HBM4EU science and policy for a healthy future

Briefing on HBM4EU work on pesticides

This briefing provides an overview of HBM4EU work on pesticides. It is intended to inform the work of the European Commission in developing the Farm to Fork Strategy for sustainable food, foreseen to be delivered under the European Green Deal.

The forthcoming Farm to Fork Strategy aims to "increase the level of ambition to reduce significantly the use and risk of chemical pesticides, as well as the use of fertilisers and antibiotics. In parallel, the Commission's regulatory framework will need to reflect scientific evidence on the risk posed by chemicals such as endocrine disruptors."

1. How will HBM4EU support the Farm to Fork Strategy?

HBM4EU - the European Human Biomonitoring Initiative - is co-funded by the EU under the European Commission's Horizon 2020 programme and is investigating the exposure of the European population to 18 priority chemical substances and substance groups including pesticides and resulting impacts on human health. These active substances were identified through a prioritisation exercise involving the national authorities from across 28 HBM4EU partner countries, as well as Directorate-Generals of the European Commission (GROW, EMPL, ENV, SANTE, RTD, JRC) and relevant EU agencies (EEA, ECHA and EFSA).

Diet is the main pathway of intake for several of these chemical groups, and as such, HBM4EU is generating valuable evidence of immediate relevance to the "Farm to Fork" strategy. HBM4EU can also inform on the relative weight of the different exposure routes which is valuable in pesticide hot spot areas.

As mentioned in the European Commission's roadmap for the Farm to Fork Strategy, the current food system needs to be changed to significantly reduce the use of pesticides and fertilizers and the related environmental and human health risks. A number of policy measures aiming at reducing pesticide use and managing risks to human health and the environment are already in place in the EU. Based on an understanding of current human exposure to pesticides and health impacts, it may be relevant to strengthen these policy ambitions to deliver a high level of protection of human health.

In order to secure the trust of the regulated communities, stakeholders and the public, it is essential to demonstrate the effectiveness of existing policies through monitoring activities. **Human biomonitoring can be used to establish a baseline of exposure for the European population against which to measure the effectiveness of future risk management measures aimed at minimising human exposure to pesticides under the Farm to Fork Strategy.**

Conducted through harmonised approaches at European scale, human biomonitoring provides a tool for monitoring the exposure of the European population to chemicals. In addition, comparable human biomonitoring data from across Europe can allow an understanding of regional differences and can help to identify vulnerable groups, in order to inform targeted measures to reduce exposure. Internal exposure data provides a complete picture of human exposure, including via diet as well as via other exposure pathways. As such, **human biomonitoring data can be used to improve chemical risk assessment by providing information on actual human exposure via multiple exposure pathways.**

HBM4EU work on pesticides focusses primarily on the following pesticides: chlorpyrifos, glyphosate and pyrethroids. The exposure to pyrethroids is expected to be increasing as they have replaced organophosphates (OPs) in biocidal products and to some degree also as insecticides in agriculture.

HBM4EU is **generating data on current internal exposure levels of the EU population** to those specific prioritised pesticides, including new data on children's exposure in different EU-countries through the **aligned studies**[1], by analysing urine samples.

In relation to body weight, children eat more than adults, and expressed per kg body weight, they are therefore more exposed to pesticide residues in food. In consultation with EFSA's CONTAM panel, intake calculations based on the main dietary sources of exposure and measurements of pesticide residues in food items will be related to internal exposure levels. Potential health risks will be characterised with special focus on pregnant women and children as vulnerable population groups.

In addition, HBM4EU is monitoring environmental exposure to **mixtures of pesticides** in urine samples from residents living in **hotspot areas,** close to agricultural fields (orchards), and in control areas in five European countries. The advantage of hotspot area analysis is different exposure pathways that may be involved, such as air, diet and water. This monitoring is based on an innovative suspect screening approach, aiming to characterize exposure patterns to multiple pesticides by looking at detection frequencies for several hundreds of pesticide related exposure markers.

HBM4EU has also provided input to a request from DG SANTE on copper (Cu) compounds, through the Rapid Response Mechanism available on the website, related to their renewal as active substances for plant protection products. HBM4EU was requested to assess human biomonitoring data and to establish whether all Cu compounds are similarly absorbed and excreted, whether Cu compounds used as plant protection products are contributing more to the burden than other sources and whether a cumulative exposure assessment can realistically bring an added-value to the risk assessment of Cu. Aggregated HBM data on copper from 13 different countries were collected from HBM4EU National Hubs or data owners. Parameterisation of the generic PBTK model of the INTEGRA platform was also performed for integrated exposure assessment. The HBM data provided no strong conclusions and additional toxicokinetic data are needed to cover higher exposure patterns related to occupational exposure, as well as to capture the short exposure regime dynamics and how they may affect copper homeostasis.

2. What policy questions will HBM4EU answer?

HBM4EU reviews available evidence and generates new data on human exposure and associated health impacts in order to answer key policy questions. Regarding the Farm to Fork Strategy, key questions are listed below.

1. What are the current exposure levels and patterns of the EU general population to pesticides?
2. What other exposure pathways are important?
3. Are the current exposure levels of vulnerable groups, such as pregnant women and children, and inhabitants of hotspot areas, of health concern?

3. How is HBM4EU answering these policy questions

HBM4EU is implementing a broad range of activities in order to address policy questions on human exposure to chemicals. Activities are briefly outlined below against a more detailed set of questions.

1 The aligned studies aimed at collecting HBM samples and data from (national) studies to derive current internal exposure data representative for the European population/citizens across a geographic spread.

What are the current internal exposure levels of the EU general population to the prioritised pesticides?

- Collecting and assessing existing biomonitoring data on priority pesticides from across European partner countries
- Analysis of children's urine samples from studies aligned to produce comparable data across 8 European countries
- Analyses of pooled data from countries across the European Union in order to assess time-trends, differences between countries and population groups, including identification of subpopulations with high exposure levels (occupational exposure will not be assessed due to the lack of HBM data in Europe)

Which are the most suitable biomonitoring methods and exposure biomarkers?

- Evaluation and selection of biomarkers of exposure best suited for assessing human exposure to priority pesticides
- Harmonisation of methods for analysing urinary metabolites of pyrethroids, chlorpyrifos and glyphosate across partner countries to obtain comparable values and limits of detection (LODs), and testing new biomonitoring assessment of a large set of pesticides using a suspect screening approach

What are the main dietary sources of exposure across the member states?

- Model HBM data in relation to monitoring data on residues in food samples to 1) compare and complement exposure assessment performed by EFSA

What are the important non-dietary pathways of exposure?

- Exposure to pyrethroids via indoor use as biocides may be assessed via the aligned studies
- Assessing the internal exposure of populations living close to agricultural land treated with pesticides through hotspot studies coupling HBM and integrated exposure modelling

What are the cumulative risks of exposure to mixtures of pesticides?

- Assessing the exposure patterns of multiple pesticides among populations living in "hotspot areas" close to agricultural land where pesticides are applied, compared to a control group
- Assessing the combined exposure to pyrethroids using a common group-specific urinary metabolite, 3-phenoxybenzoic acid (3PBA), and investigating associations between 3PBA and health effects in the aligned studies

Are the exposure levels of health concern for vulnerable groups or high exposure population groups?

- Investigate associations between human biomonitoring data and health outcomes obtained in relevant studies, including prospective birth cohorts
- Identify key mechanisms and adverse outcome pathways (AOPs) for relevant health outcomes, including neurodevelopment and endocrine disturbances and develop a dedicated webserver
- Identify and suggest relevant effect biomarkers

- Further develop the mixture risk assessment methodologies (D15.4 Report on approaches to identify mixture health effects)

Is it possible to establish EU wide accepted human biomonitoring guidance values (HBM-GV) for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?

- Explore if HBM-GVs can be developed for certain pyrethroids and derive such values whenever possible (e.g. for deltamethrin, cyfluthrin or another pyrethroid)
- If no HBM-GV is available or cannot be derived in the project, the measured HBM values could be converted into model-predicted external exposure values using reverse dosimetry which then could be compared to acceptable daily intake (ADI).

Collating existing human biomonitoring data on exposure to pesticides

There is a wide variety of pesticides in use. Human biomonitoring data are available in some countries but are heterogeneous with respect to age group and substances that are measured. At EU level, the database is very fragmented. The studies indicate widespread exposure of specific substances in the general population.

The review of existing studies is summarised in the scoping document on pesticides. Moreover, it will be explored if it is possible to look into determinants of exposure in some of these data collections for which individual data are available.

Producing data on the exposure of the European population to pesticides

A principle activity under HBM4EU is the establishment of a European human biomonitoring Platform to collect data on the internal chemical exposure of citizens across Europe. Aligning national studies across Europe to deliver comparable, coherent datasets has proven a challenging task.

Under HBM4EU, human biomonitoring studies across 21 countries[2] have been harmonised in terms of the sampling, survey and analytical approach, known as the **aligned studies**. This will deliver comparable data on human exposure to pesticides across Europe, an important input to the assessment and management of pesticides risks to human health.

For this purpose, **Europe was divided into North (with approx. 21% of the population), South (28% of the population), East (11% of the population) and West (41% of the population) according to the United Nations geoscheme of Europe.** Studies are collecting samples from three age groups of interest, including:

- children aged 6-11 years old
- teenagers aged 12-19 years old
- adults aged 20-39 years old

Each country provides 300 samples, except for a few smaller countries that are providing a lower number of samples.

[2]Of the 28 partner countries, 21 countries were able to align ongoing studies to the HBM4EU approach in order to generate comparable exposure data.

For the pesticides, samples from **children will be analysed to assess exposure to glyphosate, pyrethroids and chlorpyrifos in Norway, Slovenia, Italy, Cyprus (to be confirmed), France, Germany, Belgium, the Netherlands and Israel.**

For **adults, analysis will be done in samples from Switzerland and Israel.** Participation of other countries has not confirmed yet.

For an overview of the pesticide study distribution please see Table 1 and Table 2 in the following page.

Figure 1 below provides the timeline for the generation of harmonised datasets on population exposure to pesticides in Europe

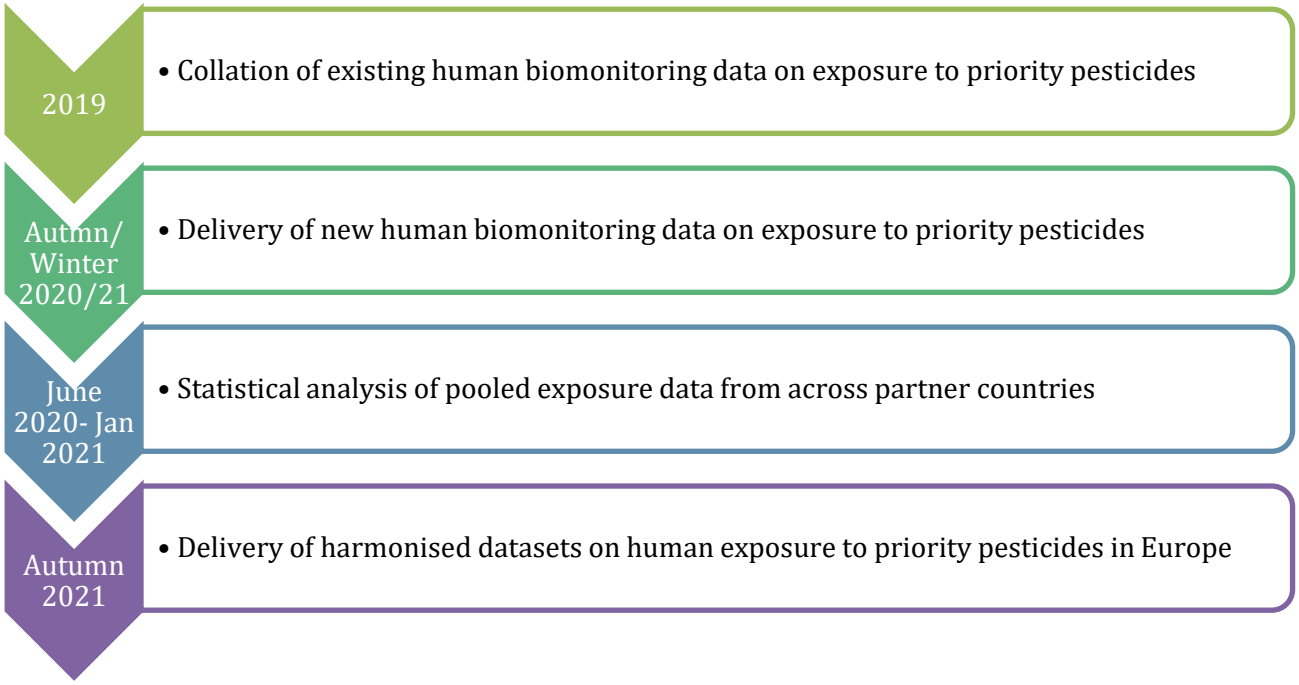

Figure 1 Timeline for the delivery and analysis of exposure data produced under the national studies

In December 2019, the standing committee on plants, animals, food and feed (SCOPAFF) voted against the renewal of the authorisation for both chlorpyrifos and chlorpyrifos-methyl, which was due to expire on 31st January 2020. However, the population will still be exposed from residues in goods produced outside the EU.

It is likely that other organophosphates (OPs) still in the market will replace chlorpyrifos and chlorpyrifos-methyl and therefore discussions are taking place to include dialkyl phosphates (DAPs, a common urinary metabolites of OPs) in HBM4EU studies. This would allow **for future HBM programs to monitor if DAPs will decrease along with the specific metabolite of chlorpyrifos and chlorpyrifos-methyl (TCP_Y).**

Analysing for TCP_Y in samples from the aligned studies will provide a baseline value on human exposure prior to the ban, allowing for an evaluation of the efficacy of the ban in reducing exposure.

Table 1 Children's (6-11 years old) pesticide analysis in urine. √ = new analysis, - = no analysis, (√) = data already available no new analyses planned

CHILDREN										
	North	East	South			West				Other
	NEBII	POLAES	SLO CRP	NAC II	Organiko	ESTEBAN	GerES V	3xG	Dutch SPECIMEn (control)	RAV MABAT
	Norway	Poland	Slovenia	Italy	Cyprus	France	Germany	Belgium	The Netherlands	Israel
	NIPH	NIOM	JSI	EPIUD	MOH-CY	ANSP	UBA	VITO/PIH	RIVM	MOH-IL
Pesticides (chlorpyrifos, pyrethroids and the metabolite TCPy)	√	-	(√) *TCPy, 3PBA, 4F3PBA*	√	√	(√) *chlorpyrifos 3-BPA F-BPA*	√	√	√	√
Pesticides (Glyphosate/AMPA)	√	-	(√) *Glyphosate, AMPA*	√	√	(√) *Glyphosate, AMPA*	(√) *AMPA*	√	-	√

Table 2 Adult's (20-39 years old) pesticide analysis in urine. √ = new analysis, - = no analysis, (√) = data already available no new analyses planned

ADULTS									
	North		East		West				Israel
	CPHMINIPUB (parents)	Diet_HBM	POLAES	INSEF-ExQAP	ESTEBAN		ESB	Oriscav-Lux2	RAV MABAT
	Denmark	Iceland	Poland	Portugal	France	Switzerland	Germany[3]	Luxembourg	Israel
	RegionH	UI	NIOM	INSA	ANSP	SWISS TPH	UBA	LNS	MOH-IL
Pesticides (chlorpyrifos, pyrethroids and the metabolite TCPy)	-	-	-	-	-	√	-	-	√
Pesticides (glyphosate and AMPA)	-	-	-	-	-	√	-	-	-

[3] Germany's ESB study is not representative of the population.

Assessing exposure to mixtures of pesticides in hotspots

HBM4EU is running a survey of human internal exposure to mixtures of pesticides across five of our partner countries: Hungary, Czech Republic, Spain, Latvia and the Netherlands. Switzerland will also collect urine samples, with a slightly different design. **The main aim is to generate new exposure data across Europe on a broad combination of pesticides and to assess possible local contributions (i.e. hotspot areas) and within-person variation.** This survey, entitled 'SPECIMEn', explores bystander exposure to pesticides and focusses on residential areas or "hotspots" close to agricultural fields where pesticides are applied. The survey is designed to assess concomitant/combined exposure to multiple pesticides in hotspot and control areas using human biomonitoring. A detailed description of the study protocol can be found in AD15.7 "Updated General Study protocol SPECIMEn study".

Hotspot areas are defined residential areas within a range of 250 meters to agricultural fields where pesticides are actively applied, specifically focussing on orchards (apples and pears), citrus groves, olive groves and vineyards. From each household from both areas, control and hotspot, urine samples of one parent and a child (6-11 years) will be collected. These samples will be subjected to, a pesticide suspect screening method, capable of detecting multiple pesticide related markers (parent compounds and metabolites) in a single assay in a semi-quantitative way (determination of detection frequencies, patterns of exposure, and variability). These suspect screening approaches are built on non-selective analytical workflow and allow the qualitative monitoring of several hundred (up to several thousands) of exposure markers, including various pesticide classes under their parent or metabolite form.

This suspect screening approach, based on HPLC-full-scan-HRMS and developed under harmonized conditions and consolidated QA/QC provisions, will be used to gain insight into the occurrence of extended exposure patterns of pesticide-biomarkers, differences across the countries participating in SPECIMEn, differences between two seasons (spraying season with active application, and non-spraying season with no active application) and/or location (living close to agricultural areas or not). The results obtained will also support mixture effect investigations and contribute to the prioritization of certain substances in terms of further exposure and risk assessment, and to possibly generate early warning information.

Understanding exposure pathways

To enable translation of HBM data into external exposure levels for comparison with e.g., ADI values, information on toxicokinetic properties is needed. Physiology-based toxicokinetic (PBTK) modelling will be used to link external exposure to internal dose in humans (e.g., concentration in urine) by describing the process of absorption, distribution, metabolism and excretion (ADME) that undergoes a substance in living organisms. A comprehensive review of human PBTK models available for the prioritized pesticides, has been performed. Most models concern adults while toxicokinetic data on sensitive populations (pregnant women, foetuses or children) are still missing.
Existing PBTK models for humans have been identified for four pyrethroids: deltamethrin (type II pyrethroids), permethrin (type I pyrethroids), cypermethrin (type II pyrethroids) and cyfluthrin (type II pyrethroids). Interestingly, one of the models for deltamethrin predicted a considerably higher brain concentration in humans than rats due to an almost six-fold higher cardiac output to the brain in humans. A generic model for pyrethroids has also been proposed as a tool to interpret the combined exposure to pyrethroids reflected by non-specific urinary biomarkers. Several PBTK models were also identified for chlorpyrifos and other organophosphate insecticides while no specific PBTK-models for neither humans or animal species were identified for glyphosate, the co-formulant polyethoxylated tallow amine (POEA) or fipronil.

The PBTK models identified above will be parameterised and used for forward or reverse dosimetry to either translate HBM data for the pesticides into external exposure doses for comparison with established guidance values for risk assessment (e.g., ADI) or to estimate the biologically effective dose of the compounds. However, most HBM studies on adverse health effects related to pyrethroid exposure are based on the group specific urinary metabolite 3PBA (3-phenoxybenzoic acid) reflecting the combined exposure to pyrethroids. The reason is that the detection frequency of more specific metabolites is typically very low because different pyrethroids are used alternately and they have short biological half-lives. Thus, besides comparing HBM concentrations of specific metabolites with the derived HBM-GV values, we shall investigate how group-specific biomarkers, such as 3PBA, can be translated into external exposure levels and integrated in assessment of cumulative risks of pesticide mixtures. Finally, in combination with food consumption and residue contamination data from EFSA, apportionment of exposure levels to different sources and pathways of exposure to pesticides will be performed to support effective regulatory decision- and policymaking.

Assessing impacts on health

Most of the prioritised pesticides are neurotoxicants (OPs, pyrethroids, fipronil) and some also have endocrine disrupting (ED) or genotoxic/carcinogenic properties. The main health concerns are adverse effects on neurodevelopment and/or endocrine disturbances affecting reproduction and metabolism.

HBM4EU will assess human biomonitoring (HBM) data from EU studies, e.g. from birth cohort studies, with health outcomes to explore exposure levels of health-relevance for vulnerable groups. Based on extensive literature search, several review publications are in process in which epidemiological evidence on adverse health outcomes (including neurodevelopment and reproductive/endocrine disturbances) will be integrated with mechanistic knowledge to identify plausible toxic mechanisms, potential AOPs, and suggestion of effect biomarkers. If possible, meta-analyses will be performed but is hampered by considerable differences in methods used for assessing the outcomes (e.g., neurodevelopment). A text mining tool (AOP-helpFinder) has been developed at INSERM. This tool screens automatically abstracts from the PubMed database. It has been applied to the HBM4EU prioritised pesticides to identify linkages with Adverse Outcome Pathway (AOP) events existing in the AOP wiki database. This tool will be applied to help identifying relevant AOP events. The data are included in a publicly available webserver (AOP4EUpest, http://www.biomedicale.parisdescartes.fr/aop4EUpest/search.php).

Besides, associations between HBM data and health outcomes are analysed within aligned study cohorts, e.g., between exposure in pregnancy and child health outcomes in the Odense Child Cohort and between glyphosate and DNA damage and immunological effects in the FLEHS IV cohort.