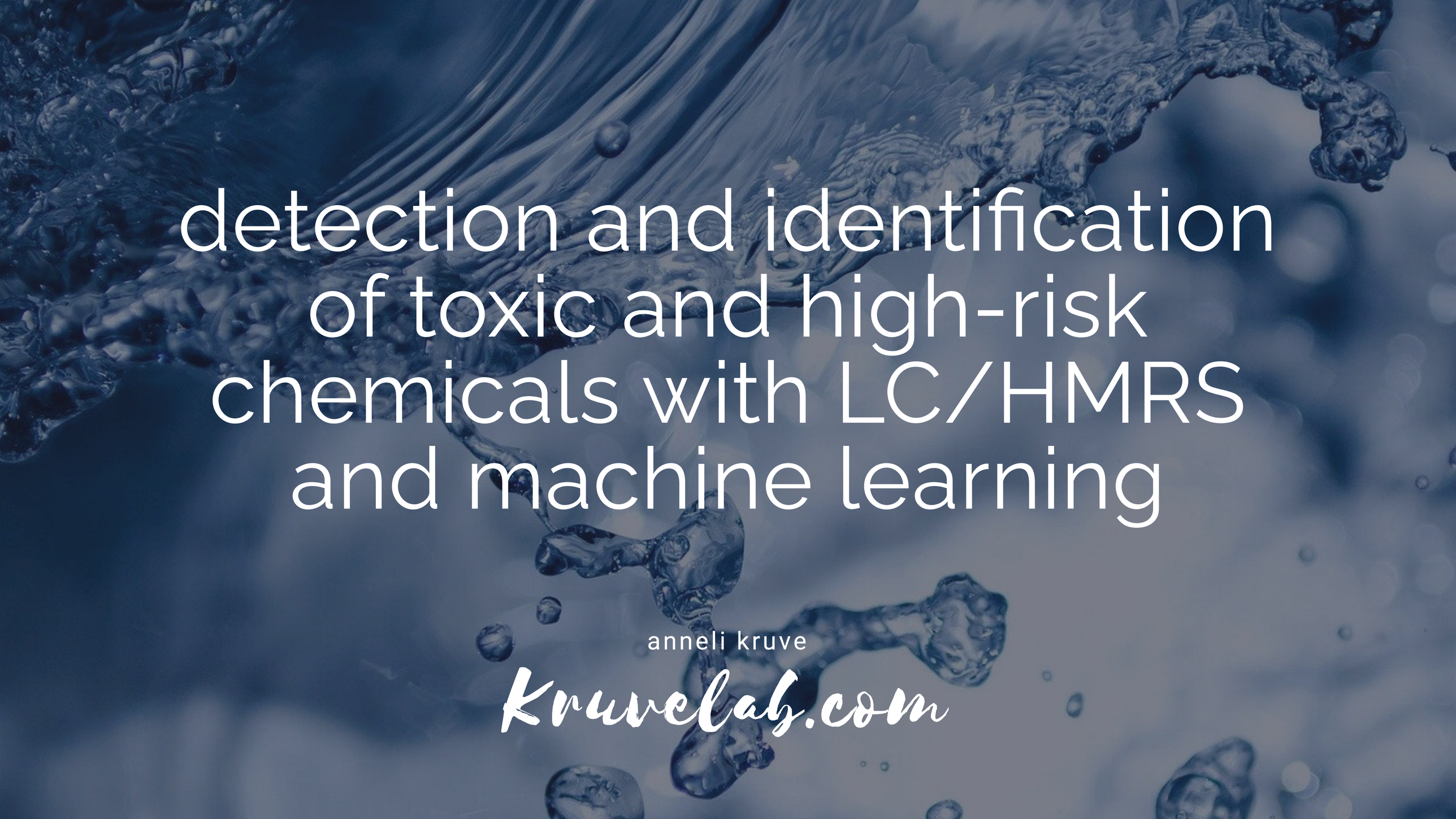

detection and identification of toxic and high-risk chemicals with LC/HMRS and machine learning

anneli kruve

Kruvelab.com

increased threat to Swedish drinking water

an offence more than every fifth day

anneli kruve

DAGENS NYHETER.

SVERIGE

Ökat hot mot svenskt dricksvatten – brott mer än var femte dag

Uppdaterad 2024-12-02 Publicerad 2024-12-01

water analysis

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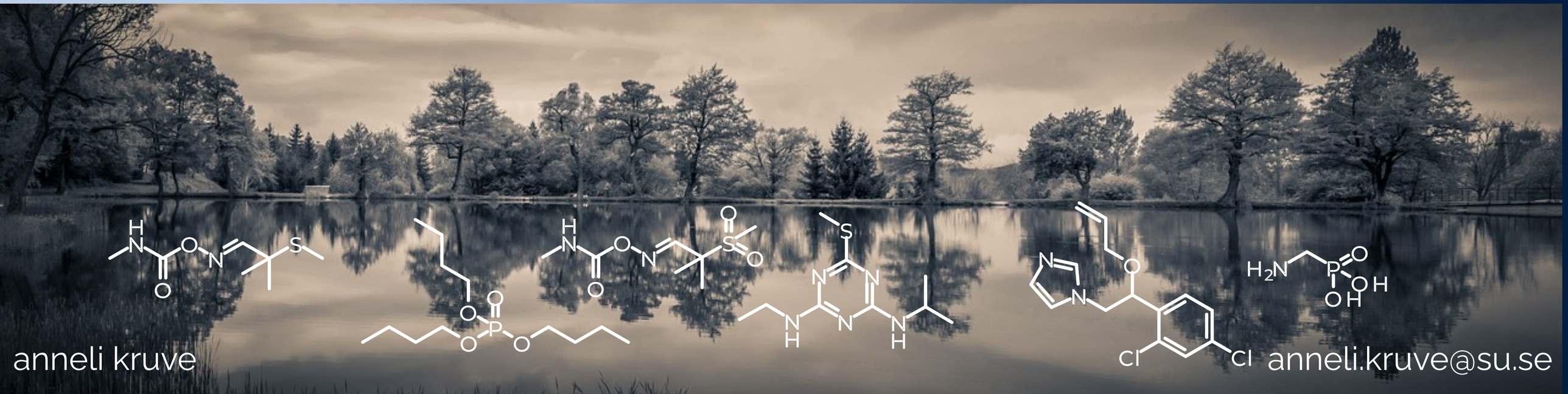
water analysis



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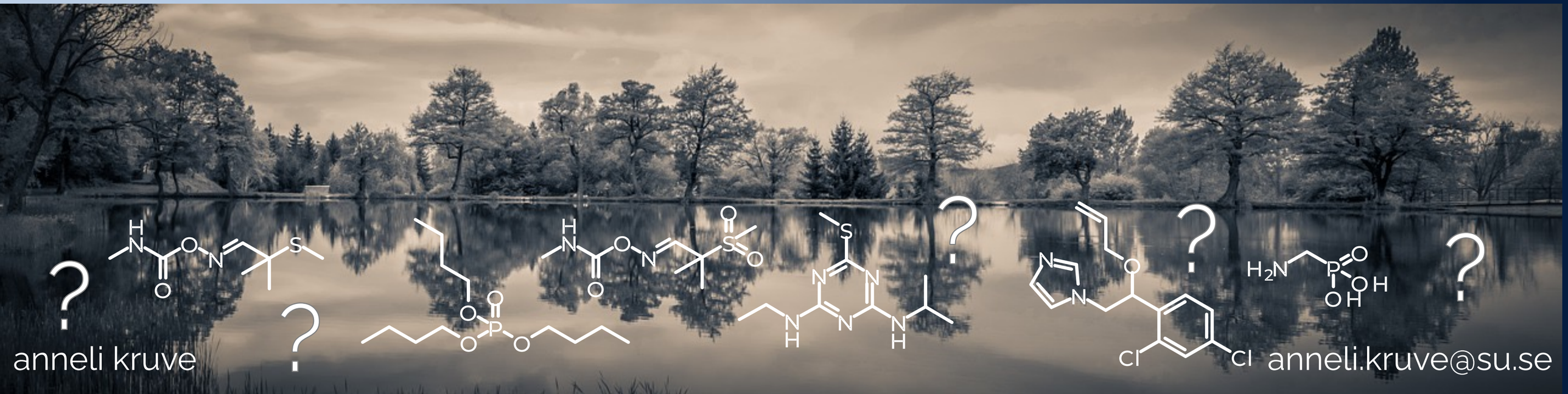
water analysis

thousands of chemicals detected



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water analysis

thousands of chemicals detected

Machine Translated by Google

BioCell ANALYTICA

Table 1. The following BEQ values were measured in the current samples:

	Nrf2 activity	AntiÿAR activity	AR activity	ER activity	AhR activity
	µg/L (tBHQ equivalents)	ng/L (OHF equivalents)	ng/L (DHT equivalents)	pg/L (E2 equivalents)	ng/L (TCDD equivalents)
Reference to sample 1	<LOD	<LOD	<LOD	<LOD	<LOD
Sample 1	<LOD	<LOD	79300	784	0.0814
Reference to sample 2	21.1	73.6	<LOD	21.7	<LOD
Sample 2	992	2670	<LOD*	<LOD*	<LOD*
Detection limit	8.34	43.8	0.122	12.5	0.0196
detection limit*			6.93	50.0	0.156

Table 2. Genotoxicity.

	Genotoxic?
Reference to sample 1	No
Sample 1	No
Reference to sample 2	No
Sample 2	Could not be determined*

*Due to extensive cytotoxicity, despite repeated analyses, it could not be determined whether sample 2 was genotoxic or not. The sample was tested down to the concentration REF 12.5, but even then was too cytotoxic to be able to determine if it was genotoxic.

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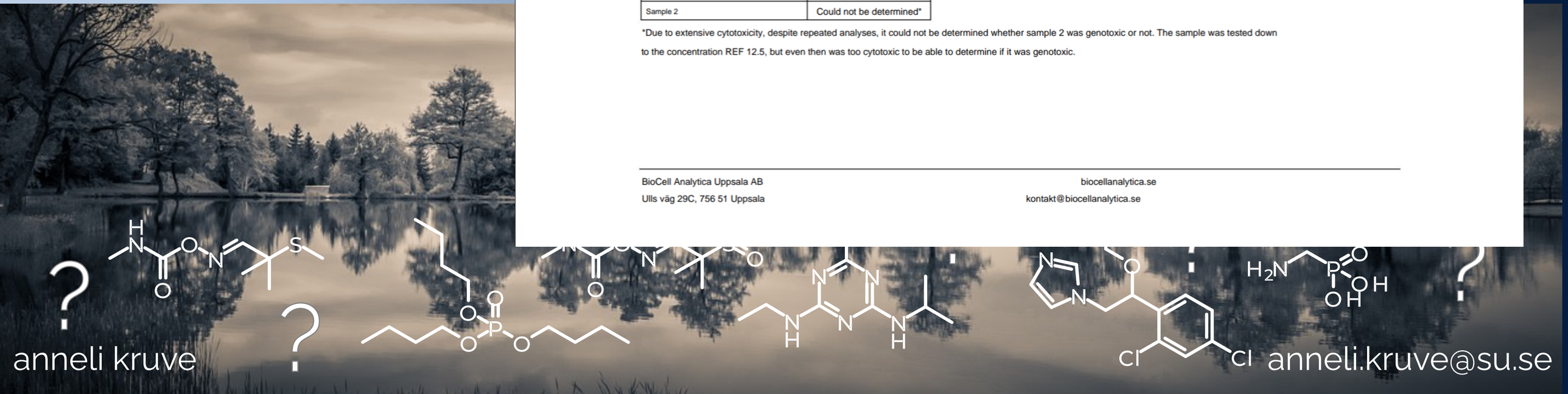

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how to ...

PRIORITIZE

risk

IDENTIFY

structure

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how to ...

PRIORITIZE

risk

IDENTIFY

structure

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prioritization of chemicals

TOXICITY

ecotoxicity and endocrine disruptors

CONCENTRATION

exposure to potentially toxic chemicals

RISK

$$\text{PriorityScore} = \frac{C_{\text{predicted}}}{AC_{50}^{\text{5th percentile}}}$$

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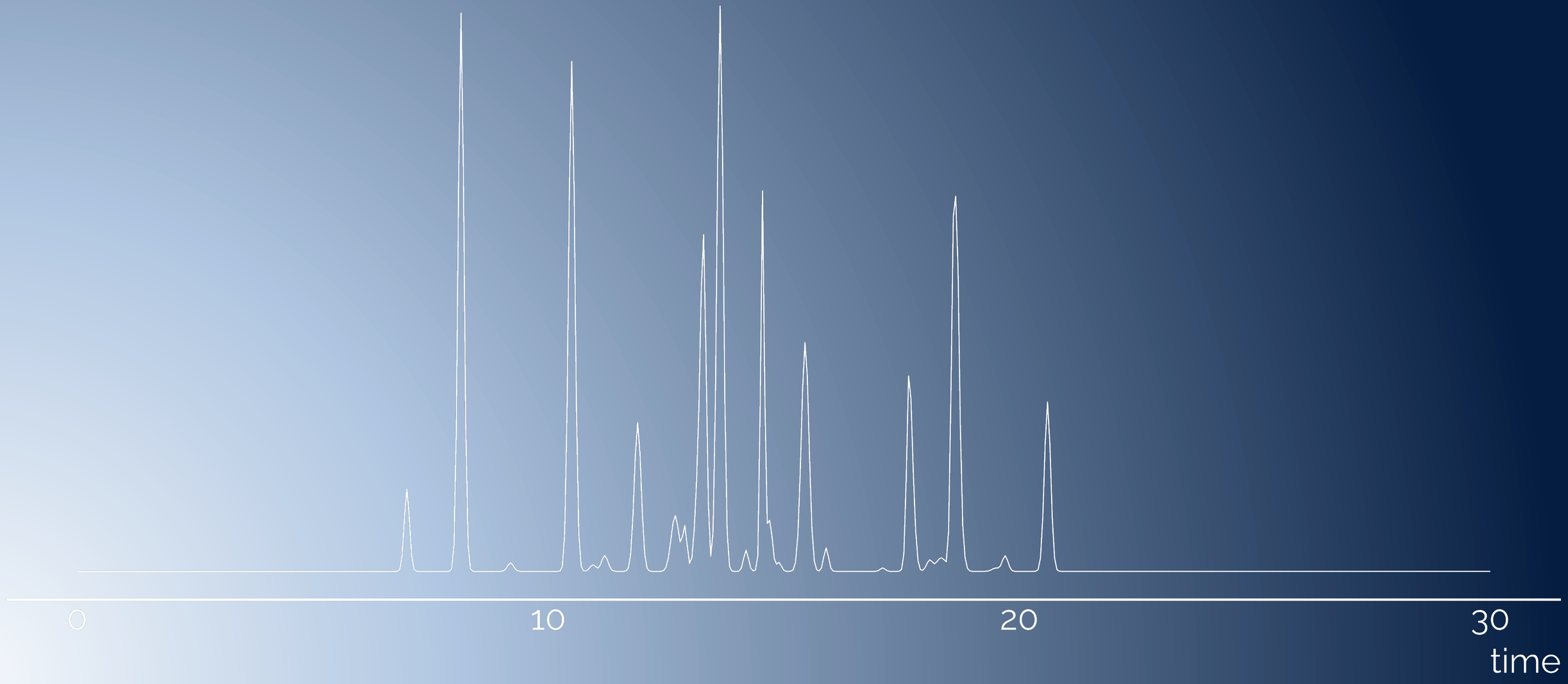
toxicity

of detected chemicals

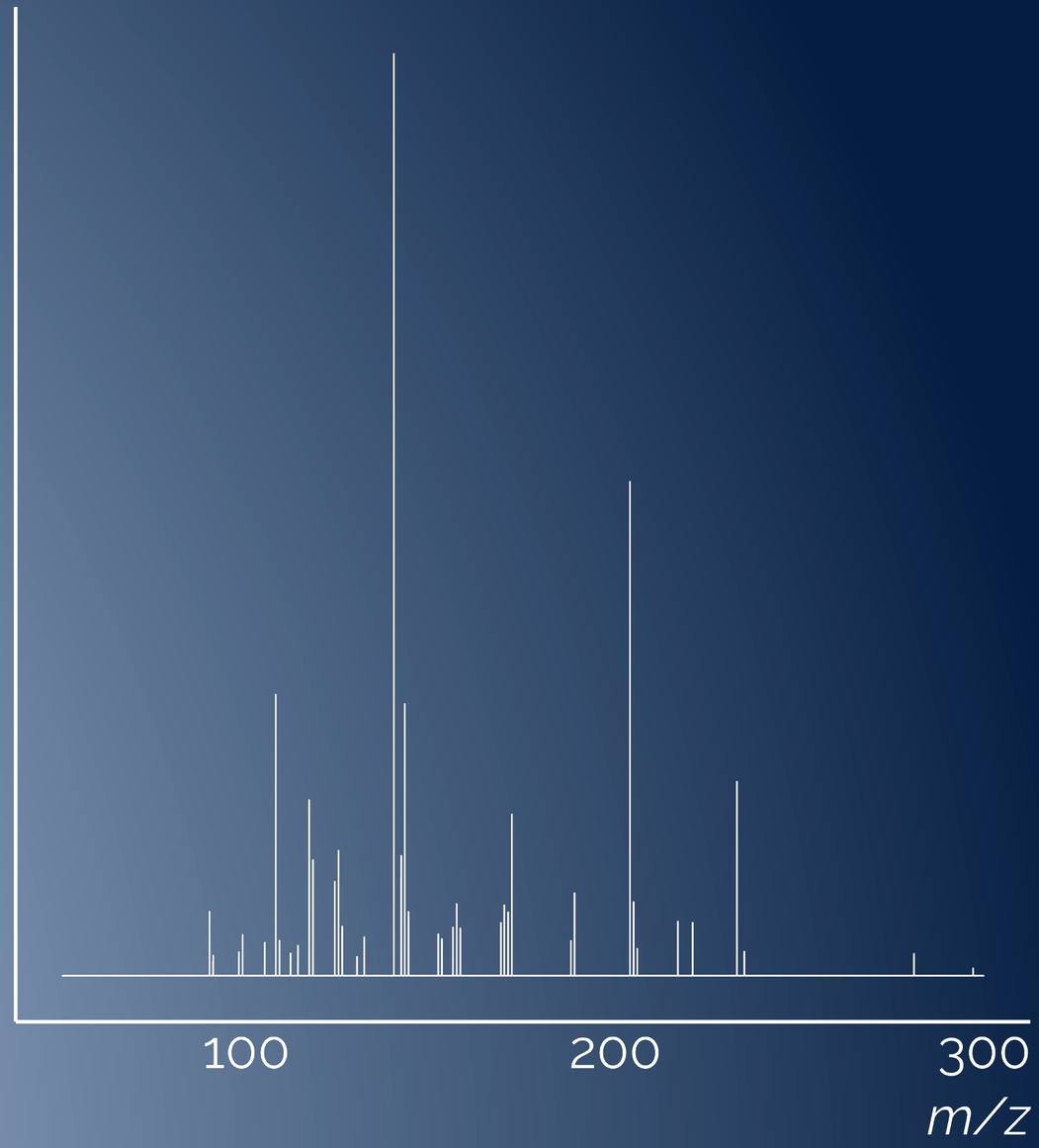
nontarget screening

with LC/HRMS



toxicity assessment

from spectra
to structure
to toxicity



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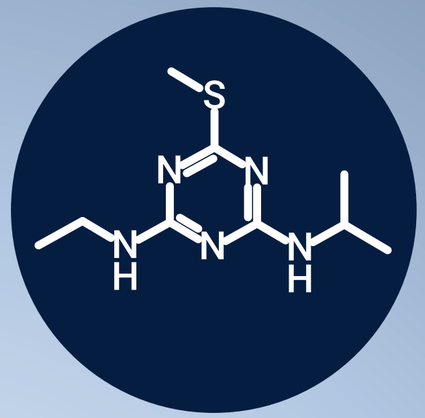
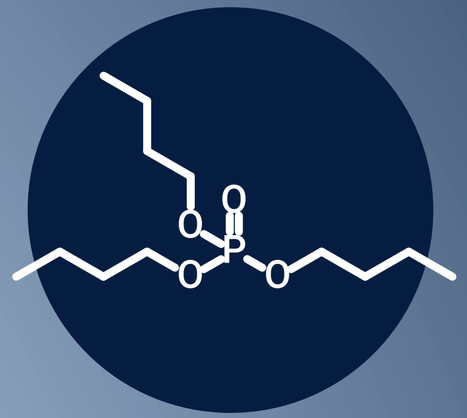
toxicity assessment



LC_{50} = 9.3 mg/L

known structure
known toxicity



LC_{50} = ? mg/L

known structure
unknown toxicity

LC_{50} = ? mg/L

unknown structure
unknown toxicity

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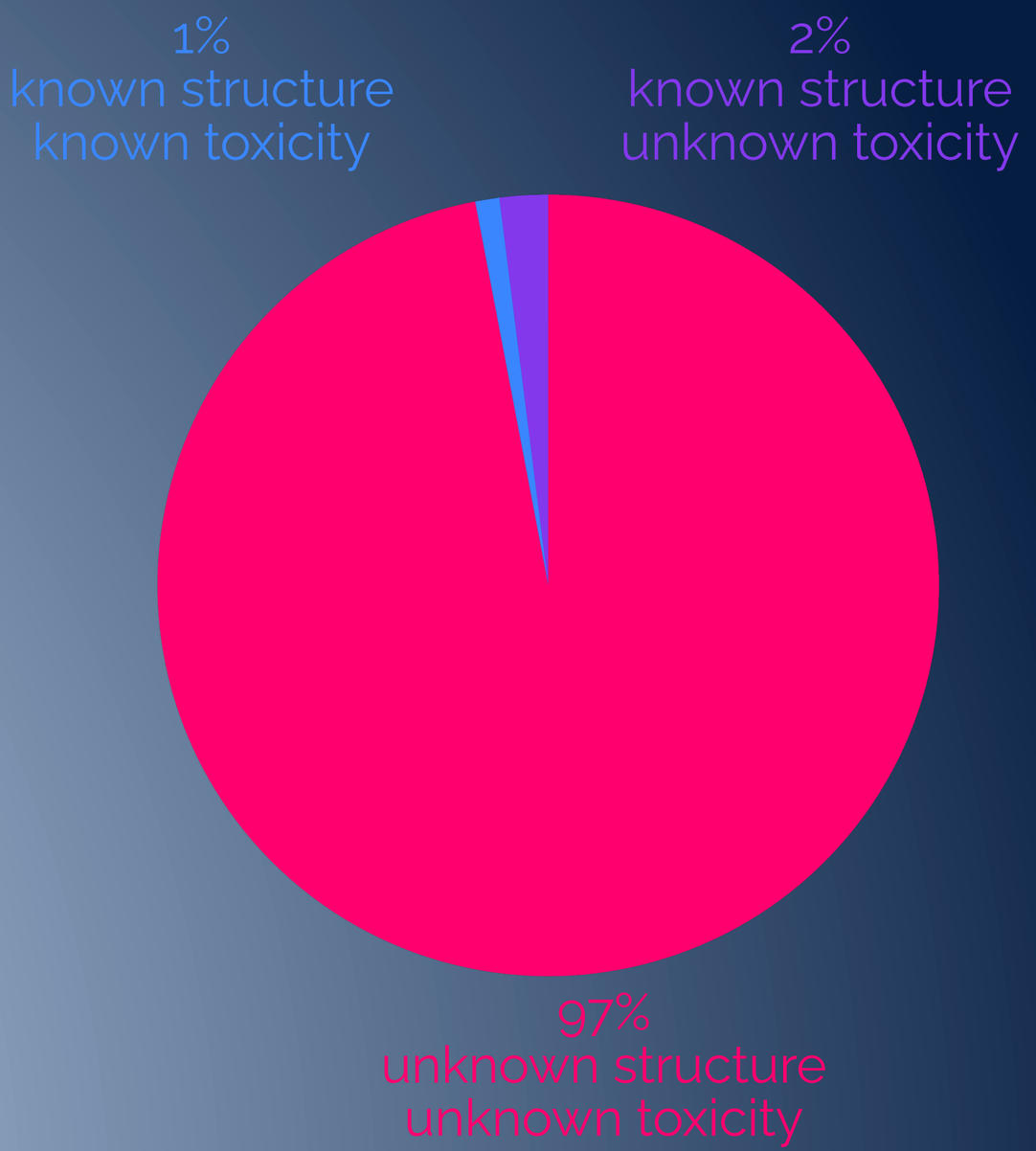
toxicity assessment

vast majority of detected chemicals remain unknown



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predicting toxicity

of detected chemicals

endpoints

ECOTOXICITY

fathead minnow, bluegill,
and rainbow trout

ENDOCRINE DISRUPTION

AhR, AR, ER, MMP, P53, ...

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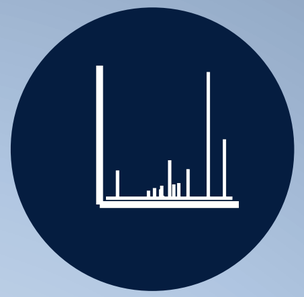
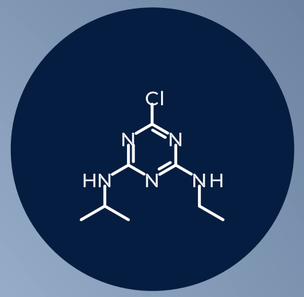
workflow



MS² spectra



structure as SMILES

molecular descriptors

toxicity prediction

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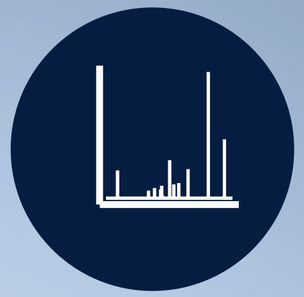
workflow



MS^2 spectra

molecular descriptors

toxicity prediction

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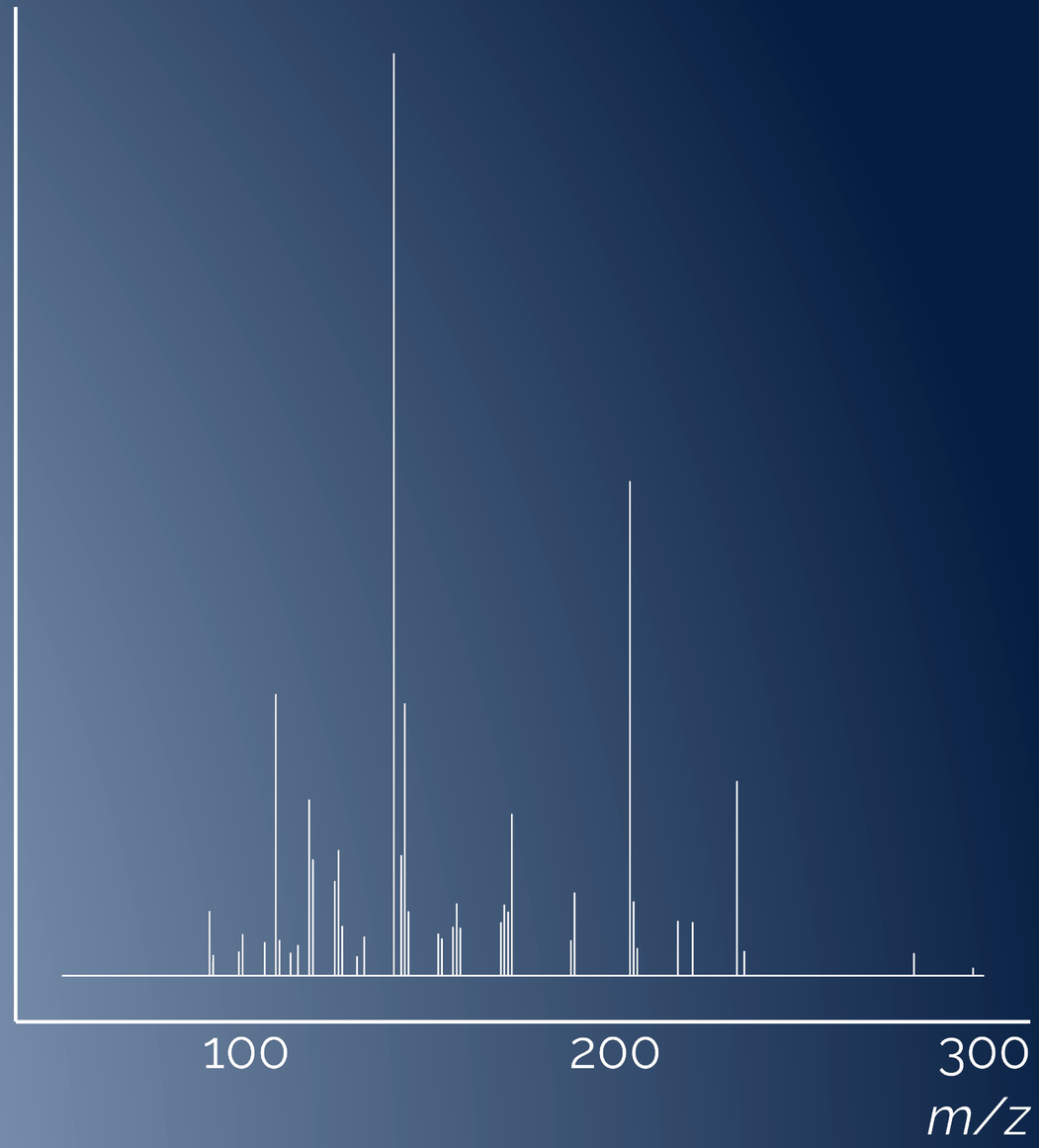
information available

in mass spectra



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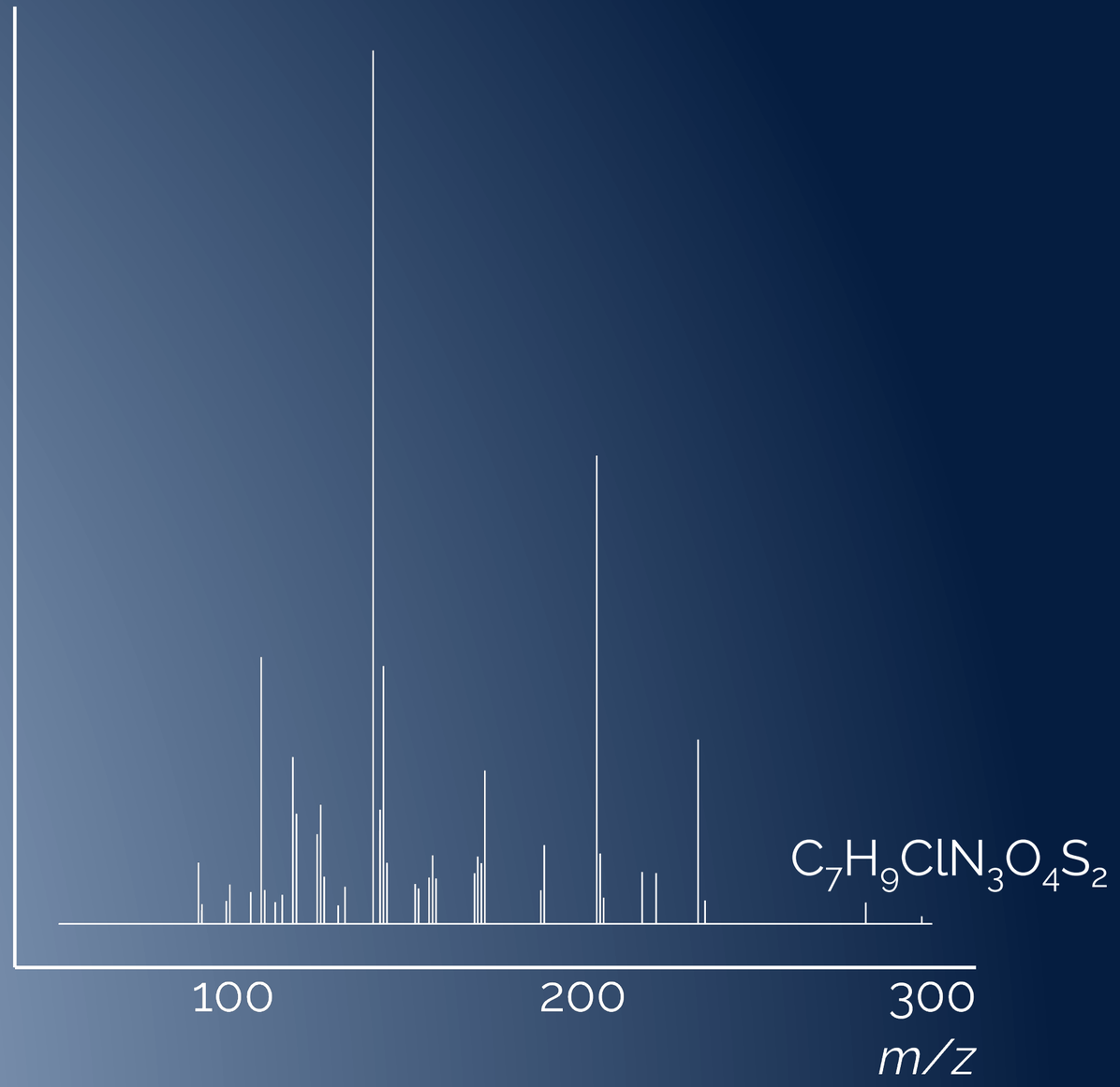
information available

in mass spectra



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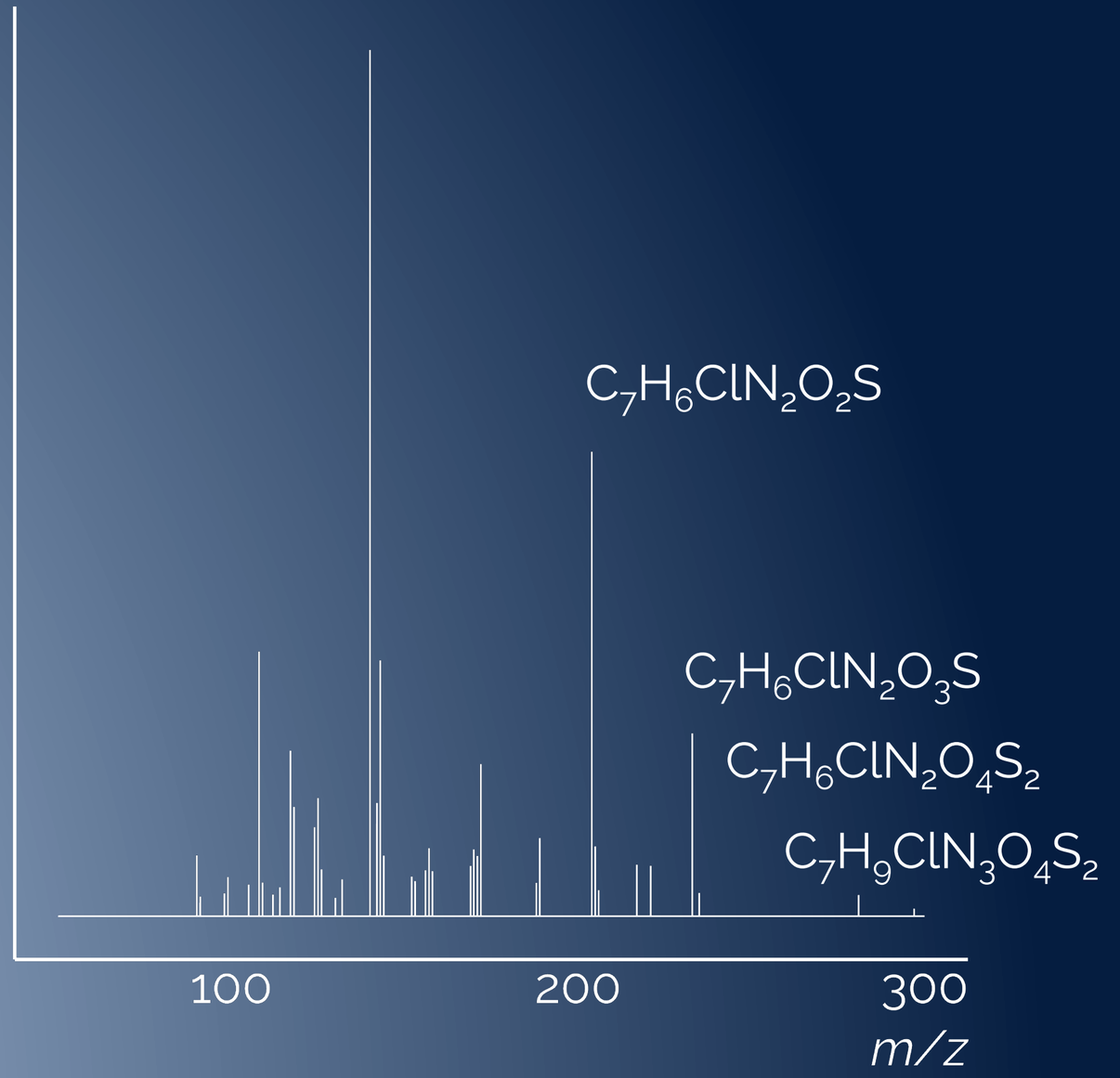
information available

in mass spectra



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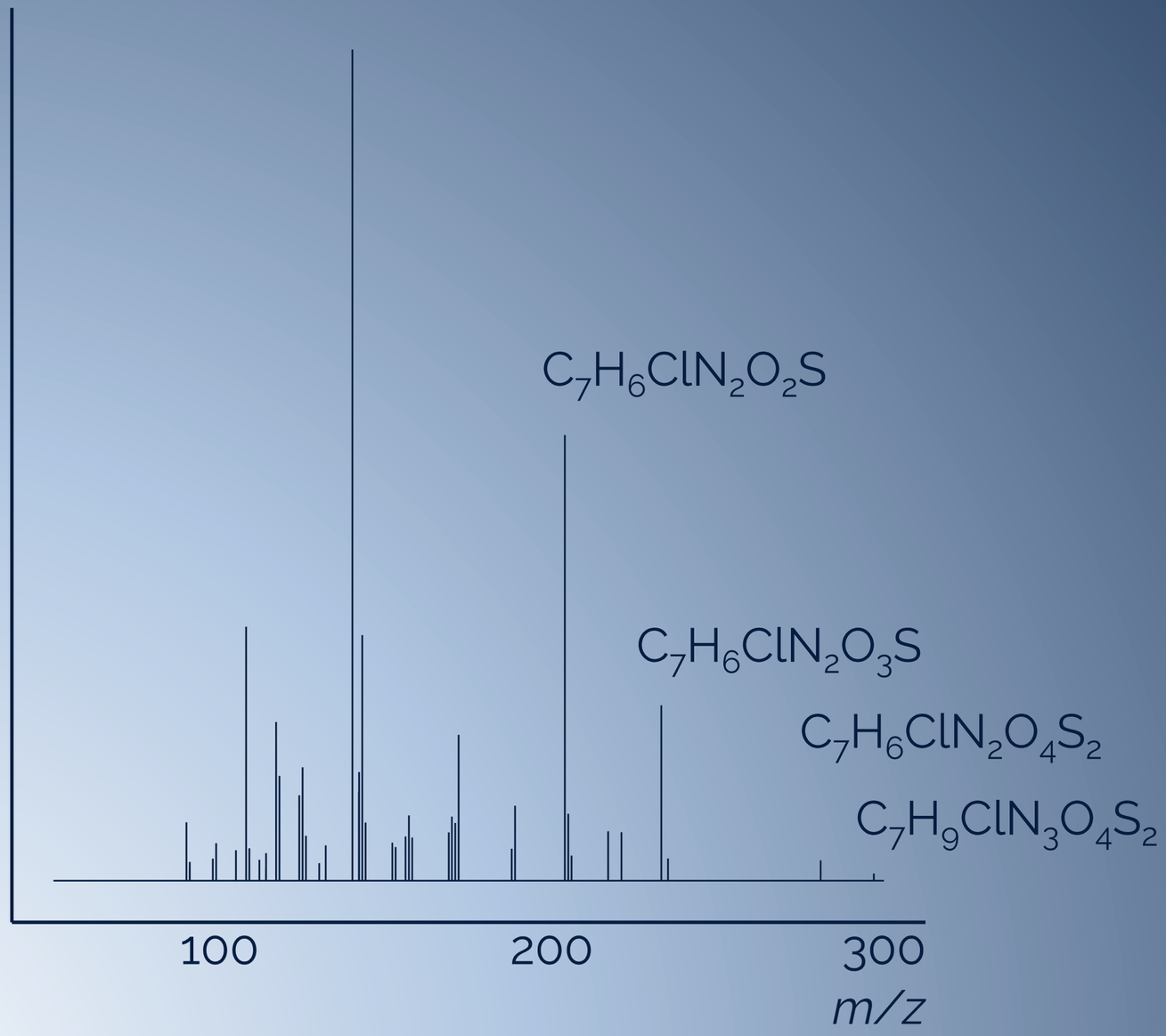
information available

in mass spectra



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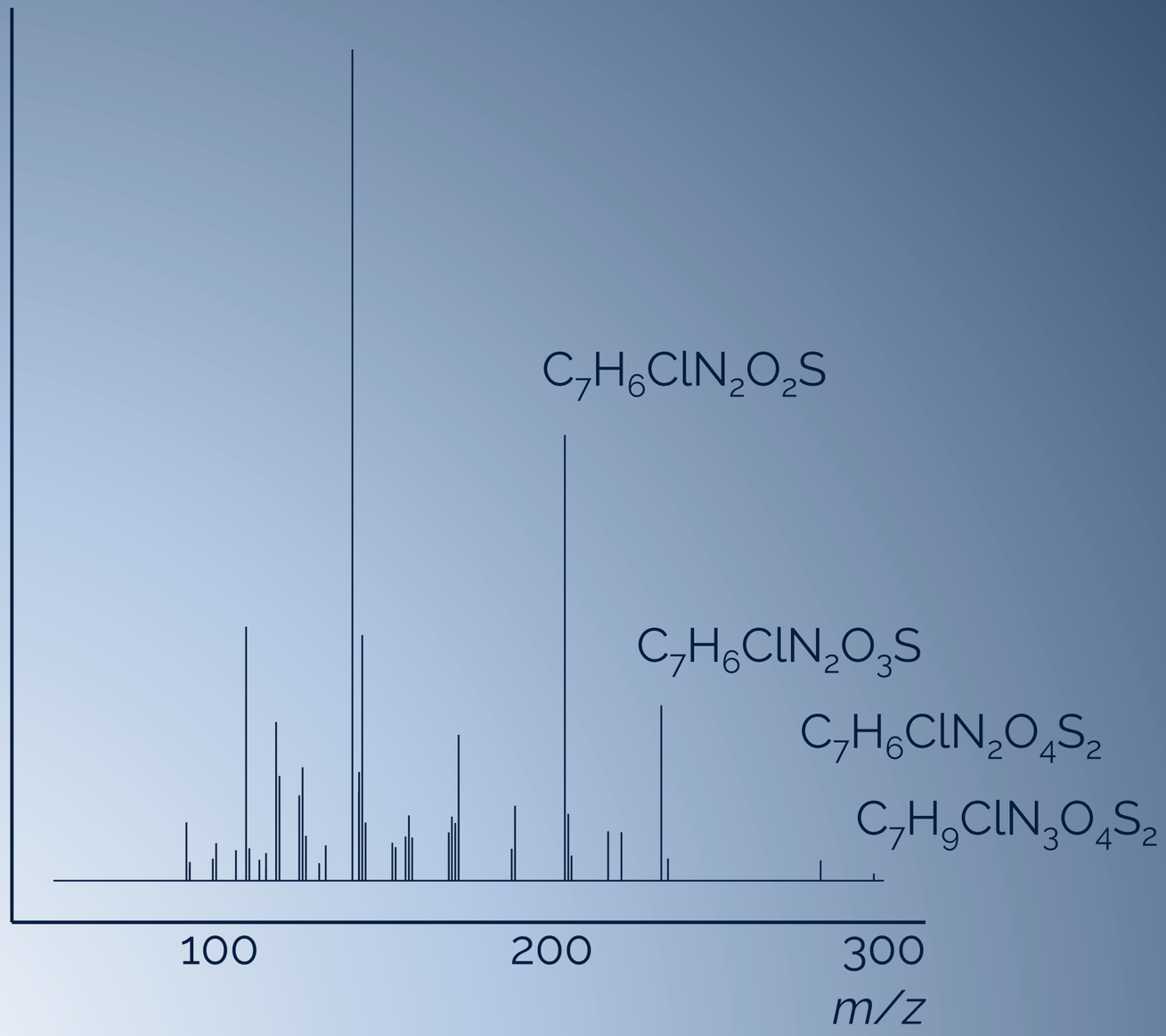

predict toxicity

for unknown chemicals

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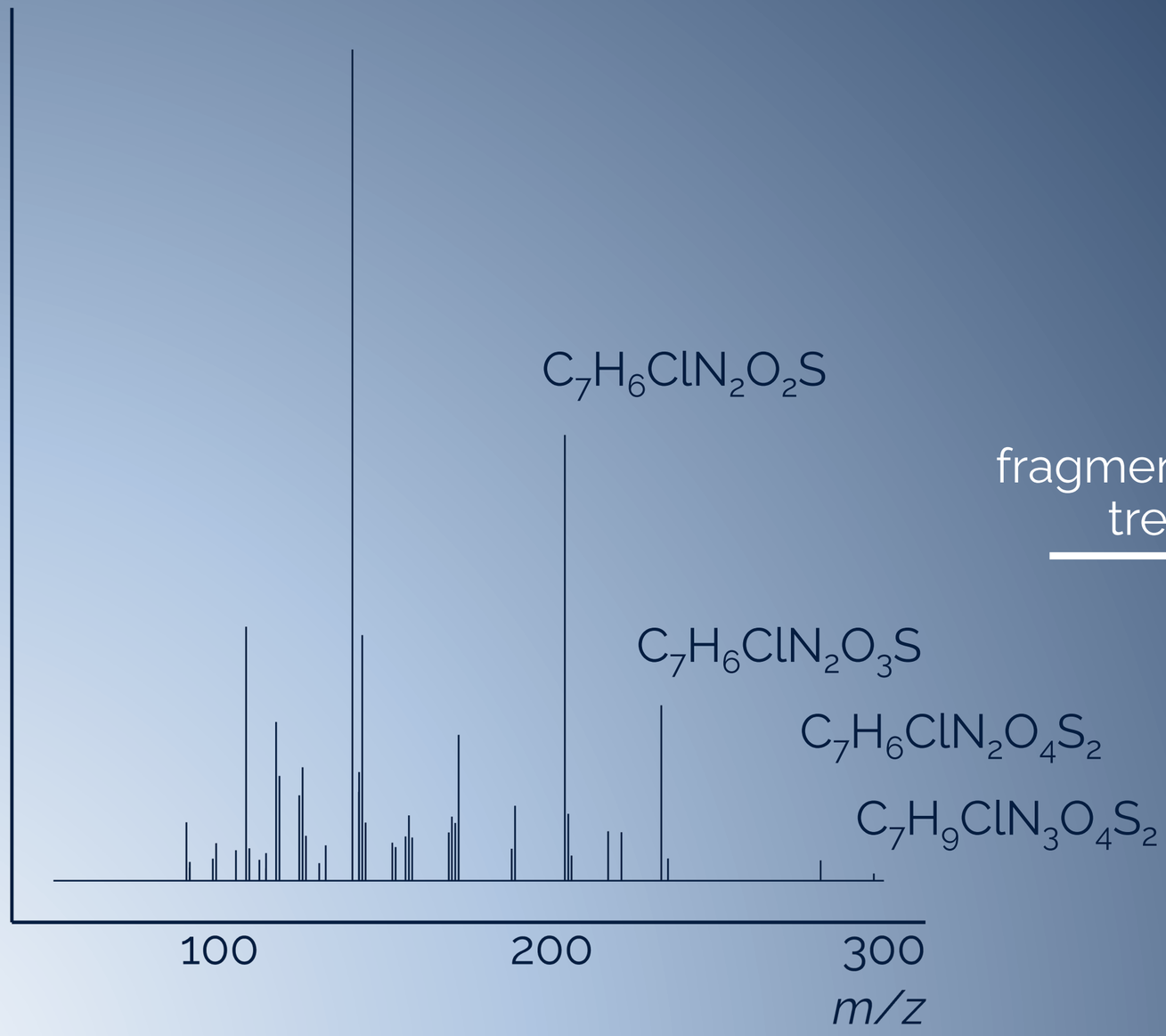
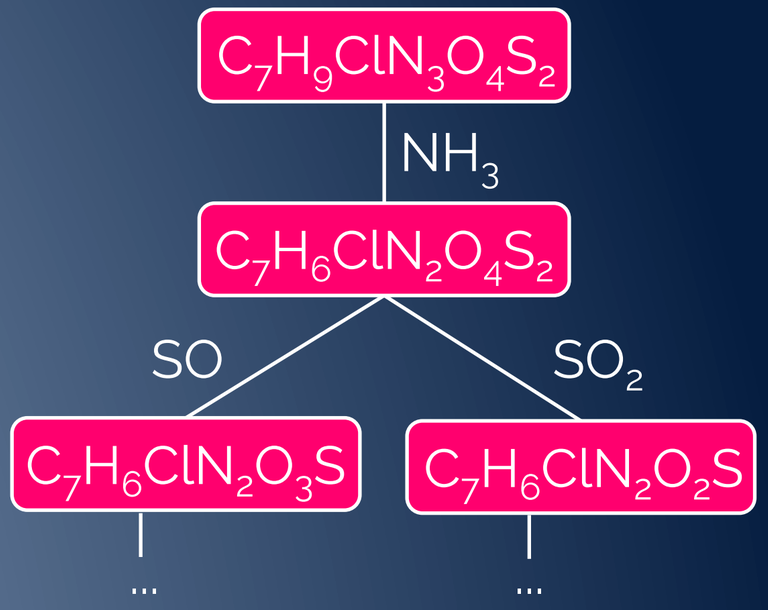
Dührkop et al. SIRIUS + CSI:FingerID



fragmentation tree



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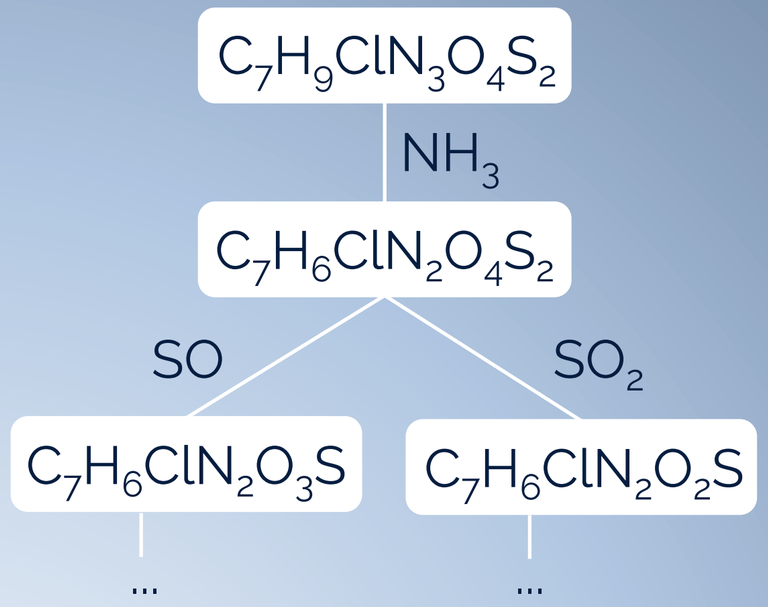
Dührkop et al. SIRIUS + CSI:FingerID



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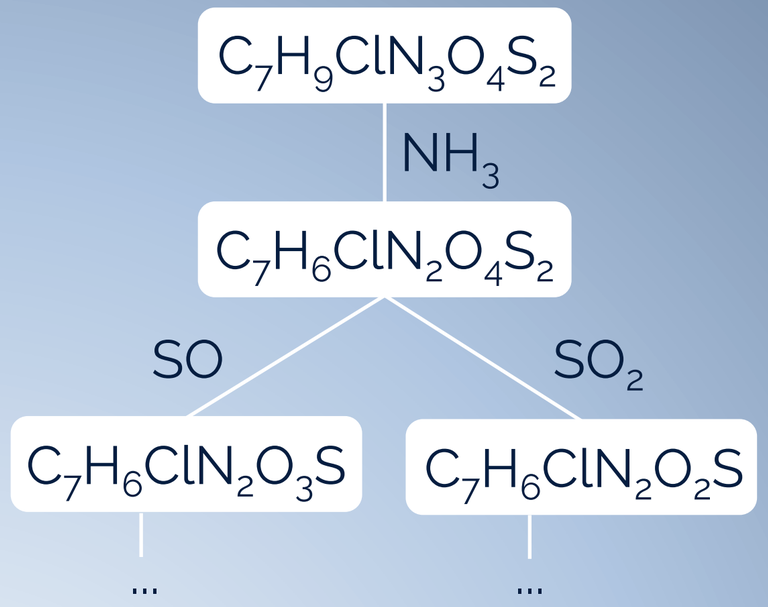
Dührkop et al. SIRIUS + CSI:FingerID



machine learning →

0.08	O-P
0.87	S=O
0.15	C-F
0.93	NH_2
0.99	O

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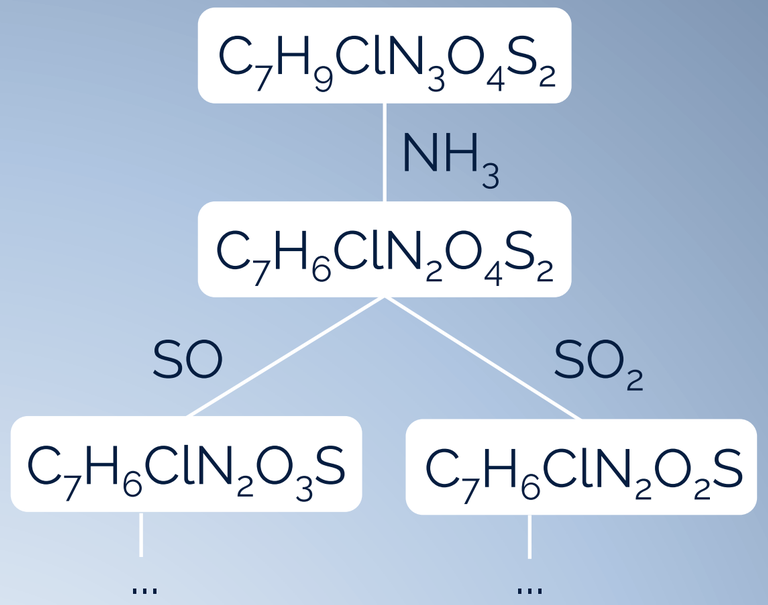

machine learning →

0.08	O-P
0.87	S=O
0.15	C-F
0.93	NH_2
0.99	O

machine learning →

p(AhR active)= 0.83

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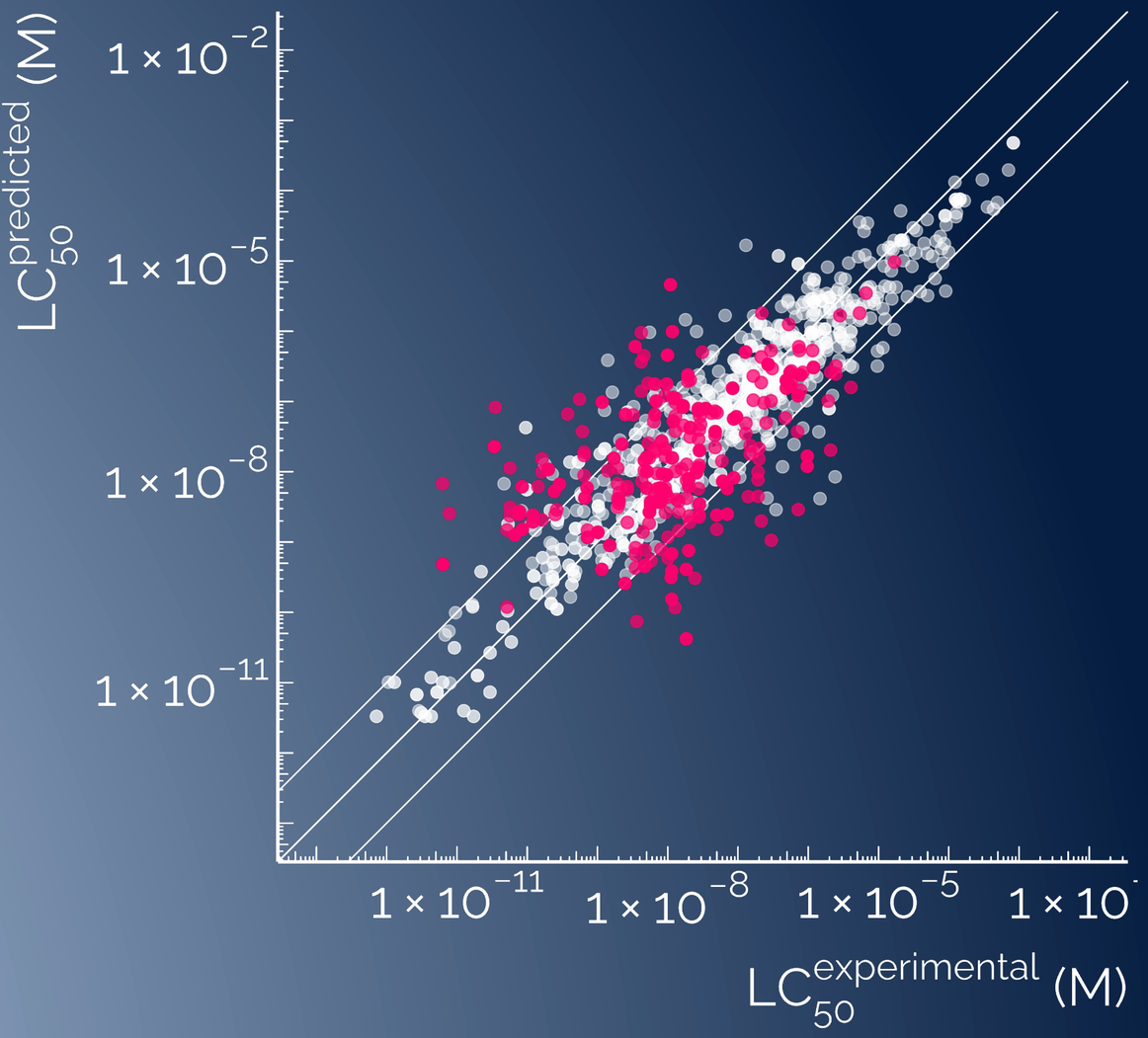
LC_{50} predictions

Peets et al. ES&T 2022

fish LC_{50}



validation on MassBank

$RMSE_{model}$ 0.88 log(M)

$SD_{experimental}$ 0.44 log(M)

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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

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endocrine disruption

true label	
active	non-active

Rahu et al. JCIM 2024
Tox21 endpoints

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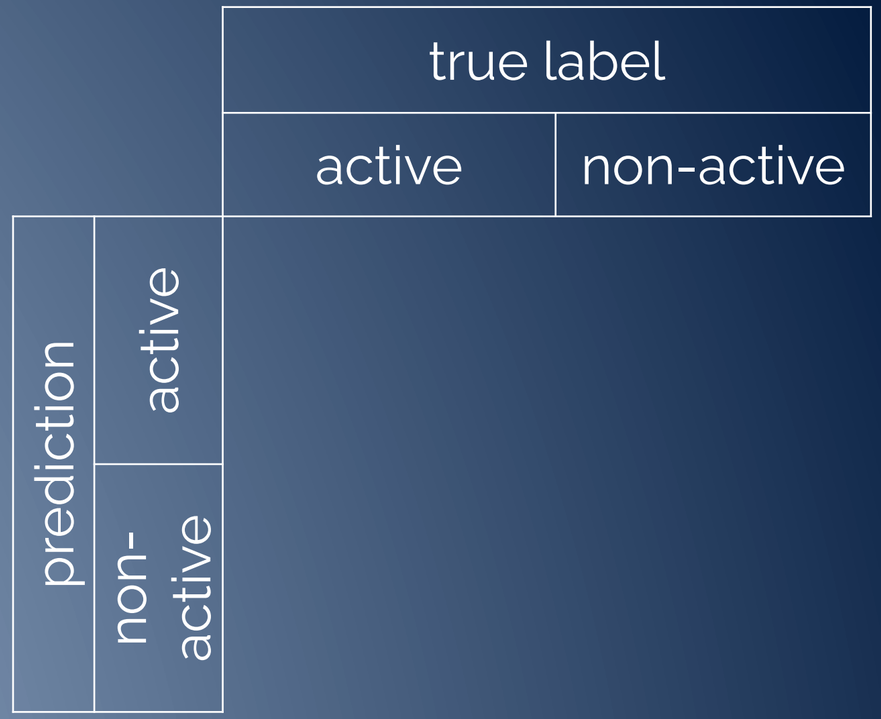
endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints



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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	
	non-active		TN

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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	FP
	non-active		TN

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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	FP
	non-active	FN	TN

which is more dramatic:
FP or FN?

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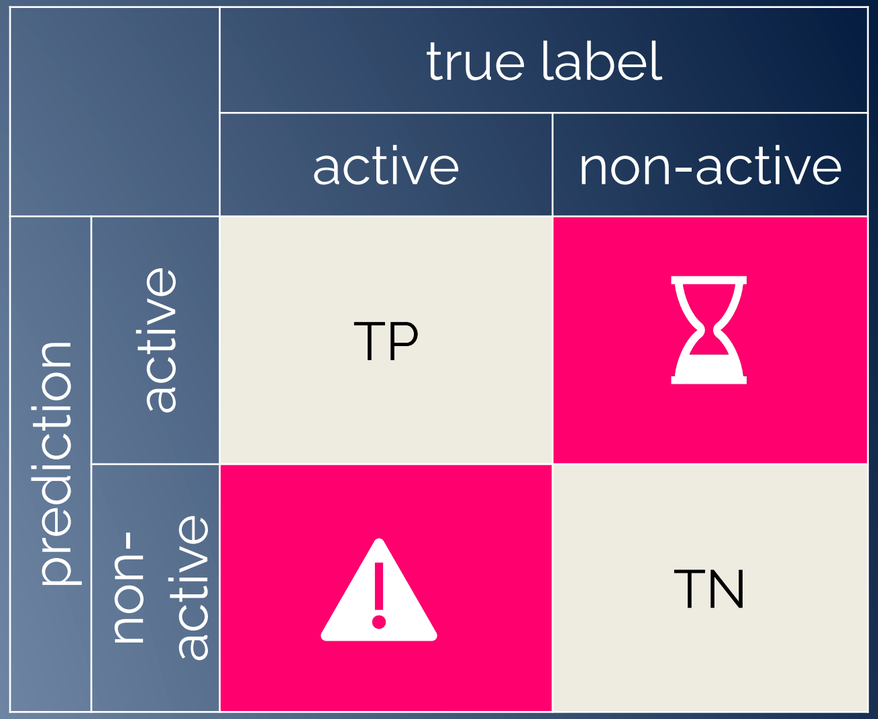
endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints



which is more dramatic:
FP or FN?

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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

		true label	
		active	non-active
prediction	active	TP	FP
	non-active	FN	TN

FPR @ TPR = 0.9

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endocrine disruption

Rahu et al. JCIM 2024
Tox21 endpoints

bioassay	FPR
sr.mmp	25.1%
sr.p53	25.4%
nr.ahr	41.8%
...	...
nr.ar	82.4%
nr.er	85.0%

MassBank & MoNA
748 chemicals

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case study: interlaboratory comparison

water analysis

thousands of chemicals detected in the environment

BioCell ANALYTICA

Table 1. The following BEQ values were measured in the current samples:

	Nrf2 activity	AntiÿAR activity	AR activity	ER activity	AhR activity
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detection limit*			6.93	50.0	0.156

Table 2. Genotoxicity.

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Reference to sample 1	No
Sample 1	No
Reference to sample 2	No
Sample 2	Could not be determined*

*Due to extensive cytotoxicity, despite repeated analyses, it could not be determined whether sample 2 was genotoxic or not. The sample was tested down to the concentration REF 12.5, but even then was too cytotoxic to be able to determine if it was genotoxic.

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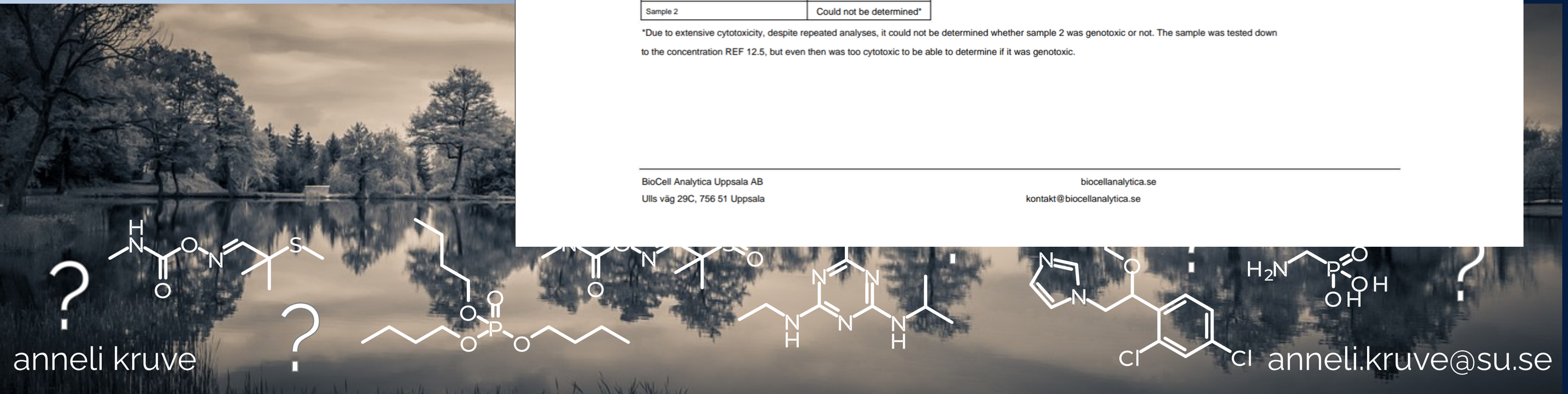

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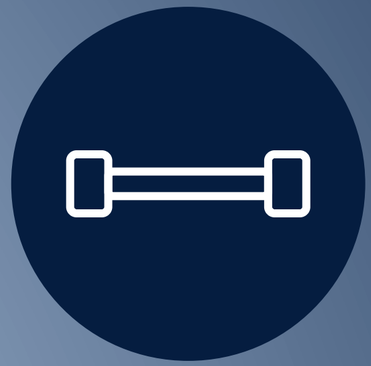
interlaboratory comparison

Sandberg, Rahu, in preparation

SAMPLES

spiked water samples



ANALYSIS

HRMS, etc. characterization

DATA PROCESSING

AhR activity

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results

4700

LC/HRMS features detected

238

features with MS^2 spectra

55

features predicted active

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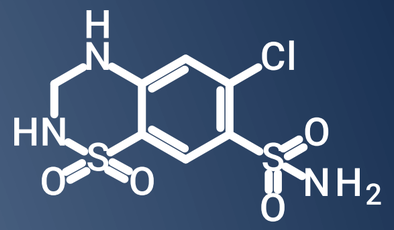
AhR active



hydrochlorothiazide

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alternative approaches

Kreutzer et al. in preparation
Tox21 endpoints

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molecular networks

Kreutzer et al. in preparation
Tox21 endpoints

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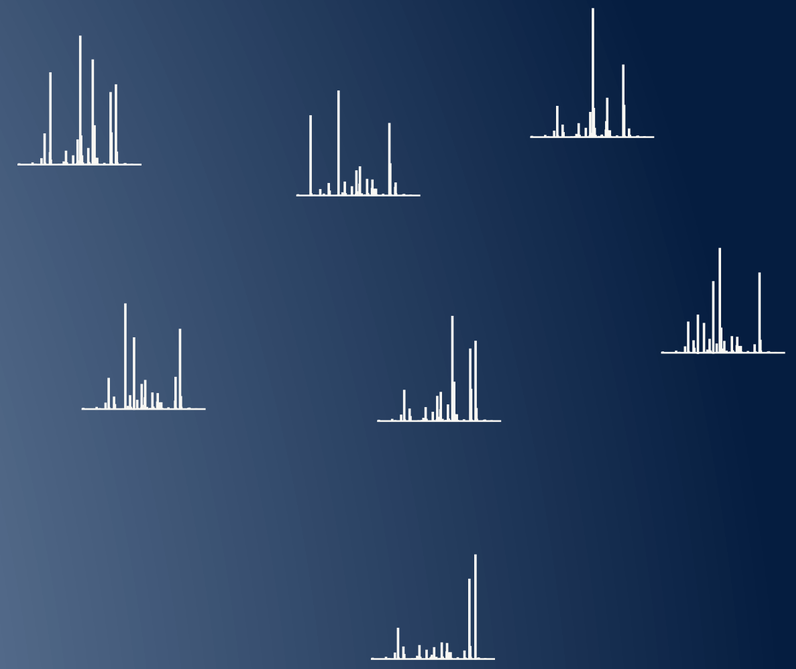
molecular networks

Kreutzer et al. in preparation
Tox21 endpoints



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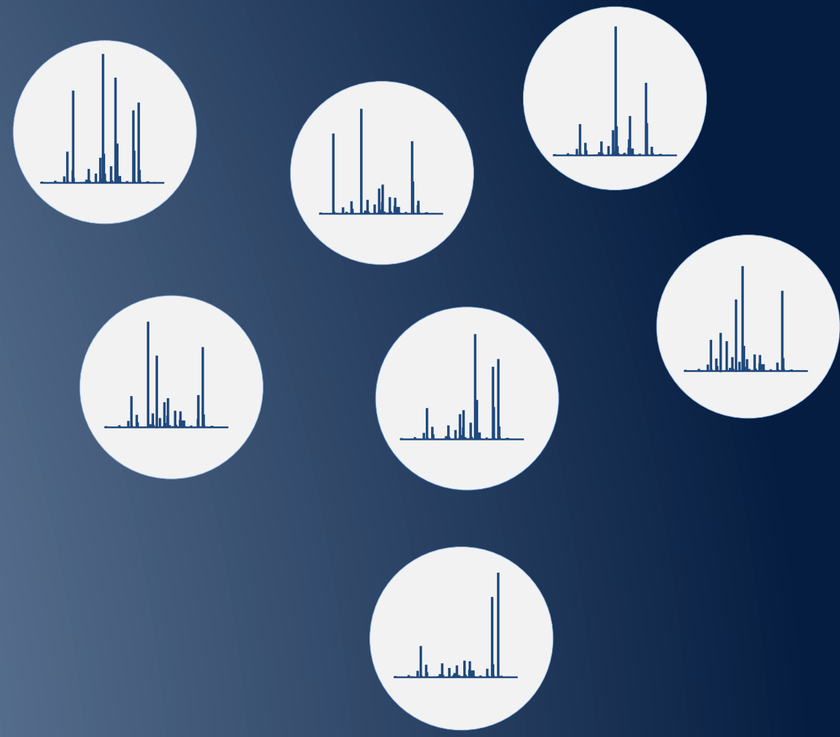
molecular networks

Kreutzer et al. in preparation
Tox21 endpoints



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molecular networks

Kreutzer et al. in preparation
Tox21 endpoints

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molecular networks

Kreutzer et al. in preparation
Tox21 endpoints

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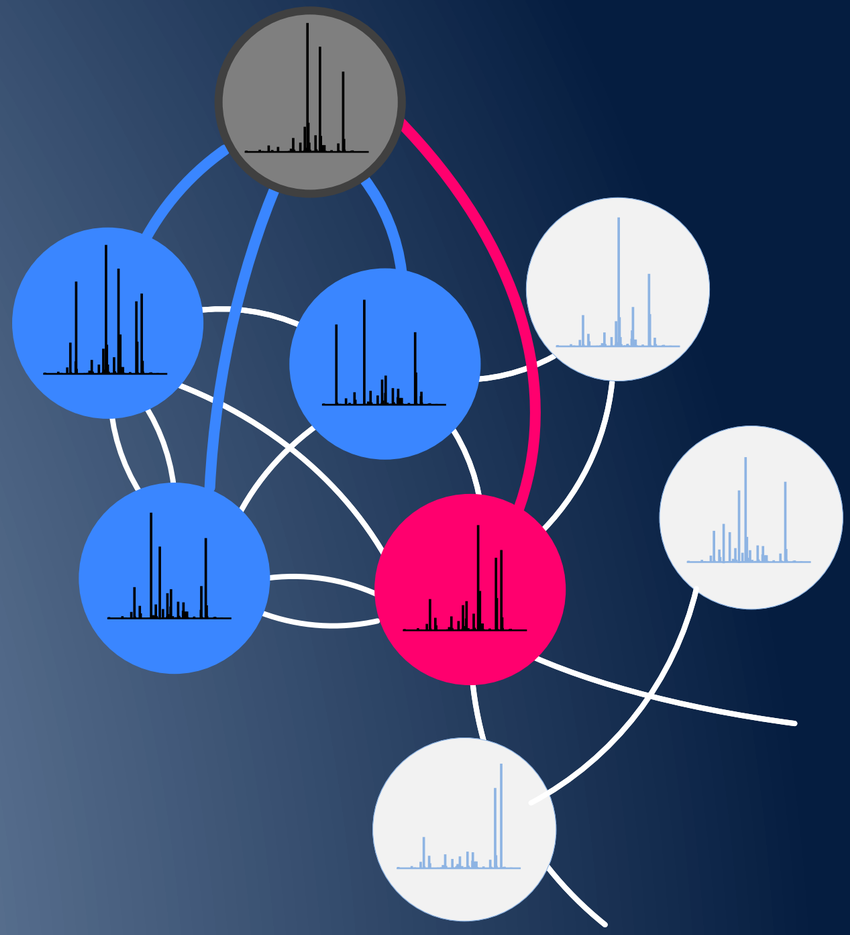
molecular networks

Kreutzer et al. in preparation
Tox21 endpoints



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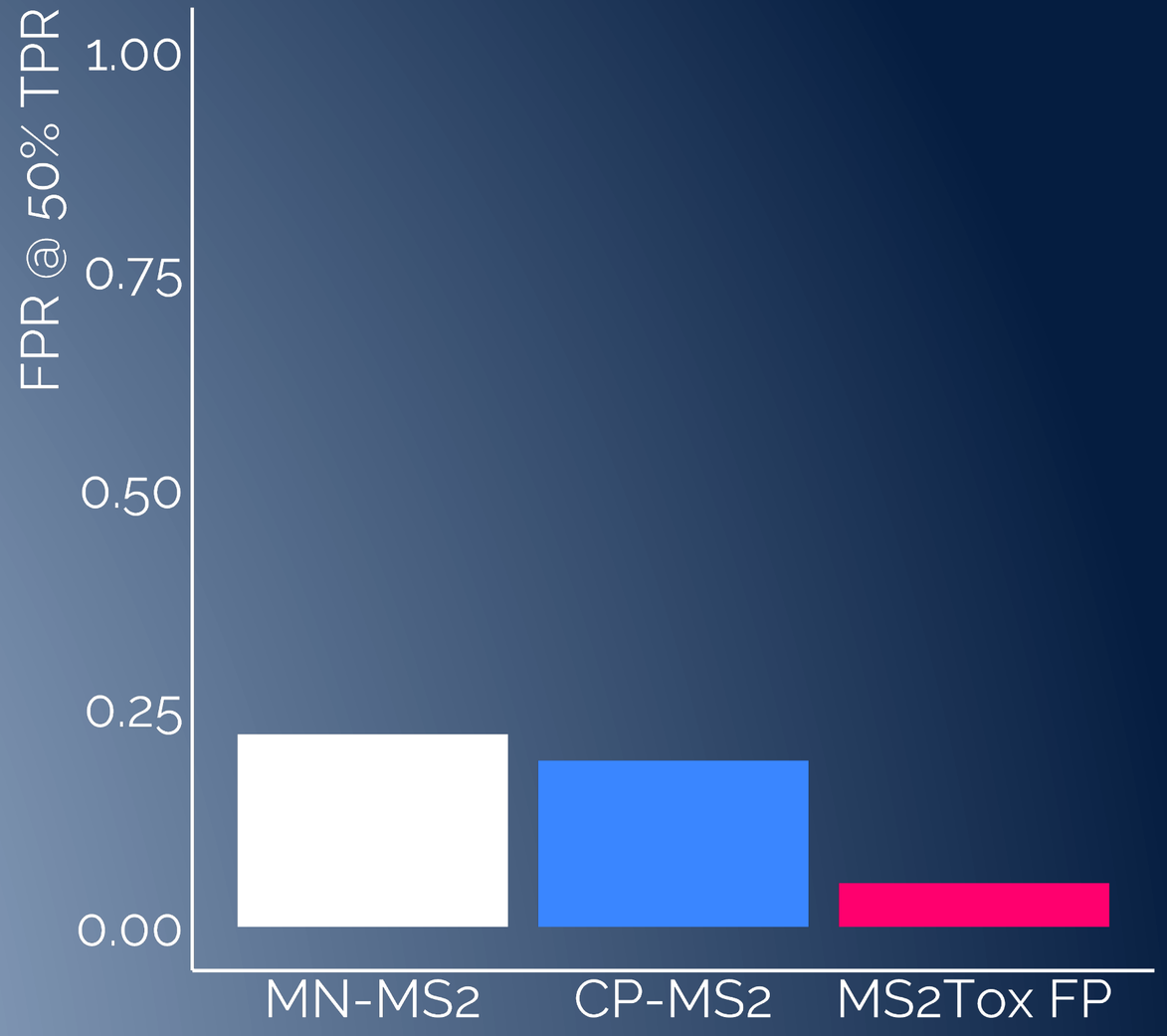
AhR activity predictions

Kreutzer et al. in preparation
Tox21 endpoints



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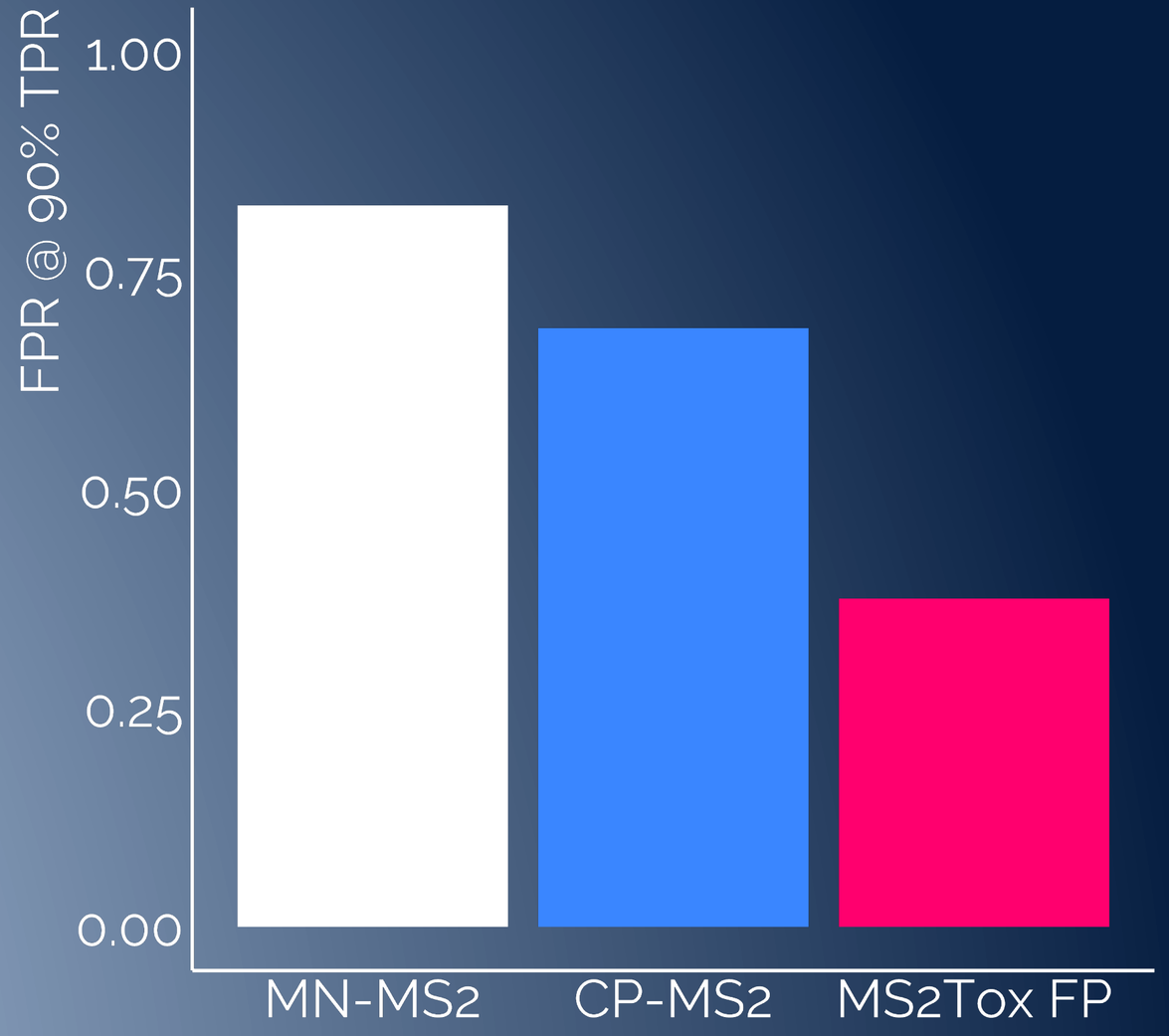
AhR activity predictions

Kreutzer et al. in preparation
Tox21 endpoints



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application:
wastewater analyses

LC/HRMS features

986 features

LC/HRMS features

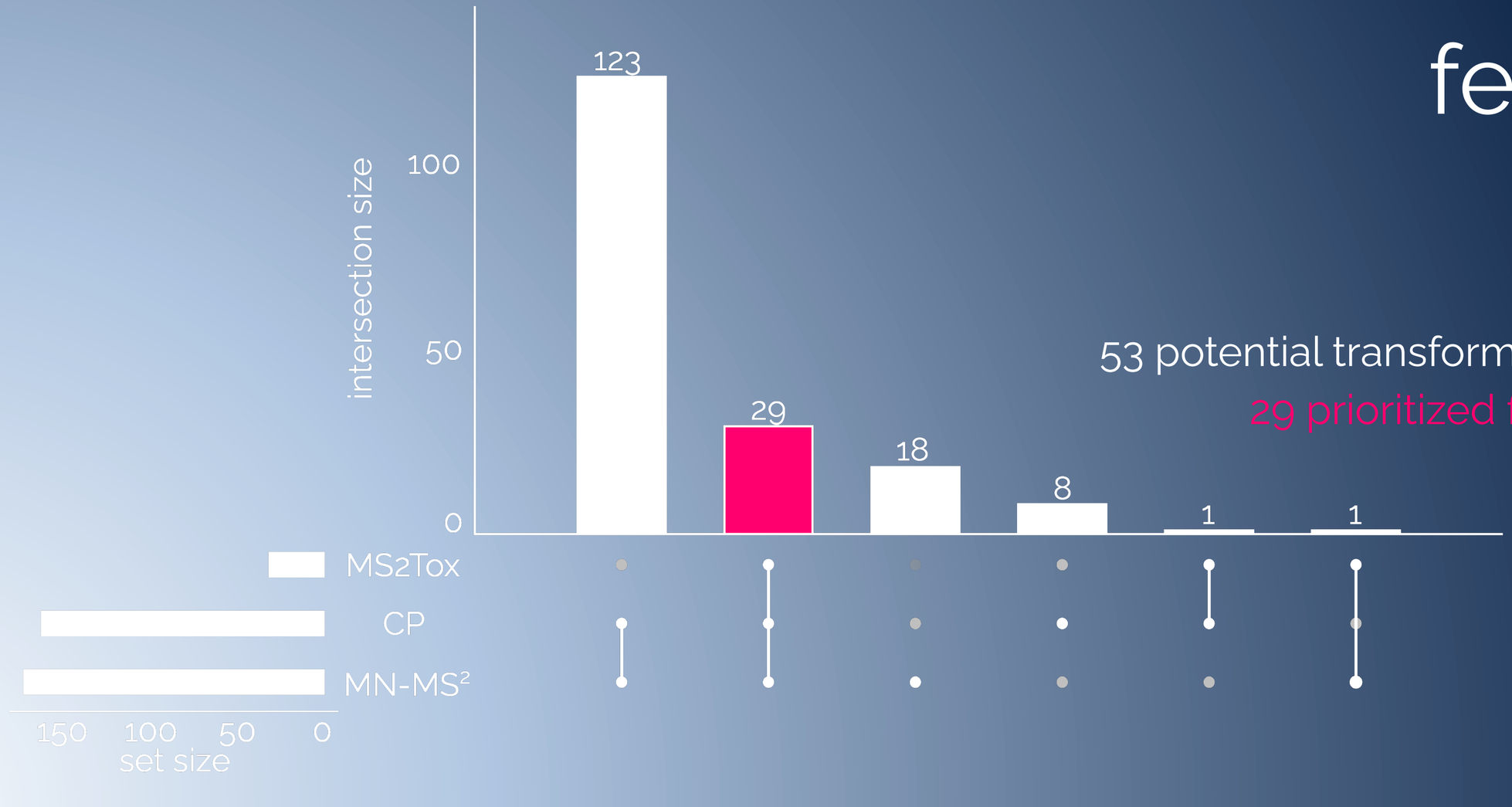
986 features
136 persistent

LC/HRMS features

986 features
136 persistent
53 potential transformation products

LC/HRMS features

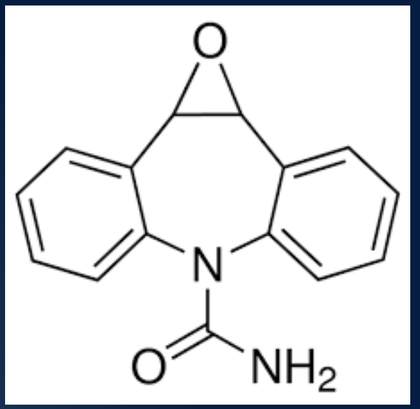
986 features
136 persistent
53 potential transformation products
29 prioritized for AhR activity

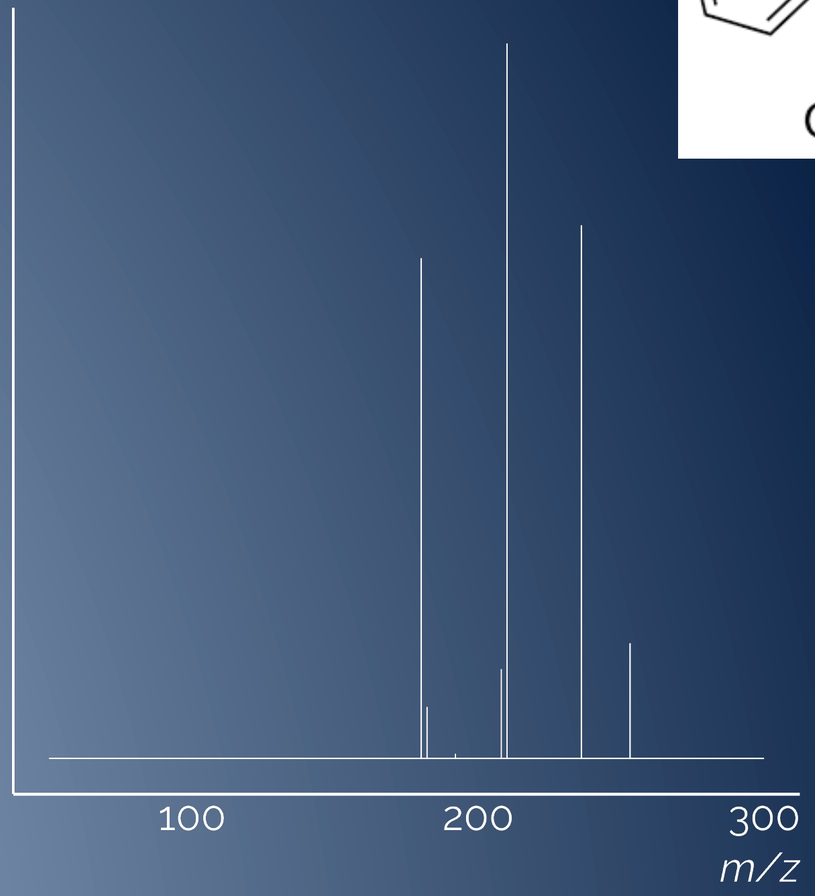


carbamazepine-10,11-epoxide

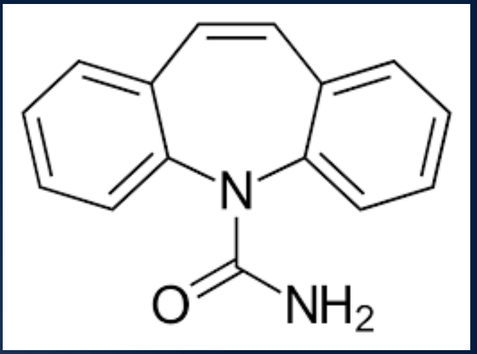
RT 8.01 min *m/z* = 252.090

confirmed with analytical standard





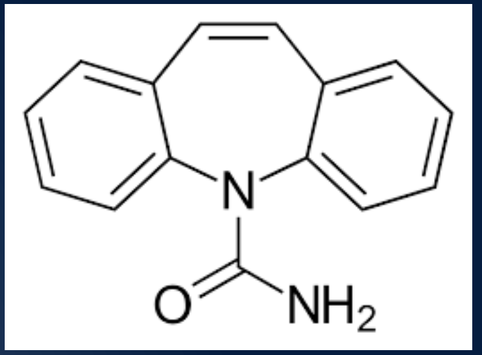
carbamazepine

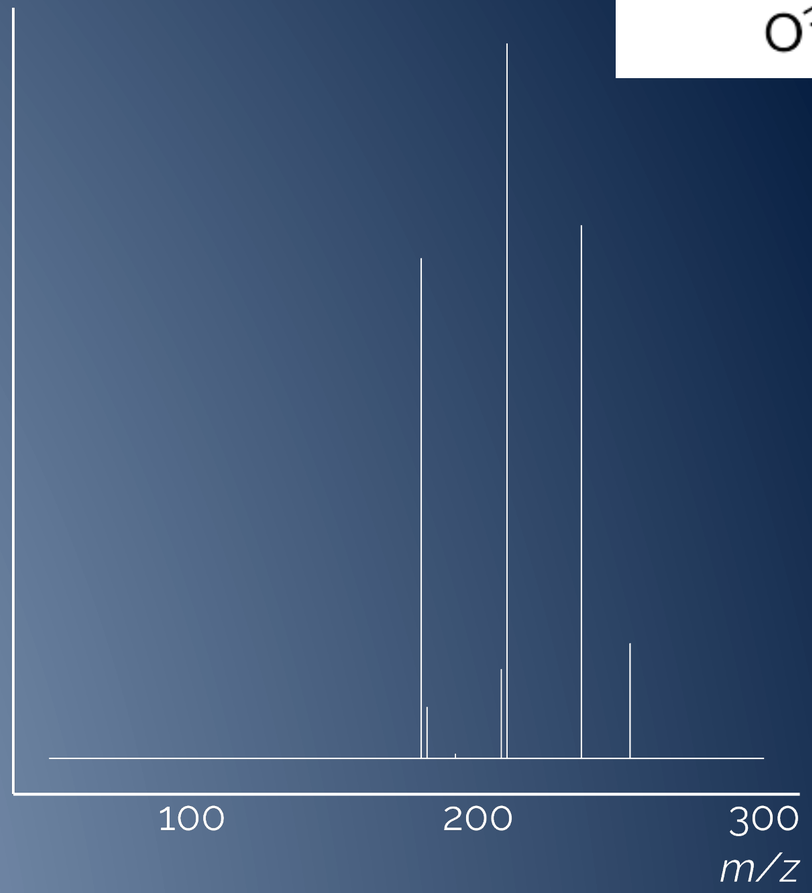


carbamazepine

RT 9.42 min *m/z* = 236.095

confirmed with analytical standard



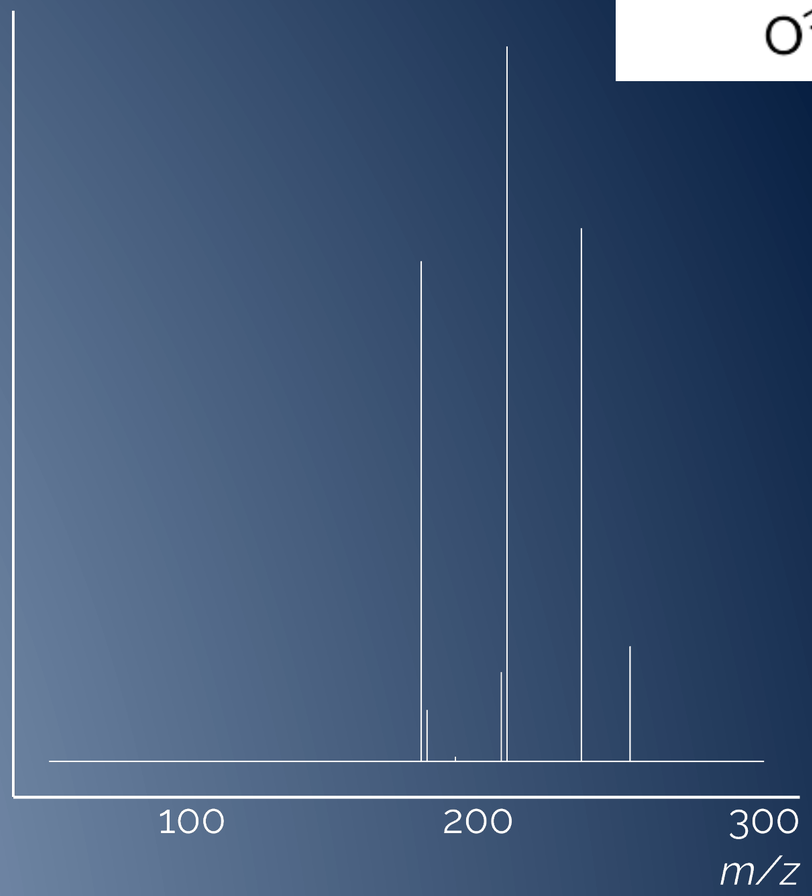


carbamazepine

RT 9.42 min *m/z* = 236.095

confirmed with analytical standard

prioritized by MS2Tox & MN-MS2

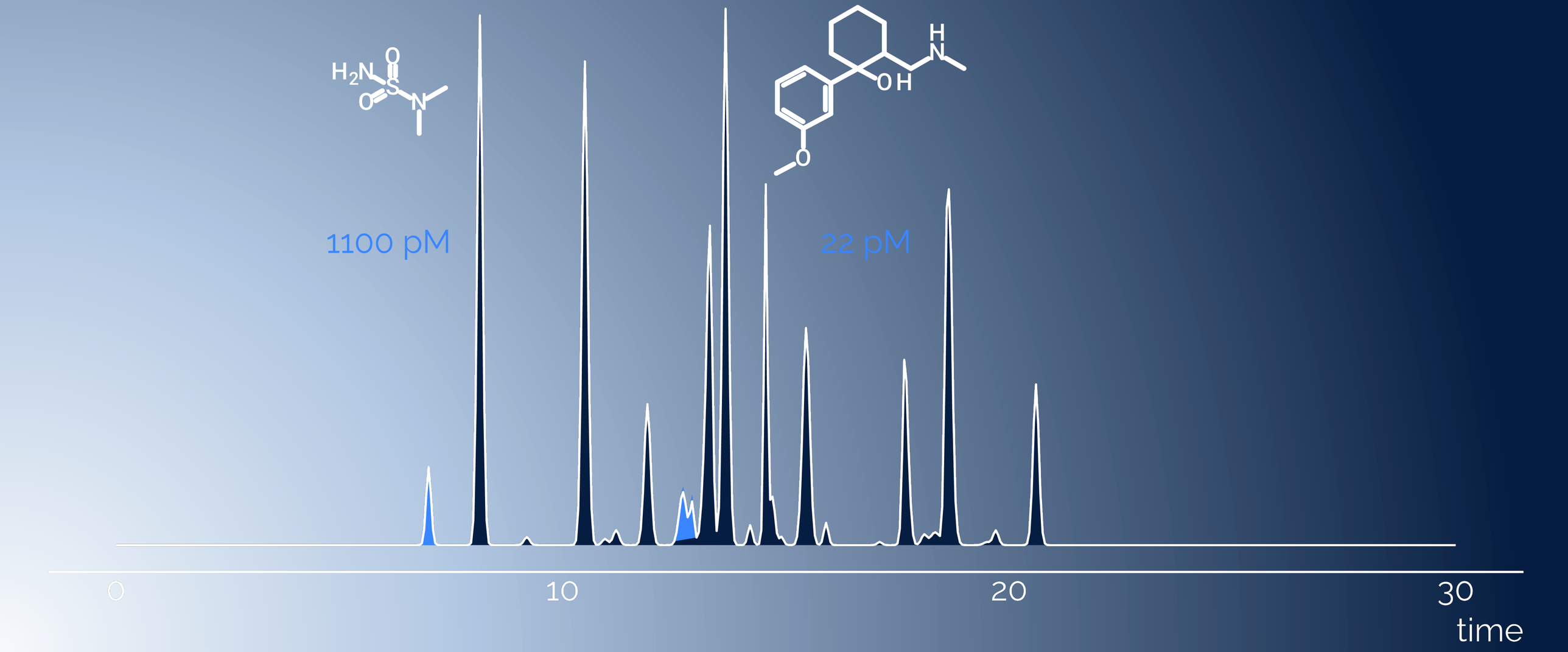


quantification

of detected chemicals

quantification in ESI/HRMS

Malm et al. Molecules 2021



electrospray

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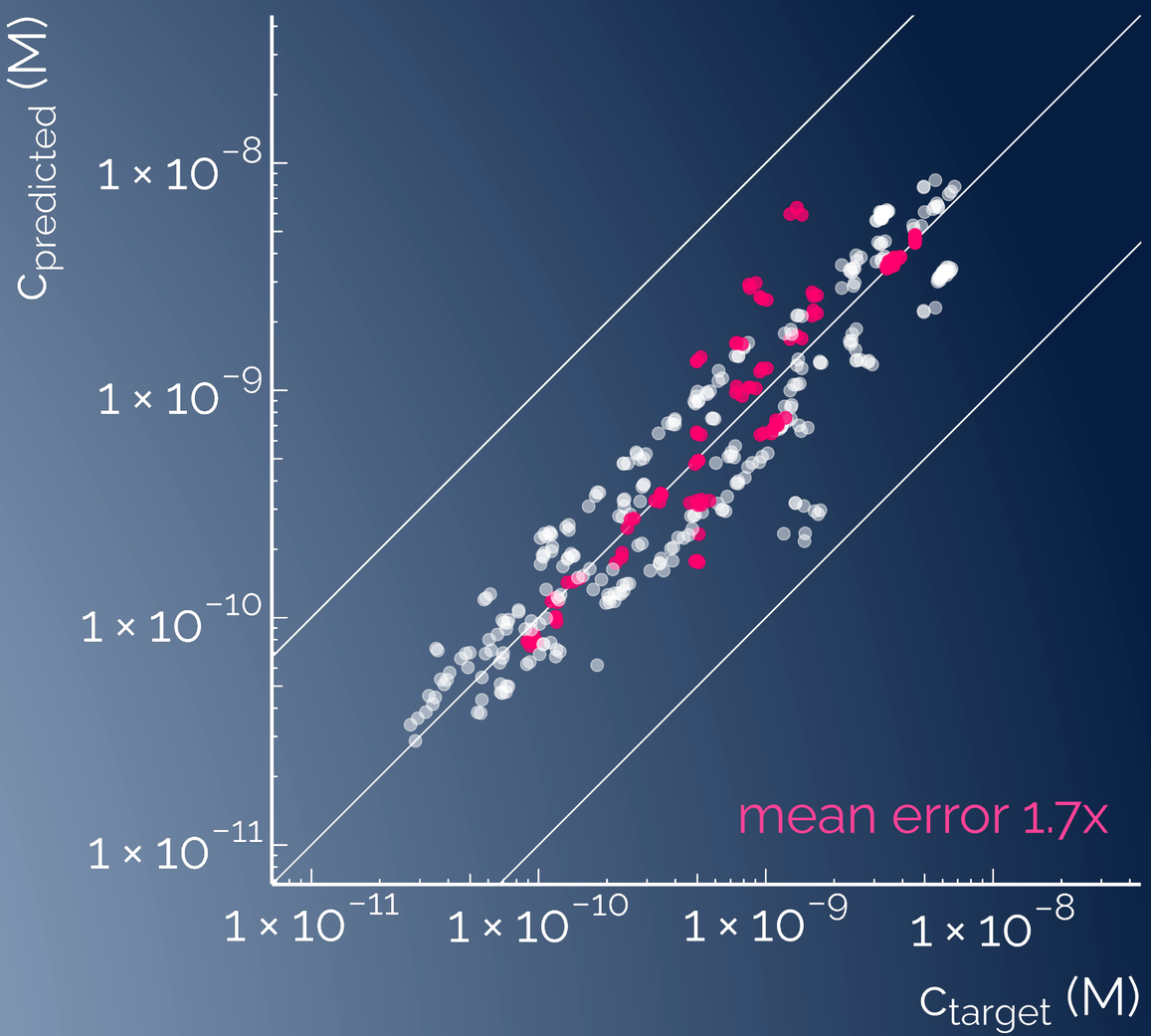
quantification

from structure

Been et al. Water Research 2021



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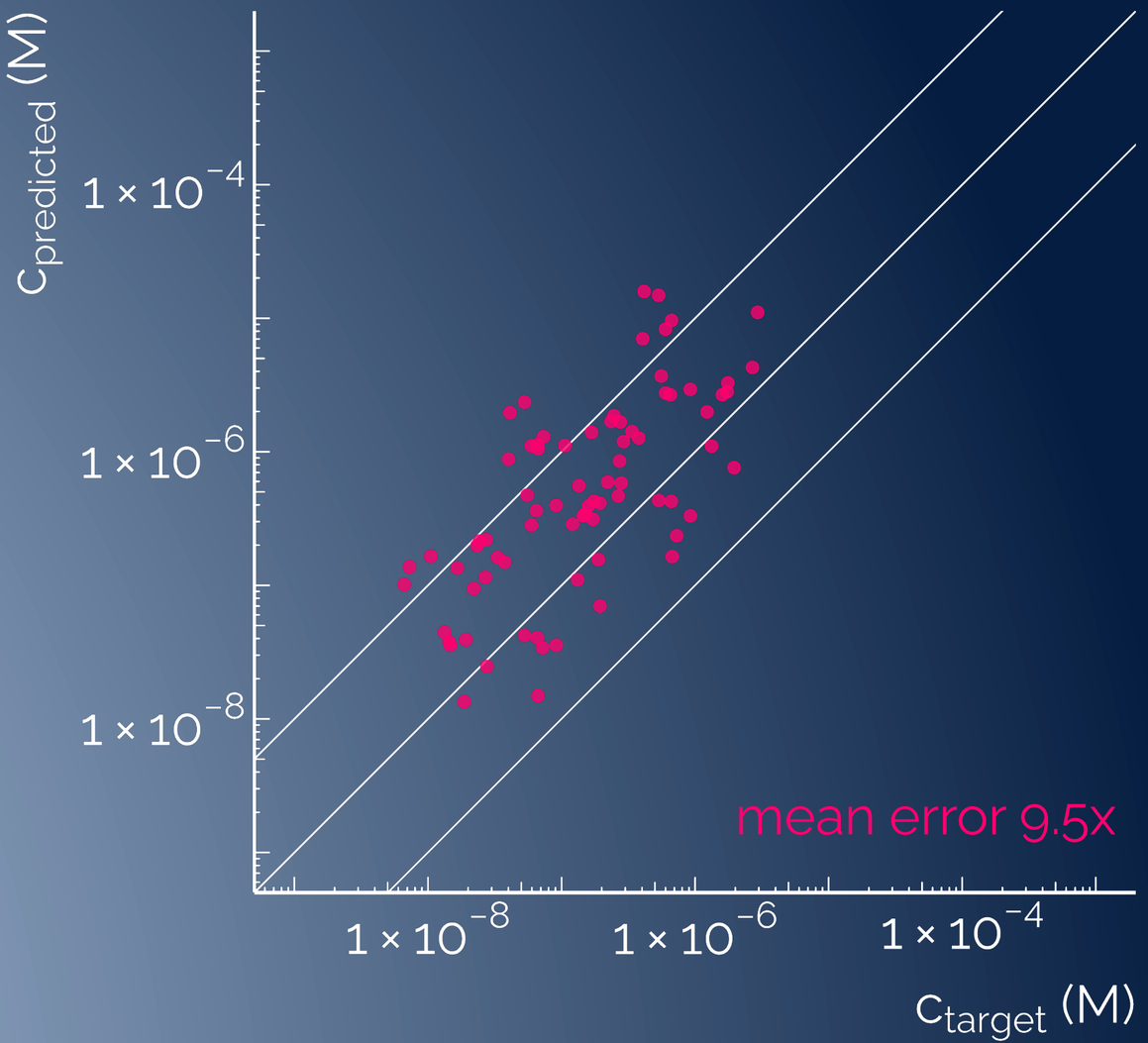
quantification

from MS² spectra

Sepman et al. Anal Chem 2023



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risk

based prioritization

prioritization of chemicals

TOXICITY

ecotoxicity and endocrine disruptors

CONCENTRATION

exposure to potentially toxic chemicals

RISK

$$\text{PriorityScore} = \frac{C_{\text{predicted}}}{AC_{50}^{\text{5th percentile}}}$$

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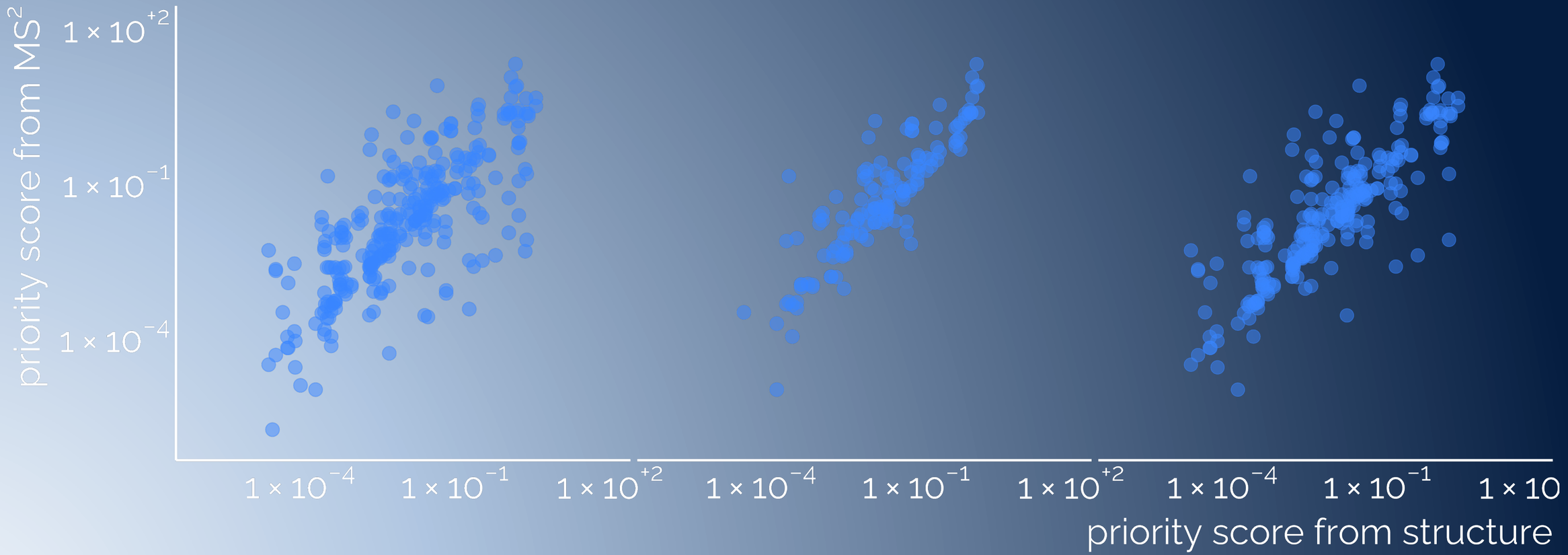
wastewater analysis

Sepman, in preparation



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how to ...

PRIORITIZE

risk

IDENTIFY

structure

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how to ...

PRIORITIZE

risk

IDENTIFY

structure

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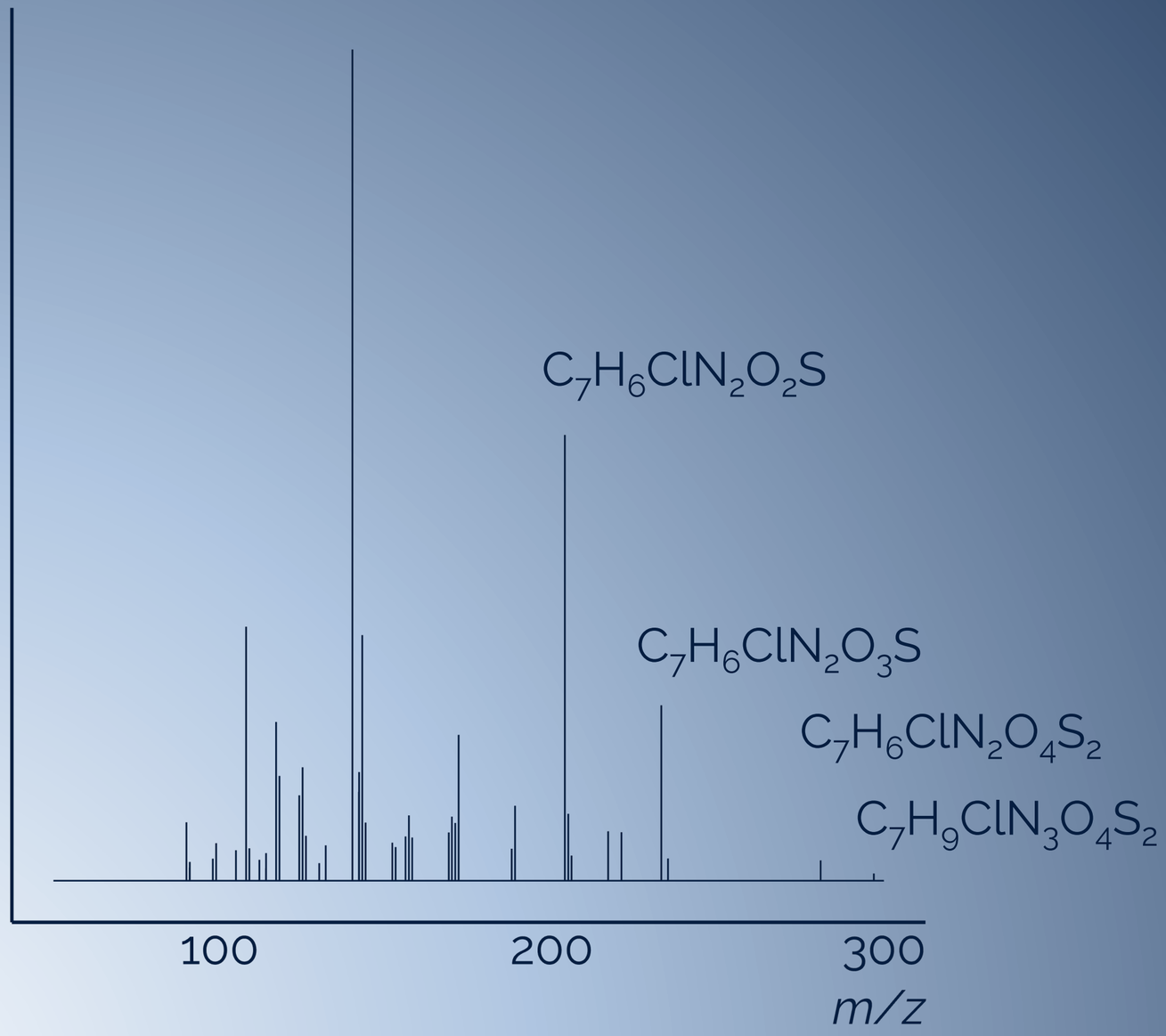
structural identification

from MS^2 spectra



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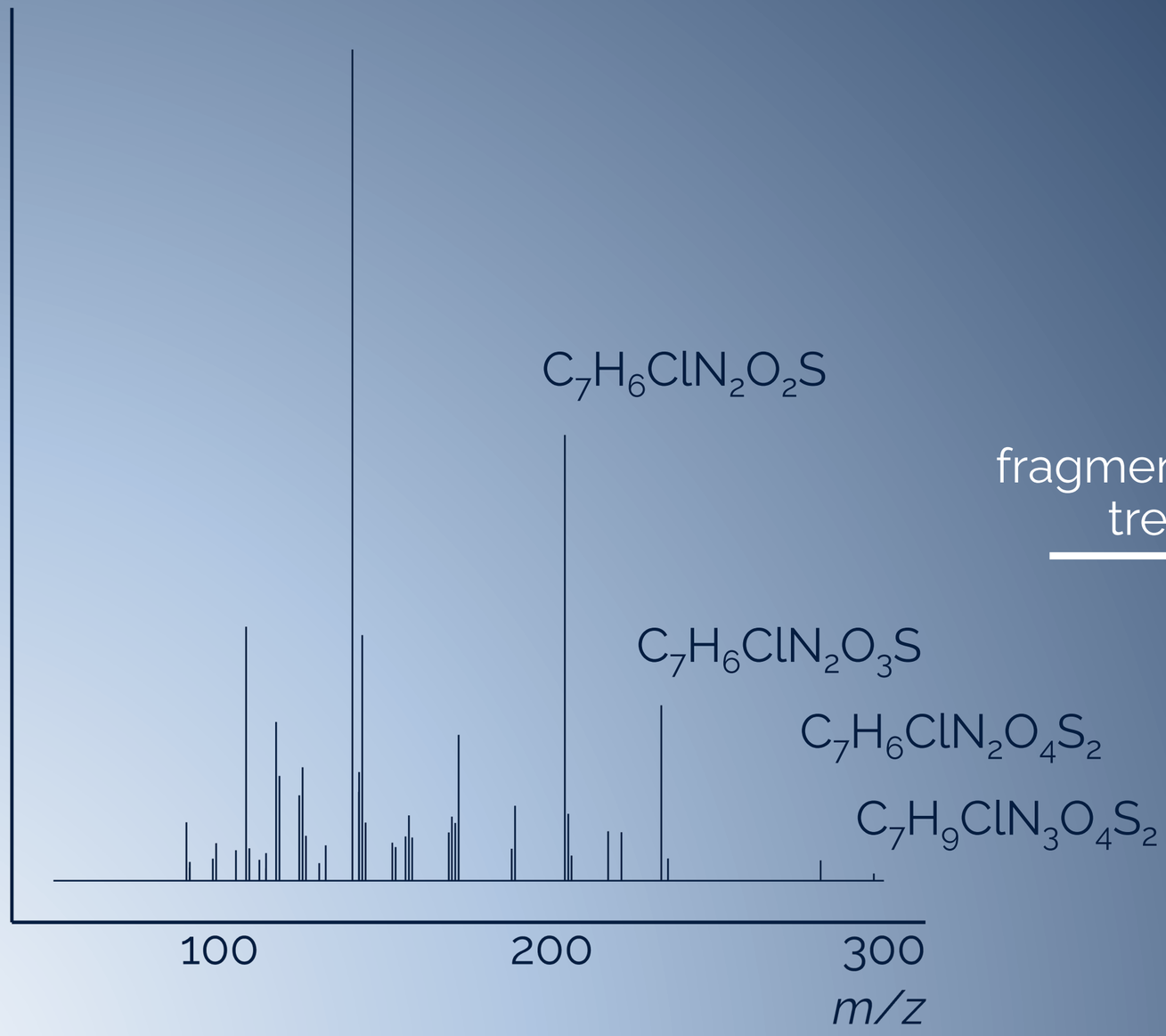
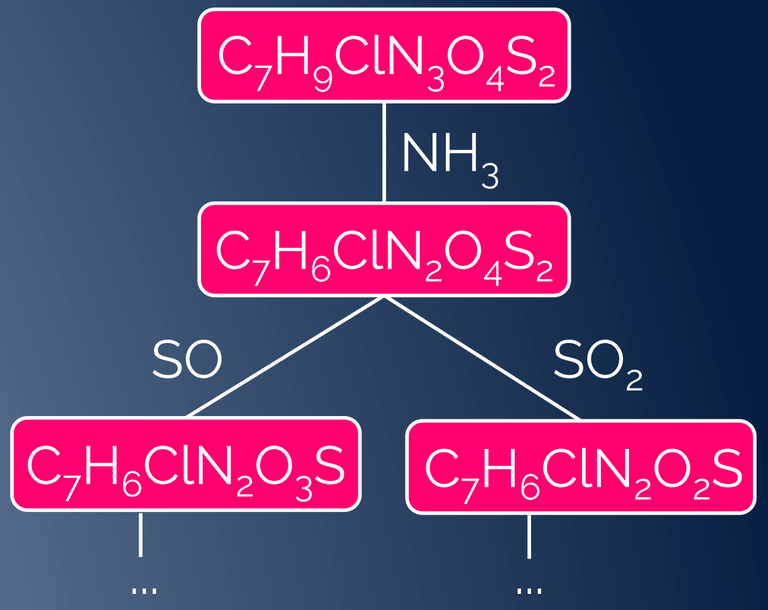
Dührkop et al. SIRIUS + CSI:FingerID



fragmentation tree



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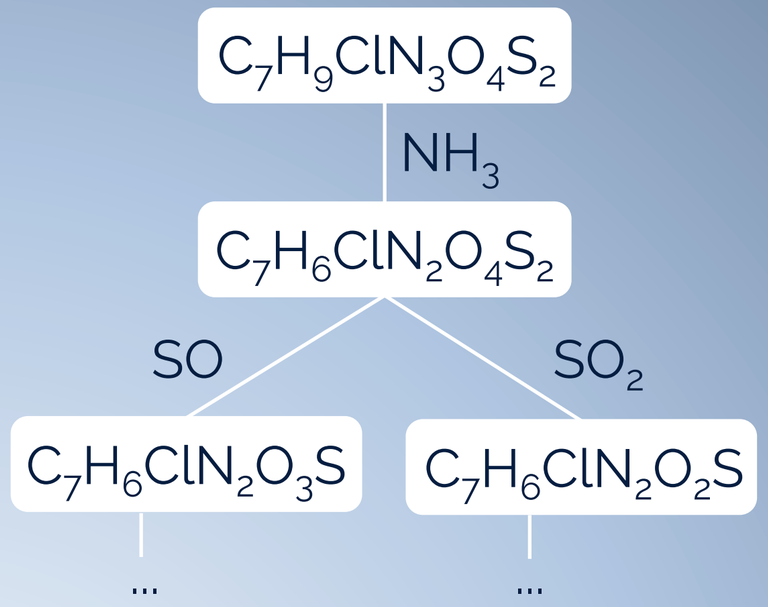

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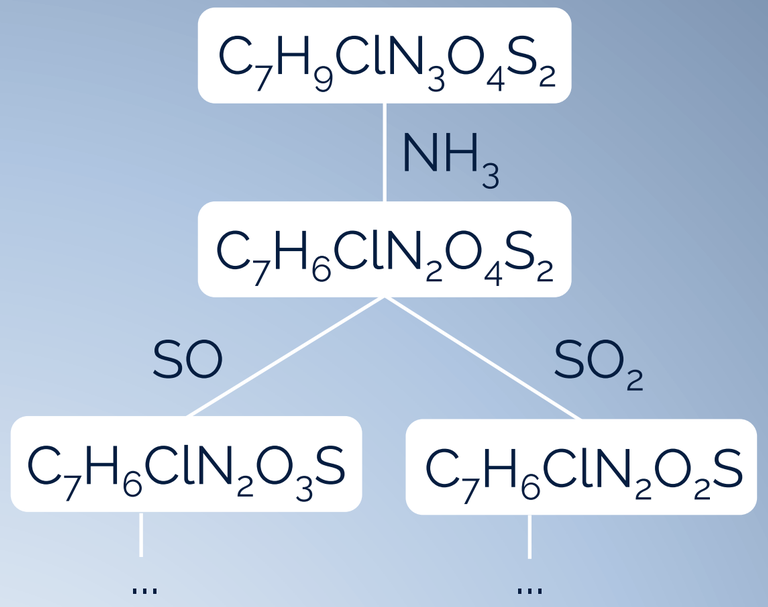
Dührkop et al. SIRIUS + CSI:FingerID



machine learning →

0.08	O-P
0.87	S=O
0.15	C-F
0.93	NH_2
0.99	O

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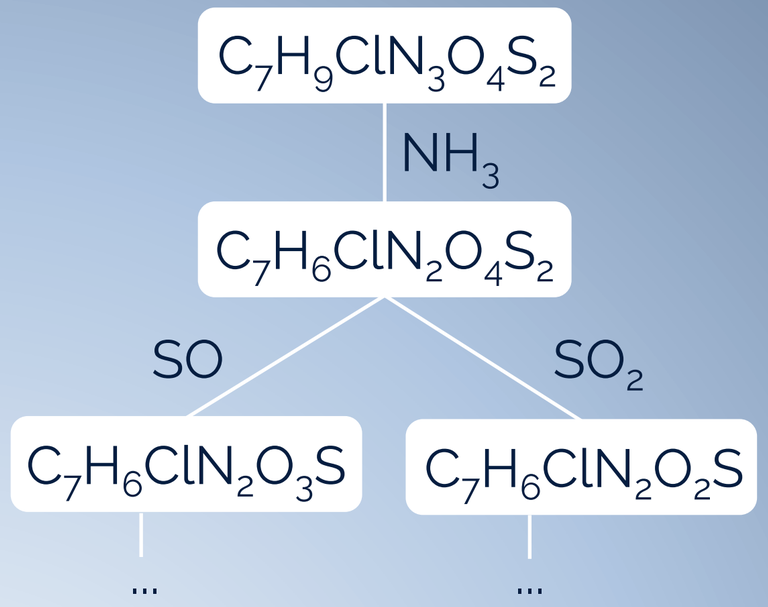
Dührkop et al. SIRIUS + CSI:FingerID



machine learning →

0.08	O-P
0.87	S=O
0.15	C-F
0.93	NH_2
0.99	O

structure →

database matching

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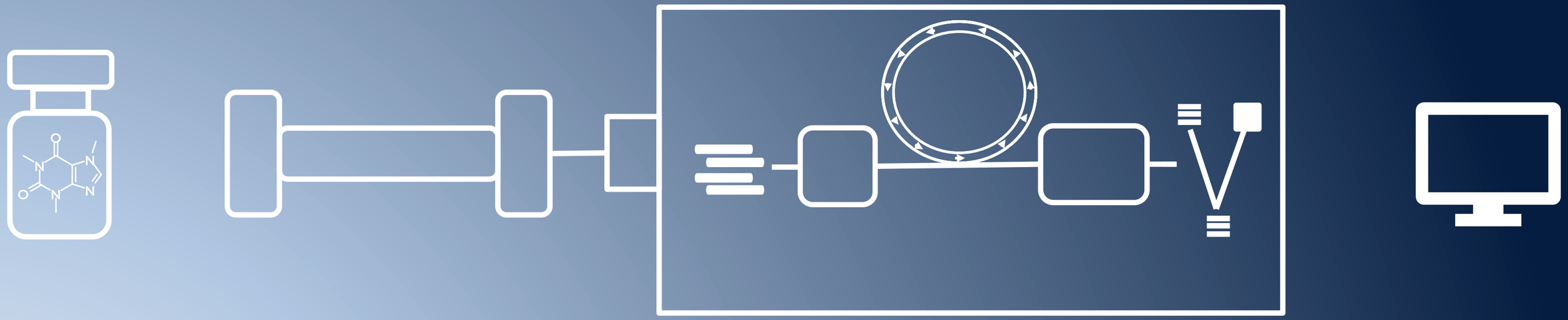
orthogonal separation

Akhlaqi et al. Anal Bioanal Chem 2023



chromatography ion mobility MS^2

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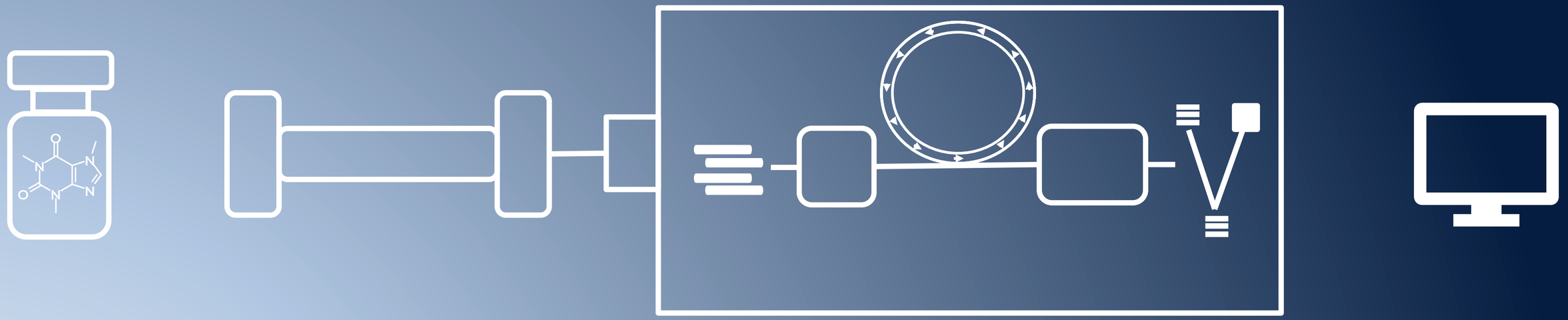
orthogonal separation

Akhlaqi et al. Anal Bioanal Chem 2023



14
isomeric
chemicals

C_{18} RP

Cyclic IMS
&
MS^2 with ToF

SIRIUS+
CSI:FingerID
&
CFM-ID

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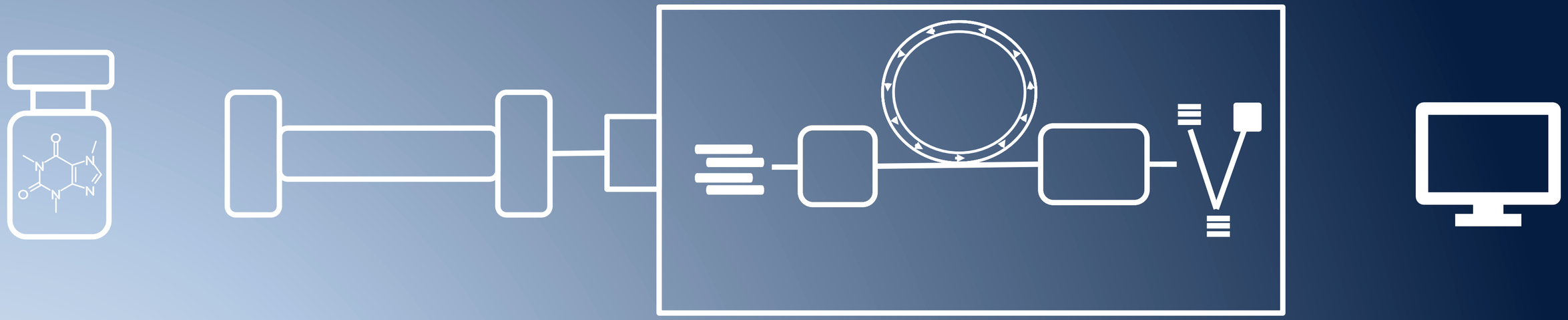
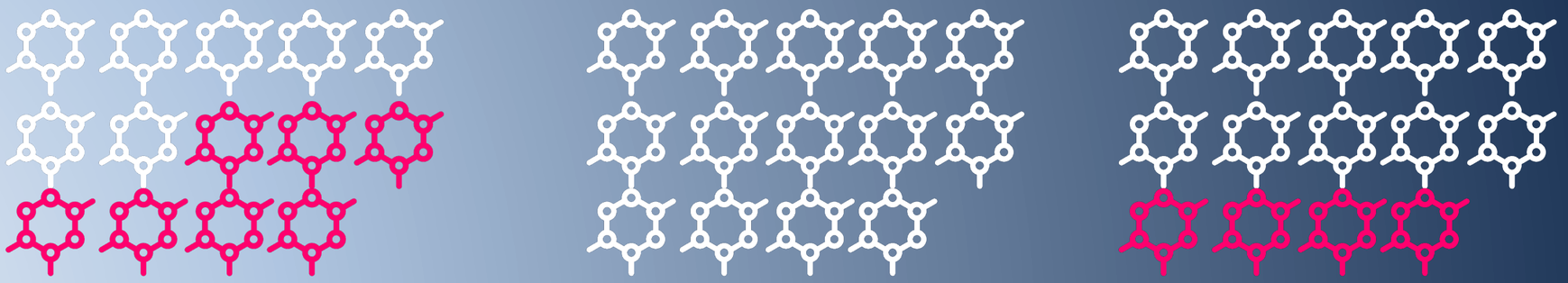
orthogonal separation

Akhlaqi et al. Anal Bioanal Chem 2023



t_R *CCS* MS^2



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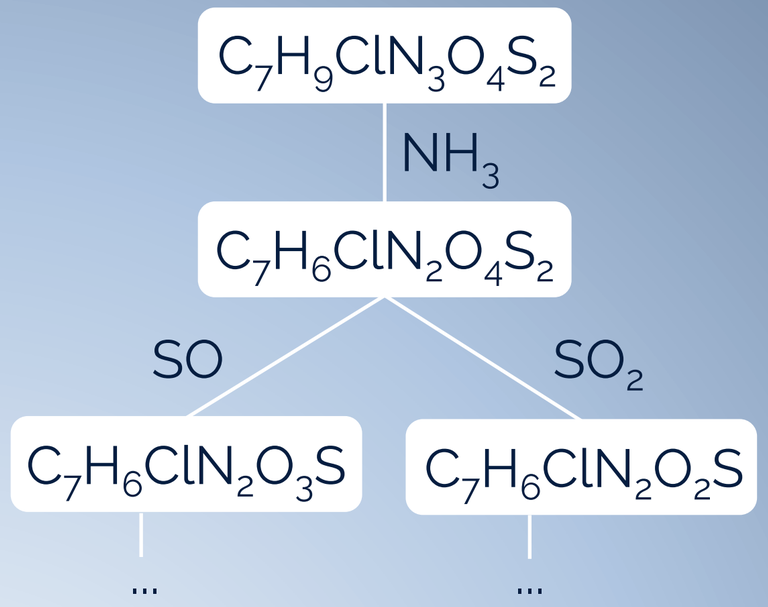
Dührkop et al. SIRIUS + CSI:FingerID
Stravs et al. MS Novelist



machine learning →

0.08	O-P
0.87	S=O
0.15	C-F
0.93	NH_2
0.99	O

structure →

database matching

De Novo

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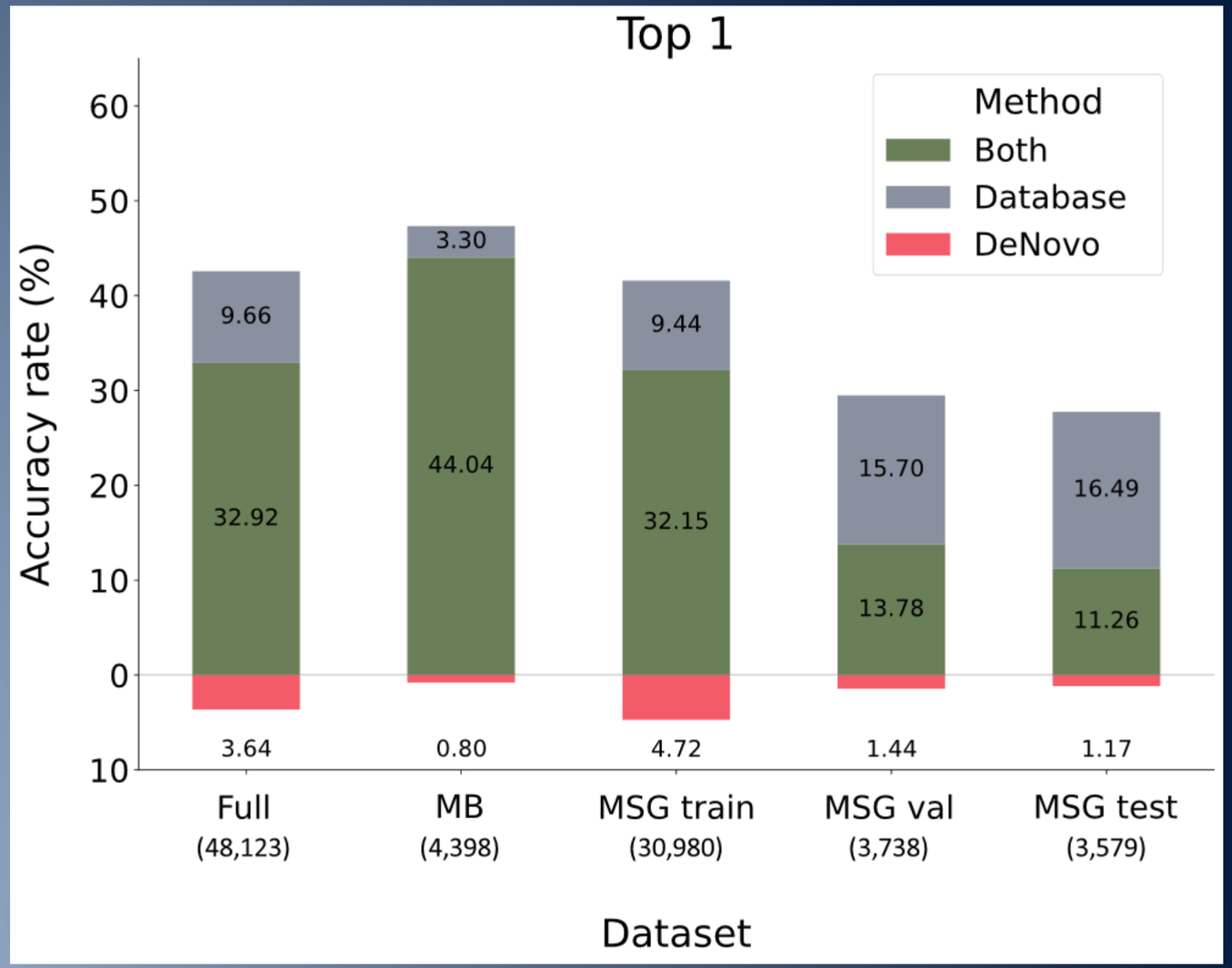
structural identification

from MS^2 spectra

Hättestrand et al., in preparation



in training set 36.0%

not in training 8.3%

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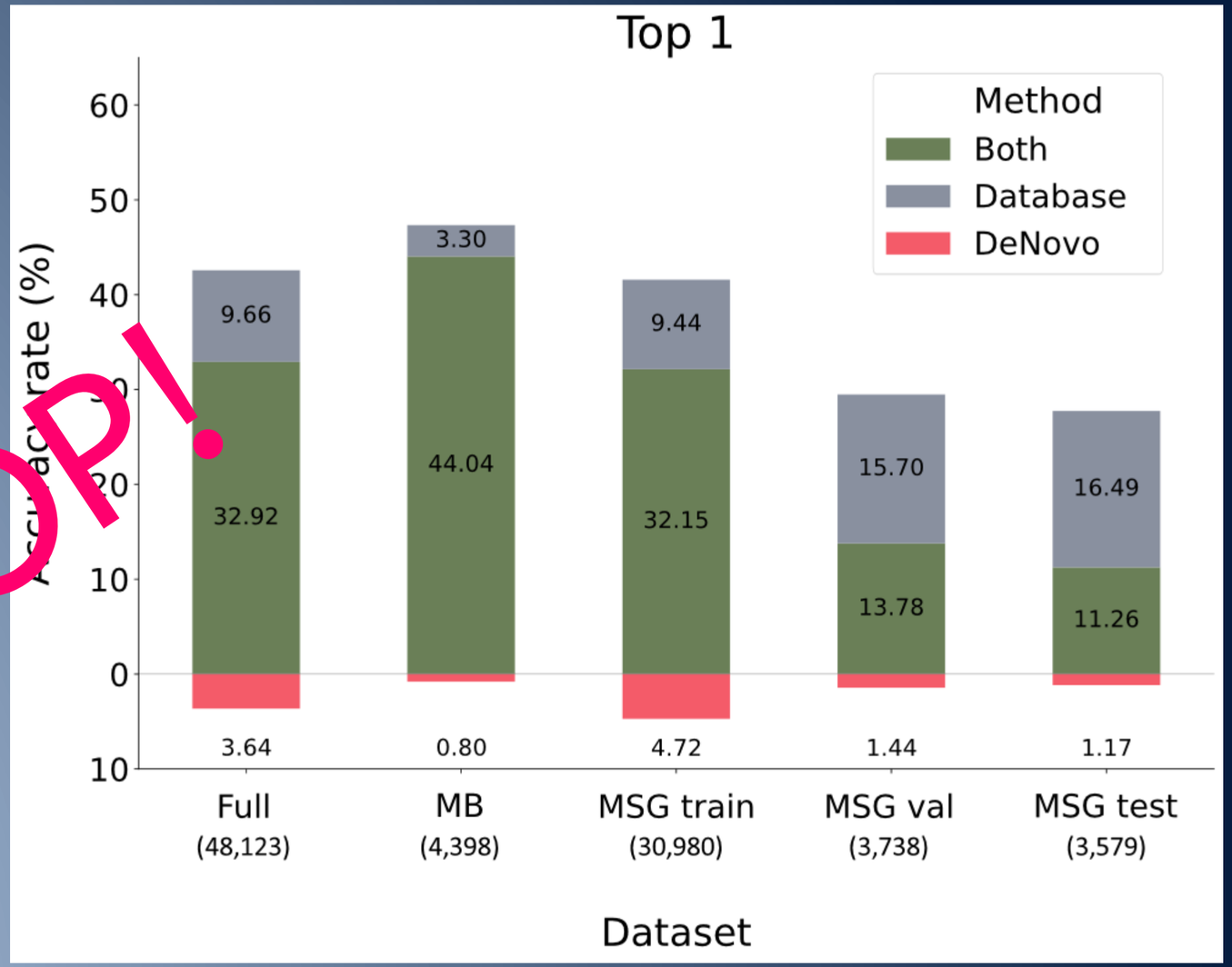
structural identification

from MS^2 spectra

STOP!



in training set 36.0%

not in training 8.3%

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take-home message

PRIORITIZE

(1) measuring performance is very important!
(2) chemistry still matters!

IDENTIFY

(1) all wrong structures are NOT false positives!
(2) multi-technique approach is important!

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