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High-resolution mass spectrometry-based untargeted approach: A cutting-edge analytical technique for the detection and identification of organophosphorus synthesis sources

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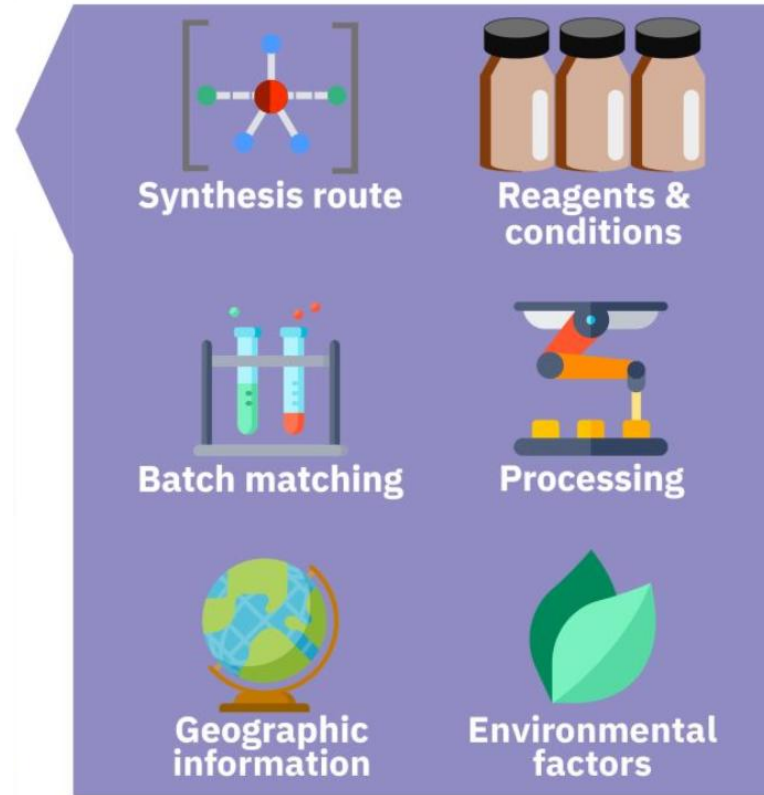
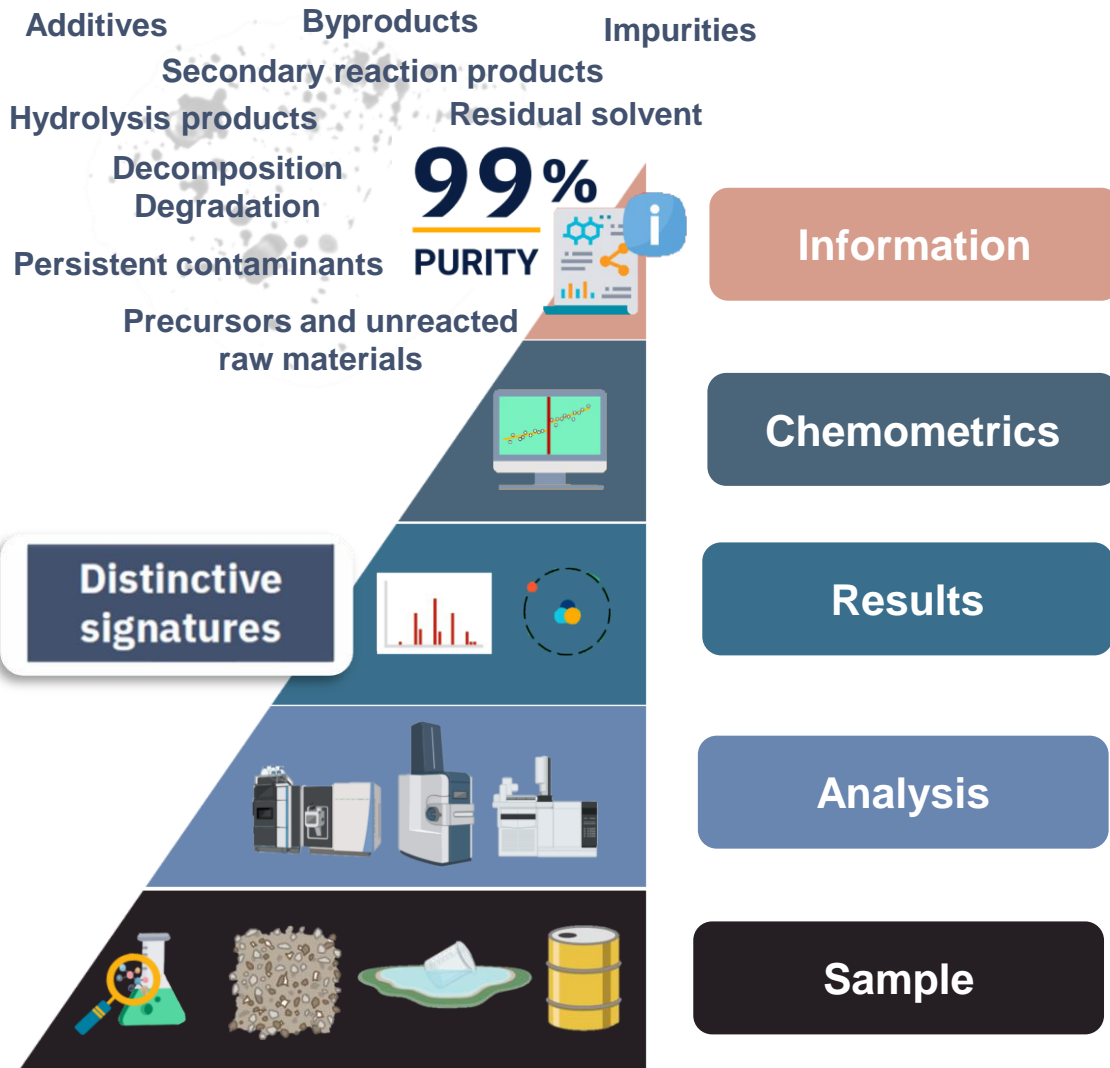
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➤ High-resolution mass spectrometry-based untargeted approach

A cutting-edge analytical technique for the detection and identification of organophosphorus synthesis sources

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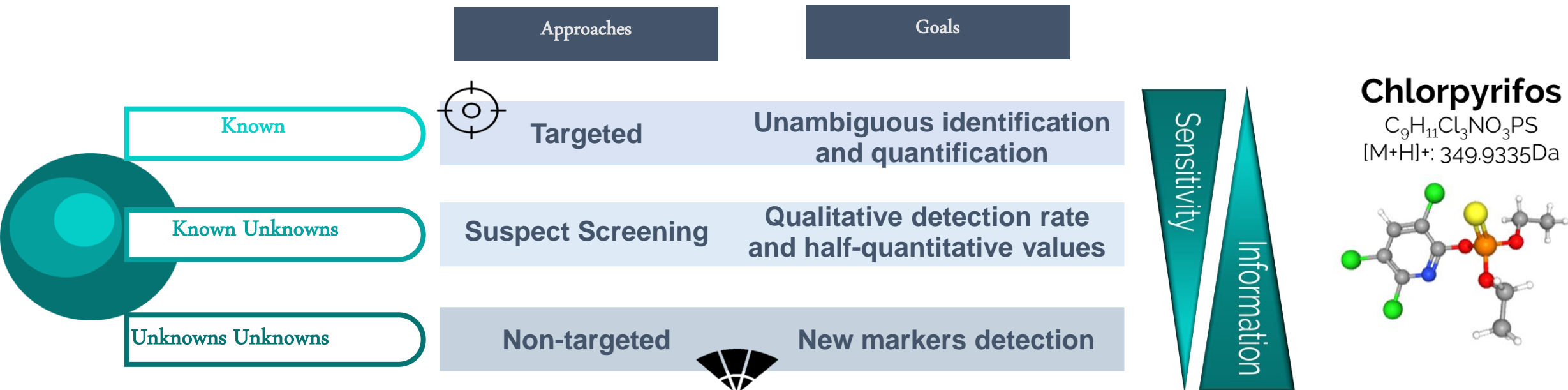
➤ Sourcing and impurity profile



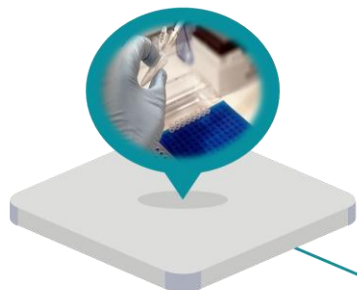
**Chemical
Attribution
Signature**

Distinctive signatures can reveal well-founded findings about a chemical's production history, to strengthen forensic investigations.

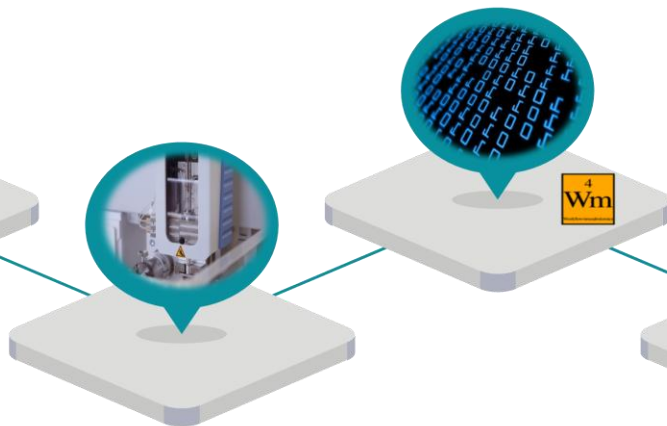
Metabolomics-based CAS discovery strategies



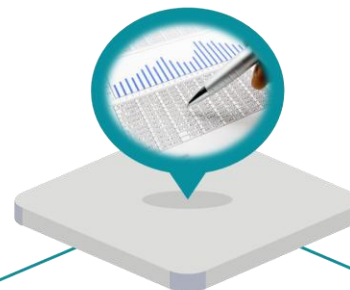
Sample Preparation



Data Processing and Curation



Features detection



Chemical Attribution Signature



RT, accurate mass, isotopic pattern, ion mobility

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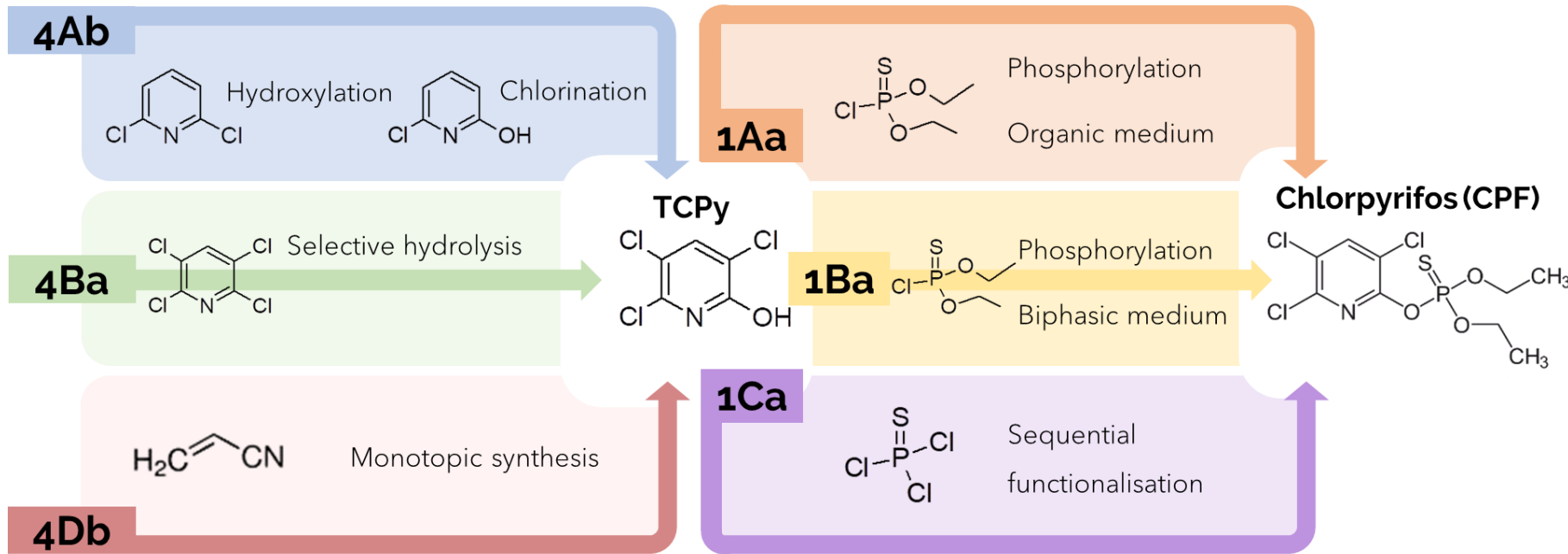
UHPLC-HRMS

Chemometrics

Multivariate analysis

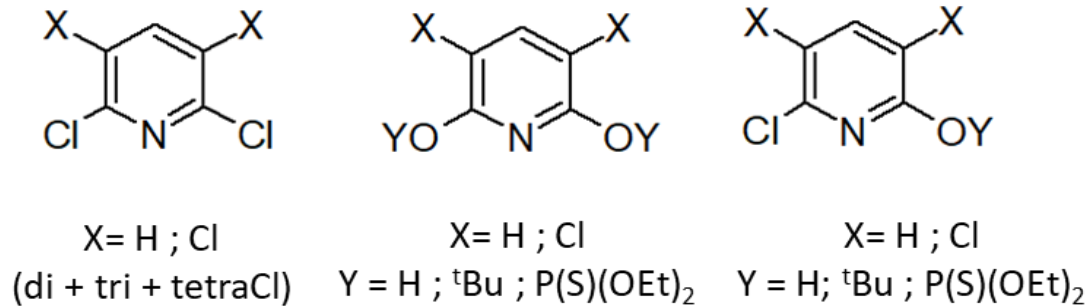
MS/MS identification

➤ Our use-case: chlorpyrifos (various synthetic routes)



Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba

S1



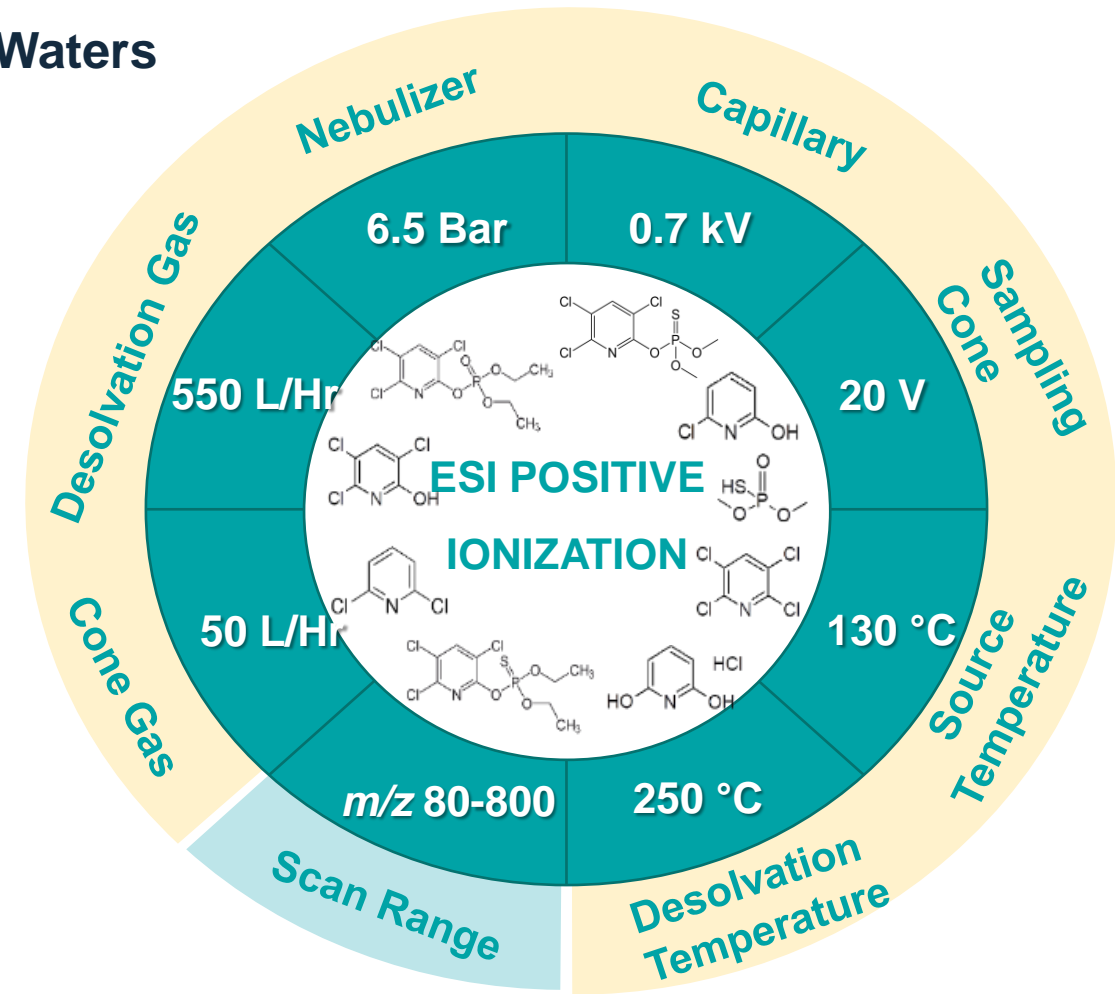
Wide range of compounds and possible impurities combinations

➤ Analytical LC-HRMS conditions



ACQUITY UPLC I Class
QToF Synapt G2-Si HRMS Waters

Column	Hypersil Gold C18 (100 x 2.1mm, 1.9µm)
Mobile phase	A) H ₂ O/CH ₃ OH/AcOH (95/5/0.1) B) CH ₃ OH/AcOH (100/0.1)
Flow rate and Oven temperature	0.3mL/min at 40°C
Injection volume	10µL



• MS Resolution : ~ 15000

➤ Untargeted data processing



Workflow4metabolomics



Analytical and blank noise
RSD pool features

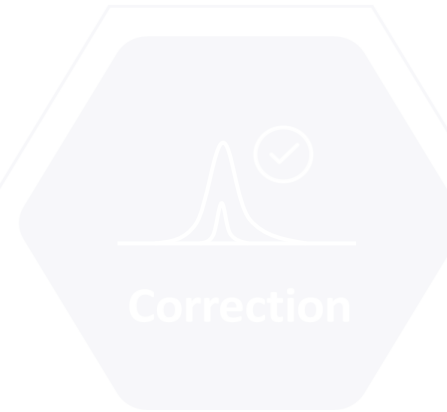
Univariate and multivariate



Universal data
format
(mzML)



Peak detection and grouping
Retention time alignment
Integrating missing data



Batch and analytical drift
Standardization



Data matrix

	Samples
Features (m/z + RT)	Intensity

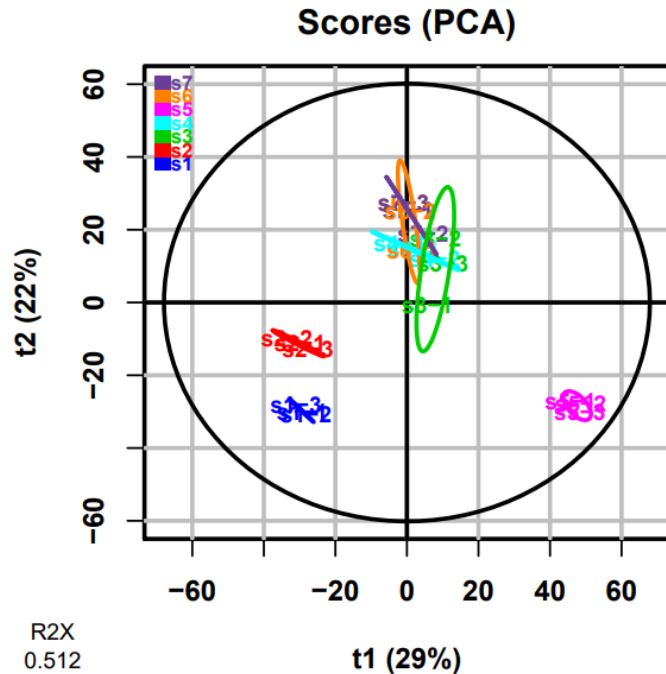


➤ Chemometrics

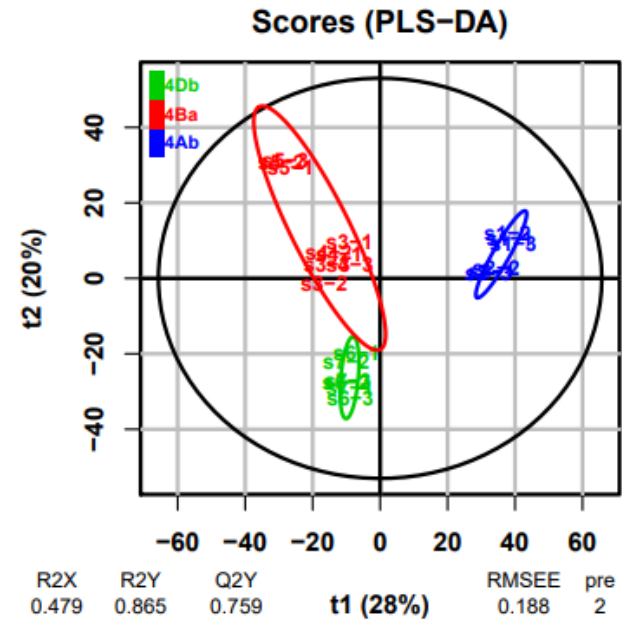
Multivariate methods suitable to provide a more complete description of studied phenomena

- Major sources of variability → PCA (unsupervised)
- Specific sources of variability → PLS (supervised)

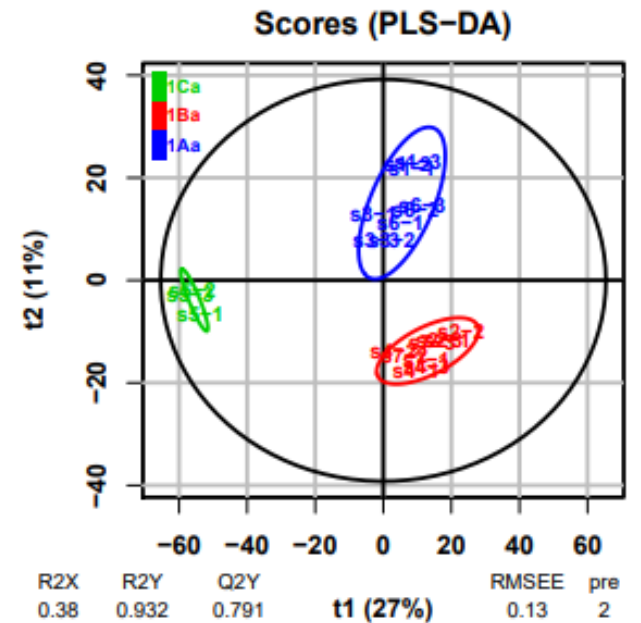
Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba



Precursor



Reactant



PCA: First overview of variability between samples and correlation between features

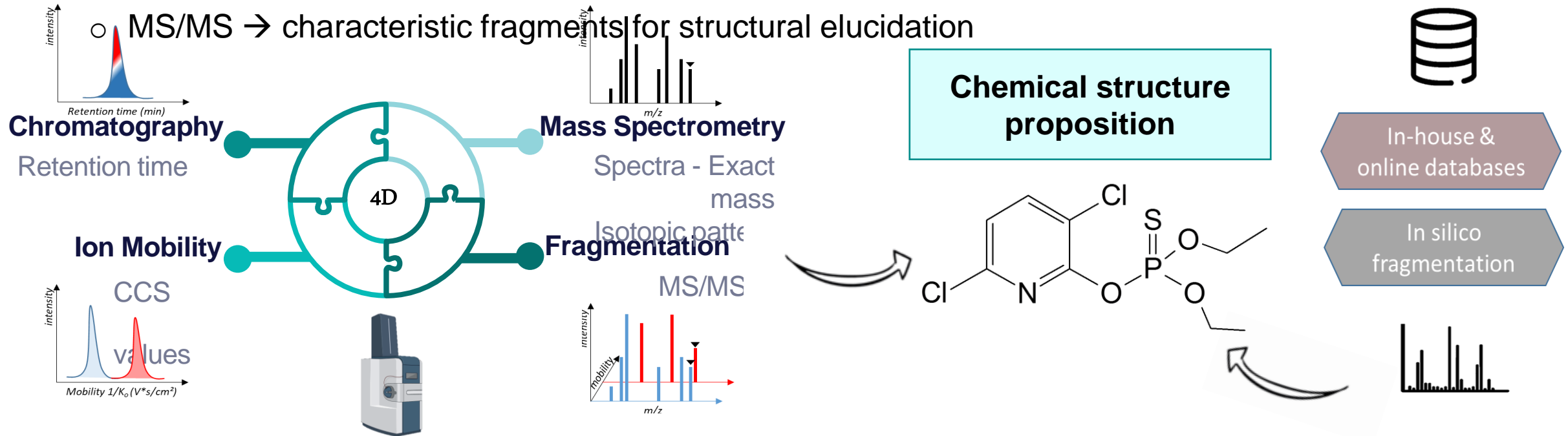
PLS-DA: Discrimination of various used synthetic sources

Quality of the model assessed with $Q^2Y > 0.4$ and permutation test

Annotation Challenges

HRMS: sensitive and selective detection of discriminating impurities

- Features (PLS-DA; VIP>1) annotated using LC (RT), HRMS, MS/MS and IMS data to propose a putative structure



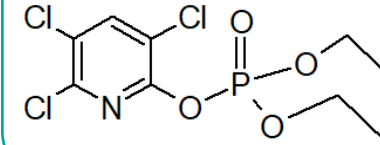
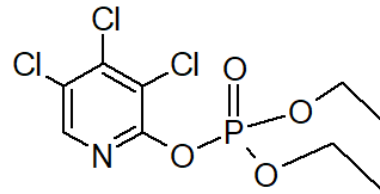
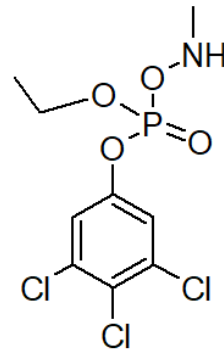
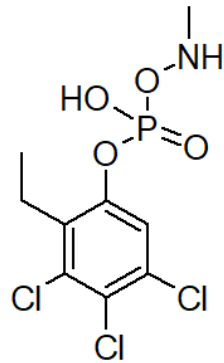
[1] Confirmed Structure	[2] Probable Structure	[3] Tentative Candidate	[4] Unknown
5	72	36	9

➤ Ion mobility spectrometry: a valuable aid for annotation



m/z 333.9468
Rt: 20 min
 $C_9H_{11}Cl_3NO_4P$

CCS measurement: 162.7 Å²



**Chlorpyrifos-oxon
identified with
standard MSMS**

Predicted CCS	166.1 Å ²	165.5 Å ²	165.1 Å ²	165.5 Å ²
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5% accuracy acceptance

CCS: close values between structure proposals
Support to annotation → remove hypothesis, putative information of structure

➤ Analysis of unknown samples

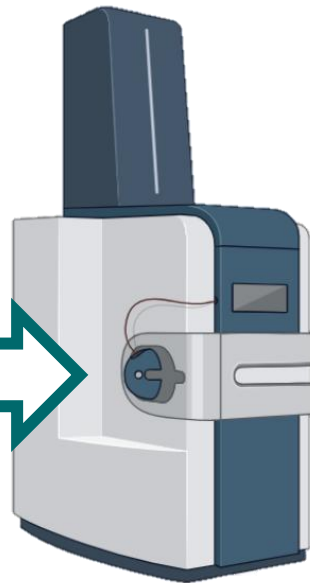
4D

RT, accurate mass, MS/MS, ion mobility



Coupled separation technique

(LC)
Annotation with database
(RT)



Methodology validation



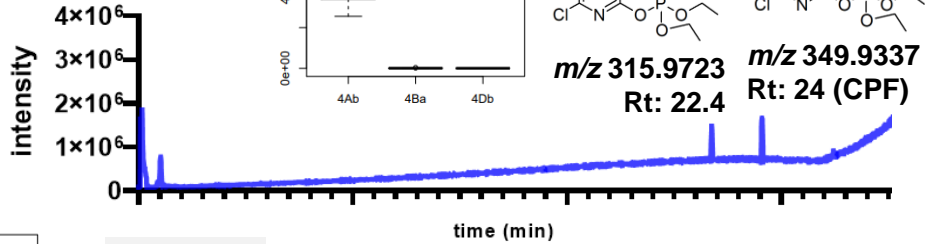
Blind analysis of new unknown samples to **classify** their **synthesis route** according to their **discriminating impurities** with the **constructed model**

Analysis of unknown samples

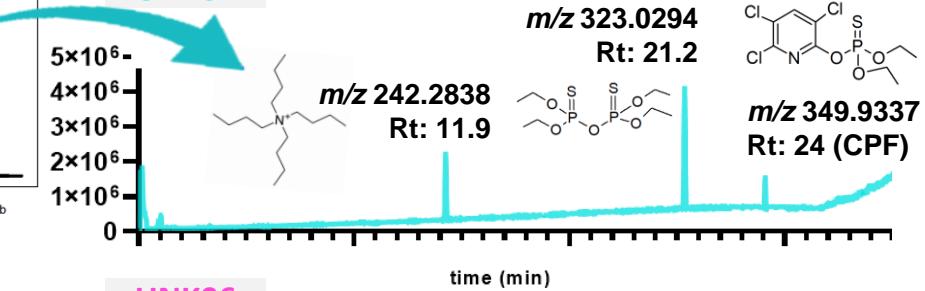


Unknown Samples

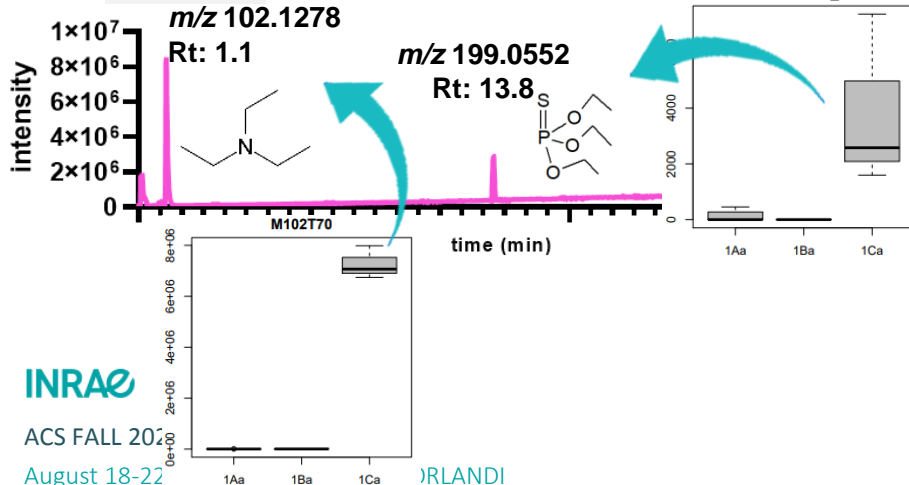
UNK04



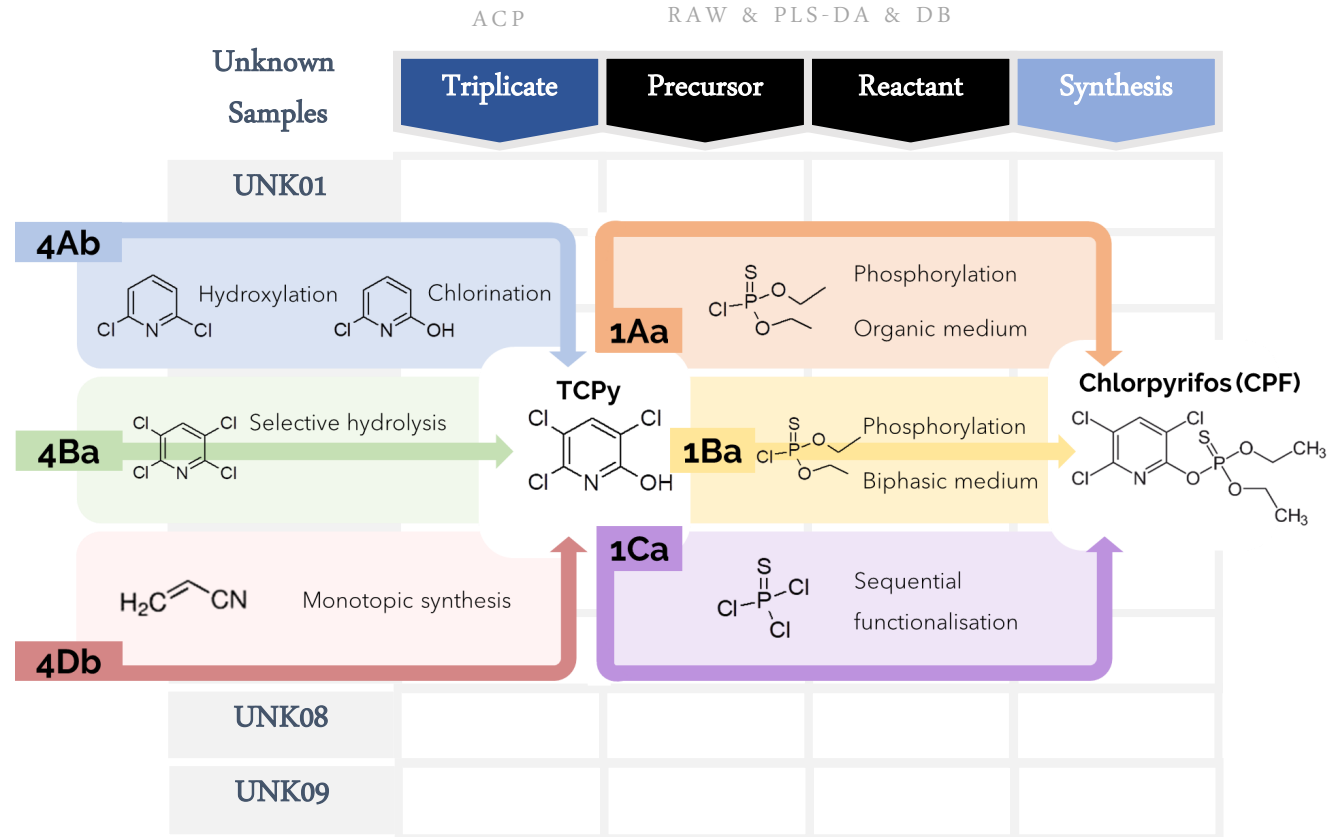
UNK02



UNK06

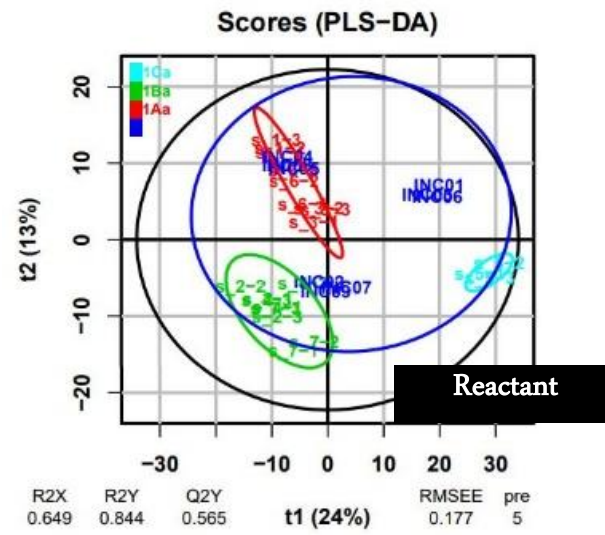
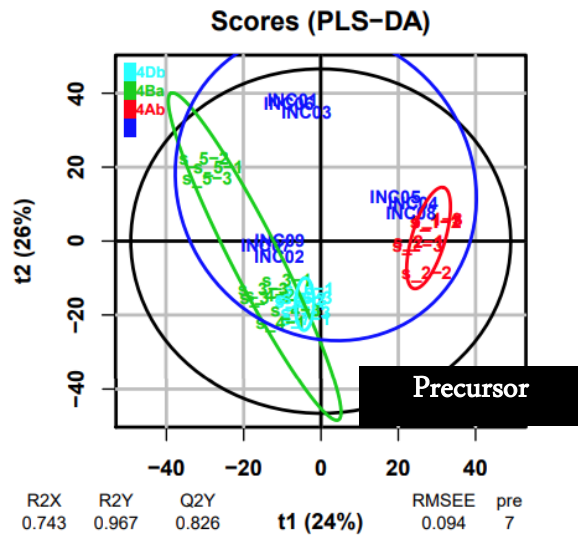
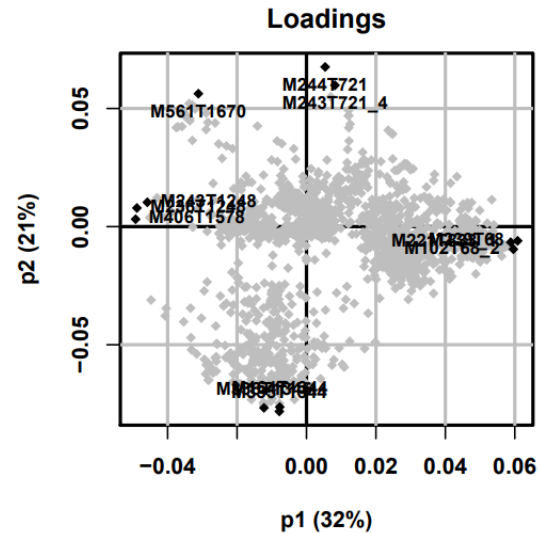
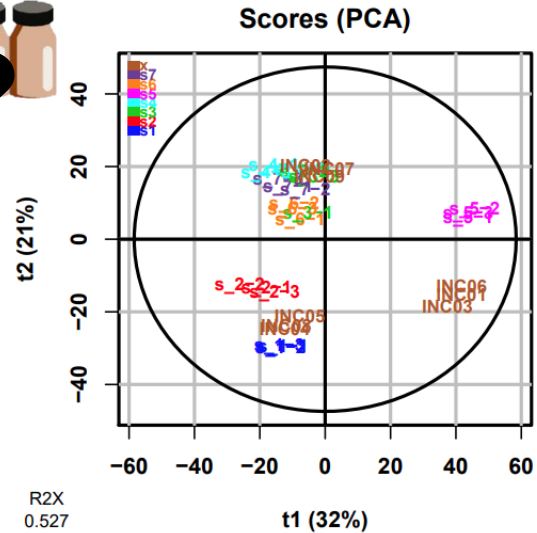


Methodology validation



Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba

Analysis of unknown samples



Methodology validation

Unknown Samples	ACP	RAW & PLS-DA & DB		
	Triplicate	Precursor	Reactant	Synthesis
UNK01	♥		1Ca	
UNK02	♣	4Ba ^{4Ba}	1Ba	S4
UNK03	♥		1Ca	
UNK04	♦	4Ab ^{4Ab}	1Aa	S1
UNK05	♦	4Ab	1Aa	S1
UNK06	♥		1Ca ^{1Ca}	
UNK07	♣	4Ba	1Ba	S4
UNK08	♦	4Ab	1Aa	S1
UNK09	♣	4Ba	1Ba	S4

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba



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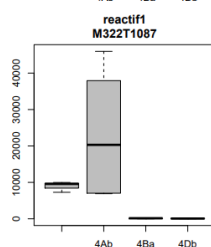
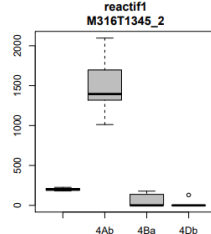
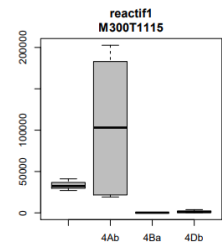
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➤ What about UNK01,03,06 « Precursor »?



Targeted Approach

Discriminant features	Presence?
Precursor 4Ab	<i>m/z</i> 299.9963 <i>rt</i> 18.5min YES
	<i>m/z</i> 315.9736 <i>rt</i> 22.4min YES
	<i>m/z</i> 321.9771 <i>rt</i> 17.6min YES
	<i>m/z</i> 287.9421 <i>rt</i> 12.7min YES
Precursor 4Ba	<i>m/z</i> 242.2849 <i>rt</i> 12min NO
Precursor 4Db	<i>m/z</i> 554.7456 <i>rt</i> 27.5min NO
	<i>m/z</i> 504.8106 <i>rt</i> 26.6min NO
	<i>m/z</i> 532.8596 <i>rt</i> 26.2min NO
	<i>m/z</i> 424.9442 <i>rt</i> 21.9min NO



Methodology validation

Unknown Samples	ACP	Raw & DB	PLS-DA & DB	Synthesis
	Triplicate	Precursor	Reactant	
UNK01	♥	4Ab	1Ca	S8
UNK02	♣	4Ba ^{4Ba}	1Ba	S4
UNK03	♥	4Ab	1Ca	S8
UNK04	♦	4Ab ^{4Ab}	1Aa	S1
UNK05	♦	4Ab	1Aa	S1
UNK06	♥	4Ab	1Ca ^{1Ca}	S8
UNK07	♣	4Ba	1Ba	S4
UNK08	♦	4Ab	1Aa	S1
UNK09	♣	4Ba	1Ba	S4

Synthesis	Precursor	Reactant
S1	4Ab	1Aa
S2	4Ab	1Ba
S3	4Ba	1Aa
S4	4Ba	1Ba
S5	4Ba	1Ca
S6	4Db	1Aa
S7	4Db	1Ba
S8	4Ab	1Ca

New combination of synthesis pathways discovered

> Conclusions

Methodology to support **attribution of the origin** of a **toxic threat agent** according to the **chemical signature of substances** resulting from its **production pathway**

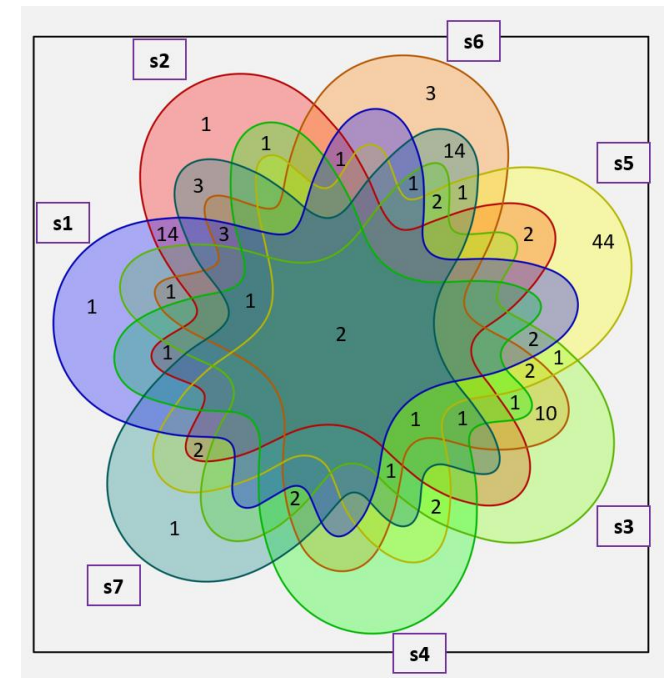
- ⊕ Chemical footprints were linked to a manufacturing process from synthetic raw materials
- ⊕ Methodology validated under blinded conditions with unknown new samples

Untargeted Approach

Discriminant impurities

Targeted Approach

Shotgun and specific analysis

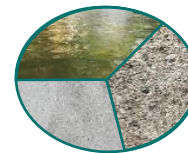


Applicable to others scientific fields: biomarkers of chemical exposure on health and sourcing of toxic treat agents

« *Better understand how it is produced, better understand how it works!* »

> Perspectives

⊕ **Ruggedness:** Apply the methodology to complex samples



⊕ **Structural Elucidation:** Trap and isolate impurities (2D-LC-HRMS) to complete elucidation by NM



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Thank you for your attention

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