aldehyde or Ketone
N-substituted Urea derivative -> Urea derivative + Aldehyde or Ketone
N,N-disubstituted Urea derivative -> N-substituted Urea derivative + Aldehyde or Ketone

Aerobic Likelihood: Neutral

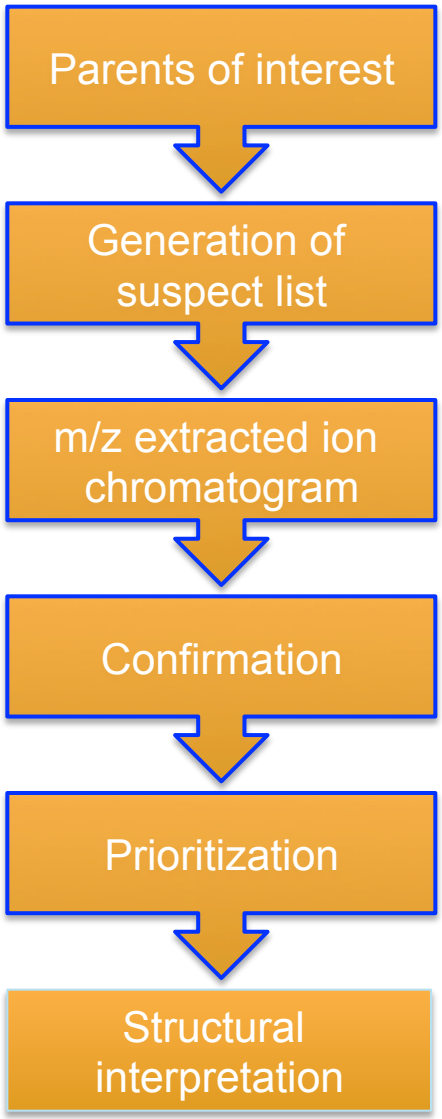
EAWAG-BBD Reaction(s):

Alachlor -----> Formaldehyde + 2-Chloro-2',6'-diethylacetanilide (reacID# r1677)
Caffeine -----> Paraxanthine (reacID# r1251)
Caffeine -----> Theobromine (reacID# r1247)
Diazepam -----> 7-Chloro-5-phenyl-3H-1,4-benzodiazepin-2-one (reacID# r1760)
2-Hydroxy-2',6'-diethyl-N-acetanilide -----> Formaldehyde + N-(2,6-Diethylphenyl)-2-hydroxyacetamide (reacID# r1694)
Hydroxymonodemethylisoproturon -----> Formaldehyde + 4'-(2-Hydroxyisopropyl)phenylurea (reacID# r0894)
N-Isopropylacetanilide -----> Acetanilide + Acetone (reacID# r0914)
Isoproturon -----> Formaldehyde + Monodemethylisoproturon (reacID# r0892)
1-Methylxanthine -----> Xanthine (reacID# r1331)
3-Methylxanthine -----> Xanthine (reacID# r1329)
Monodemethylisoproturon -----> Formaldehyde + Didemethylisoproturon (reacID# r0897)
Paraxanthine -----> 7-Methylxanthine (reacID# r1257)
Theobromine -----> 7-Methylxanthine (reacID# r1248)
Theophylline -----> 1-Methylxanthine (reacID# r1327)
Theophylline -----> 3-Methylxanthine (reacID# r1325)

www.eawag-bbd.ethz.ch/predict
envipath.org

Suspect screening

Transformation products in natural waters



52 highly used biocides, pesticides and pharmaceuticals

1794 possible transformation products from literature and Eawag-PPS

1057 peaks found in at least on of 7 water samples

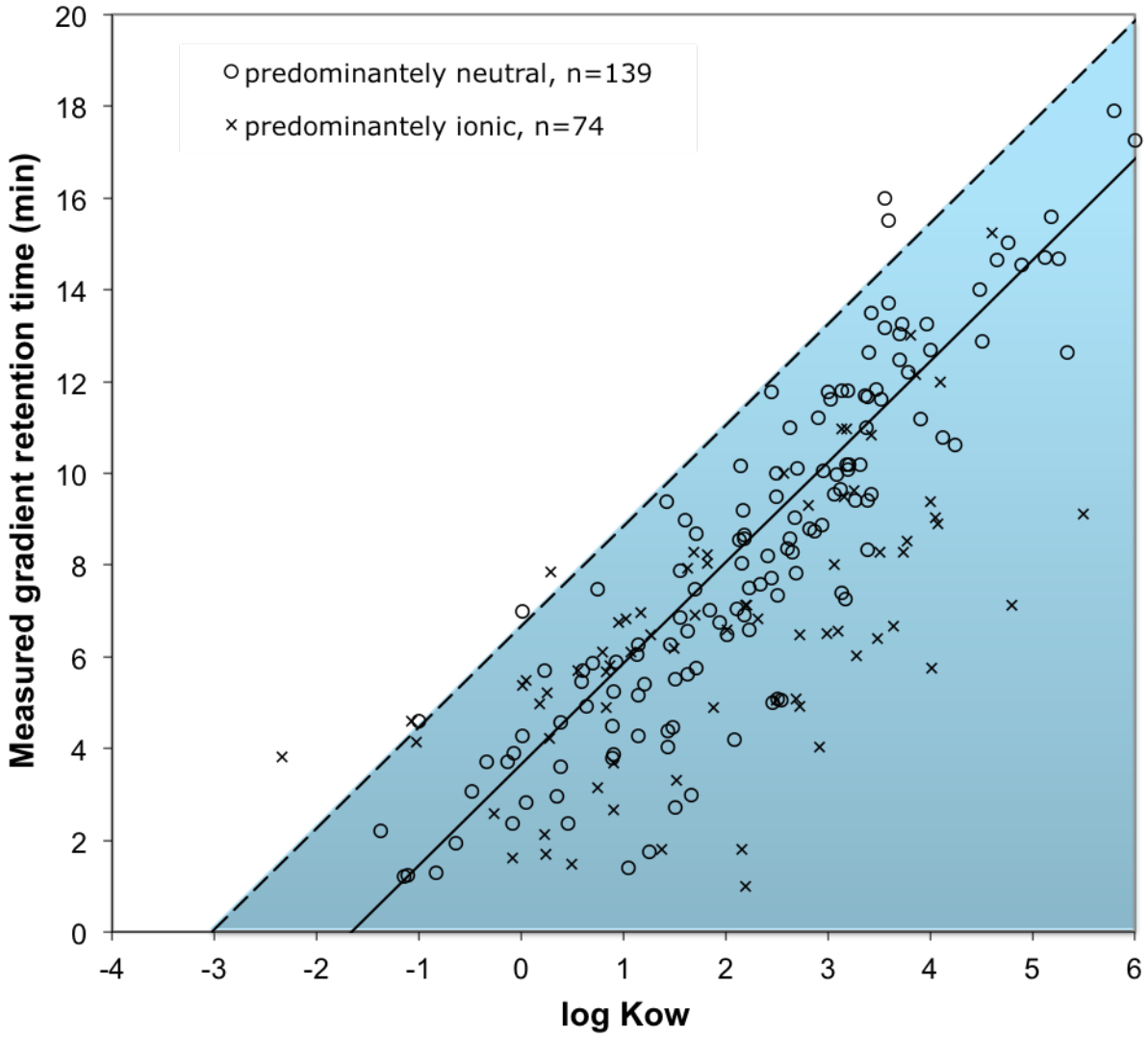
957 without blank peaks
640 peaks intensity $\geq 10^5$
438 reasonable retention time
415 peaks with correct isotope pattern
371 peaks with correct ionization mode

Data-dependent MS/MS, interpretation of fragmentation patterns

Kern et al., 2008, ES&T

Retention time filter

Crude, realistic, but still efficient



Reversed phase X-Bridge, MeOH-water gradient

Kern et al., 2008, ES&T

Suspect screening

Transformation products in natural waters

Parents of interest

52 highly used biocides, pesticides and pharmaceuticals

Generation of suspect list

1794 possible transformation products from literature and Eawag-PPS

m/z extracted ion chromatogram

1057 peaks found in at least on of 7 water samples

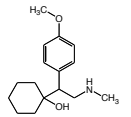
Confirmation

957 without blank peaks
640 peaks intensity $\geq 10^5$
438 reasonable retention time
415 peaks with correct isotope pattern
371 peaks with correct ionization mode

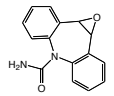
Prioritization

Structural interpretation

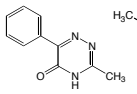
19 proposed structures



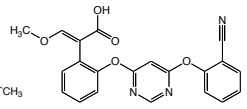
N-desmethyl venlafaxin



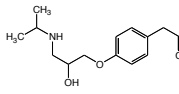
Carbamazepine-10,11-epoxide



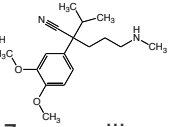
Metamitron-desamino



Azoxystrobin acid



Atenolol acid



D617 (Verapamil TP)

...

Suspect screening & case-control

Transformation products along river

Parents of interest

Generation of suspect list

m/z extracted ion chromatogram

Confirmation

Prioritization

Structural interpretation

32 organic micropollutants (out of 2560) with decreasing trends along 4 river stretches

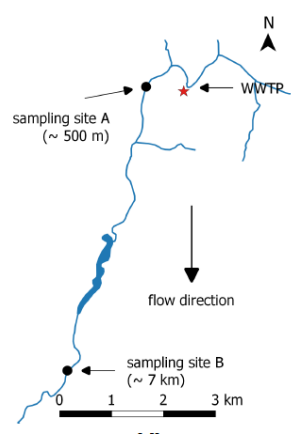
1315 possible transformation products from Eawag-PPS (3 generations)

54 after blank subtraction, intensity filter, and isotope pattern check

19 with increasing trends ($Att_x < 0$)

8 after retention time filter (ΔlogKow vs ΔRT) and ionization plausibility check

8 proposed structures

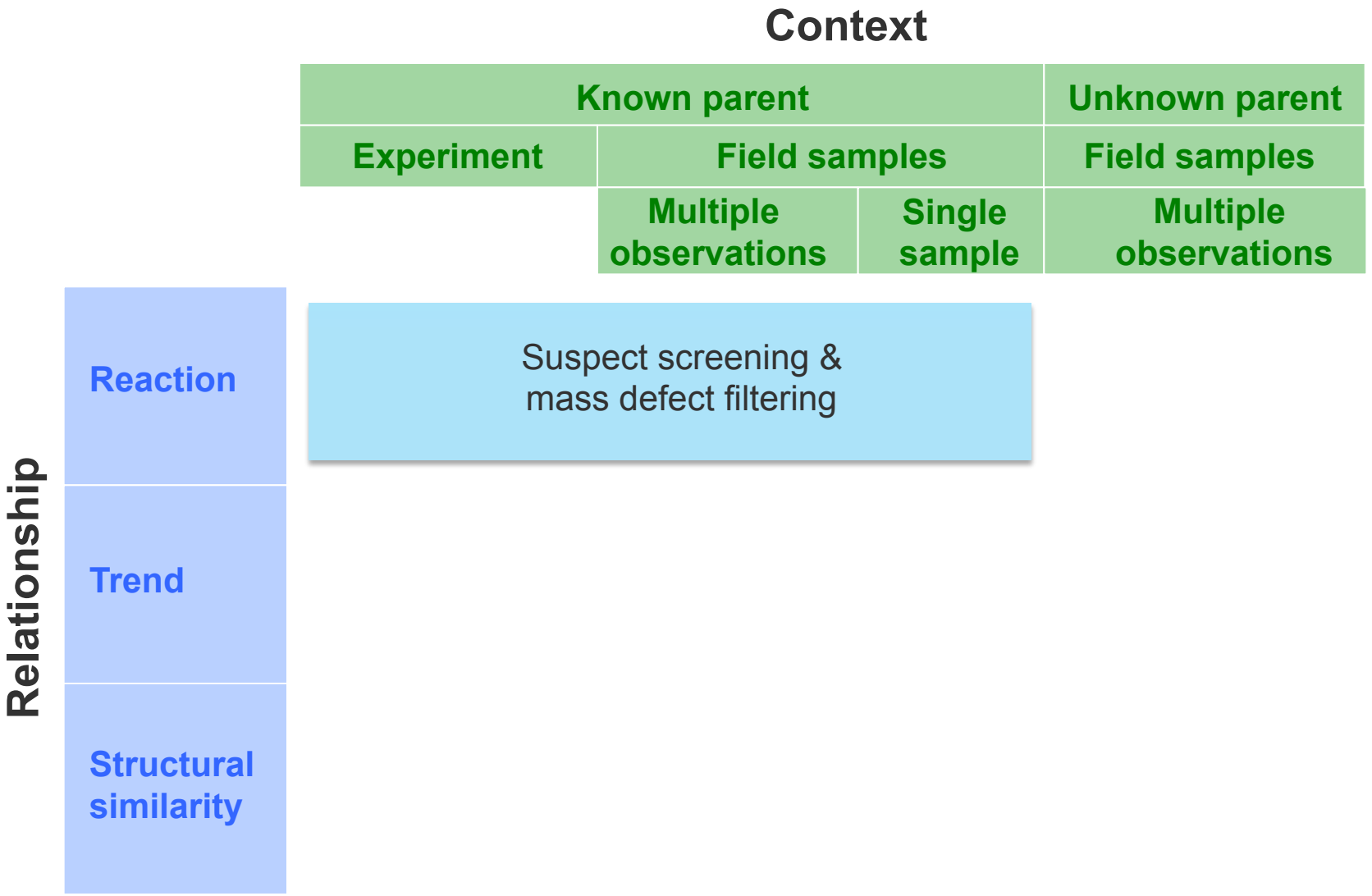


$$Att_x = 1 - \frac{\frac{Area_{x,siteB}}{Area_{x,siteA}}}{\frac{Area_{REF,siteB}}{Area_{REF,siteA}}}$$

Li et al., 2016, manuscript in preparation

TP search with high-resolution MS

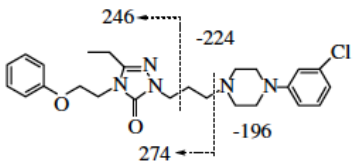
Overview of strategies



A few words on mass defect filtering

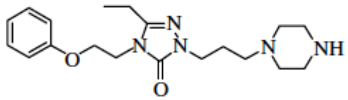
"Extended suspect screening", example nefazodone

MDF template for metabolite search[a]



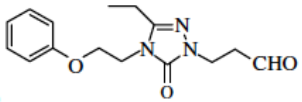
(A)

$MH^+ = 470.2323$
$(C_{25}H_{33}O_2N_5Cl)$



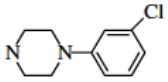
(B)

$MH^+ = 360.2399$
$(C_{19}H_{30}O_2N_5)$



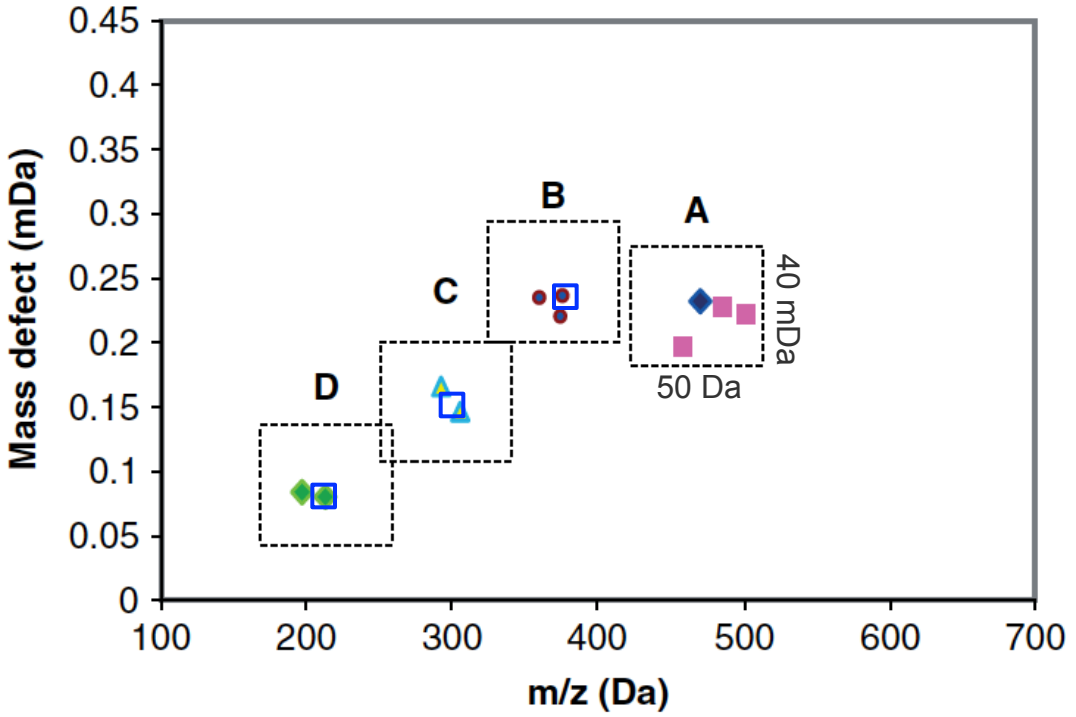
(C)

$MH^+ = 290.1505$
$(C_{15}H_{20}O_3N_3)$



(D)

$MH^+ = 197.0845$
$(C_{10}H_{14}N_2Cl)$

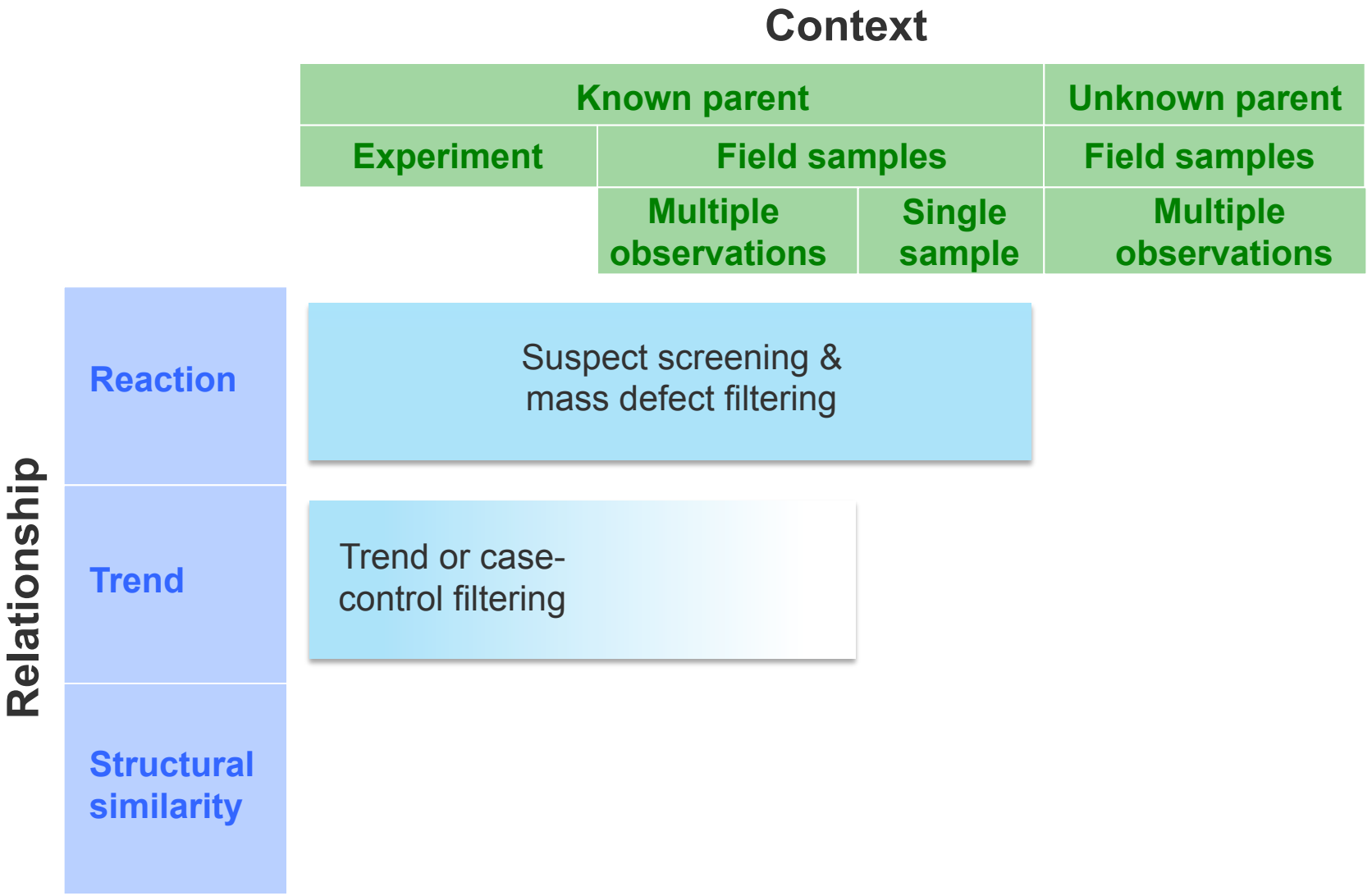


□ : First generation suspects B, C and D

Zhang et al., 2009, J. Mass. Spectrom.

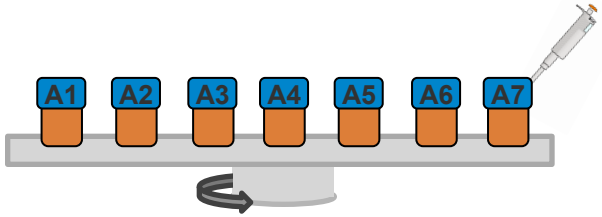
TP search with high-resolution MS

Overview of strategies

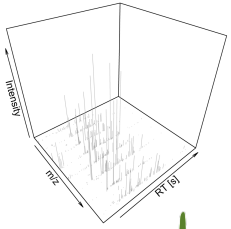


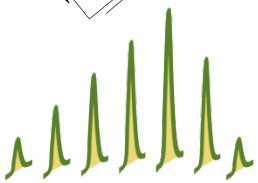
Trend screening in spike experiments

Comprehensive enumeration of TPs, pathway elucidation



t=0 1h 2h 4h 8h 1d 2d





t=0 1h 2h 4h 8h 1d 2d

Sample time series

Extracted ion chromatogram (XIC)

HR-MS full scan of sample(s), automated peak detection, blank subtraction

Trend filter

Increasing or increasing/decreasing trend, absence in matrix controls

Further prioritization

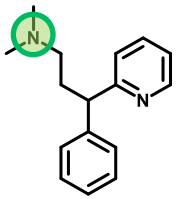
Componentization; reasonable molecular formula , m/z and RT relative to parent compound

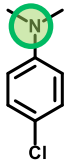
Structural interpretation

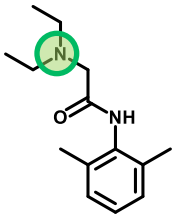
Helbling et al., 2010, ES&T
Gulde et al., 2016, ES&T

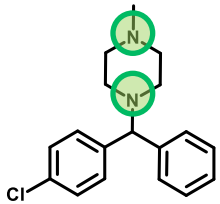
Trend screening in spike experiments

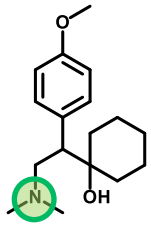
Comprehensive enumeration of amine transformation pathways

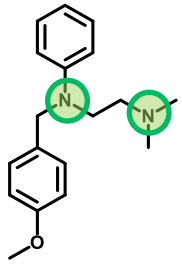

Pheniramine

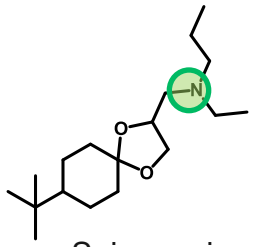

N,N-dimethyl
-p-chloroanilin

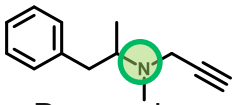

Lidocaine

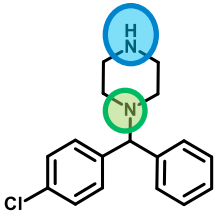

Chlorcyclizine

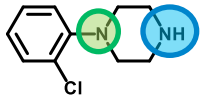

Venlafaxine

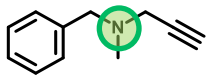

Pyrilamine

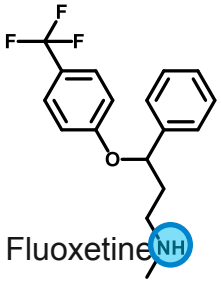

Spiroxamine

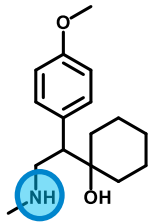

Deprenyl

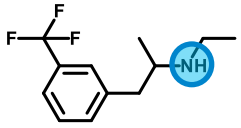

1[(4-
chlorophenyl)
phenylmethyl]
piperazine

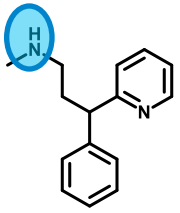

ortho-
chlorophenyl
piperazine

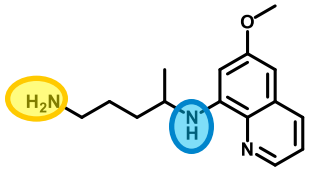

Pargyline

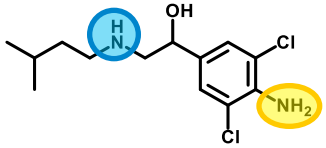

Fluoxetine

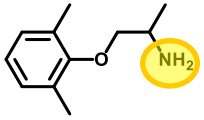

N-demethyl
venlafaxine

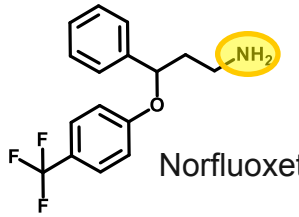

Fenfluramine


N-demethyl
pheniramine


Primaquine


Clenisopenterol

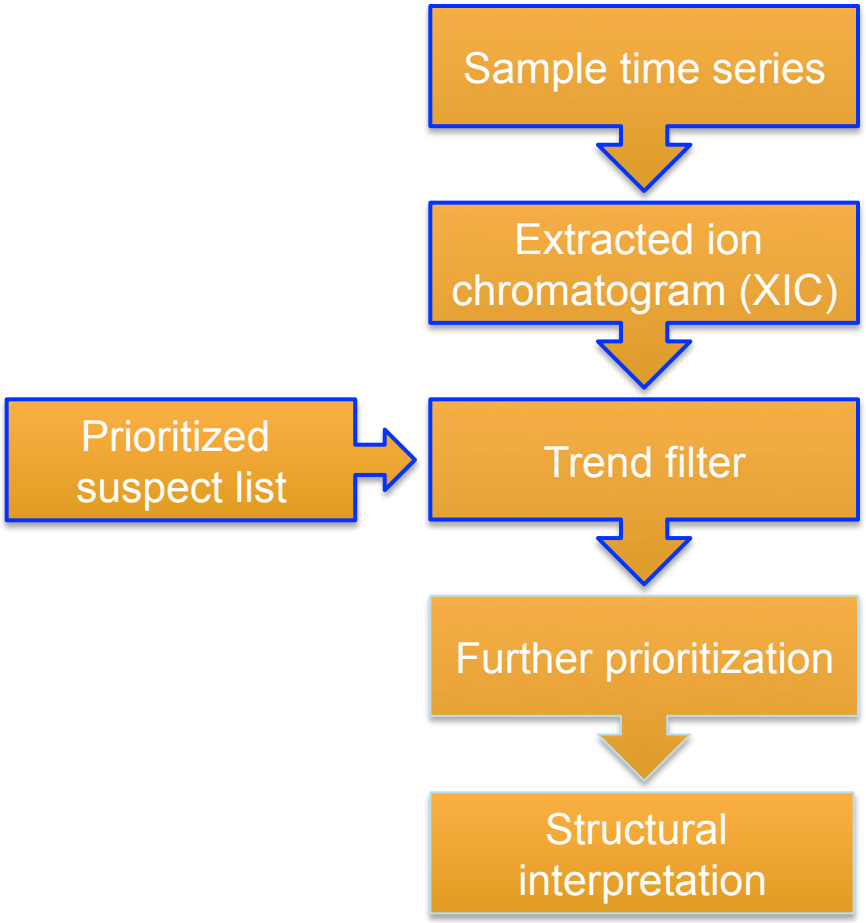

Mexiletine


Norfluoxetine

Gulde et al., 2016, ES&T

Trend screening in spike experiments

Comprehensive enumeration of TPs, pathway elucidation



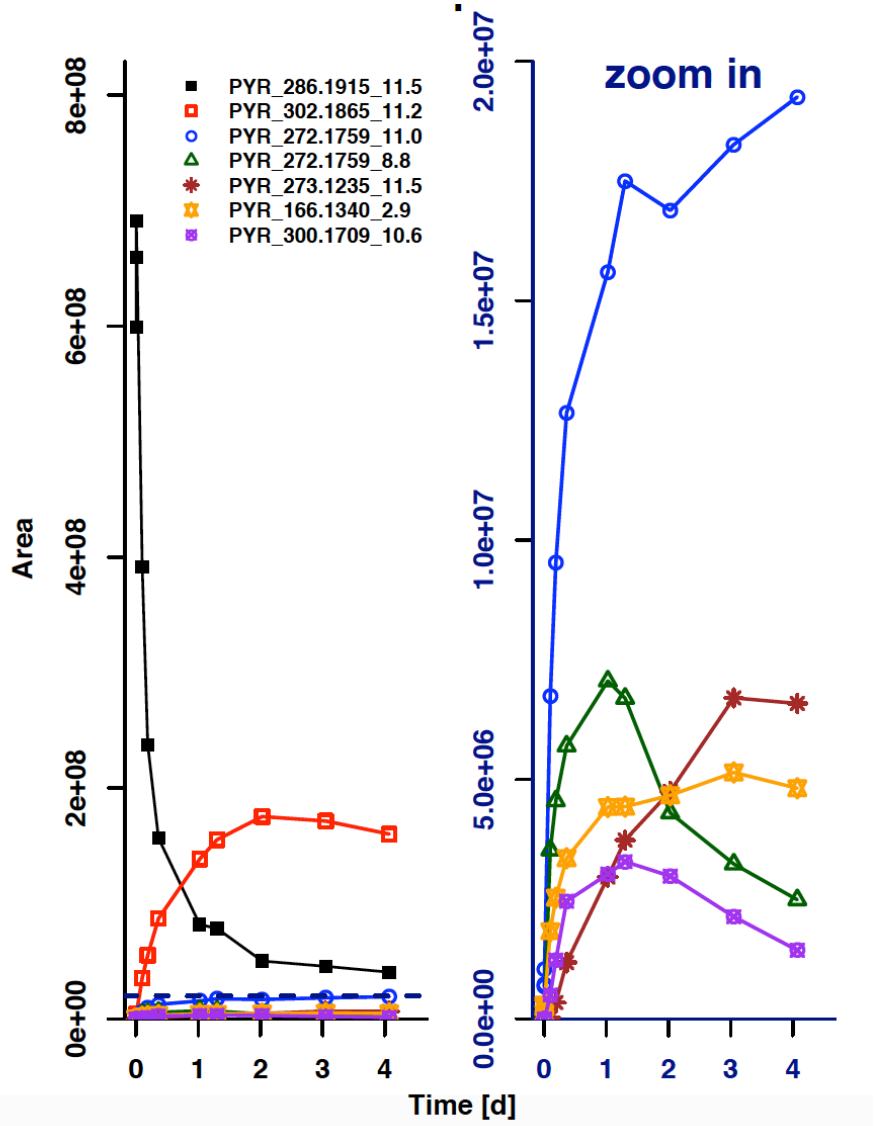
Triplicates of 19 bioreactors spiked with separate compounds, 9 time points per compound/reactor

144 peaks (or components) with expected trend and plausible molecular formula

Helbling et al., 2010, ES&T
Gulde et al., 2016, ES&T

Trend screening in spike experiments

Exemplary results for pyrilamine

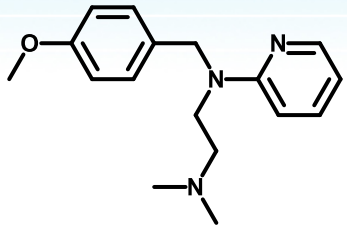


Gulde et al., 2016, ES&T

Structure elucidation

Communicating confidence

Pyrilamine (parent):



Confidence levels:

Level 1: ***Confirmed structure***
by reference standard

Level 2: ***Probable structure***
a) by library spectrum match
b) by diagnostic evidence

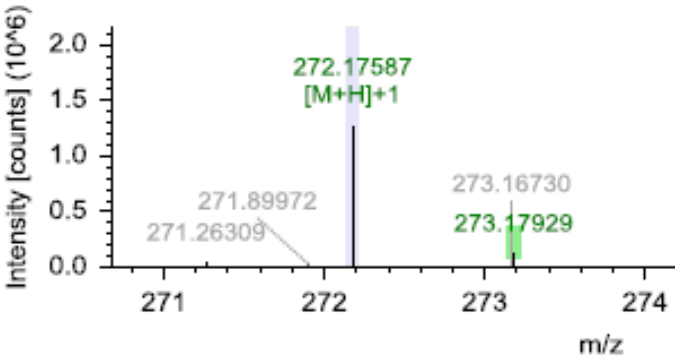
Level 3: ***Tentative candidate(s)***
structure, substituent, class

Level 4: ***Unequivocal molecular formula***

Level 5: ***Exact mass*** of interest

Data for TP:

MS spectrum



Prediction of sum formula based on likely atoms; consistent isotopic pattern
→ Molecular formula: $C_{16}H_{21}N_3O$ **Level 4**

Difference to parent compound:
→ $-CH_2$

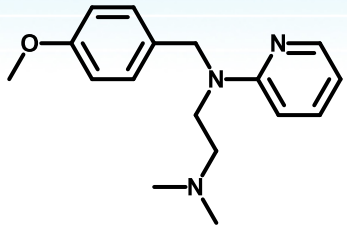
Demethylation at ether or tertiary amine group **Level 3**

Schymanski et al., 2014, ES&T

Structure elucidation

Communicating confidence

Pyrilamine (parent):



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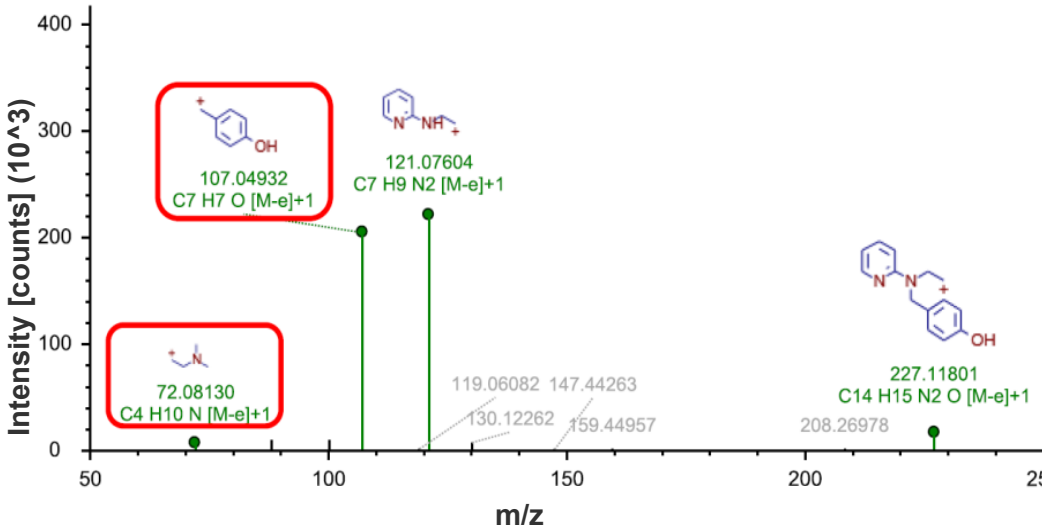
Level 3: ***Tentative candidate(s)***
structure, substituent, class

Level 4: ***Unequivocal molecular formula***

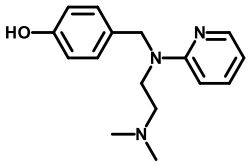
Level 5: ***Exact mass*** of interest

Data for TP:

MS^2 spectrum



Marked fragments provide diagnostic evidence for demethylation at the ether group



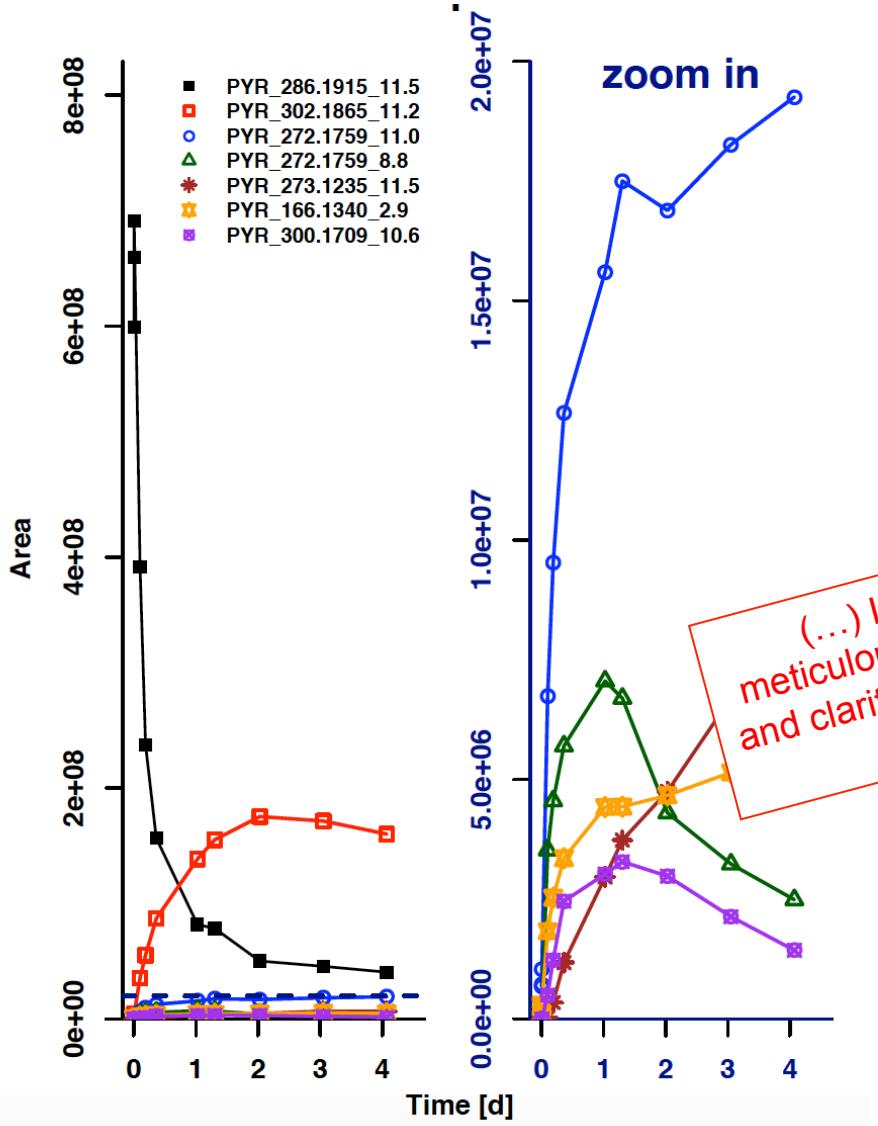
→ Structure identifiable with high confidence

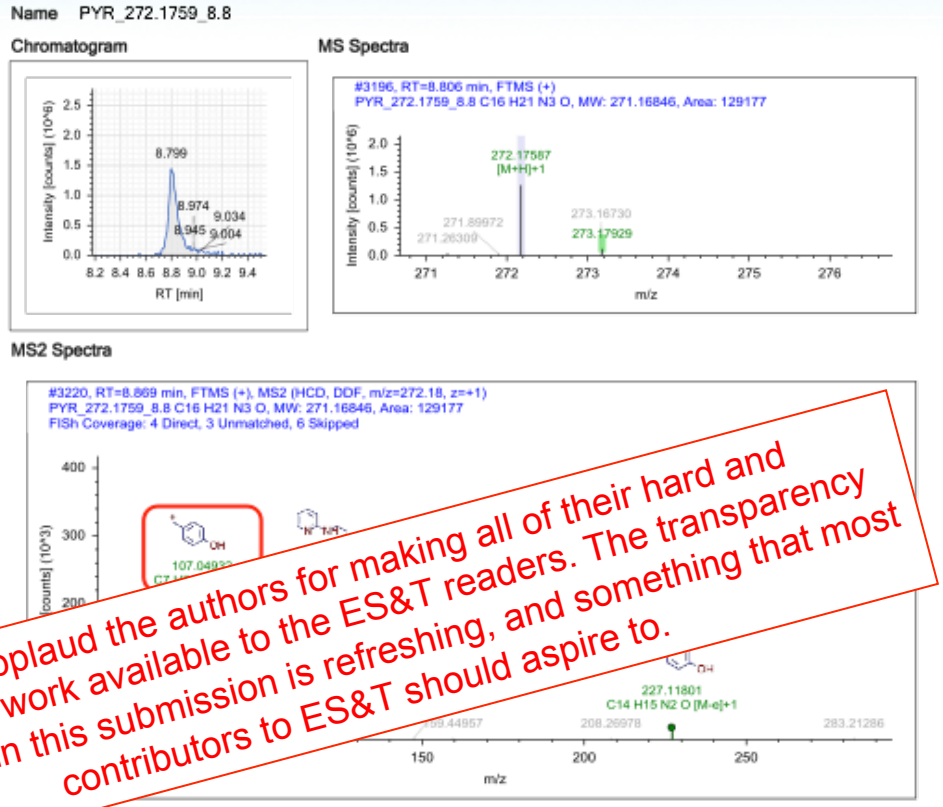
Level 2b

Schymanski et al., 2014, ES&T

Trend screening in spike experiments

Exemplary results for pyrilamine



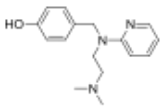


(…) I applaud the authors for making all of their hard and meticulous work available to the ES&T readers. The transparency and clarity in this submission is refreshing, and something that most contributors to ES&T should aspire to.

Formula
C16 H21 N3 O

Atomic Modification
-CH2

Proposed Structure



Confidence Level
Level 2b,
diagnostic evidence

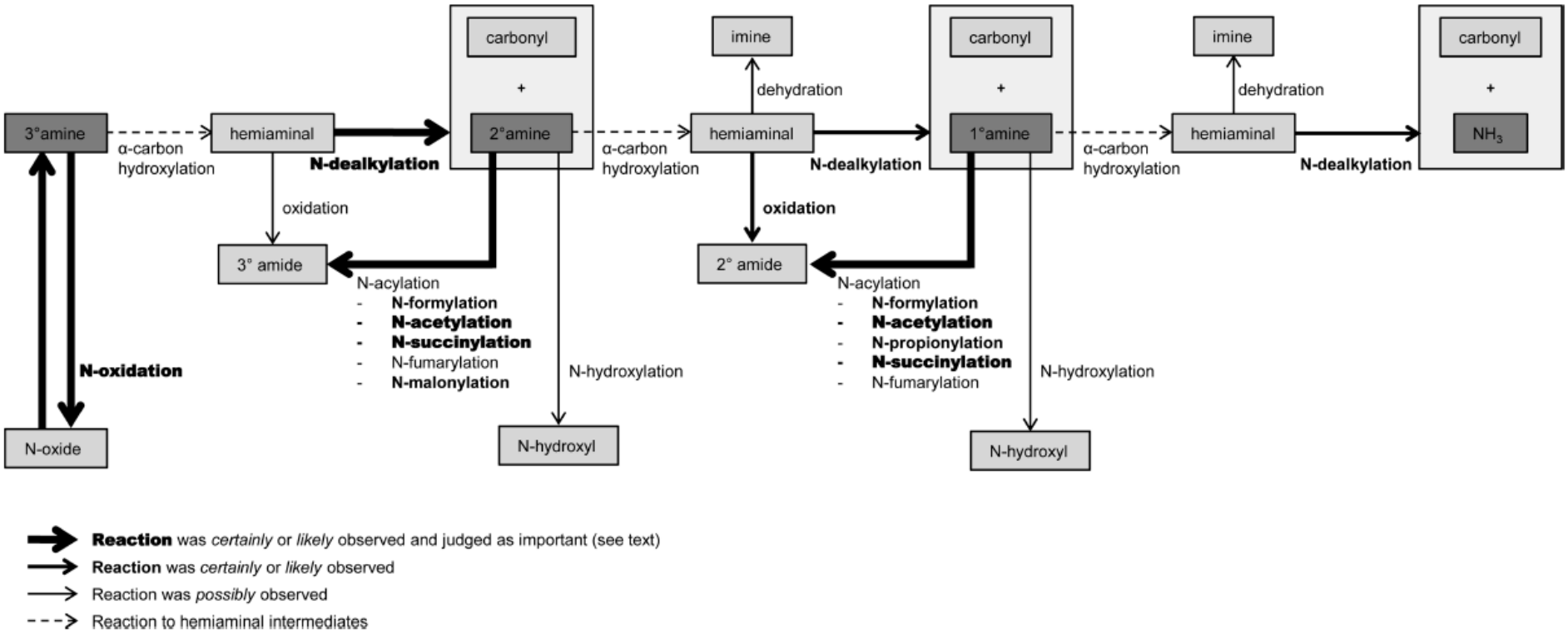
Additional Evidence for Structure Interpretation
The atomic modification of -CH2 indicates the loss of a methyl moiety. There are two methyl moieties that can be lost easily, one is an amine substituent, the other an ether substituent. The MS2 fragment at the nominal mass 72 was observed for the parent compound and indicates that the dimethyl amino group remains unaltered. The MS2 fragments at the nominal masses 72 and 107 are diagnostic evidence that the demethylation occurred at the ether moiety. The loss of an amino methyl was observed for TP PYR_272.1759_11.0.

Attributed Reaction from the Parent Compound to this TP
It is *certain* that this TP was formed via an O-demethylation reaction.

Gulde et al., 2016, ES&T

Amine biotransformation pathways

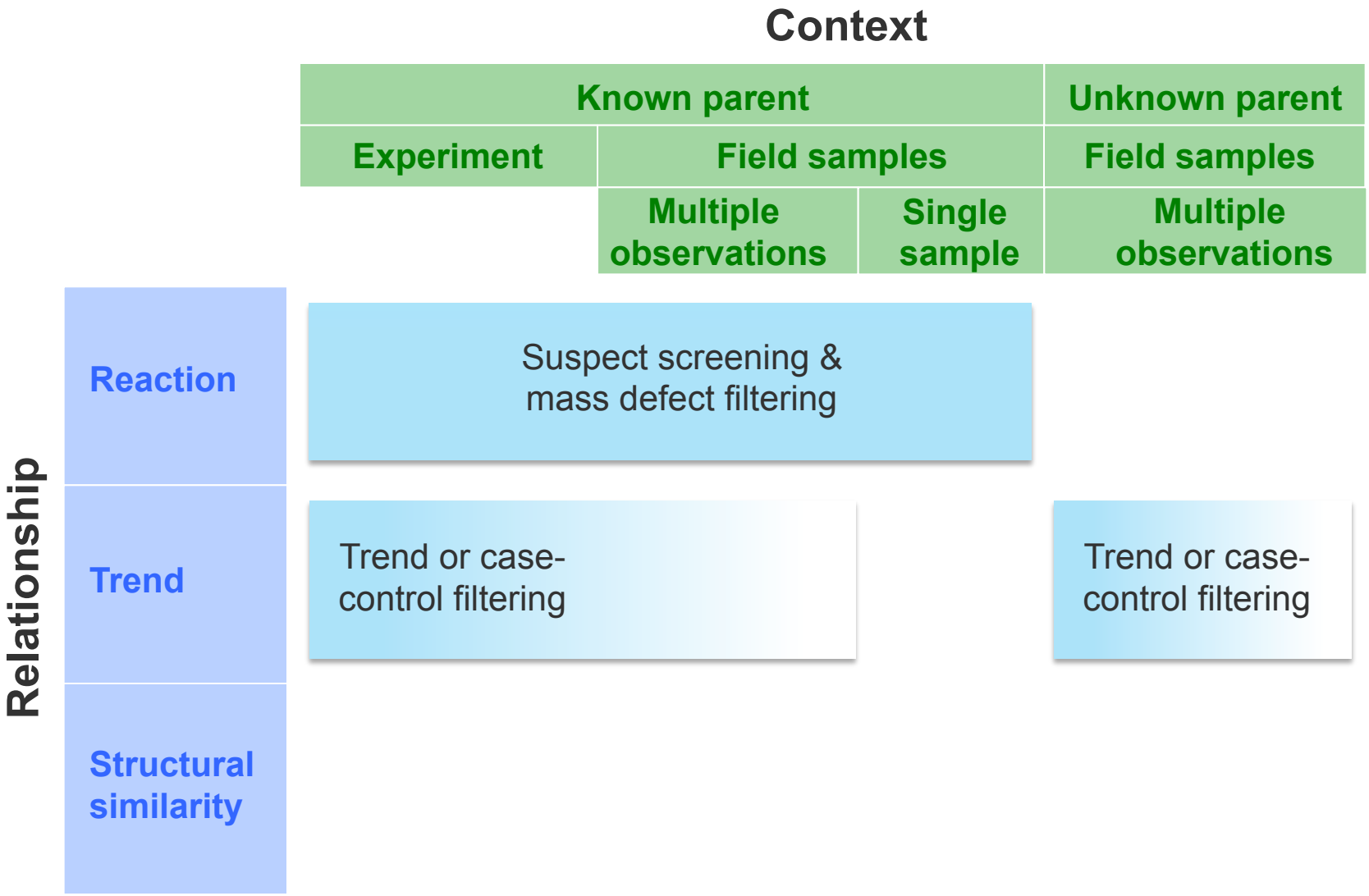
Multiple, but consistent transformations across 19 amines



Gulde et al., 2016, ES&T

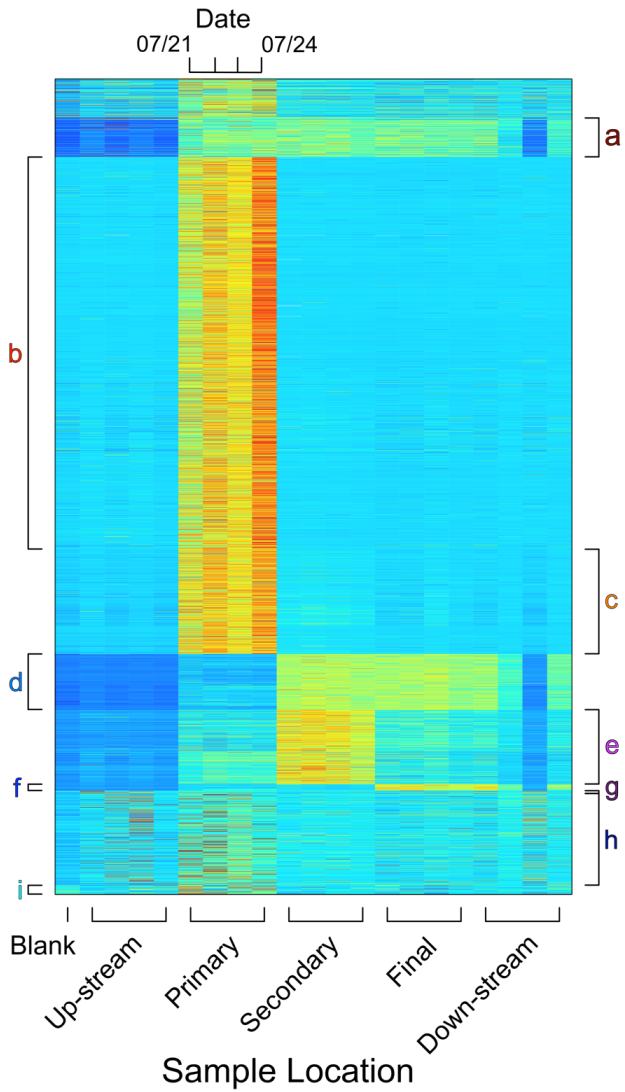
TP search with high-resolution MS

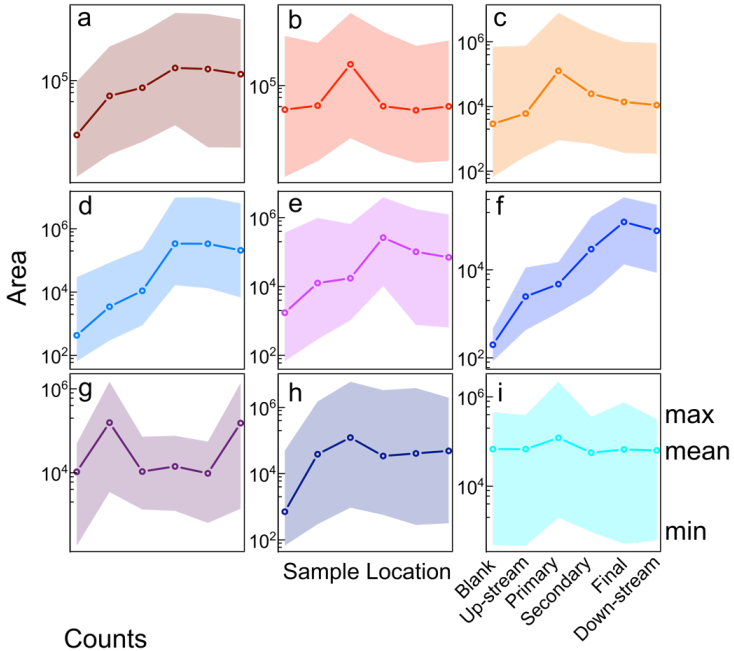
Overview of strategies

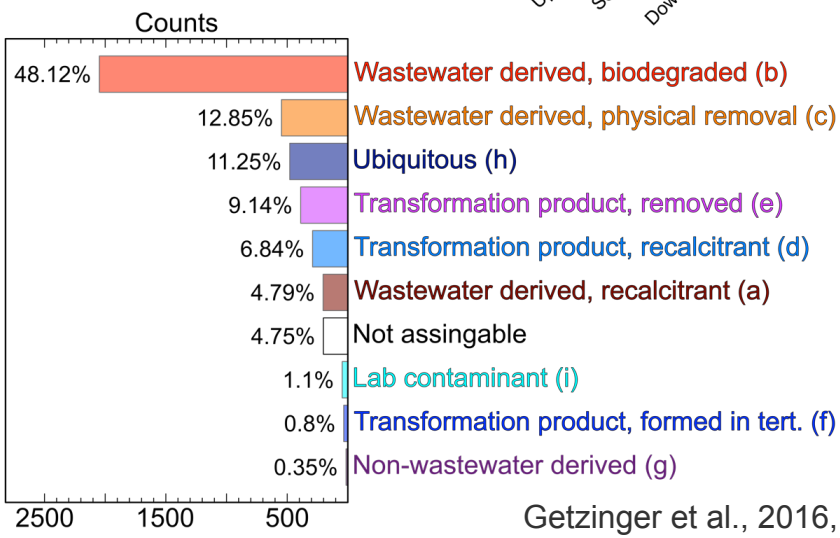


Trend screening in process studies

Characterization of processes, removal/formation of micropollutants



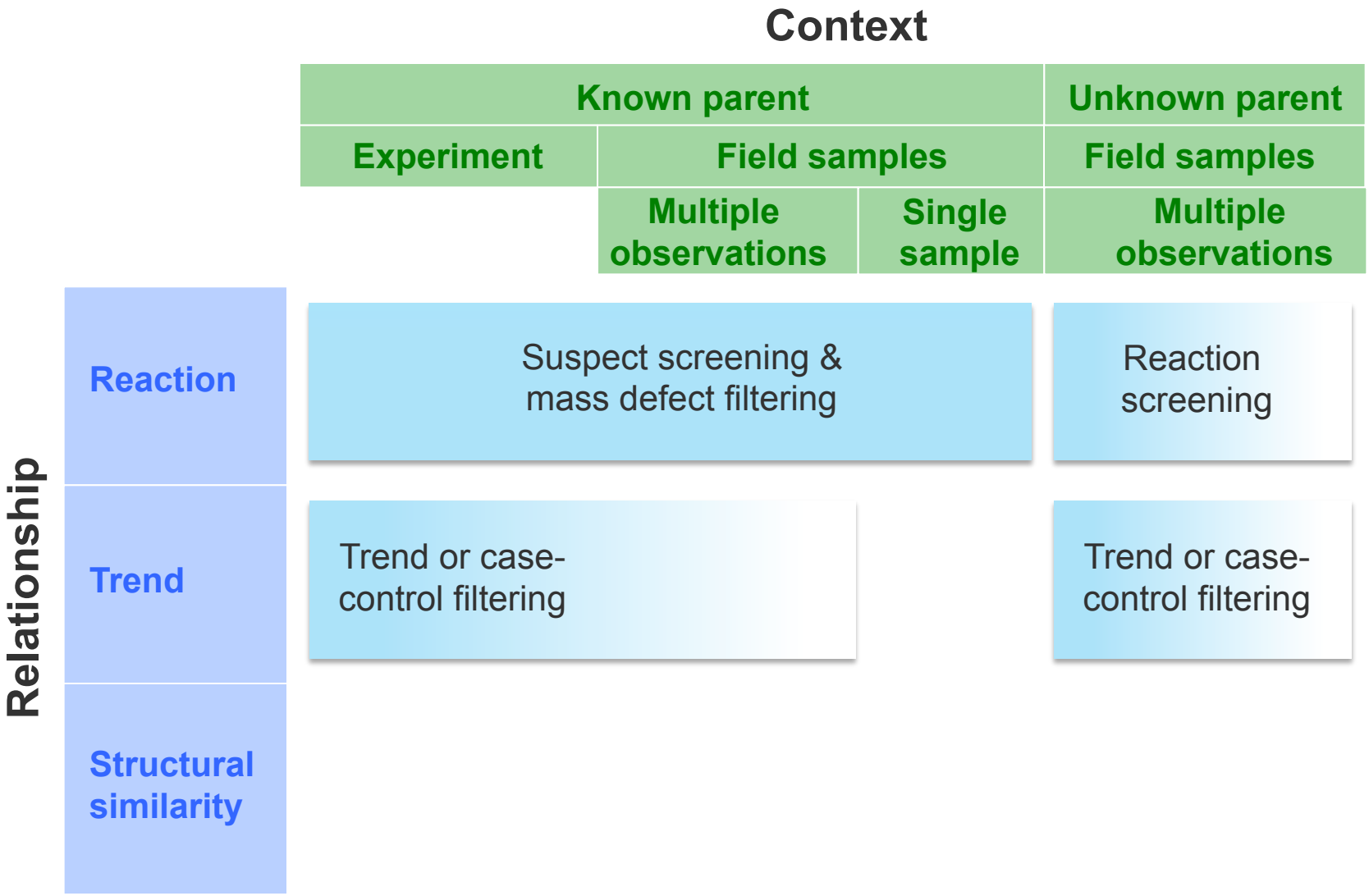




Getzinger et al., 2016, personal communication

TP search with high-resolution MS

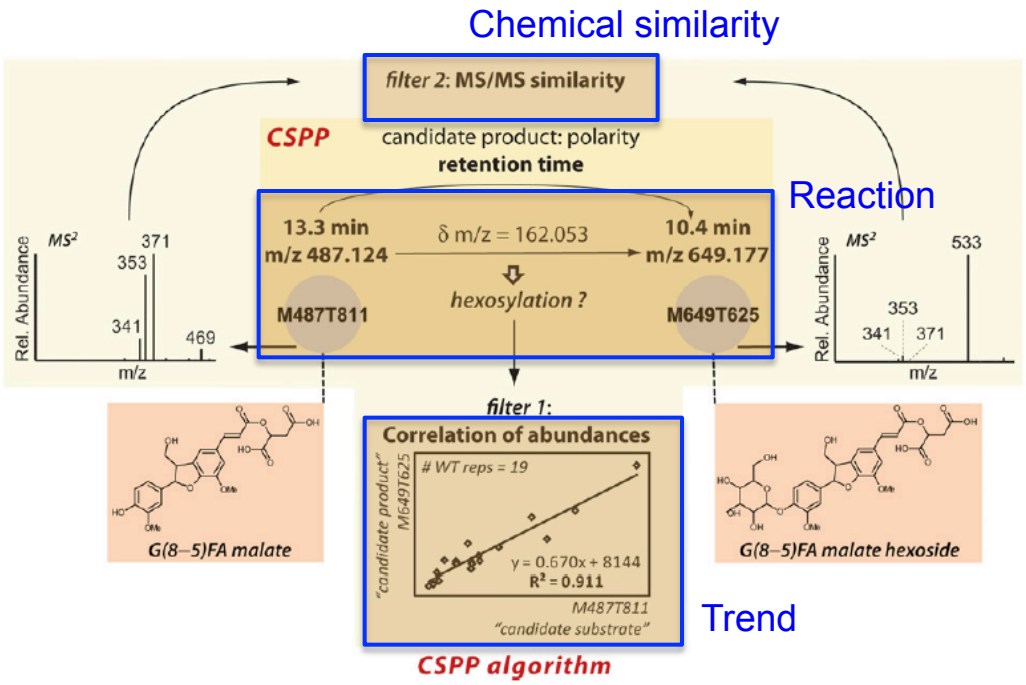
Overview of strategies



Reaction screening

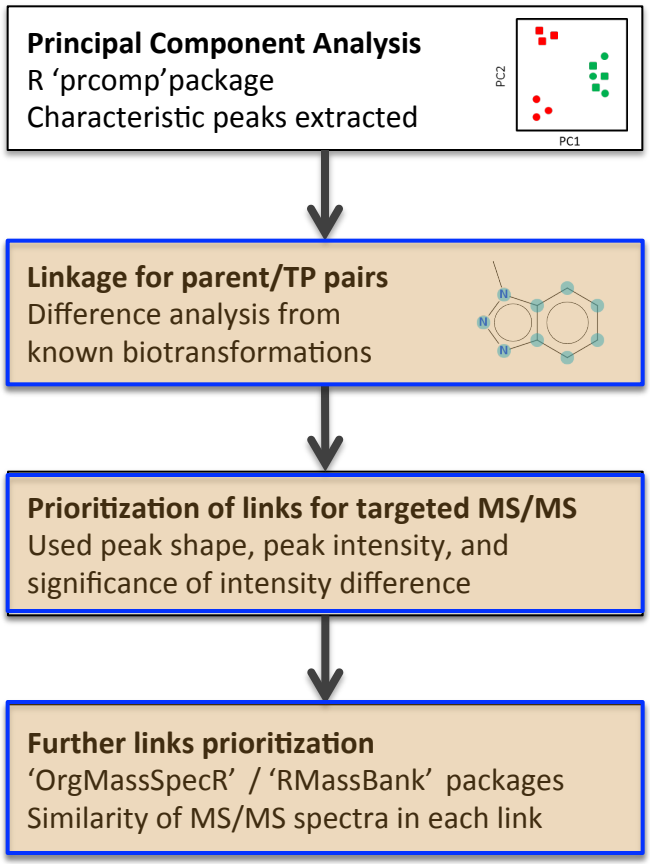
Process evaluation, finding unknown parent-TP pairs

Systems biology:
Structural Characterization of Metabolites in *Arabidopsis* via Candidate Substrate-Product Pair Networks



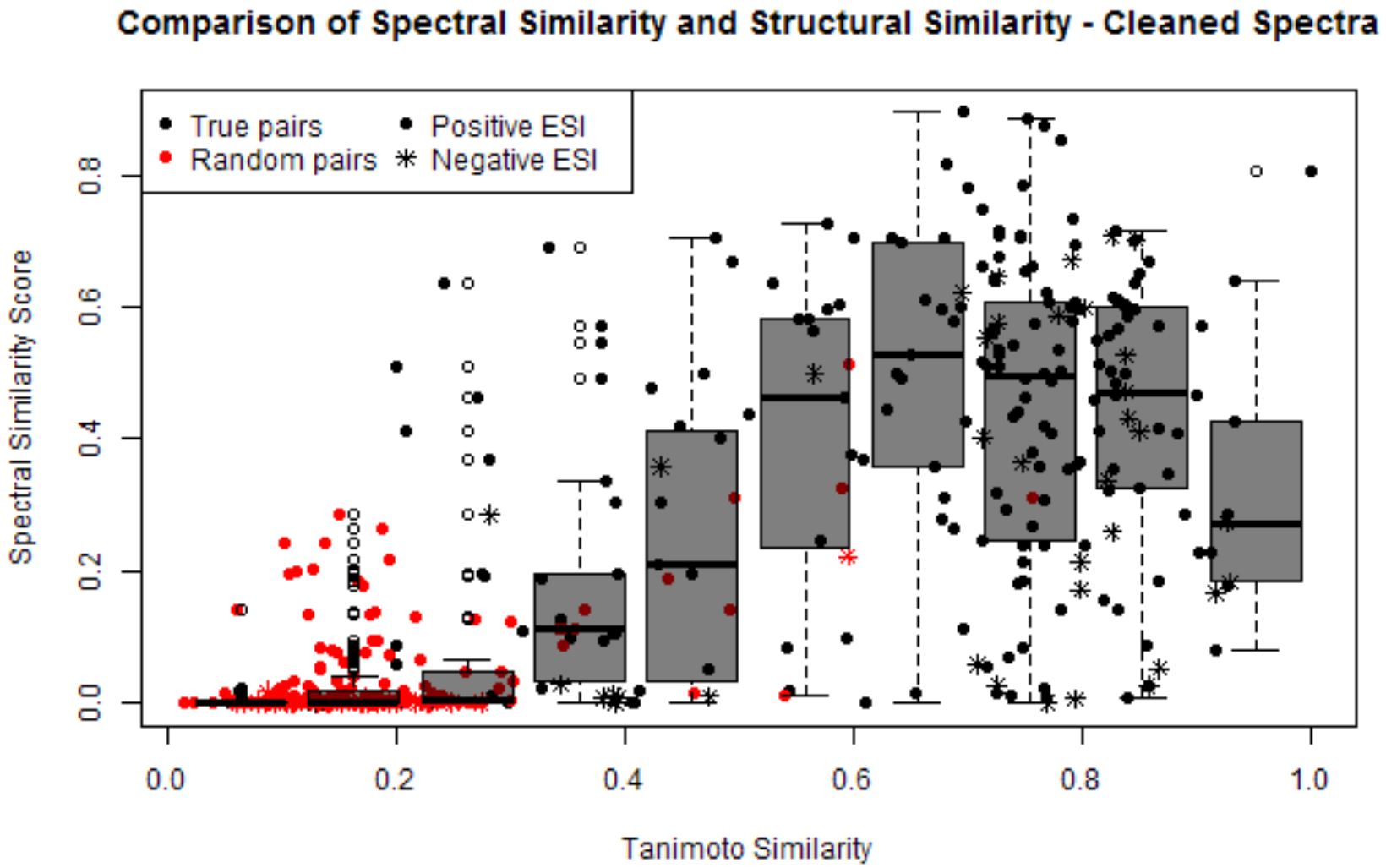
Morreel et al., 2014, Plant Cell

Environmental chemistry:
Finding PC-TP pairs in EICs from process samples (ozonation, WWTP)



Schollée et al., 2016, Anal. Chem.

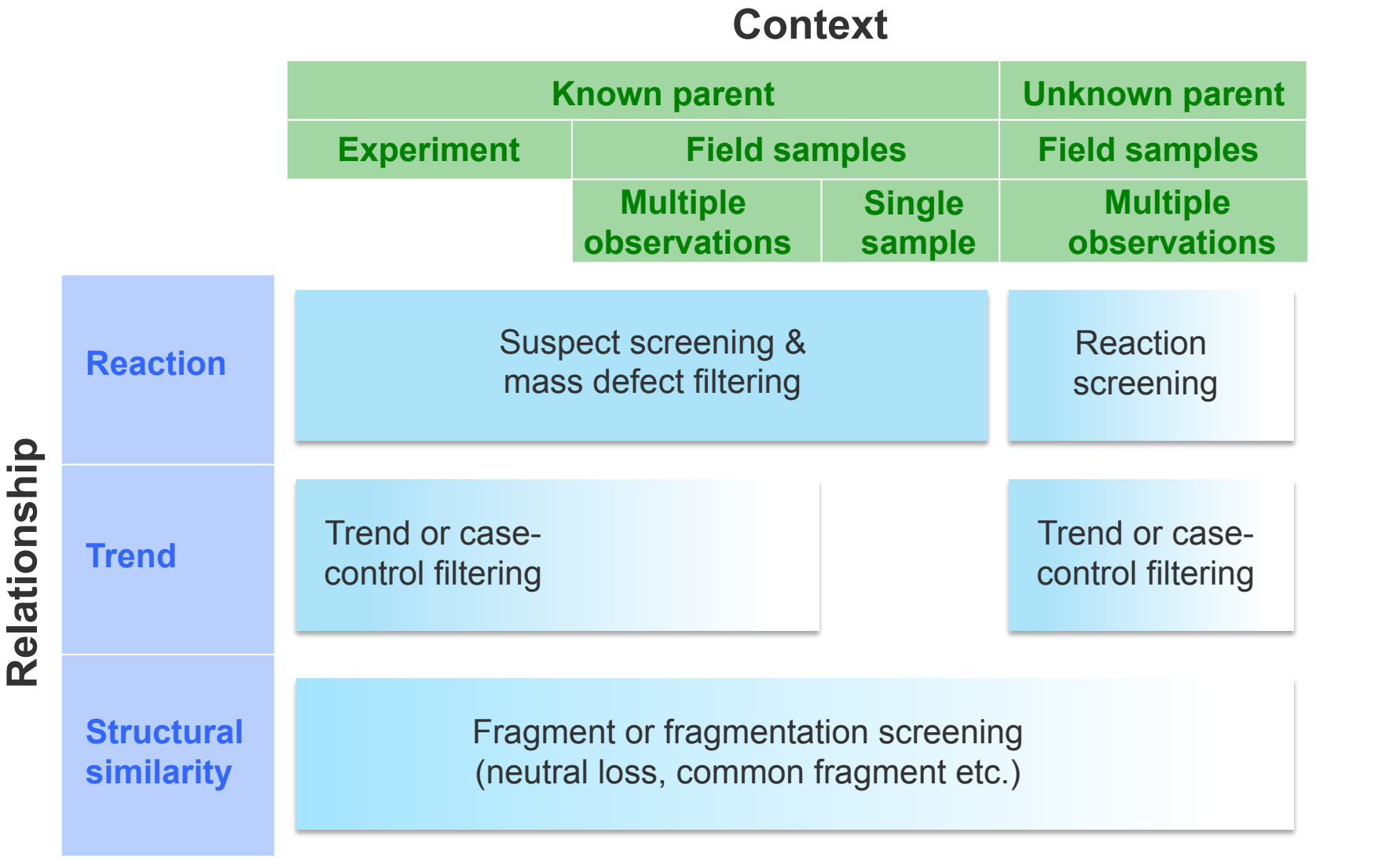
MSMS similarity as PC-TP pair filter



Schollée et al., 2016, submitted to Anal. Chem.

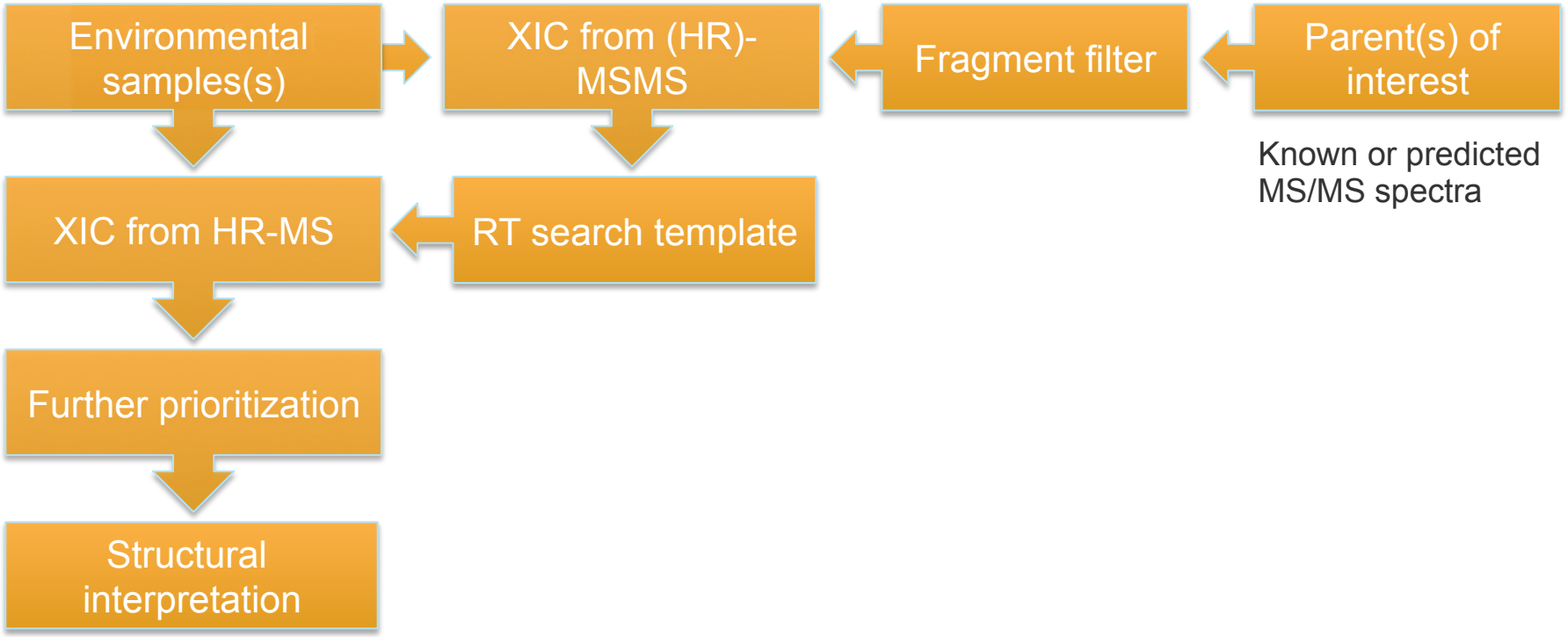
TP search with high-resolution MS

Overview of strategies



Fragment screening

Comprehensive enumeration of TPs, pathway elucidation



Mistrik et al., 2010, Drug Metabol. Rev.

Fragment screening

Comprehensive enumeration of TPs, pathway elucidation

WWTP & SW samples(s)

XIC from HR-MS

Further prioritization

Structural interpretation

RT search template

New RT of interest for venlafaxine

New parent ion mass at that RT

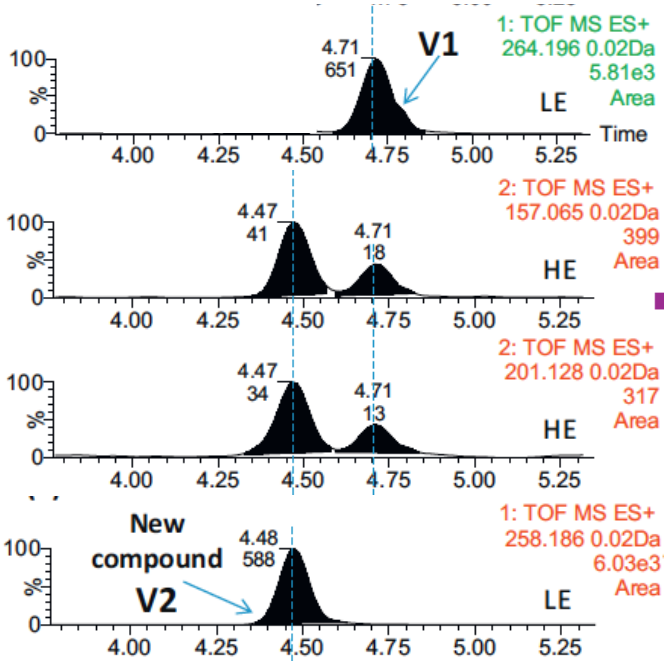
XIC from (HR)-MSMS

Fragment filter

3-4 known fragment ions of parent and/or known TP

Parent(s) of interest

5 pharmaceuticals with high production volume and significant removal in WWTP

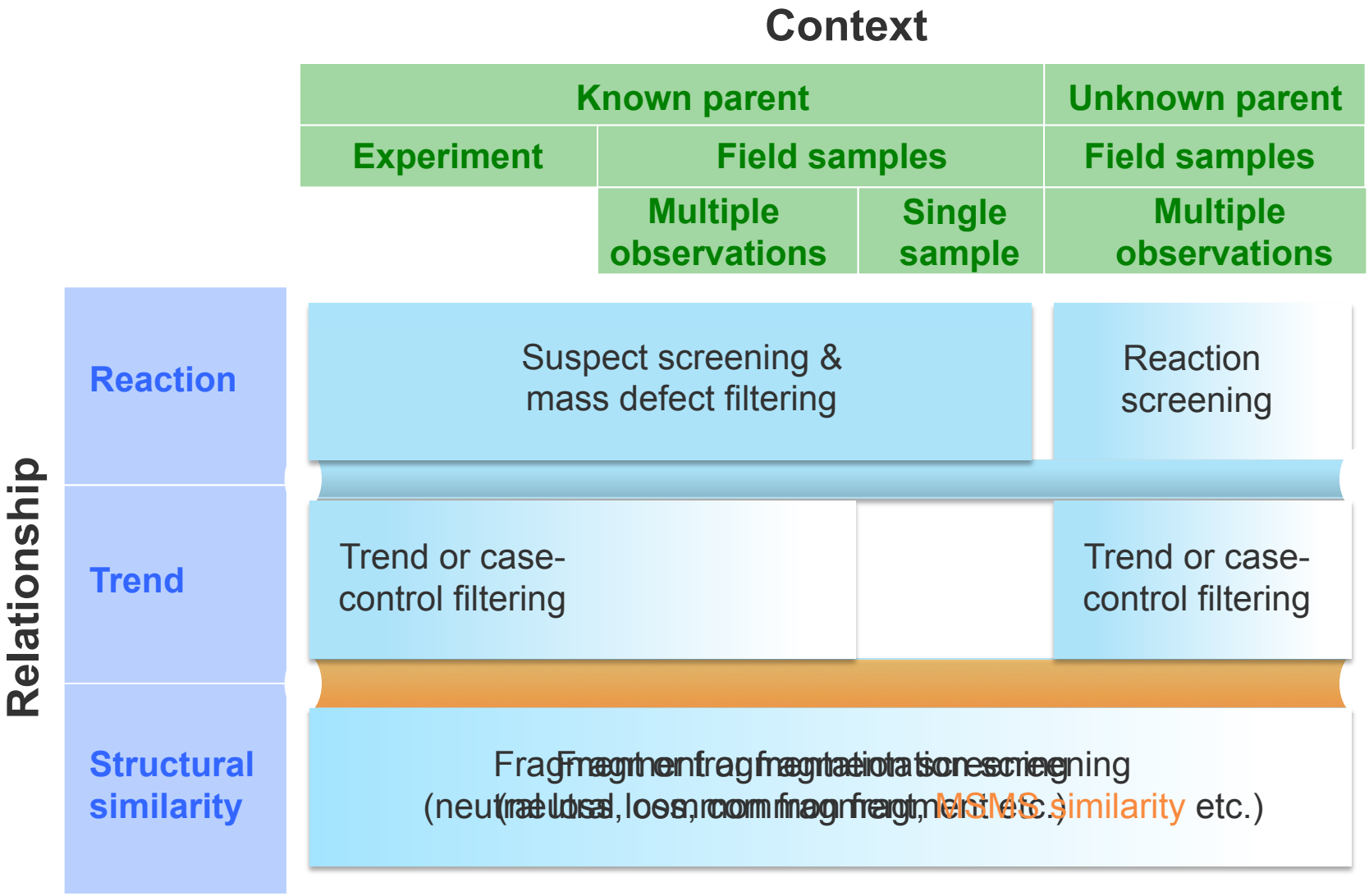


Boix et al., 2016, J. Hazard. Mat.

Other example: Zonja et al., 2016, ES&T

TP search with high-resolution MS

Conclusions



New biotransformation database & prediction tool

enviPath (https://envipath.org, former Eawag-BBD)

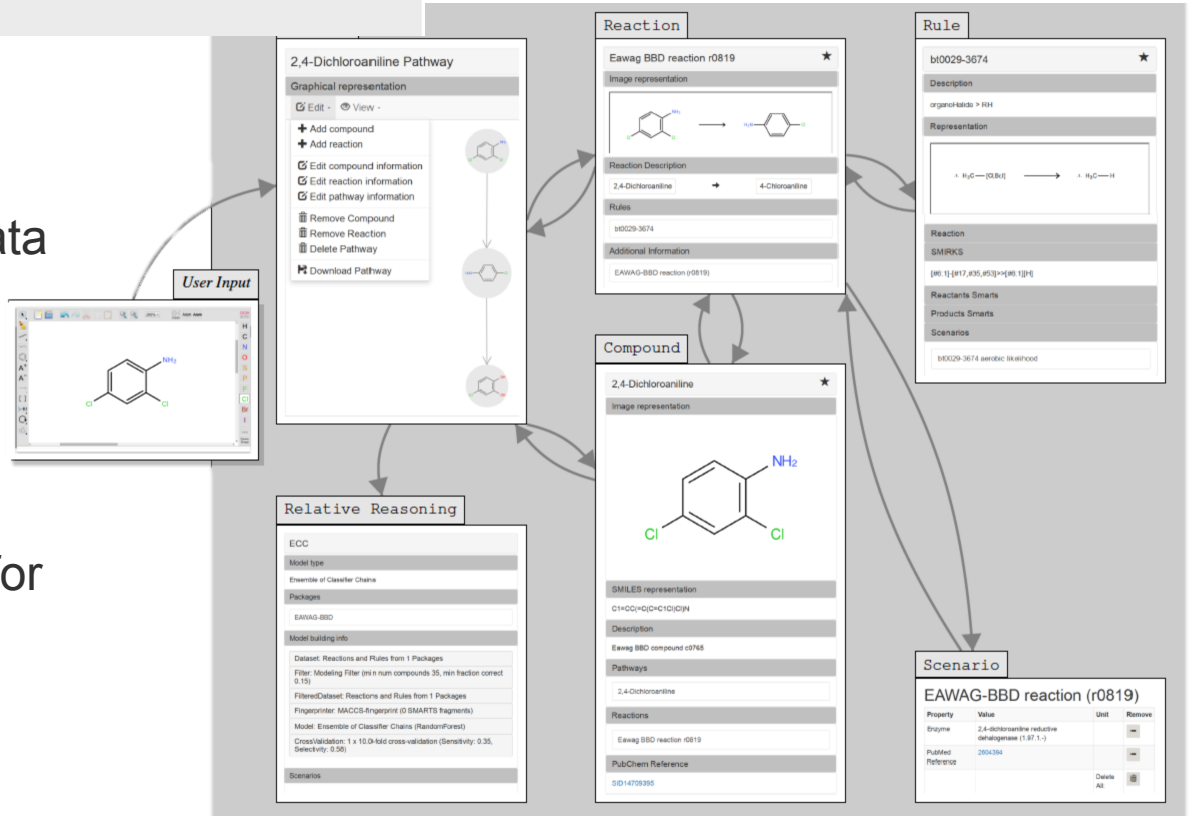
enviPath | THE ENVIRONMENTAL CONTAMINANT BIOTRANSFORMATION PATHWAY RESOURCE

enviPath is a database and prediction system for the microbial biotransformation of organic environmental contaminants. The database provides the possibility to store and view experimentally observed biotransformation pathways. The pathway prediction system provides different relative reasoning models to predict likely biotransformation pathways and products. You can try it out below.

Learn more >>

- Central resource for contaminant biotransformation pathways
- Database and pathway prediction

- Public and private data
- Easy data entry and metadata annotation
- Biotransformation rules for pathway prediction
- Relative reasoning models
- Machine-learning methods for training own models



Wicker et al., *Nucleic Acids Res.*, 44, 2016

The road ahead…

- Data-independent or all ion fragmentation experiments
- Data processing workflows to sort together fragment ion and parent ion peaks
- Efficient data processing software solutions
- Complementary chromatographic methods to capture polar and ionic TPs

Thanks…

PhD students:
Michele Stravs

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Philipp Longrée

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Damian Helbling
Martin Loos

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