

# Development and application of non-targeted approaches to characterise human internal exposure to halogenated chemicals of concern

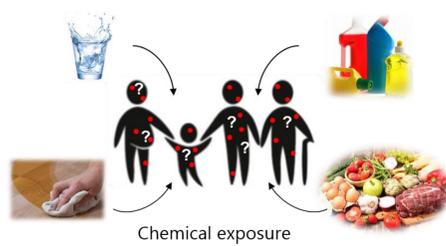
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## Introduction

Human are today exposed to a huge number of chemicals present in the environment (water, air, dust, food etc.). This wide range of contaminants still increases over years and has to be addressed through the **environment-food-human continuum**.



The **HBM4EU-WP16 “emerging chemicals”** aims to contribute to improve our knowledge about this chemical exposome, especially with regard to the reality of human exposure to chemical of emerging concern (CECs), then to act as early warning support to policy.



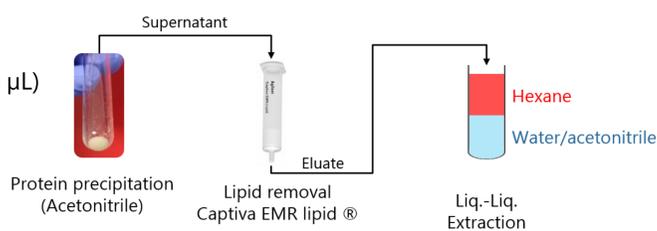
New methodological approaches based on large scale **suspect and non-targeted screening (NTS)** are then developed. In particular, a NTS analytical workflow was elaborated and applied to screen specifically **halogenated markers of exposure in human milk**.

## Experimental

### Sample preparation:

The developed sample preparation procedure aimed to find a compromise between a low selectivity to cover a wide range of markers of interest and sufficient clean-up for compatibility of the prepared extract with a reliable LC-HRMS measurement.

- Breast milk samples (100 µL)
- + Procedural blank
- + Quality control (QC)



### Analysis:

Both extracted fractions (water/acetonitrile and hexane) are analysed with **LC-HRMS** and **GC-HRMS**, respectively. The complementarity of these two techniques allows to cover a board range of molecules with different physico-chemicals properties.

### LC-HESI-Q-Orbitrap (full scan)

### GC-EI-Q-Orbitrap (full scan)



### Data processing:

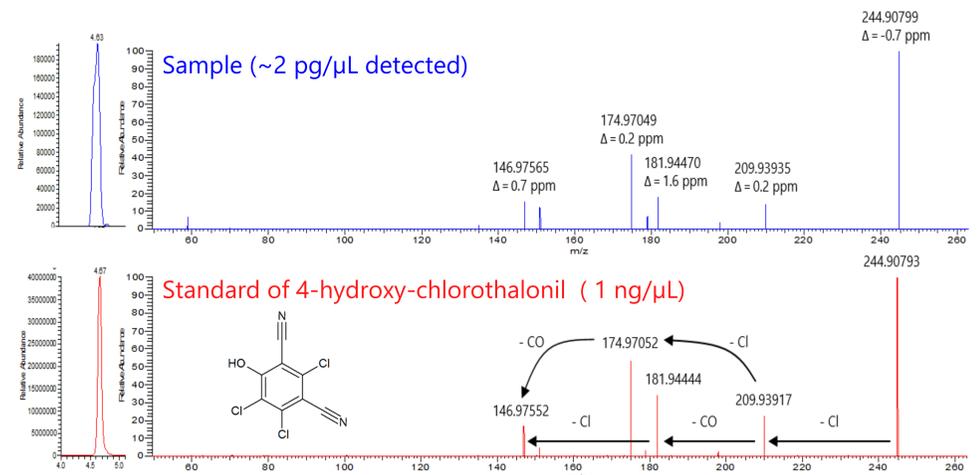
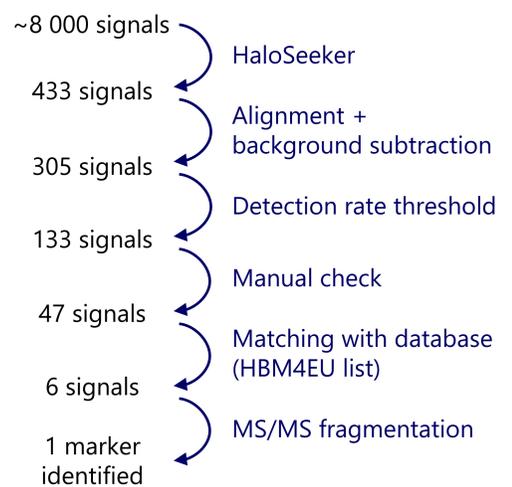
The developed user friendly software HaloSeeker (Léon *et al.*, Anal. Chem. 2019, 91:3500–3507) permits to reveal detected signals corresponding to halogenated markers, based on their typical isotopic pattern observed in high resolution MS.



## Results and discussion

The present section deals with LC-HRMS results obtained from the water / acetonitrile fraction. The methodology used to point out and prioritise potential signals of interest finally resulted in the **annotation of 13% of the detected signals**. Among which **1 signal** was **identified** after complementary MS/MS fragmentation and comparison with a reference standard. This particular marker evidenced in several analysed human milk samples with such totally without *a priori* approach was identified as **4-hydroxy-chlorothalonil**.

Annotation results obtained for a particular analysed human milk sample after filtering



Comparison of MS/MS fragmentation spectra (60 eV) for the compound detected in a human milk sample (blue) and a reference standard of 4-hydroxy-chlorothalonil (red).

This **first proof of concept** act as a promising result for non-targeted analysis of human matrices using a low volume of breast milk (100 µL) and LC-HRMS instrumentation. The GC-HRMS results are not available yet, but the complementarity of these two techniques will allow to cover a boarder range of compounds and so to better characterise samples and its related exposure.

## Conclusion

**Results:** 13 % of signals detected with a non-targeted approach were annotated, among which 1 signal was unambiguously identified.

**Why no more?** 1. Very low concentration levels of the expected markers of exposure and limited sample amount for analysis (sensitivity issue).  
2. Lack of appropriate and sufficiently extended annotation database (MS ref. library) for markers of exposure and QA/QC consolidated.

**Solutions:** 1. Improve analytical workflow (sample preparation).  
2. Develop the ambitious HBM4EU database.

**Perspectives:** 1. Generate HBM data with LC and GC-HRMS instruments  
2. Transpose this workflow to other biological matrices (meconium, placenta)

