1 Prioritised substance group: Pesticides- UPDATED

Pyrethroids (group), chlorpyrifos, dimethoate, glyphosate (including the co-formulant POE-tallow amine) and fipronil (D.4.5)

Responsible author	Helle Raun Andersen	E-mail	
Short name of institution	SDU	Phone	
Co-authors	/		

1.1 Policy-related questions

1. Which are the most suitable methods and biomarkers of exposure?
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 sources and pathways of exposure?
5. What are exposure levels among occupationally exposed workers?
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 health outcomes be assessed and integrated in regulation?
8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?

1.2 Research Activities to be undertaken

Table 1 Research activities research activities to be carried out to answer the policy questions for pesticides

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1. Which are the most suitable methods and exposure biomarkers?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	There are established and validated methods for analysing urinary metabolites as marker for the total pyrethroid exposure (3-PBA), the combined exposure to cypermethrin, permethrin and cyfluthrin (cis- and trans-DCCA), and for some specific pyrethroids (deltamethrin, cyfluthrin, bifenthrin). The detection frequency is low for most specific pyrethroid metabolites but depends on the limit of detection (LOD) which vary between different analytical approaches and labs. Furthermore, pyrethroids are often metabolised to several different metabolites with low fractions of each specific metabolite. There are available methods for analysing the metabolite, TCPy, which is specific for chlorpyrifos and chlorpyrifos-methyl and for group-specific urinary organophosphate metabolites, i.e., dialkyl phosphates (DAPs) as a marker for the total OP exposure level. DAPs are divided into diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate. A method exist to measure some pyrethroids (total and some specific) and chlorpyrifos simultaneously. Glyphosate is primarily excreted in urine as unchanged parent molecule. Humans are also exposed to AMPA which is the main metabolite found in water. Both glyphosate and AMPA can be measured in urine with established methods.	Activities: 1. Evaluation and selection of best suited biomarkers of exposure (Y3-Y4) (WP9) 2. The methods for analysing urinary metabolites of pyrethroids, chlorpyrifos, organophosphates (DMPs and DEPs), and glyphosate, need to be harmonised within partner countries to obtain comparable values and LODs (WP9) 3. Development/validation of methods to include more specific pyrethroid metabolites could be considered based on expected prevalent exposure and whether major specific metabolites are formed (WP9).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POEA)	Because dimethoate and the specific metabolite are rapidly metabolised to DMPs, establishment of a sensitive specific urinary biomarker for dimethoate is not possible. Dimethoate will be included in the DMPs (see above for Cat B) After fipronil exposure, the major metabolite, fipronil sulfone, is rapidly formed. This metabolite is rather persistent and toxic in mammals. A method to measure fipronil sulfone in serum is available and seem to be the best biomarker for fipronil exposure. Recently, a method to measure hydroxyl-fipronil in urine was developed using rat urine but this method has not yet been applied on human samples.	Activities: 1. If prioritised to include fipronil (Q2), urine will be the preferred matric, allowing analyses of all the pesticides in the same samples. Thus, a method to measure its metabolite in human urine should be further developed and validated (WP9) 2. If prioritised to include POEA (Q2), the first step will be to collect available information on toxicokinetic i.e., absorption after different exposure routes, metabolism, and major urinary metabolites to evaluate if it is possible to establish a sensitive and reliable biomarker method (WP9).
2. What are the current exposure levels of the EU general population to the prioritised pesticides?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	HBM studies including these substances have been performed in some EU countries but not EU-wide. The studies indicate widespread exposure in the general population. The exposure to pyrethroids is expected to be increasing as they replace organophosphates (OPs) in biocidal products and to some degree also as insecticides in agriculture. Children have higher food intake per kg body weight leading to higher exposure levels from pesticide residues in food as also confirmed in previous HBM-studies	Gaps: Few studies have been performed after 2010 and data are lacking for many EU countries. More data are needed to evaluate differences between countries and population groups, time trends, and age-related differences in exposure. Activities: 1. Collecting, comparison, and evaluation of existing biomonitoring data in the EU and integration into IPCHEM (Y3-Y4) (WP10) 2. Identify and prioritise knowledge and data gaps and related research needs (Y3) (WP4) 3. Planning and analysing supplementary urine samples from the alignment studies preferentially from children and from studies with available information on dietary habits and/or residential use of pesticides (Y3-Y5) (WP8) 3. Data-analyses of time-trends and differences between countries and population groups, including identification of subpopulations with highest exposure levels (Y3-Y5) (WP10).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POE-tallow amine (POEA))	Studies from the US reported very low detection frequencies (< 1%) for dimethoate and omethoate, because they are rapidly metabolised to unspecific dimethyl phosphates (DMPs). Urinary DMPs and diethyl phosphates (DEPs) have been included in many studies as biomarker for the total OP exposure. Thus, including DMPs and DEPs will allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies. A recent case of fipronil misuse caused large scale contamination of chicken eggs but otherwise fipronil is seldom detected in commodities at the EU market. Fipronil is approved as biocide and for veterinary use but no longer for agricultural use in EU. There is reliable evidence that POEA increase the toxicity of some glyphosate formulations. Although POEA was recently banned in the EU, exposure from residues in food items (imported or due to contaminated soils) is very likely but there is no monitoring data from commodities or other potential human exposure sources to underpin the relevance of HBM.	Gaps: No EU HBM studies have included urine concentrations of dimethoate or its specific metabolite omethoate. There is no HBM data from EU on fipronil or POEA. Activities: 1. Include DMPs and DEPs in the analyses of supplementary urine samples from the alignment studies, as suggested above for the cat. B substances, to allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies (Y3-Y5) (WP8). 2. Prior to method development for POEA and fipronil (see Q1) it should be considered whether to prioritise to monitor these substances in human matrices (preferentially urine) at present (WP4). 3. If prioritised, methods for analysing POEA and fipronil in urine has to be developed (WP9) – see Q2 for Cat C substances below, and samples from the alignment studies or from targeted studies will be analysed for fipronil and/or POEA (WP8)

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
3. What are the main dietary sources of exposure across the member states?	Cat B and C (all substances)	Residues in the diet is the main continuous exposure source for pesticides in the general population. Pesticide residues in food is measured under coordinated control programmes (EUCP) and the national control programmes (NP). The coordinated multiannual control programme for 2018, 2019, and 2020 (Regulation (EU) 2017/660) includes many of the HBM4EU selected pesticides (i.e., 12 different pyrethroids, chlorpyrifos/chlorpyrifos methyl, dimethoate, glyphosate and fipronil). These data are collected and stored by EFSA (European Food Safety Authority 2017, 2018). Human breast milk samples are not included in the control programmes. Chlorpyrifos and pyrethroids have been found in breast milk samples from other countries (e.g., USA, India, Brazil and Colombia) sometimes in concentrations exceeding the MRL of 0.01 mg/kg for food for infants and young children (Directive 2006/141/EC). Only six samples from EU (Spain) have been analysed. Methods to analyse pyrethroids and chlorpyrifos in human milk samples are available.	Activities: 1. Analyse/model HBM data in relation to monitoring data on residues in food samples (EUCP) to 1) compare and complement exposure assessment performed by EFSA and 2) identify the major dietary exposure sources across member states (Y3-Y5) (WP12) 2. Consider if possible to perform a pilot study analysing selected pyrethroids and chlorpyrifos (parent compounds) in existing bio-banked milks samples (Y3-Y5) (WP9, WP8).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
4. What are other sources and pathways of exposure?	Cat B and C (all substances)	Living near agricultural areas where pesticides are applied may enhance the exposure level to pesticides due to drifting, as demonstrated in studies from the US. No HBM data from EU are currently available. A targeted survey including families (children and adults) living close to pesticide treated agricultural areas (3-5 countries) using a new developed multi-target screening of multiple pesticides in urine samples is planned in WP15 and WP16 (Survey on Pesticide Mixtures in Europe, SPECIMEn) Indoor use of pyrethroids and/or fipronil as biocides has been shown in studies from the US to contribute markedly to the exposure level – especially among children.	Activities: 1. Analysing the urine samples from the WP15/16 survey using the above-mentioned methods, will allow quantification of these pesticide metabolites (WP9) and 2. subsequent data analyses to compare the levels with those obtained from the alignment studies (WP10) and 3. comparison with the result obtained by the multi-target screening method (WP10) Data gap: Biocidal use of pyrethroids might be increasing in the EU but there is no HBM studies investigating this exposure situation. Activities: 1. A targeted study, focusing on children living in homes with repeated residential use of biocides would be highly relevant, e.g., with urine sampling before and fixed time points after treatment (WP8). 2. If a targeted study is not prioritised it may be possible to get some information by analysing HBM data from the alignment studies (including additional analyses of urine samples from children) in relation to questionnaire information on residential use, if such data are available (preferentially with information on time interval between sampling and treatment). Data on authorisation and sale of biocidal products might also be included in data analysis of HBM data from the alignment studies to investigate exposure differences between member states (Y3-Y5) (WP10).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
5. What are exposure levels among occupationally exposed workers?	Cat B and C (all substances)	Occupational exposure to agricultural workers who mix and/or apply pesticides onto crops can be substantial, with dermal exposure considered the most important pathway, although inhalation may also be important. Also, workers handling crops/plant after treatment have enhanced exposure and, since many young women in fertile age groups, are employed in agriculture/horticulture/floriculture they constitute a special risk group. Further, workers employed in companies applying biocides (pyrethroids and/or fipronil) in dwellings and institutions might have high dermal and inhalation exposure.	Data gap: There is no HBM data from EU covering occupational exposure of the selected pesticides. Investigating occupational exposure levels is important to identify high exposure groups. Activities: A targeted study addressing occupational exposure levels is highly relevant (WP8) as this Q cannot be answered based on the alignment study. The WP15/WP16 mixture survey will provide data on exposure profile/level among residents (children and mothers) close to agricultural fields (orchards) in five EU-countries. Including urine sampling also from agricultural workers who mix and/or apply the pesticides in this survey would allow additional information on occupational exposure (WP15, WP16, WP8)
6. Are the exposure levels of health-relevance/concern for vulnerable groups or high exposure population groups?		Most of the prioritised pesticides are neurotoxicants (OPs, pyrethroids, fipronil) and some also have ED or genotoxic/carcinogenic properties. The main health concerns are adverse effects on neurodevelopment and/or endocrine disturbances affecting reproduction, metabolism etc.	Activities: 1. Combining HBM data from EU studies, e.g., from birth cohort studies with health outcomes – if possible using meta-analysis (WP13) 2. Identify/suggest adverse outcome pathways (AOPs) for relevant health outcomes, including neurodevelopment and endocrine disrupting effects (WP13). 3. Identify/suggest relevant effect biomarkers (WP14)
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?		Assumed additivity within the pesticide groups (similar mode of action; (e.g. pyrethroids) but also across groups (similar adverse effects; e.g., neurotoxicity of pyrethroids and OPs)	Input from WP15

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1 8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?			Comparison of HBM values with toxicologically derived guidance values (ADI values) and findings on associations with health outcomes in (Y3-Y5) (WP5 and WP15) Input from WP5 and WP15
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?			Input from WP5