National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Human biomonitoring for the identification of real-life mixtures

Mirjam Luijten

Mirjam.Luijten@rivm.nl

Chemical mixtures: the issue

- People are continuously exposed to a **multitude of substances** from different environmental sources, via different routes and duration of exposure

- Exposure to chemical mixtures may **increase health risks of individual substances** due to potential combination effects

- Due to the large number of chemicals present in the environment, **mixture risk assessment is complex** and poses a number of challenges for scientists, risk assessors and managers

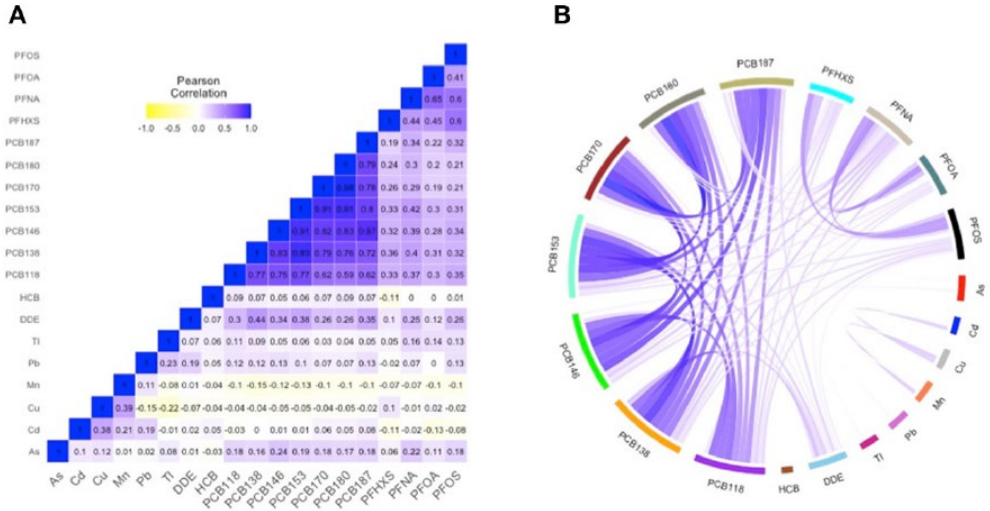
Human biomonitoring

- Within HBM4EU we aimed to achieve a better understanding of the exposure to chemical mixtures and the resulting health effects in Europe through human biomonitoring

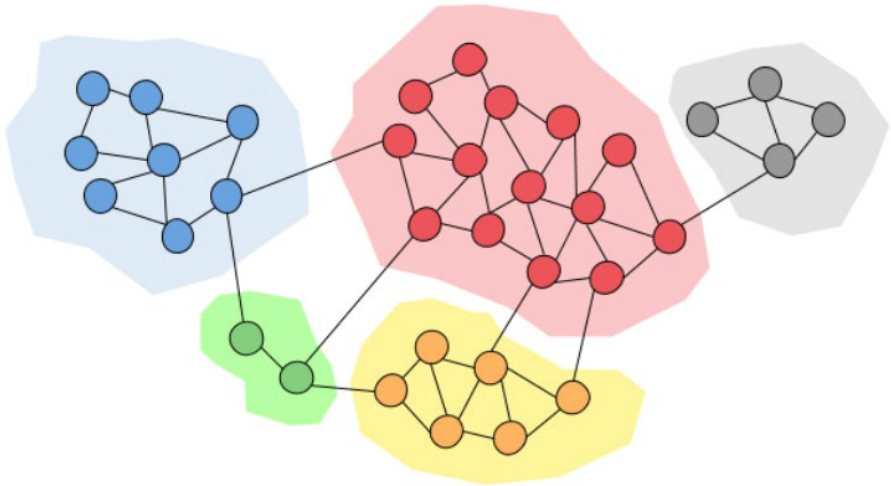
- Therefore, we
  - determined **real-life mixtures** by making use of existing data from studies across Europe
  - generated new data on **pesticide exposure** using suspect screening analyses
  - (developed an advanced workflow for assessing mixture health effects)

Network analysis in HBM

- For the identification of real-life mixtures we evaluated existing HBM data using correlation network analysis
- Multiple exposure biomarkers are measured in the same individuals in HBM studies, but correlation patterns among biomarkers have been so far largely ignored.
- There are several ways to present correlation information, but the interpretation is often difficult.
- Networks provide a graphical method to represent groups or communities in the data, which has been used widely in other research fields.



From: Ottenbros et al, 2021.
DOI: 10.3389/fpubh.2021.590038



Slide modified from Jelle Vlaanderen

Network analysis in HBM4EU

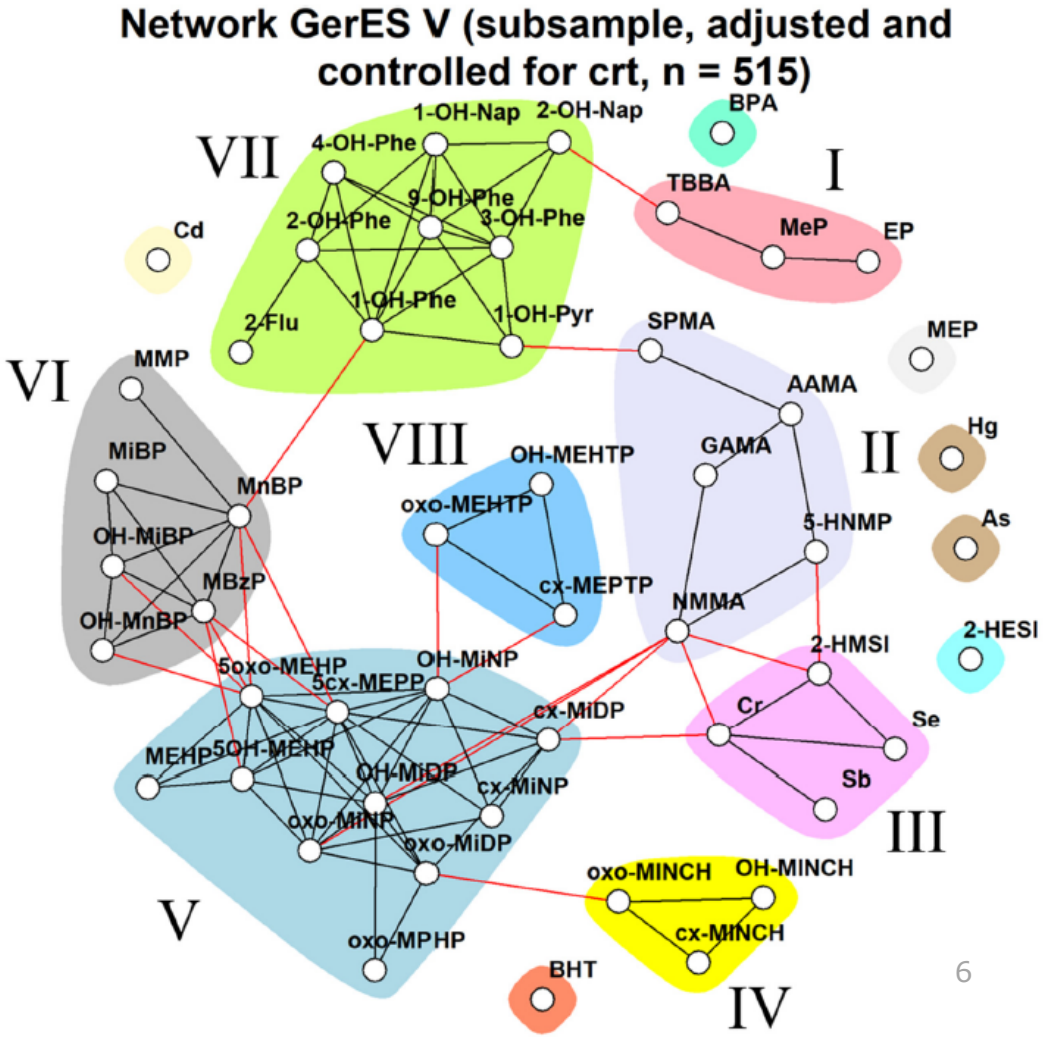
- Proof of principle was developed on simulated data, and subsequently tested on FLEHS data set

- Network analysis was applied to datasets from four different countries across Europe, i.e. Germany, Belgium, Spain and the Czech Republic

- The four studies are quite diverse, both in terms of the exposure biomarkers measured and in study design and population groups involved

Network analysis in HBM4EU - Example

- Example shown for data set from GerES V subsample (morning urine, participants age 3-17 years)
- Network analysis reveals 'communities': groups of correlated exposure biomarkers, reflecting co-occurrence of chemicals
- Observed communities include a) PAHs; b) acrylamide, benzene, aprotic solvents and methylisothiazolinones; c) parabens and lysmeral
- Remarkably, some substances are not part of a community, e.g. BPA and mercury



From: Rodriguez Martin et al, 2023. DOI: 10.3390/toxics11030204

Network analysis: conclusions

- Network analysis of existing human biomonitoring studies reveal that **combined exposures** to chemical substances are **common** and occur in all population groups

- Identified communities consist of substances from **different chemical classes** (which may be regulated under different regulatory frameworks)

- Integration with toxicological and concentration data is crucial for further interpretation: which communities are of higher concern, i.e. have a combined body burden that is of **potential health concern**?

Network modeling: toxicity weighing

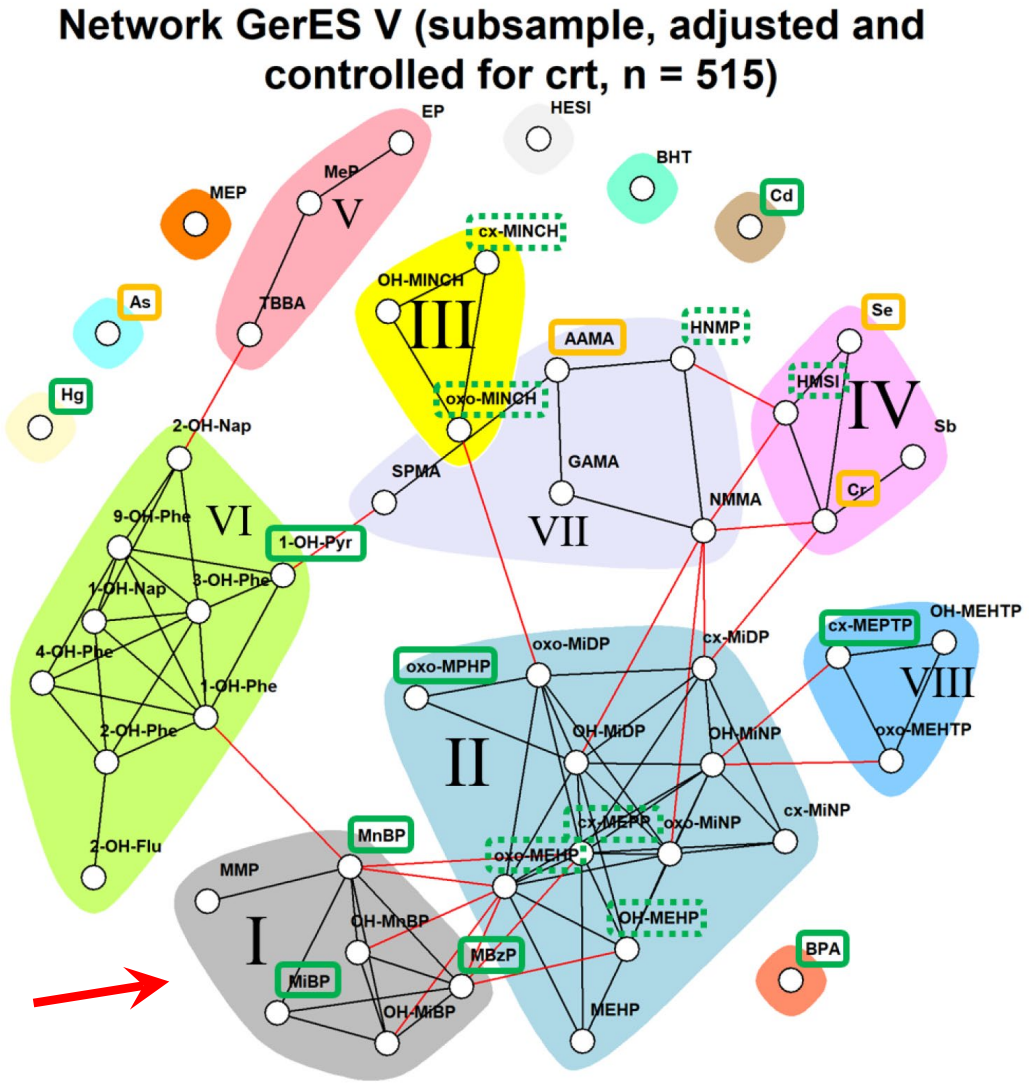
- To address this issue, we applied the Hazard Index (HI) approach

- A database of HBM - health-based guidance values (HBM-HBGVs) and equivalents was developed and used for deriving Hazard Quotients (HQ) for each substance:

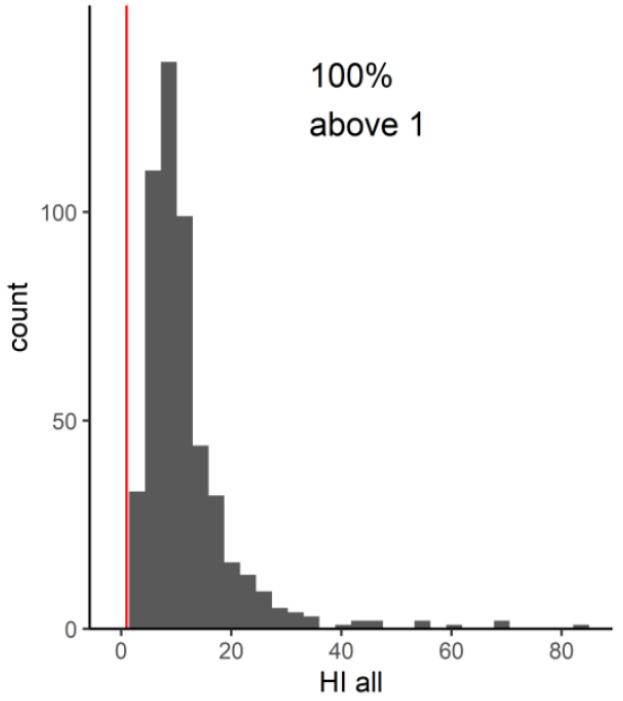
$$HQ_i = C_i / \text{HBM-HBGV}_i$$

- The resulting HQ values were used for deriving the Hazard Index (HI) across substances: $HI = \sum_{i=1}^{n} HQ_i$

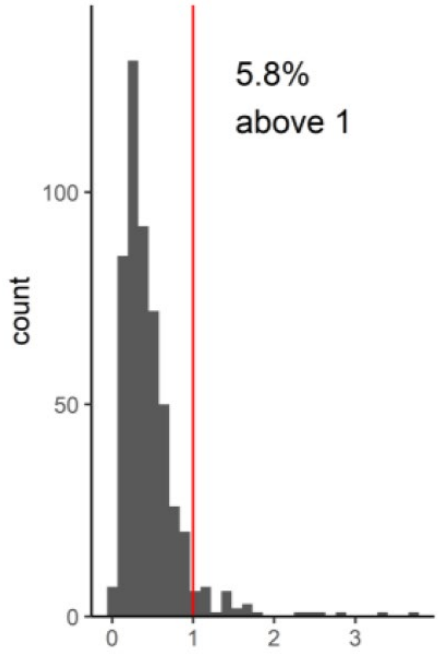
Network modeling: toxicity weighing



All substances



Phthalates



From: Loh et al, 2023. DOI: 10.3390/toxics11050408

Network analysis: conclusions

- Network analysis of existing human biomonitoring studies reveal that **combined exposures** to chemical substances are **common** and occur in all population groups

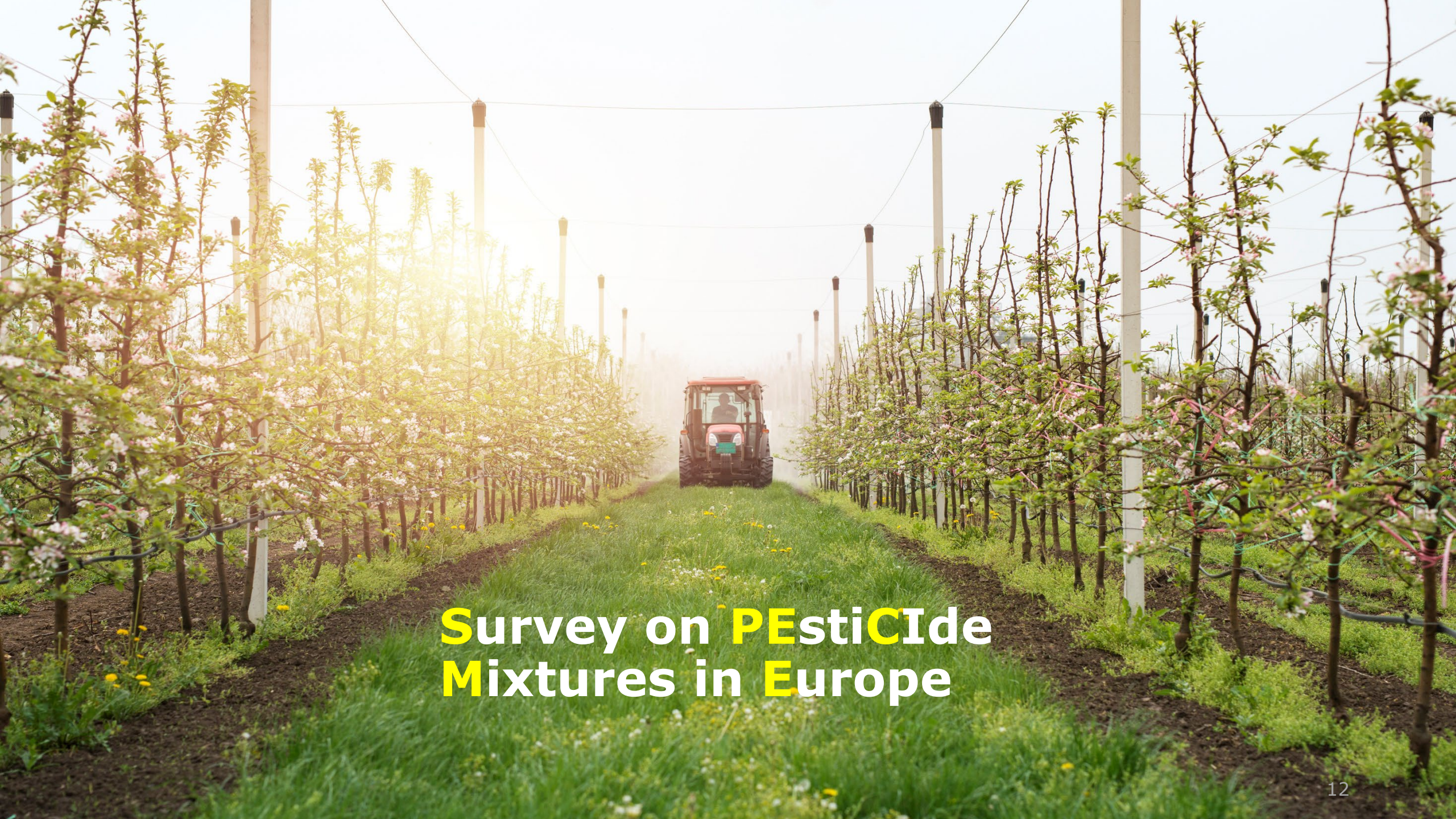
- Identified communities consist of substances from **different chemical classes** (which may be regulated under different regulatory frameworks)

- Integration with toxicological and concentration data is crucial for further interpretation: **toxicity weighting** can be applied at the level of identified communities, but its effective use is limited by the lack of HBM-HBGVs.

Human biomonitoring

- Within HBM4EU we aimed to achieve a better understanding of the exposure to chemical mixtures as well as the resulting health effects in Europe through human biomonitoring

- Therefore, we
  - determined **real-life mixtures** by making use of existing data from studies across Europe
  - generated new data on **pesticide exposure** using suspect screening analyses
  - (developed an advanced workflow for assessing mixture health effects)


Survey on PEstiCIde
Mixtures in Europe

SPECIMEn study

Focusing on pesticides, HBM4EU aimed:

- To generate **new exposure data** across Europe on a **broad combination of pesticides**
- To assess **possible local contributions**

Specific research questions were as follows:

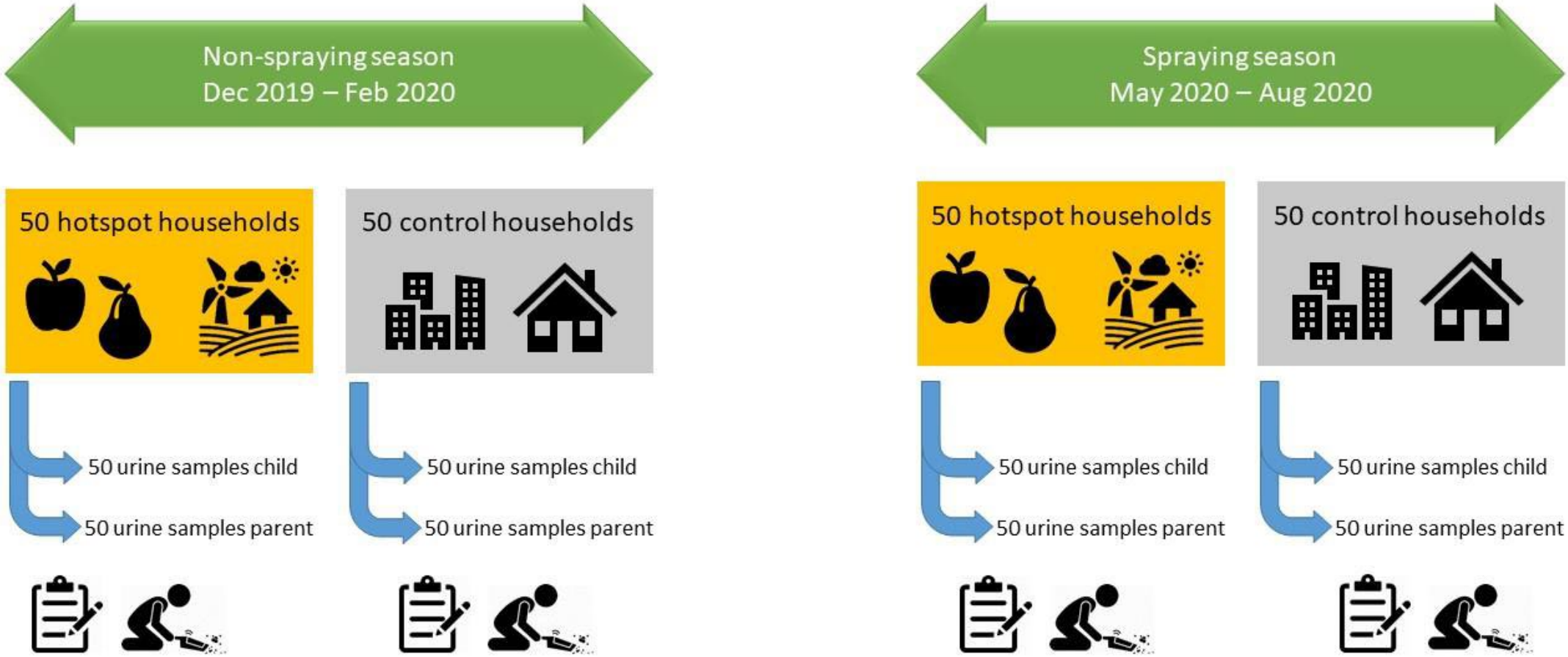
1. Which combinations of pesticides are most commonly detected?

2. Can we identify **hotspots?** Do people living close to pesticide application sites have higher exposure levels compared to people living further away?

3. Do these combinations differ **between seasons** (spraying *versus* non-spraying)?

4. Do these combinations differ **between age groups and study populations** in different countries?

SPECIMEn study



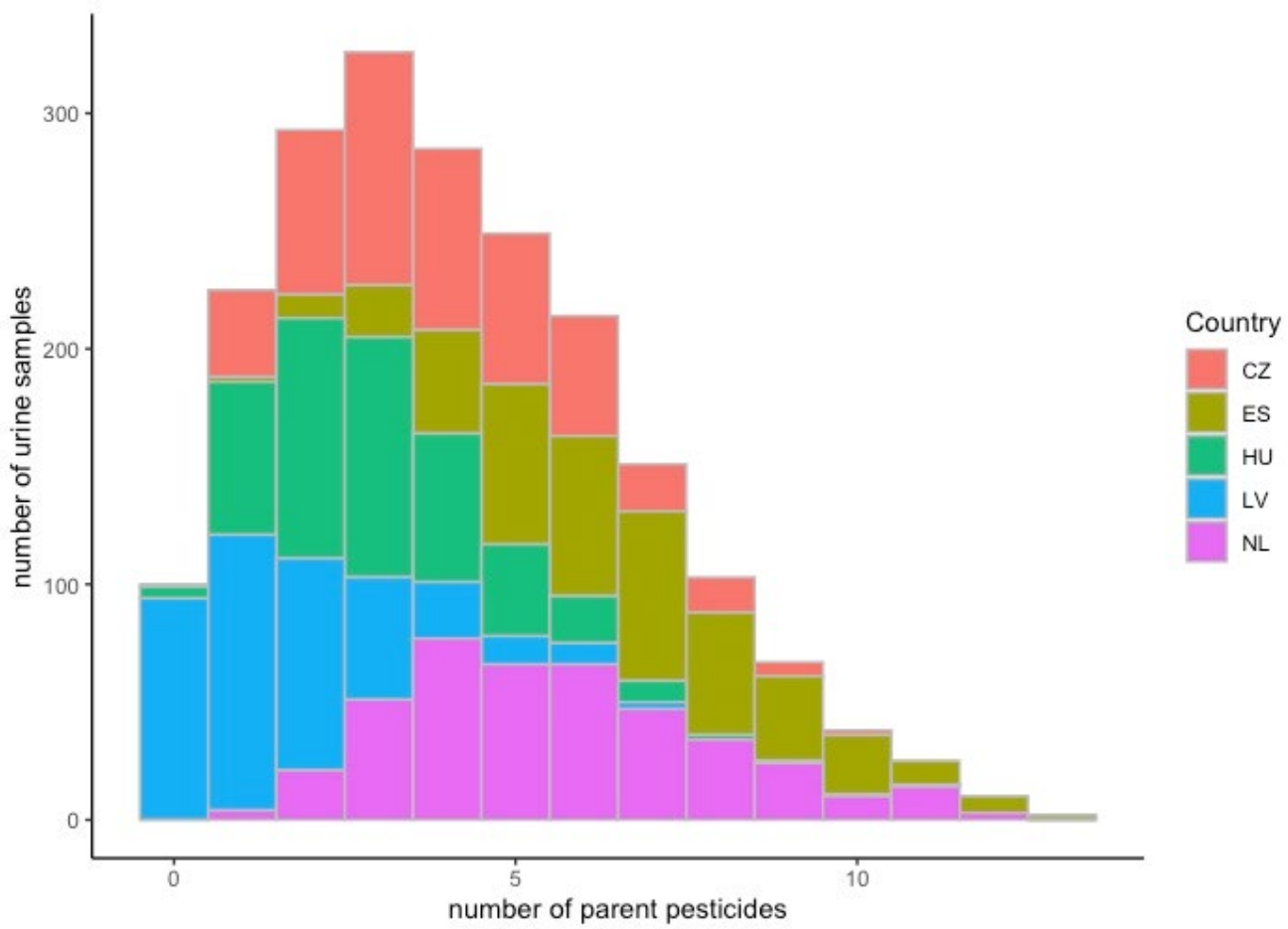
Hotspot: <250 meters from agricultural areas

SPECIMEn results

- Urine samples were subjected to a **consolidated** and **harmonized methodological workflow** for suspect screening (Jean-Philippe Antignac; INRAE)

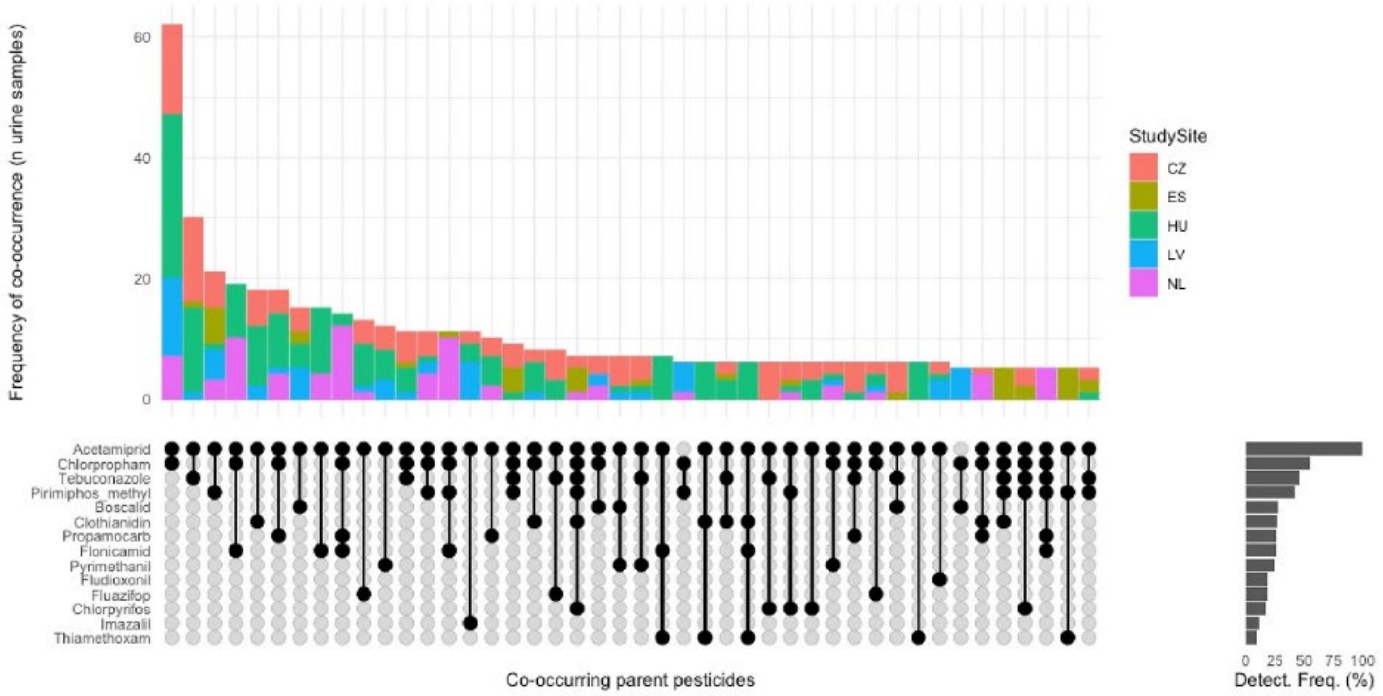
- In total **95 pesticide-related markers** were identified; of these, nearly half was identified with a high level of confidence.

- Pesticide-related markers identified with a high level of confidence relate to **29 parent compounds**. Examples include acetamiprid, chlorpropham, boscalid, and clothianidin.



From: Ottenbros et al, 2023. DOI: 10.1016/j.ijheh.2022.114105

SPECIMEn results



From: Ottenbros et al, 2023. DOI: 10.1016/j.ijheh.2022.114105

- Detection frequencies for parent pesticides varied substantially between countries.
- Many of the pesticides identified showed differences in detection rates when comparing hotspot areas *versus* control areas, samples collected in summer *versus* winter, and children *versus* adults; however, differences were in many cases not significant. The significant differences were not consistent across countries.

SPECIMEn: Conclusions

- Using suspect screening analyses, HBM4EU generated **valuable exposure data** across Europe on a **broad combination of pesticides.**

- We did **not observe consistent strong contributions from agricultural application** to detection rates in hotspots or in spraying season.

- **Suspect screening** is a **valuable** approach to get a broader overview and a semi-quantitative evaluation of substance exposures across the EU. This allows prioritization of substances for targeted analysis and comparison of the suspect screening data with reported substance usage.

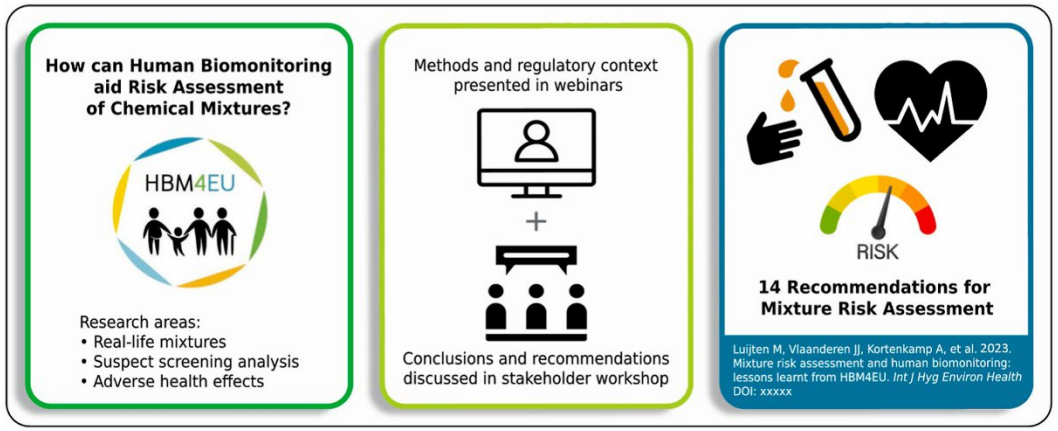
- **Mixture risk assessment** would strongly benefit from a **strategy for the measurement of multiple exposure and effect biomarkers in the same subject in HBM programmes**. This requires the development of an inclusive HBM/exposome infrastructure in Europe.

Mixtures and HBM4EU: Lessons learnt

- Topics addressed:
  - Network analyses to identify real-life mixtures
  - Suspect screening analyses
  - Health effects due to exposure to mixtures

- HBM4EU outcomes were used for drafting conclusions and recommendations, which have been discussed in a stakeholder workshop

- Final result: 14 recommendations for Mixture Risk Assessment



Useful links

Publications

- Network analysis
  - **Ottenbros** et al. 2021, doi: 10.3389/fpubh.2021.590038.
  - **Rodriguez Martin** et al. 2023, doi: 10.3390/toxics11030204.
  - **Loh** et al. 2023, doi: 10.3390/toxics11050408.
- Suspect screening
  - **Huber** et al. 2022, doi: 10.1016/j.envint.2022.107452.
  - **Ottenbros** et al. 2023, doi: 10.1016/j.ijheh.2022.114105.
- Lessons learnt: **Luijten** et al. 2023, doi: 10.1016/j.ijheh.2023.114135.

Webinars

- https://www.hbm4eu.eu/result/events/trainings/

Especially to all partners contributing to mixture activities in HBM4EU, as well as all HBM participants

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.