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

Martin Krauss & Carolin Huber
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

All compounds in a sample

Extraction / enrichment / clean-up

Chromatography

Ionisation

Compounds in a target method

What we can see in our screening method
Non-target screening: workflow

Modified from Hollender, Schymanski, Singer & Ferguson, 2018, ES&T Feature, 51:20, 11505
Non-target screening: workflow

Site-specific contamination
Real-time monitoring
Before/after comparison
Retrospective analysis

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
Integration!

Identification

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

• Xenobiotic compounds are typically small peaks against the biological background
• Severe matrix effects
• MS$^1$ and MS$^2$ spectra have a high background noise

Urine samples (1 batch), direct injection after filtration

>180'000 peaks
Challenges in NTS of biological samples

Different strategies

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

We start with a suspect screening approach

Massive (metabolome) annotation and expanding MS\(^2\) 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, Rebeka 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

- Suspect search: m/z and predicted RT
- No occurrence in blank samples?
- Is ESI+/ESI- expected?
- Are expected isotopes found?
- MS2- Generation
- Plausibility check of fragment masses with suspect formula
- Similar Database MS2 Spectra?
- Spectral similarity with in silico fragmentation software?
- Standard available?
- MS2 and RT match?
- Confirmed result

Confidence level by Schymanski

1. Confirmed structure
2. Probable structure
3. Tentative candidate
4. Unequivocal molecular formula
5. Exact Mass

Human liver S9 Experiment shows same MS2 and RT? Circumstantial evidence
Human S9 liver experiments

Monoisotopic mass: 223.0845 u

S9 metabolism of Bendiocarb

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

2000 samples
Thank you very much for the attention!

Helmholtz Centre for Environmental Research
Department of Effect Directed Analysis
Department Leader Werner Brack

martin.krauss@ufz.de
carolin.elisabeth.huber@ufz.de

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