



Strategies for suspect and non-targeted screening of new emerging chemicals in human biomonitoring

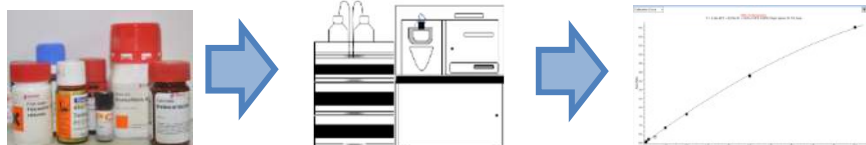
Martin Krauss & Carolin Huber



HELMHOLTZ
CENTRE FOR
ENVIRONMENTAL
RESEARCH – UFZ

General concepts

- Target analysis/ target screening



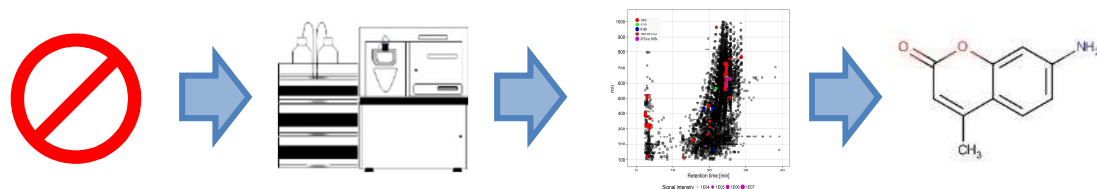
Quantify
10s-100s of
known compounds

- Suspect screening of expected contaminants



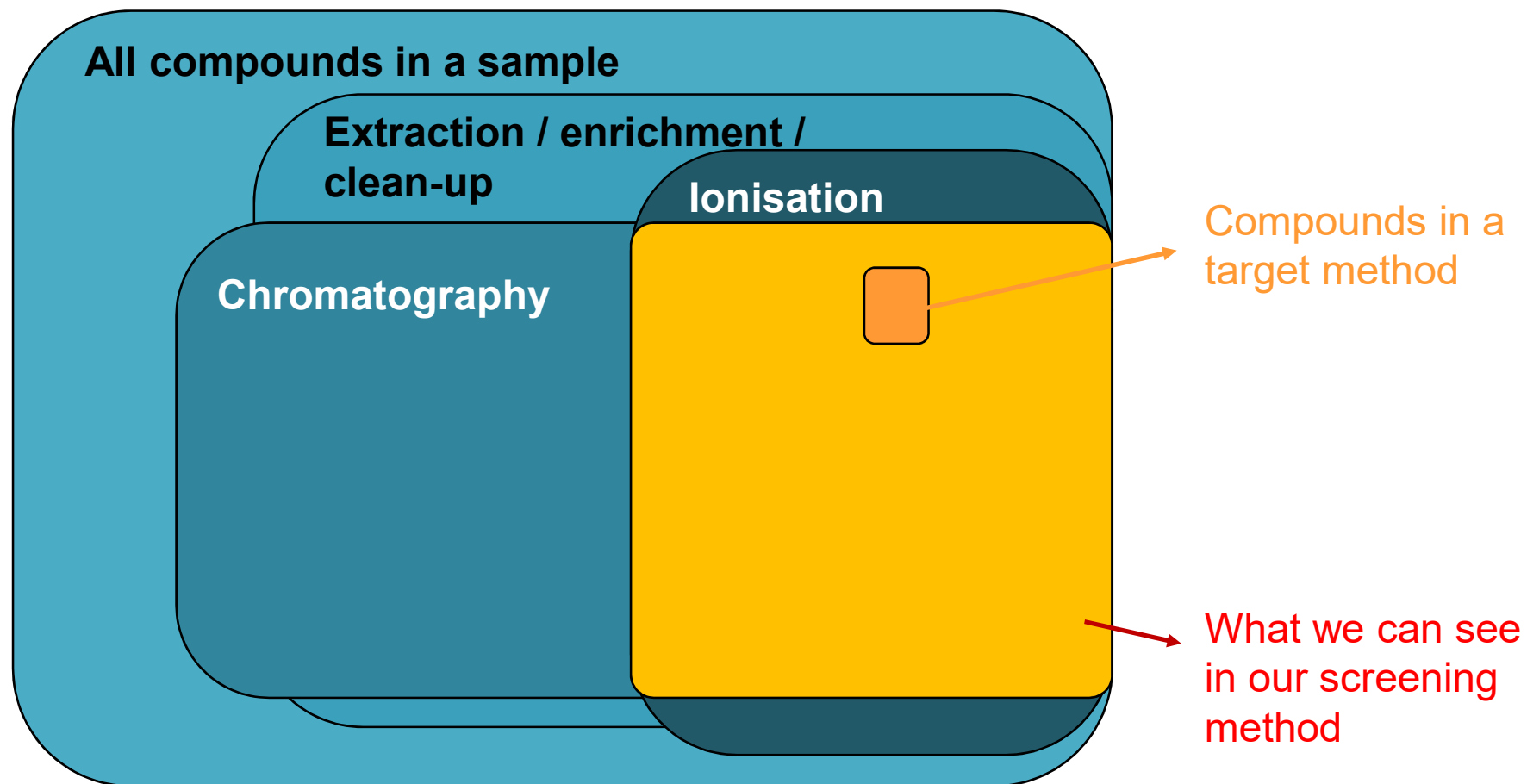
Detect & confirm
100s (1000s?) of
known compounds

- Non-target screening (NTS) for fingerprinting of mixtures and discovery of new chemicals

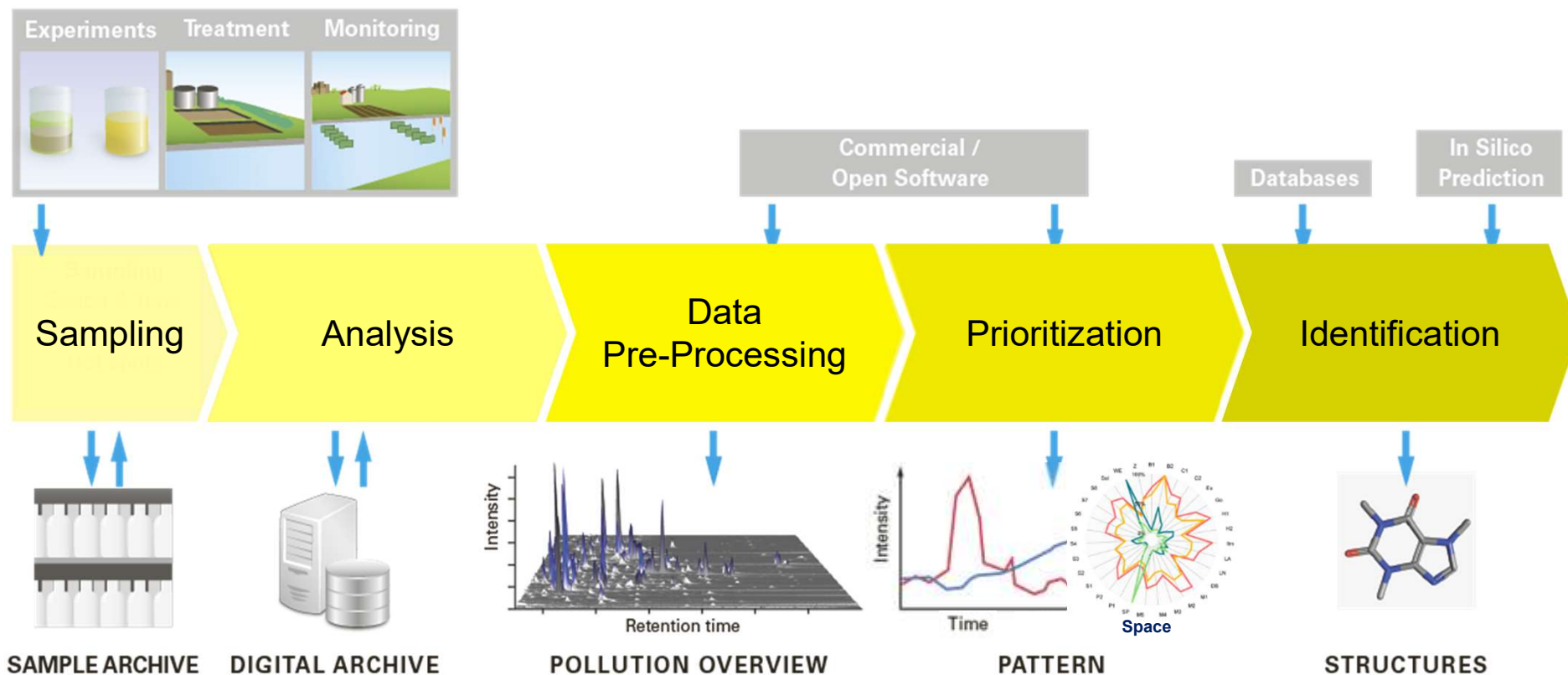


Detect “**ALL**”
known & **unknown**
compounds

Screening methods & compound domains

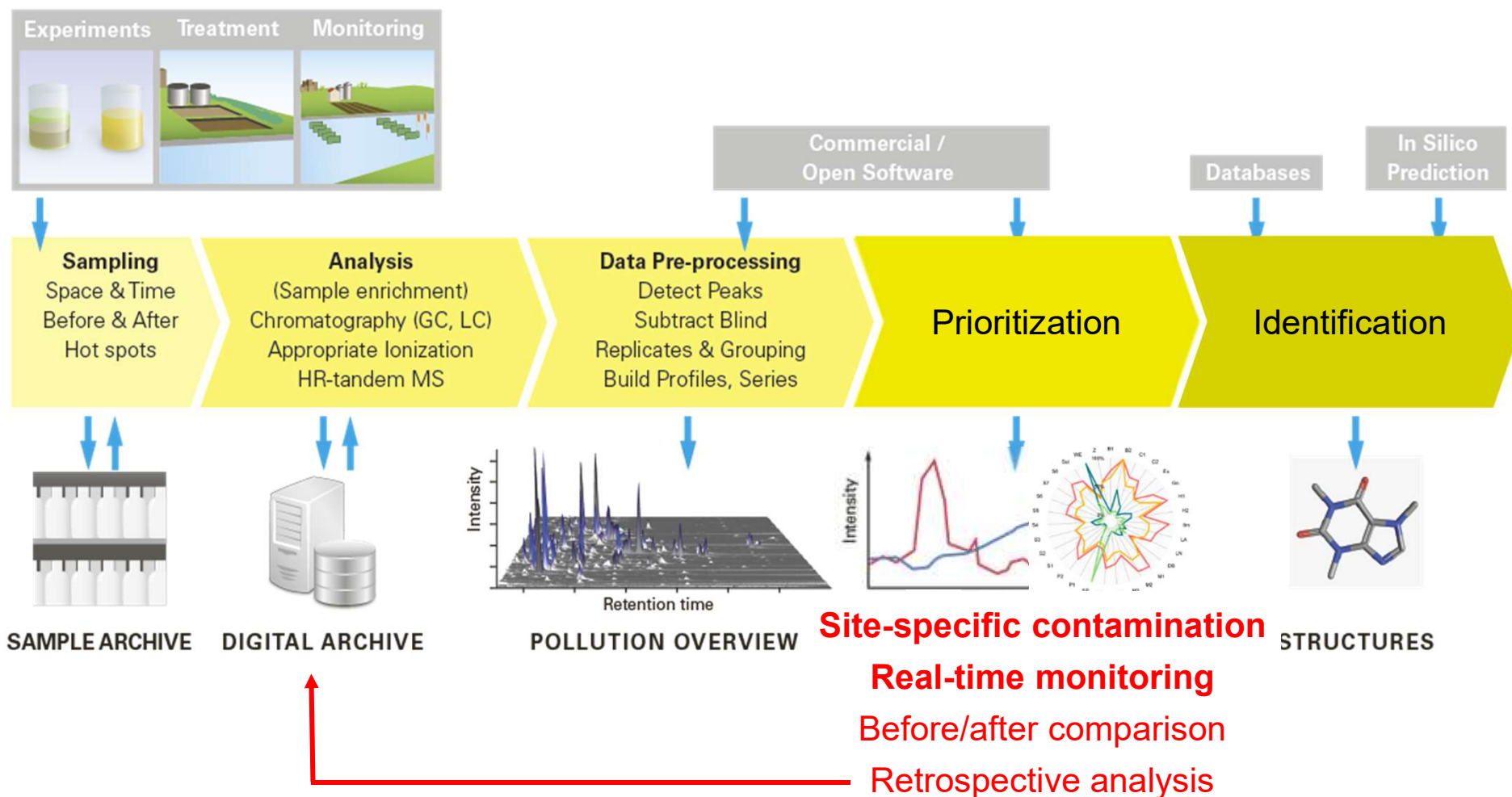


Non-target screening: workflow



Modified from *Hollender, Schymanski, Singer & Ferguson, 2018, ES&T Feature, 51:20, 11505*

Non-target screening: workflow

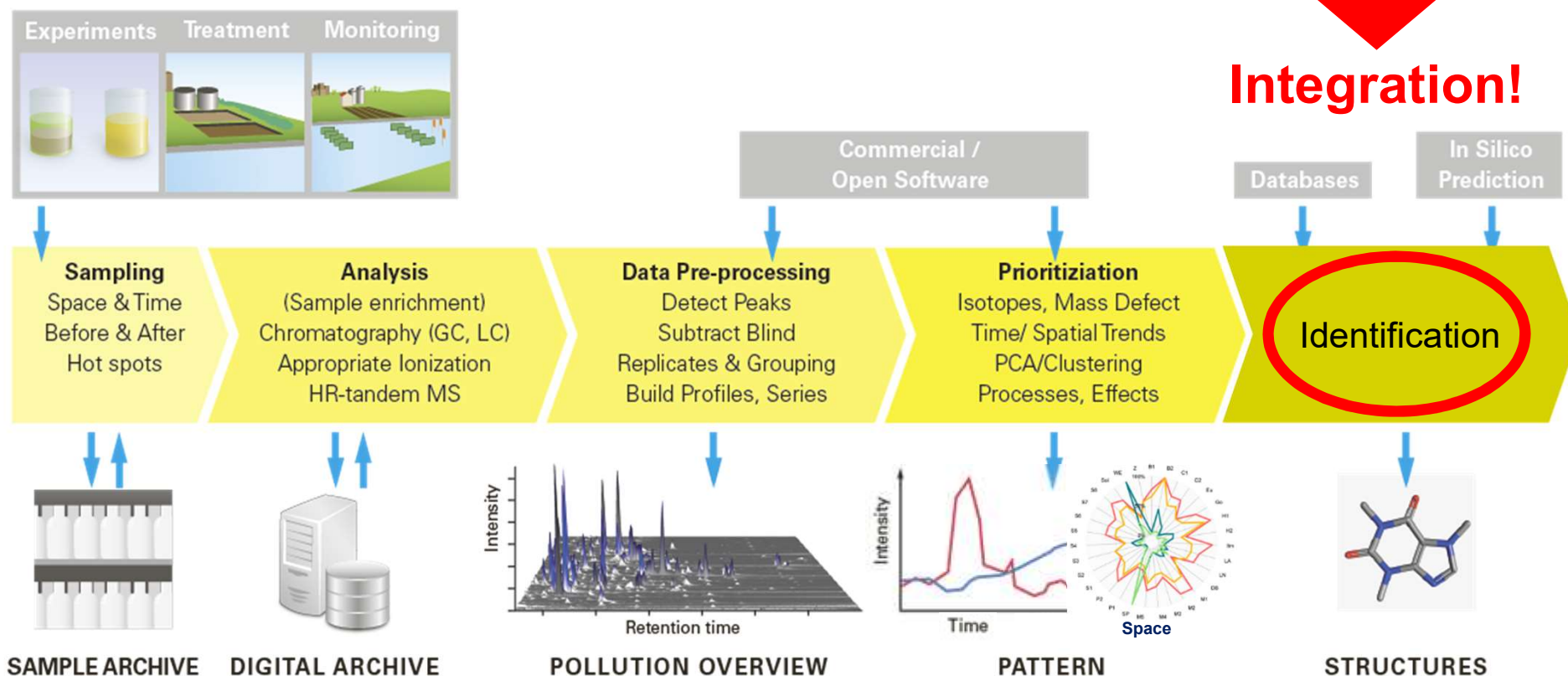


Modified from Hollender, Schymanski, Singer & Ferguson, 2018, ES&T Feature, 51:20, 11505

Non-target screening: workflow

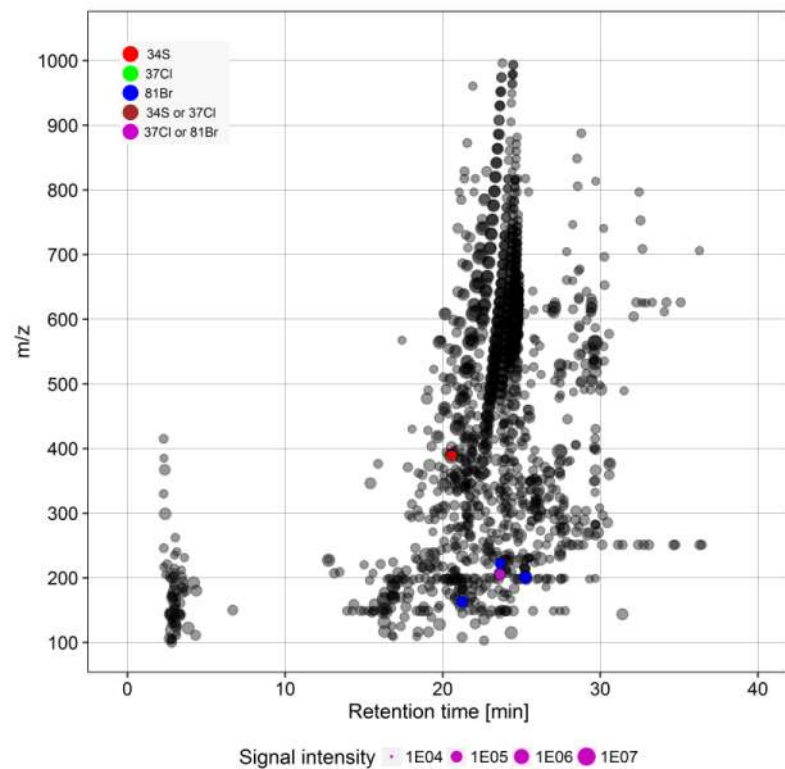
The limiting step!

**Lots of work
Many software tools**

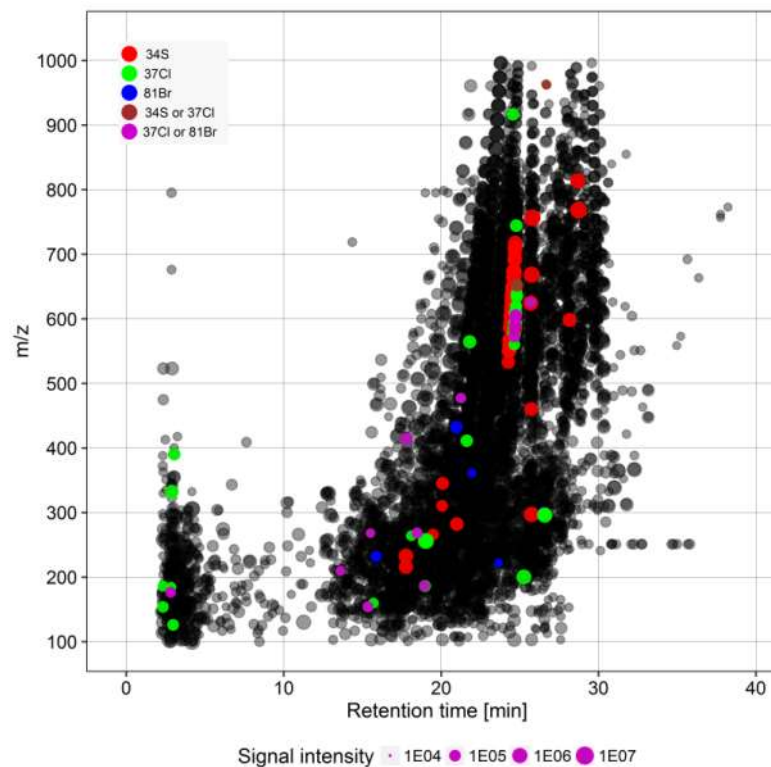


Modified from Hollender, Schymanski, Singer & Ferguson, 2018, ES&T Feature, 51:20, 11505

NTS of water samples



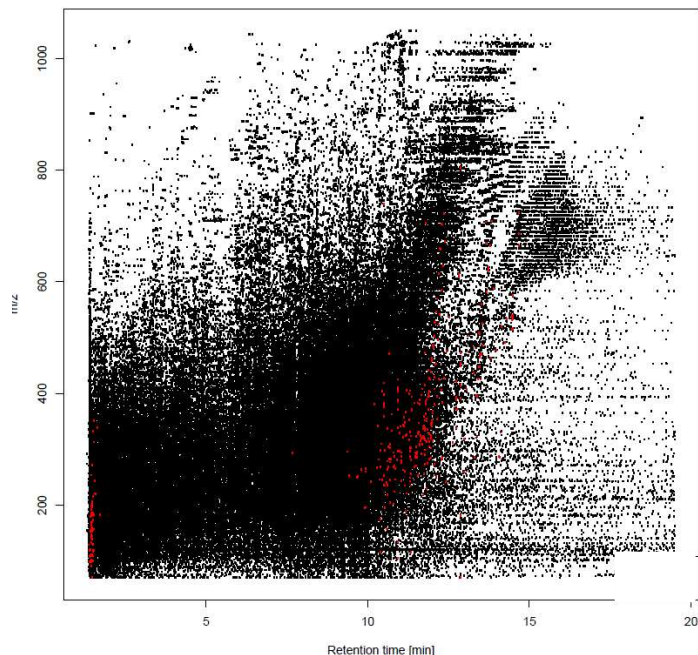
Small river *upstream*
of wastewater treatment plant



Small river *downstream*
of wastewater treatment plant

Large peaks are (mainly) anthropogenic contaminants

Challenges in NTS of biological samples

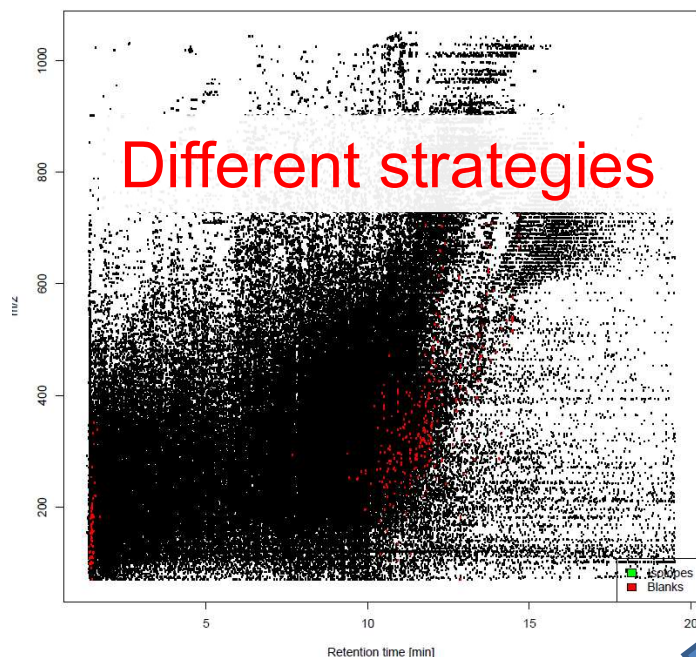


*Urine samples (1 batch),
direct injection after filtration*

>180'000 peaks

- Xenobiotic compounds are typically small peaks against the biological background
- Severe matrix effects
- MS¹ and MS² spectra have a high background noise

Challenges in NTS of biological samples



We start with a suspect screening approach

Search for halogenated compounds based on isotopologue pattern (e.g., HaloSeeker)

Léon et al., Anal. Chem. 91:3500

Search for compounds with time trends in time-series samples

Plassmann et al., Anal. Bioanal. Chem. 408, 4203

Compound-class specific analysis (diagnostic fragments/neutral losses)

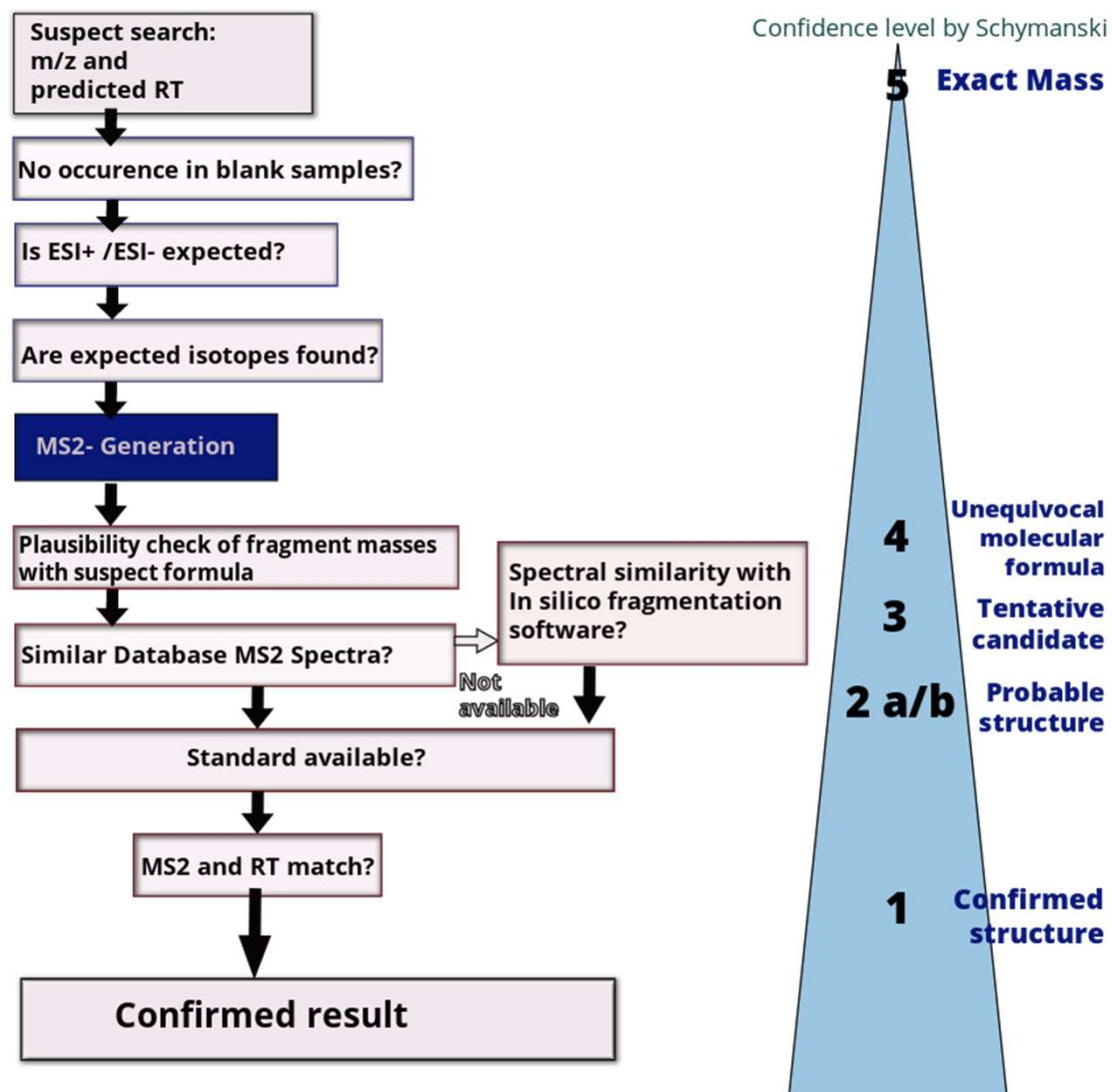
Comparison of exposure and control groups

Massive (metabolome) annotation and expanding MS² libraries

Current activities at UFZ

- Automated high throughput suspect screening for heterocyclic and aromatic amines and their conjugates in direct injected urine samples based on tandem MS information
- High throughput screening strategy for biomarkers of pesticide exposure using in-vitro generation of metabolites

Suspect screening workflow at UFZ

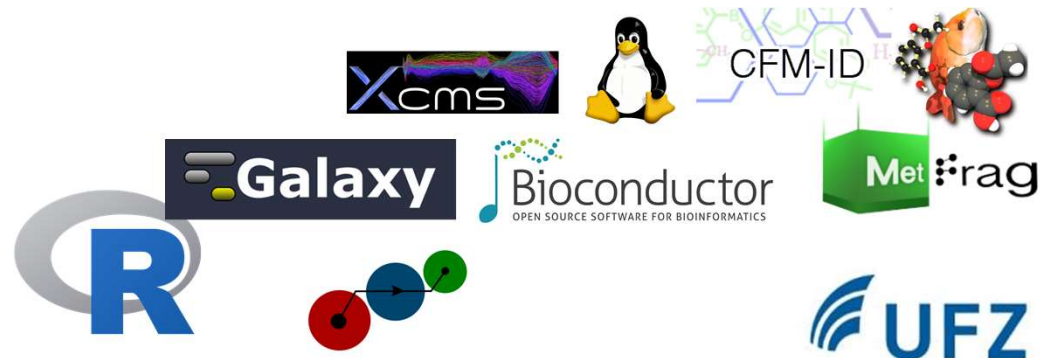


Identifying Small Molecules via High Resolution Mass Spectrometry:
Communicating Confidence

Emma L. Schymanski,^{*,†} Junho Jeon,[†] Rebekka Gulde,^{†,‡} Kathrin Fenner,^{†,‡} Matthias Ruff,[†]
Heinz P. Singer,[†] and Juliane Hollender^{*,†,‡}

Computational processing of HRMS data

- Development of workflows based
- on open source software packages
- Use of high performance computing
Linux (centOS7) based calculation cluster
2564 cores / 25.8 TB RAM
- Example:
 - ~ 20 000 detected features per sample
 - >100 000 aligned features per batch (25 samples + QC)



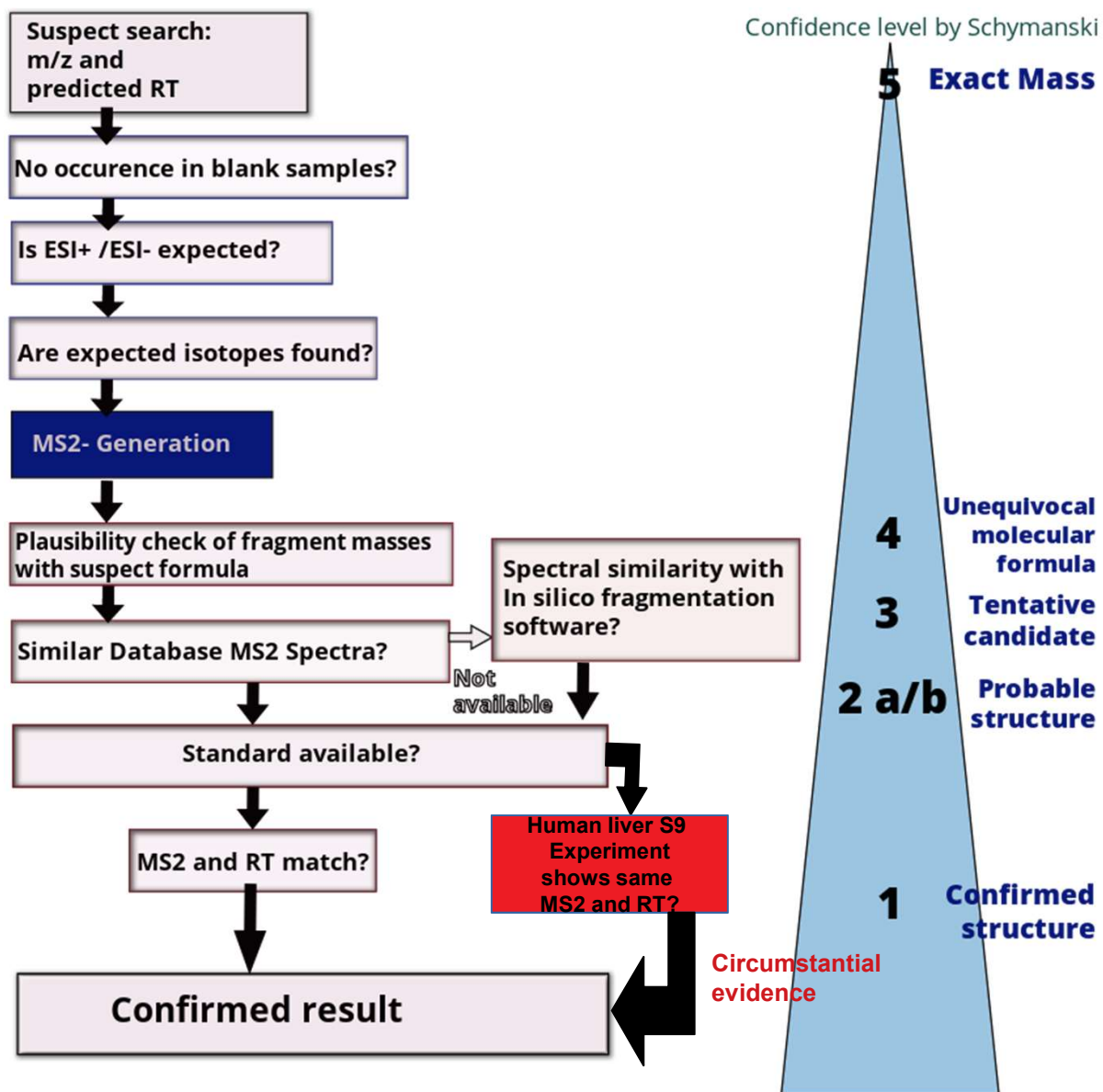
Obtaining metabolite information for possible biomarkers

- Strategy I:
Literature Search => registration dossiers of pesticides
~130 parent compounds => List of ~800 suspects

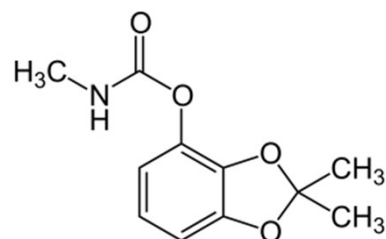
⇒ only a few of them are commercially available at reasonable prices
- Strategy II:
S9 human liver experiments with parent compounds

⇒ Will also facilitate identification
(RT and MSMS data generation of statistically relevant features)

Getting higher confidence for candidates



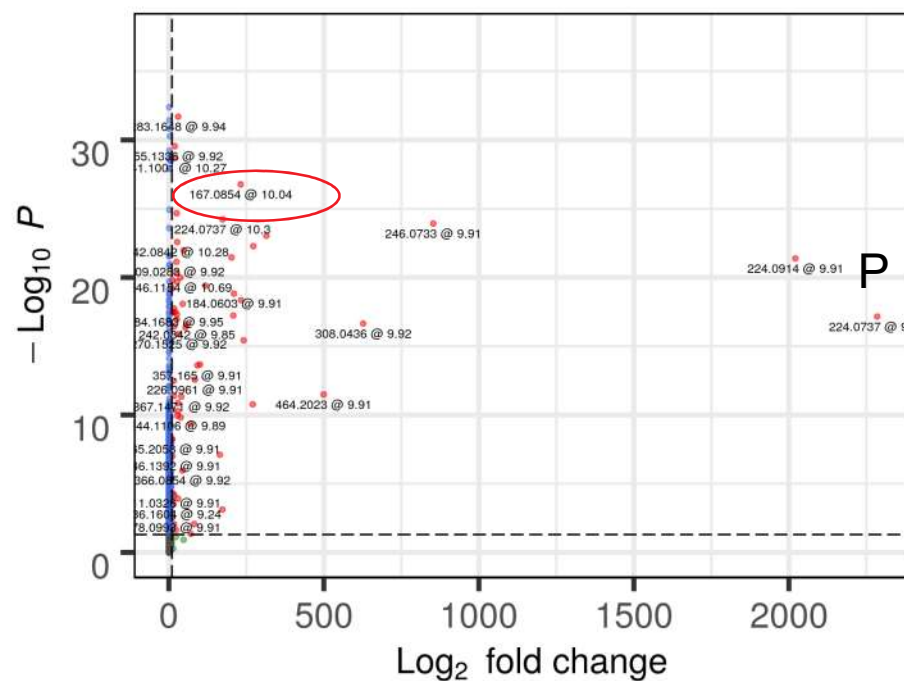
Human S9 liver experiments



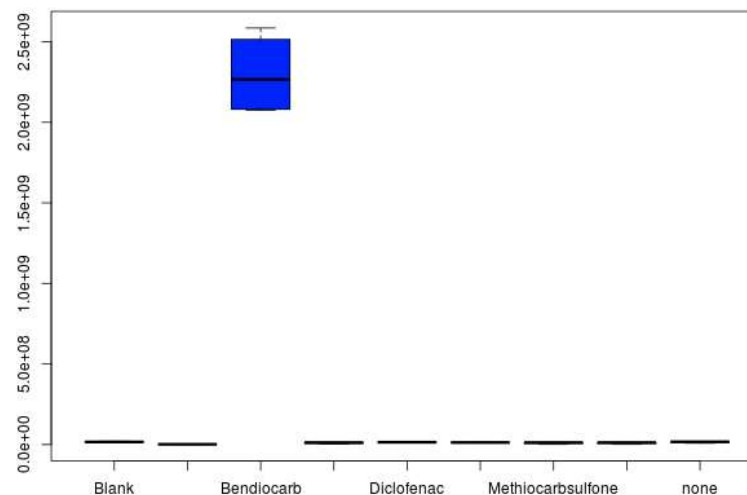
Monoisotopic
mass:
223.0845 u

S9 metabolism of Bendiocarb

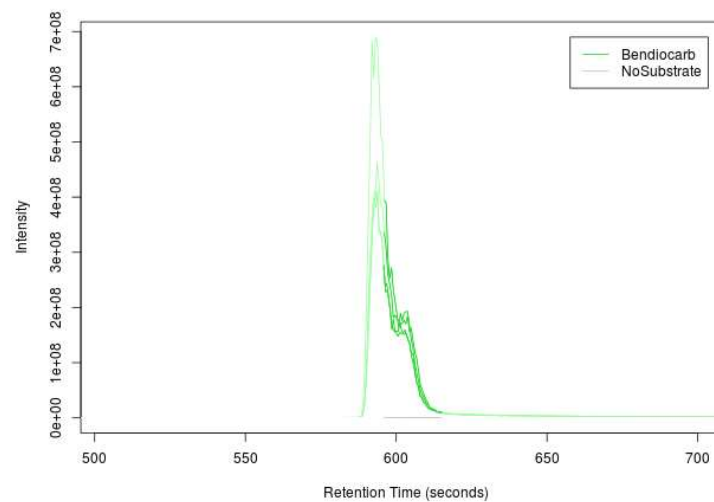
● NS ● Log2 FC ● P ● P & Log2 FC



Feature 167.1/603



Extracted Ion Chromatogram: 167.0851 - 167.0858 m/z



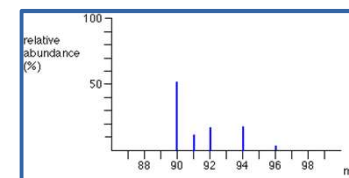
Current activities within work package 16

- LC/GC-HRMS profiling of a exchange of existing EU cohort studies

⇒ Review of results 20-21/05/19



- Expanding HBM-relevant capabilities MS/MS libraries



- Curation of a suspect list of emerging contaminants for HBM (including metabolites)

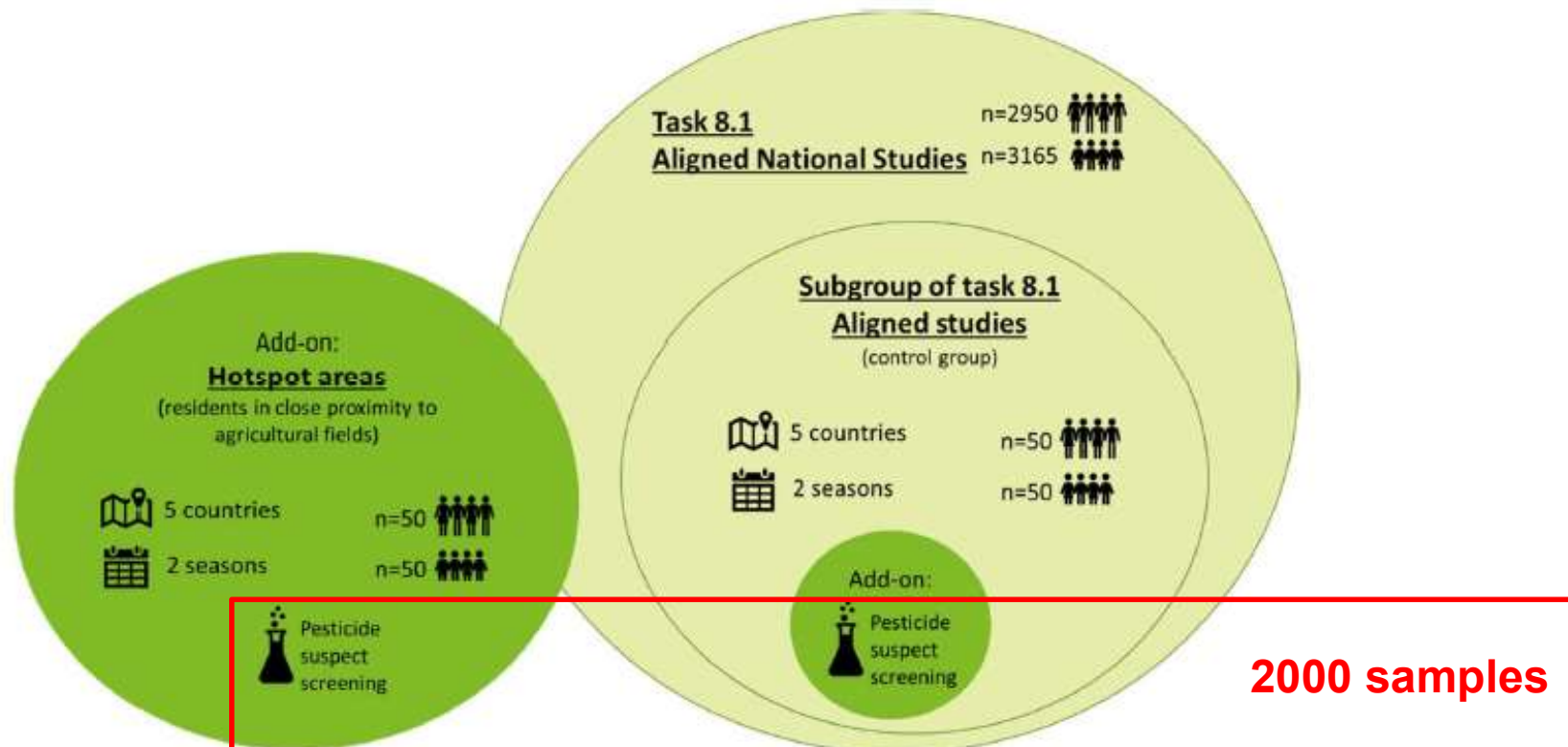


- Comparison of existing/developed methods for
- quality assessment of screening procedures



Upcoming activities in WP 16

- WP 15-16 interaction: Joint survey on pesticide exposure in hotspots and control areas



Need to share work among four groups ⇒ Harmonisation

Thank you very much for the attention!

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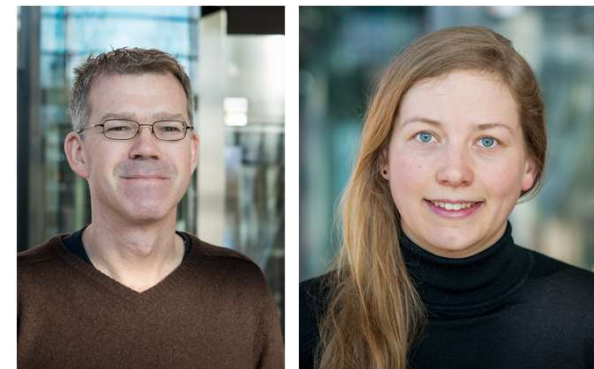
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WP16 HMB4EU (WP Leader Jean Phillippe Antignac, Laberca Nantes)