Prioritised substance group: Mycotoxins

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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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<tr><th>Policy Question</th><th>Short Summary of Results</th></tr>
<tr><td>1. Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?</td><td>An inventory has been made on biomarkers for deoxynivalenol (DON), fumonisin B1 (FB1) and aflatoxins and analytical methods, in WP9.1, deliverable report D9.5 “Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances”, v1.1 27th May 2019. An update version is foreseen for (June 2020). It has been decided that within HBM4EU only DON will be included in analysis of samples from aligned studies. Total DON (after deconjugation) in urine has been chosen as biomarker for exposure to DON and its derivatives (3/15-acetyl-DON, DON-3G). Harmonised methods and certified reference materials are not available. A list of 13 candidate laboratories for the chemical analysis of DON was elaborated after consulting the CGLs for suggestions. The list was included in the deliverable D9.6 ”Database of candidate laboratories for the 2nd prioritisation round of substances and update for the 1st round”. This list was used for the QA/QC program implementation in task 9.4. In order to identify expert laboratories, a questionnaire was sent to the candidate laboratories to gain more detailed information on validation status, experience and capacity to analyse HBM4EU samples. Based on these criteria, the QAU had selected 6 expert laboratories for mycotoxins to be included in a 3-round interlaboratory comparison study to assess comparability of analysis results.

However, one laboratory had to withdraw from participation (consequence of COVID-19). The third round is ongoing (samples were sent to the laboratories). Expected completion of the interlaboratory exercise is September 2020. The table below summarises the information about the QA/QC program for mycotoxins (total DON):

<table>
<tr><th>Substance group</th><th>Matrix</th><th>Compounds</th><th>Organiser</th><th>CM prep. & testing</th><th>ICI Round-1</th><th>ICI Round-2</th><th>ICI Round 3</th></tr>
<tr><td>Mycotoxins</td><td>urine</td><td>Total deoxynivalenol</td><td>RIKILT (WFSR)</td><td>RIKILT (WFSR)</td><td>6 labs (Jan-Feb 2020)</td><td>5 labs (Feb-June 2020)</td><td>5 labs (June-July 2020)</td></tr>
</table>
ICI = interlaboratory comparison investigation; consists of 3 rounds, after that a decision memo on comparability will be prepared (expected Sept 2020)</td></tr>
<tr><td>2. What are the current exposure levels of the European population to DON and FB1? Are there exposure data for other</td><td>Since no data on mycotoxins exposure is available at HBM4EU database or repository, an initial literature search on Human Biomonitoring studies in Europe related to prioritised mycotoxins and also other mycotoxins (more abundant) is on course and expected to finish by July. Literature search was performed in Pubmed, Web of Science and Scopus with the keywords: Human AND</td></tr>
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mycotoxins?	Biomonitoring AND Mycotoxins, review papers, and EFSA reports. Until now 104 references were identified and included in Mycotoxins Biomonitoring Database. Next, and within WP 10, task 10.4, a research protocol will be developed for the selected mycotoxins exposure (FB1 and DON and its glucoronides) and other main toxins, based in the mentioned literature search. The identification of existent data collections is being performed in order to determine which research questions can be explored with HBM data already available. The draft research plan was presented and discussed in the WP 10 Meeting held in November 2019, in Paris and an update was recently presented in the virtual WP10 Workshop, organised in June 2020. A biomarker of DON exposure (urine total DON) is being included in the aligned studies for the adult population across Europe that is expected to also answer this question (Decision Memo on the implementation of Mycotoxins analysis in the aligned studies of task 8.1, by WP 8, submitted 2019 by CGL).
3. Does the exposure to mycotoxins differ among different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localisation)?	Differences on mycotoxins exposure profiles as well as the geographic variations of FB1 and DON exposure will be ascertained either through the literature survey or through the new data obtained in the aligned studies.
4. Is there a time trend in human exposure to mycotoxins across Europe? Which are the identifiable factors associated with these trends (regulation related with food safety, climate change, others)?	This question will be addressed within the research protocol for mycotoxins within WP10, task 10.4.
5. Is the risk associated to human exposure to these mycotoxins characterised?	The assessment plan for mycotoxins was elaborated and described in the scope of task 5.3 (march 2020). New HBM data on mycotoxins will be used in the RA of mycotoxins, namely from i) bibliographic search, on course at WP10 (DON and FB1) and ii) through the performance of HBM4EU aligned studies (WP 8.1) in different European regions (only total DON). In the RA that will be performed, the general population (mainly exposed through food) will be the focus although occupational exposure may also be included if relevant exposure data is available. The HBM data considered in the scope of WP10 or the new HBM data obtained under the aligned study will be compared directly with the HBM-GV, if available. If not, a reverse dosimetry estimate comparing obtained data with available TDI values will be performed. The policy questions and activities mainly related to mycotoxins risk assessment were presented by CGL at the Joint Meeting HBM4EU and EFSA to exchange ideas and promote collaboration between both entities (February 2020).

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6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?	In Task 12.3, the parameterisation of the generic PBTK model developed in Task 12.1 for mycotoxins was performed. Specifically, a published human PBTK model for DON, with a focus on pharmacokinetic parameter values and methods used to estimate these values was taken into account. In addition, literature data, generally obtained experimentally (in vivo or in vitro) was also used for the estimation of parameter values. Regarding fumonisins, future efforts should be done to increase the knowledge of their toxicokinetics and consequently contributing to a better human risk assessment. Other mycotoxin PBPK model available include zearalenone and its metabolites. Summary of the human PBPK models for mycotoxins was reported in "Review of available models for the 2nd set of prioritised substances", Deliverable Report AD 12.8. As biological half-life values of mycotoxins DON and FB1 in humans were not found, data from half-life values in different biological media (plasma, liver, kidney, muscles and brain) of animals were reported in "Biological half-life and internal dosimetry of the 2nd set of priority compounds", Deliverable Report D12.7 (submitted June 2020).
7. Is it possible to set HBM guidance values for mycotoxins?	Based on literature survey and after consulting experts from WP 5, task 5.2 (from UBA, RIVM and ANSES) and the CGLs, the possibility of deriving a HBM guidance value for DON was identified and the work is currently being carried out. A "Decision Memo on the derivation of HBM-GVs for DON, provided by WP 5 (task 5.2), was submitted to the Management Board (June 2020) involving a close collaboration between RIVM, ANSES and INSA.
8. Which are the key events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?	A focus group led by RIVM was organised for mycotoxins aiming at addressing this policy question. The first outcomes have been communicated to EFSA and was included in an interim report as AD13.6-2020 (AD13.6 - Answers from WP13 to exposure-health policy questions for the 2nd priority compounds; december 2019). Two draft Adverse Outcome Pathways (AOPs) for DON and FB1 were prepared last year, however, only for one of the mycotoxins will the development of an AOP be continued, since a link must be evident between the AO and adverse effects relevant for humans. A human effect, neural tube defects, has been identified after chronic exposure to FB1. No human health effects have been identified after chronic exposure to DON. Moreover, a biomarker of effect has been identified for FB1, which matches the AOP.
9. Which are the most reliable and informative AOP- based effect biomarkers for prioritised mycotoxins?	A working group (INSA and MU) addressed the effect biomarkers reported in HBM studies for the selected mycotoxins, FB1 and DON. The most relevant health outcomes were selected for each mycotoxin, based on the effects reported in several epidemiological and animal studies. In addition, the mechanisms of action that are deemed to underlie their toxicity (in vitro/ex vivo and in vivo studies) were considered, in an attempt to link the identified health outcomes to central molecular, cellular or tissue/organ key events. This knowledge is relevant to try to establish the molecular initiation event and the key events in order contribute to production of AOP for FB1 and hepatotoxicity, nephrotoxicity or carcinogenicity and for DON and immunotoxicy, reprotoxicity or endocrine disruption. These will liaise with the work performed in WP13. Increases in sphinganine and decrease in sphingosine levels and their ratio were identified as effect biomarkers in 8 HBM studies and they may be considered as specific markers linked to key cellular events of FB1 action (disruption of sphingolipids metabolism). Other works have pointed to some endpoints and methodological approaches, e.g., gene expression or gene methylation analyses that deserve to be further explored to discover novel effect biomarkers. Regarding DON, a single study referring the use off effect biomarkers related to its immunotoxicity and autism was retrieved. The information was included in Deliverables 14.5 (Selection criteria

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	and inventory of effect biomarkers for the 2nd set of substances) and 14.6 (Report on the state of development of Task 14.3)
10. Which research needs and gaps on Human Biomonitoring activities related to prioritised mycotoxins?	The work developed in the context of several WPs and tasks has evidenced several research needs and gaps on HBM studies that should be addressed in future studies, such as: ○ validation and harmonisation of analytical methods for mycotoxins, ○ standards and reference materials availability, ○ updated of HBM exposure data, to perform a more accurate RA and risk characterisation as well as to try to identify time trends and possible relation with climate changes (since exposure to some mycotoxins are expected to increase in Europe due to changes in climatic parameters), ○ determination of HBM GV and reference values for FB1, ○ further development of toxicokinetic models, ○ further epidemiological research on novel effect biomarkers for FB1 and validation of the existing ones; identification of effect biomarkers and AOP development for DON, ○ identification, characterisation and risk assessment of mycotoxin mixtures.