Prioritised substance group: Pesticides

Lead author	Helle Raun Andersen (SDU - DK)

Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

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1. Which are the most suitable methods and biomarkers of exposure?	In WP9 a prioritised list of biomarkers, matrices, and analytical methods has been elaborated (D9.5). The list present 9 suitable biomarkers for the category B pesticides (chlorpyrifos, glyphosate and pyrethroids and 4 biomarkers for the category C pesticides (dimethoate and fipronil). Urine is the selected matrix for all the compounds except fipronil for which serum is the preferred matrix. Glyphosate is excreted both unchanged and as metabolites in urine. One urinary metabolite, aminomethylphosphonic acid (AMPA), is also the main environmental degradation product. Since AMPA and glyphosate has similar toxic profile, it is advised to measure both glyphosate and AMPA in urine as biomarker for the total glyphosate exposure. Chlorpyrifos is metabolised to TCPy (3,5,6-trichloro-2-pyridinol) and two unspecific diethyl phosphate biomarkers (DEP and DETP) which are all excreted in urine. TCPy is the most specific biomarker for chlorpyrifos exposure although two other pesticides, chlorpyrifos-methyl and triclopyr, are also metabolised to TCPy. The biomarkers for pyrethroids include a group-specific biomarker, 3PBA (3-Phenoxybenzoic acid), representing the combined exposure to many pyrethroids, 5 semi-specific biomarkers representing exposure to two-five pyrethroids, and a specific biomarker for deltamethrin exposure. Two of the biomarkers have not been measured in large population studies, while the remaining pyrethroid biomarkers have been developed and included in different large biomonitoring programs. Dimethoate is rapidly metabolised to unspecific dimethyl phosphates (DMP, DMTP, and DMDTP) which are excreted in urine. No suitable specific biomarker for dimethoate was available. The sum of the molar urinary concentrations of the three dimethyl phosphates will provide an estimate of dimethoate and other methylated organophosphate pesticides. By also including the three diethyl phosphates (DEP, DETP, and DEDTP) an estimate of the total exposure to organophosphates (including dimethoate and chlorpyrifos) will be achieved. All six metabolites (dialkyl phosphates, DAPs) are normally analysed in the same analytical run. Fipronil is mainly metabolised to fipronil sulphone which can be measured in serum/plasma. This biomarker has only been used in few studies and most of these are animal studies. LC-MS/MS was evaluated to be the most suitable analytical method for analysing all the biomarkers except for the pyrethroid biomarkers, CIF3CA (Cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid) and trans-CDCA (trans-chrysanthemumdicarboxylic acid) for which GC-MS/MS and GC-HRMS, respectively, were assessed to be more suitable.

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	No information was found for biomarkers for the glyphosate co-formulant Polyethoxylated tallow amine (POEA) or the pyrethroid co-formulant piperonyl butoxide (PBO) and therefor no methods could be selected. A database of candidate laboratories for analysing the 2nd round of substances has been elaborated (D9.6) and 36 laboratories were listed as candidates for analysing pesticides. Regarding the ICI/EQUAS programme for the 2nd round of substances, it was decided that that the approach used for the 1st set of substances would not be feasible due to time limitations. Thus, a shortened ICI/EQUAS scheme focused on the substances foreseen to be analysed in the aligned studies was established. For the pesticides, 4 expert laboratories were selected for analysing biomarkers for pyrethroids (cis-DBCA, cis-DCCA, trans-DCCA, 3-PBA, 4-F-3-PBA, ClF3CA), glyphosate (glyphosate, AMPA), and chlorpyrifos (TCPy). These labs are included in the ICI programme planned to be completed by mid-2020
2. What are the current exposure levels of the EU population to the prioritised pesticides: pyrethroids, chlorpyrifos and dimethoate, glyphosate (in combination with polyethoxylated tallow amine (POEA)), and fipronil and do the exposure levels differ between countries? **3. What are the main dietary sources of exposure across the member states?** **4. What are other potential sources and pathways of exposure?** **5. What are exposure levels among occupationally exposed workers?**	WP10 has developed a general and a substance-specific statistical analysis plan including variables, which are needed for the statistical analyses to address research questions for the pesticides on general exposure level including identification of high exposure subgroups, time trends including potential impact of regulation, geographic comparisons, and exposure determinants (D10.5). A comprehensive review of the existing HBM-studies on the prioritised pesticides (including information on occupational exposures and other exposure determinants) performed within the HBM4EU associated countries is in progress in the scope of WP10. Overall, there are relatively few studies, several were performed before 2010, they are not EU-wide, and few studies address occupational exposures. Besides, analyses of the main dietary and non-dietary sources of exposure will be analysed using existing HBM-data from selected data-collections. A protocol for this work is in preparation in WP10. For dietary exposure, an attempt to analyse/model HBM data in relation to data on pesticide residues in food will be performed with the purpose to compare and complement exposure assessments performed by EFSA. In this regard, a thorough revision of the main sources and pathways of exposure to pesticides at international level was performed within WP7 (Task 7.3), in order to develop a specific questionnaire to characterise the exposure to these substances in the study population of the aligned studies (see below). This questionnaire is aimed at supporting the identification of the main sources and pathways of human exposure to pesticides across the member states related to residential environment and home exposures, dietary habits, lifestyle and occupation (D7.6). Further, a gap analysis was performed in WP7 to get an overview of the number of studies of the 2nd round of priority substances within the participating countries based on a questionnaire distributed in 2018 (D7.5). A total of 26 studies (conducted or initiated/ongoing) reported to analyse pesticides. Most of these studies were carried out in the western European-defined region followed by the North and South regions and Israel. No studies on pesticides were reported from the East region. Children were included in 12 of the studies while adults were included in 18, adolescents in 4, and elderly in 2 studies. Of these studies, 17 reported to have samples representative at national level (7 for children, 2 for adolescents, 8 for adults, and none for elderly). Within WP10, metadata for existing HBM data collections are integrated in IPCHEM, for which 16 data collections indicated to have HBM data on pesticides. From those, 9 data collections shared harmonised aggregated data for pesticides. These data can be accessed via the HBM4EU internal webpages. These data were included and visualised by exposure distributions in D10.6. To obtain a better EU coverage for HBM exposure data a sampling framework has been developed in WP8 by including 33 studies (known as the aligned studies) from 21 countries representing north, south, east, and west Europe. For the pesticides, biobanked urine samples from children aged 6-11 years from eight different countries (Norway, Denmark, Hungary, Slovenia, Italy, France, Germany, and the Netherlands) are planned to be analysed for glyphosate, chlorpyrifos, and pyrethroid biomarkers (D8.7 and

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	D8.8). In the SPECIMEn study performed in WP15 (see AD15.7 for detailed description), urine samples are collected during the spraying and non-spraying season from children and their guardians living close (within 250 m) to agricultural fields treated with pesticides as well as from urban control populations, in five EU countries. These urine samples will be analysed by suspect screening approach developed under WP16, aiming to qualitatively detect an extended range (i.e. several hundreds to thousands) of pesticide related markers of exposure through a developed harmonised analytical workflow (from sample preparation, LC-HRMS data acquisition to data processing and reporting) applied by five laboratories/countries (FR, NL, SP, CZ, DE). The results will gain insight in the feasibility of this analytical approach, and information on differences in detection frequencies of pesticides and exposure patterns between the included countries and subpopulations. From this provided picture of the reality of human internal exposure, these results will contribute to further rationale and prioritisation with regard to certain markers for which more quantitative and targeted methods will appears necessary and/or for which the inclusion in future HBM program will appears relevant.
6. Are the exposure levels of health-relevance/concern for vulnerable groups (infants, children and pregnant women) or high exposure population groups (e.g., occupational exposure)? **7. How can cumulative risks of pesticide mixtures on sensitive health outcomes be assessed and integrated in regulation?** **8. Is it possible to establish EU wide accepted HBM guidance values for the pesticides that takes into account potential mixture effects and evidence from epidemiological studies?** **9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory**	Within WP13 and WP14, comprehensive literature reviews on health effects and toxic mechanisms have been performed. The primary aim of the reviews was to assess HBM exposure levels associated with adverse health outcomes taking into account vulnerable exposure windows (pregnant women and children), and to identify the most important mechanisms and potential adverse outcome pathways (AOPs) and suitable effect biomarkers for the health outcomes of highest concern (D13.5 and D14.5). For chlorpyrifos, dimethoate, fipronil, pyrethroids and glyphosate, several classical and novel effect biomarkers for a range of health outcomes (cardiometabolic health, the immune system, pregnancy and reproductive outcomes, neurodevelopment and cancer) has been inventoried (D14.5). In the scope of WP13, the text mining tool (AOP-helpFinder) developed at INSERM was applied to the prioritised pesticides to identify linkages with AOP events existing in the AOP wiki database. This tool screen automatically abstracts from the PubMed database. The results have been collected, structured and presented in a webserver named AOP4EUpest (AOP4EUpest: Mapping of pesticides in Adverse Outcome Pathways using a text mining tool, published in Bioinformatics (Jornod et al., 2020)). A review publication entitled "Reproductive health risks associated with environmental and occupational exposure to pesticides" has been initiated. Besides, associations between HBM data and health outcomes are being analysed within some of the aligned study cohorts, e.g., between pesticide 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. Two joint WP13/WP14 review publications are in process in which epidemiological evidence on 1) developmental neurotoxicity (DNT) and 2) reproductive/endocrine disturbances will be integrated with mechanistic knowledge to identify plausible toxic mechanisms, potential AOPs, and suggestion of effect biomarkers. The AOP4EUpest is used to help identifying AOP events. If possible, meta-analyses will be performed but is hampered by considerable differences in methods used for assessing the outcomes (e.g., neurodevelopment). Since chlorpyrifos/chlorpyrifos-methyl did not get renewal of authorisation (expired 31 January 2020), it was decided to focus on pyrethroids in these reviews. Based on examples from the 1st set of substances it was concluded in WP5 that HBM would be particularly important for performing risk assessment (RA) for substances, for which several exposure routes may contribute to the body burden and the health effects. This is exactly the case for the pesticides, especially for pyrethroids used both as biocides and as agricultural pesticides. One of the major challenges mentioned was the, most often, limited data on toxicokinetic and lack of specific and/or sensitive analytical biomarker methods (D5.5). It is planned in WP5 to derive HBM guidance values (HBM-GVs) for deltamethrin and one other pyrethroid. These values will be compared with urinary concentrations obtained from the aligned studies in 2020-21 and with concentrations reported from existing data-collections in the EU

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decision-making?	inclusive studies on adverse health outcomes collected in WP13 and WP14. To enable translation of HBM data into external exposure levels for comparison with e.g., ADI values, information on toxicokinetic properties is needed. This issue is addressed in WP12 using toxicokinetic modelling to link an external exposure to an internal dosimetry in humans (e.g., concentration in blood, urine or in tissues) by describing the process of absorption, distribution, metabolism and excretion (ADME) that undergoes a substance in living organisms. A class of toxicokinetic models, the physiologically based pharmacokinetic (PBPK) models, bases the description on the ADME processes on the physiology and the anatomy of individuals, and the biochemistry of the compounds. A comprehensive review of human PBTK models available for the HBM4EU 2nd set of compounds, including the prioritised pesticides, has been performed (AD12.8). 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 PBPK-models for neither humans or animal species were identified for glyphosate or the co-formulant Polyethoxylated tallow amine (POEA) or for fipronil. Some of the PBPK models 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 simulate the biologically effective dose of the compounds (AD12.10). 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 HBM-GV derived in WP5, it will be investigated how group-specific biomarkers, such as 3PBA, can be translated into external exposure levels and integrated in assessment of cumulative risks of pesticide mixtures. Another major activity related to cumulative risk assessment is the investigation of exposure to mixtures of pesticides among residents living close to pesticide treated areas in the SPECIMEn study performed in WP15 (results are further described under the Substance group: mixture).