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## Differentiation of synthetic sources of an organophosphorus chemical by LC-HRMS-based metabolomics

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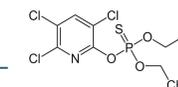
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## Overview

- Purpose** Determination of characteristic impurities of different chlorpyrifos synthetic sources.
- Method** Untargeted LC-HRMS metabolomics approaches combined with multivariate analyses.
- Results** Detection and annotation of more than 70 impurity features, under structural elucidation.

## Introduction

- Chemical attribution signatures for chemical threat agents can be used to provide an evidentiary link between the use of a given chemical and its origin to support forensic investigations [1,2]. Complementary to specific targeted compounds detection, untargeted approaches combined with multivariate analyses can provide further information to determine the source or the synthetic pathways. Organophosphorus compounds used as nerve agents, can be produced by more or less complex synthesis routes.
- To assess the specificity of by-products, impurities, and others compounds originated from a chemical synthesis, untargeted fingerprints of crude chlorpyrifos (CPF) from different synthesis ways were analyzed as a first use-case using metabolomics-based [3] trace discovery strategies [4].

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## Synthesis of chlorpyrifos

- 7 combinations of chlorpyrifos synthesis were performed in triplicate. As the physical aspects of the triplicates varied greatly, homogeneity was assessed by sampling each triplicate 3 times.

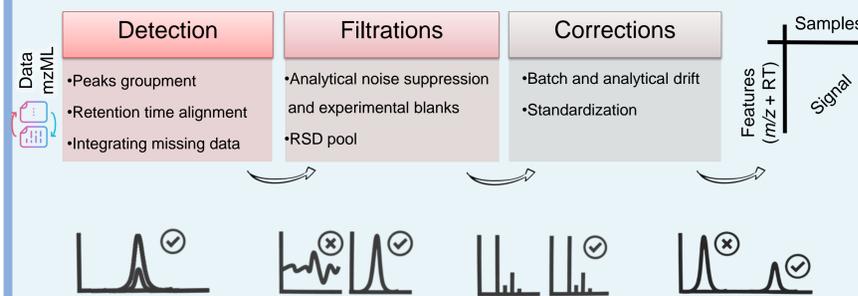
Reaction scheme for the different pathways to chlorpyrifos

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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## Features extraction & Data curation

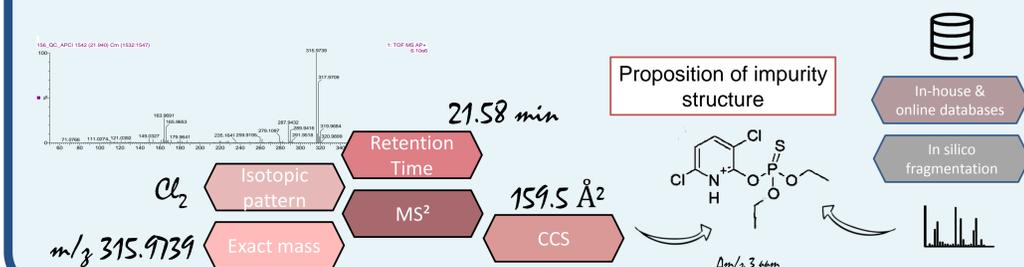
- Data were extracted and treated using XCMS under W4M [5] interface providing the final detected features matrix.



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## Features annotation

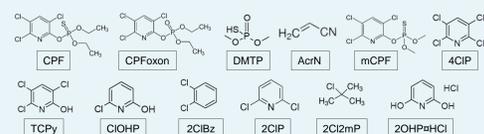
- High resolution mass spectrometric analysis allowed a sensitive and selective detection of discriminated impurities.
- Discriminating features (VIP>1, Wilcoxon test <0.05) were annotated using chromatographic, MS, MS/MS and ion mobility results to propose a putative structure.
- MS<sup>2</sup> analyses are carried out to provide characteristic fragments for their structural elucidation.



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## Optimization and analytical conditions

- Optimization of ionization conditions with standards using ESI and APCI sources.
- Syntheses analysis were performed with an UPLC-HRMS system.



standards 1ng/μL QA	ESI NEG		ESI POS		APCI POS	
	[M-H] <sup>-</sup>	[M+H] <sup>+</sup>	Retention Time (min)	Intensity	Retention Time (min)	Intensity
CPF	347.9191	349.9335	nd	nd	24.02	6.46E+05
CPF-oxon	331.9413	333.9569	nd	nd	19.92	4.91E+07
DMTP	140.9775	142.9931	1.32	7.10E+06	1.31	1.86E+06
AcN	52.0192	54.0337	nd	nd	21.90	1.31E+05
mCPF	319.8971	321.9028	nd	nd	21.90	2.66E+06
4CIP	213.879	215.8935	nd	nd	19.69	5.13E+03
TCPy	195.9129	197.9274	15.94	2.30E+07	16.02	2.34E+05
ClOHP	127.9909	130.0054	4.81	9.80E+04	4.87	1.03E+07
2ClBz	144.9617	146.9762	nd	nd	nd	nd
2CIP	145.9571	147.9715	nd	nd	10.09	2.41E+05
2Cl2mP	91.0321	93.0465	nd	nd	nd	nd
2OHPmCl	110.0248	112.0392	1.61	3.30E+06	1.62	3.15E+04



**ACQUITY I Class UPLC**

- Hypersil Gold C18 (100x2.1mm, 1.9μm)
- Mobile phases:
  - A) H<sub>2</sub>O/CH<sub>3</sub>OH/AcOH
  - B) CH<sub>3</sub>OH/AcOH
- 0.3mL/min at 40°C
- From 0 to 30 min: 0% to 100% B
- Injection volume: 10μL

**QToF Synapt G2-Si HRMS**

ESI pos & neg ionization and APCI pos  
MS spectra m/z 50 - 800  
MS resolution ~15000

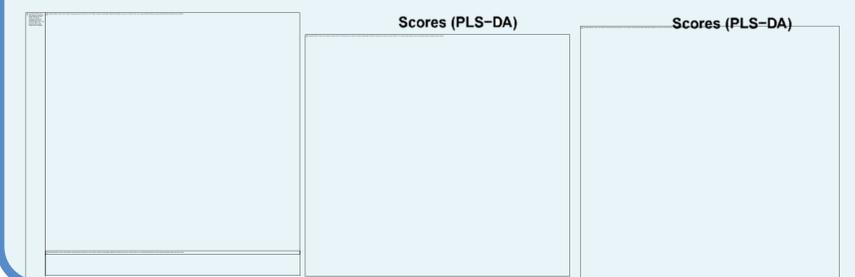
ESI parameters	NEG	POS
Capillary (kV)	0.6	0.7
Sampling Cone (V)	40	20
Source Offset	60	60
Source T (°C)	130	130
Desolvation T (°C)	300	250
Cone Gas (L/Hr)	45	50
Desolvation Gas (L/Hr)	700	550
Nebuliser G (Bar)	6.5	6.5

APCI parameters	POS
Corona μA	3
Sampling Cone (V)	30
Source Offset	60
Source T (°C)	120
Desolvation T (°C)	500
Cone Gas (L/Hr)	50
Desolvation Gas (L/Hr)	600
Nebuliser G (Bar)	6.5

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## Chemometrics

- PCA enabled a representation of variability between samples and correlation between features.
- Discrimination of synthetic sources achieved by supervised PLS-DA.
- The quality of the model was assessed with Q2 and permutations test.



## Conclusion

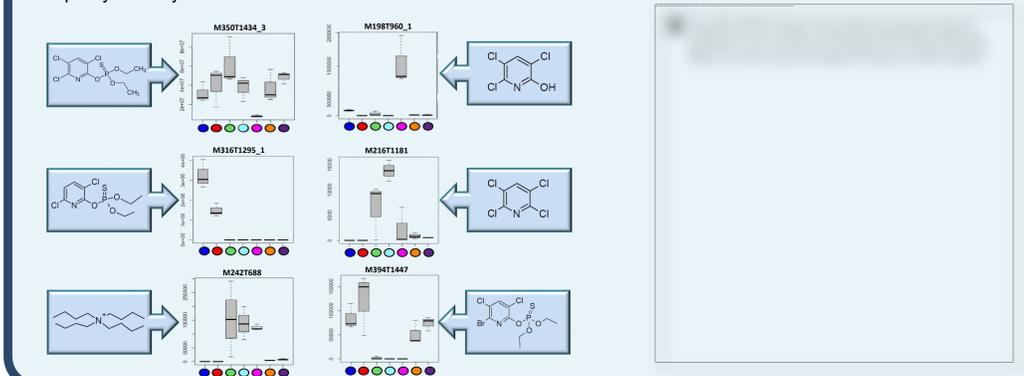
**A LC-HRMS by ESI or APCI based metabolomics methodology for sample classification of an organophosphorus pesticide (chlorpyrifos) synthesized according to several routes, was successfully set-up.**

- A combination of approaches was used to carry out this proof of concept.
- Multivariate analysis allowed to build a list of discriminating impurity features.
- Adducts, fragments and isotopic related ions were successfully annotated.

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## Chemical Attribution Signature

- Different representations can be proposed to illustrate the presence/absence and the intensity of each discriminated impurity from synthesis.



## Challenges

- Structural elucidation of the discriminating intermediates is under progress using HR-MS<sup>n</sup> analysis.
- Miniaturization of the LC method is also under progress to decrease matrix effect for analysis of "real-life" samples.

## References

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## Contact

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