Substance report

June 2022

Mycotoxins

HBM4EU science and policy for a healthy future

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Authors and Acknowledgements

Lead authors

First version (March 2021) compiled by project team at Wood: Dr. Ian Keyte, Neil Patton and Dr. Robert Whiting based on scoping documents produced by the chemical group leader (CGL) and colleagues: Nina Weindel. The EEA has since updated this document to reflect the work developed before the conclusion of HBM4EU, with the support of the CGL and other colleagues.

Maria João Silva, Paula Alvito (INSA), Susana Viegas (ENSP)

Contributors

Joana Lobo Vicente, Beatrice Grosu (EEA)

Glossary

Abbreviations	
AFB_1	Aflatoxin B_1
C&L	Classification and Labelling
CLP	Classification, Labelling and Packaging
CMR	Carcinogenic, mutagenic, and toxic for reproduction
DON	Deoxynivalenol
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FB_1	Fumonisin B_1
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HBMGV	Human Biomonitoring Guidance Value
IARC	International Agency for Research on Cancer
OEL	Occupational Exposure Limit
OTA	Ochratoxin A
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
TDI	Tolerable daily intake
WP	Work package

1 Key messages

- An exposure biomarker for DON and its derivatives was selected and several laboratories were qualified in HBM4EU.
- HBM4EU Aligned Studies[1] conducted to evaluate EU-population exposure to DON (2014-2021) have generated data for DON in 6 countries.
- HBM4EU has assessed how the European population is exposed to mycotoxins, particularly, DON and FB_1, exploring regional differences, and identifying vulnerable groups, to inform the development of targeted measures to reduce mycotoxin exposure.
- The data generated in the HBM4EU Aligned Study and the comparison with the HBM-GV defined in the scope of HBM4EU showed that the European population is exposed to DON and that a fraction of this population is, to some extent, exposed to levels that might represent a potential health concern.
- Efforts have been made to identify the key events that determine some of the health effects from chronic exposure to the target mycotoxins and the most reliable and meaningful effect biomarkers. Vulnerable groups that may be exposed to harmful levels of mycotoxins include pregnant women, babies, infants, and workers in the agricultural and food production sectors.
- Climate change is expected to increase the prevalence of warm and humid climate conditions in Europe that favour fungal growth and is likely to result in an increase in the production of mycotoxins, increasing the risk of human exposure.

2 Introduction

HBM4EU is a project funded under Horizon 2020 and runs from 2017 until 2021. It generates knowledge to inform about the safe management of chemicals and hence, protect human health and the environment in Europe. HBM4EU uses human biomonitoring (HBM) to monitor the actual human exposure to chemicals and resulting health impacts and to improve chemical risk assessment. HBM4EU compares data from across Europe which allows an understanding of regional differences and can help to identify vulnerable groups, in order to inform targeted measures to reduce exposure. The results of the HBM4EU project are aimed at supporting policy development, by providing a key evidence base in the understanding of exposure and impacts to toxic chemicals.

If you would like to read more about the project itself, please visit the HBM4EU website.

2.1 How to use this document

This document provides a summary of the known and suspected adverse human health effects of mycotoxins and describes the main exposure pathways for humans. It also indicates where HBM could be of value in the development of EU policy, along with the remaining challenges in determining human mycotoxin exposure. his substance report is intended to inform scientists, relevant stakeholders and policy makers on the value of HBM to establish the EU population's exposure to mycotoxins.

This substance report is based largely on the HBM4EU scoping document for mycotoxins, first draft produced in 2019 and regularly updated, as well as the accompanying reports on legislative

[1] The HBM4EU Aligned Studies are a survey aimed at collecting HBM samples and data as harmonised as possible from (national) studies to derive current internal exposure data representative for the European population/citizens across a geographic spread.

mapping and policy questions. Where necessary, additional information from European Chemical Agency (ECHA) documents including the classification and labelling (C&L) Inventory, and legislative text for relevant EU policy areas, have also been used for this report.

2.2 Overview of mycotoxins

General introduction to mycotoxins

This section provides a brief general introduction to the topic of 'mycotoxins'. If you would like to skip directly to the priority mycotoxins themselves, please click here.

Mycotoxins are toxic compounds that are naturally produced by certain types of mould (WHO, 2018). These substances are produced as metabolic by-products of fungi and occur as contaminants in agricultural commodities all over the world (Bennett and Klich, 2003; Wu et al., 2014). The WHO (2018) lists potential examples of foodstuffs affected my mycotoxins as cereals, dried fruits, nuts, and spices, further noting that the mould growth can occur before or after harvest and storage, particularly if warm, and humid conditions are present.

Mycotoxins are of particular concern as they are chemically and thermally stable, meaning that they can survive storage and most production processes (Koppen et al., 2010). Prior to 1985, the Food and Agriculture Organization of the United Nations (FAO) estimated as much as 25 % of global food crops may be contaminated with mycotoxins. Further studies by Eskola et al. (2020) based on around 500,000 analyses from the European Food Safety Authority (EFSA) data and global surveys from 2017 upholds this estimate.

Mycotoxins pose a public and occupational health concern as some of them have shown carcinogenic, mutagenic, reprotoxic (CMR) and endocrine disrupting effects. For food crops specific genera of fungi are key for the generation of mycotoxins, largely dominated by *Aspergillus, Fusarium,* and *Penicillium* species. These fungi are responsible for the major classes of mycotoxins linked to health concerns, in particular the aflatoxins (e.g. aflatoxin B1, AFB_1), fumonisins (e.g. fumonisin B_1, FB_1), trichothecenes (e.g. deoxynivalenol, DON), and ochratoxin A (OTA) (Cinar et al., 2019).

Climate change may mean warmer and wetter summers in the coming years, which is expected to impact fungal growth and agricultural practices (Battilani et al., 2016; Sundheim et al., 2017; EFSA, 2020; Moretti et al., 2019). Consequently, mycotoxins' concentrations and incidence in crops are expected to increase leading to an increase in human dietary exposure (WHO, 2018; Moretti et al., 2019).

2.2.1 Prioritised mycotoxins

DON and FB_1 were selected in the 2nd round of substance prioritisation under HBM4EU, with some further details provided below:

2.2.2 Deoxynivalenol (DON) (CAS 51481-10-8)

DON is one of several mycotoxins produced by fungi of the genus *Fusarium*, which is known to infect cereals (corn, wheat, barley, oats, and rice) as well as other field grains (Sobrova et al., 2010). The fungus that produces DON is able to tolerate a broad spectrum of climate conditions, with the wind as the main mechanism for transport of spores to target plants. That being the case, DON occurs as the predominant mycotoxin in the northern hemisphere and is particularly related to adverse effects on animals and animal production (Manthey et al., 2004).

Human exposure can occur both directly through contaminated cereals, but also indirectly through products of animals that have been exposed to contaminated feed (e.g., meat products such as

liver, kidneys, or offal, as well as dairy products and eggs). Importantly, studies have shown DON to be highly stable to elevated temperatures used in cooking. In studies simulating cooking conditions for pasta and noodles (i.e., boiling water for 30 minutes), DON remained chemically unchanged, while it did leach from the food into the cooking water meaning a reduction in food concentrations (Sugita-Konishi, 2006; Visconti et al., 2004; EFSA, 2014).

DON is immunotoxic (Sundheim et al., 2017), promotes intestinal inflammation and is suspected to be toxic for reproduction; EFSA considers it is devoid of genotoxic potential (EFSA, 2017). Further details on the health effects of DON are provided here.

2.2.3 Fumonisin B_1 (FB_1) (CAS 116355-83-0)

FB_1 is a mycotoxin produced by several species of fungi within the genera *Fusarium* (e.g., *F. moniliforme*, *F. proliferatum*, and *F. verticillioides*) (Stack et al., 2000; Lemmer et al., 2004). Like DON, FB_1 is predominantly found within cereal crops (particularly maize and corn), along with some meat products (particularly related to swine). FB_1 is resistant to degradation either by elevated temperatures during food processing or by light (WHO, 2000).

As with DON, human exposure can occur through ingestion of contaminated cereals (noting that maize and corn form a staple part of the diet for many European citizens), or contaminated animal products.

FB_1 causes a range of health effects in humans and animals. The International Agency for Research on Cancer (IARC) classified FB_1 as a possible carcinogen to humans (Group 2B), with *in vivo* studies showing that repeated exposure leads to tumours of the liver and kidneys in rodents. Observational studies have indicated that fumonisins exposure may be associated with esophageal cancer in humans (Sun et al., 2007). Furthermore, FB_1 has been shown to cause developmental toxicity in several animal species (IARC, 2002).

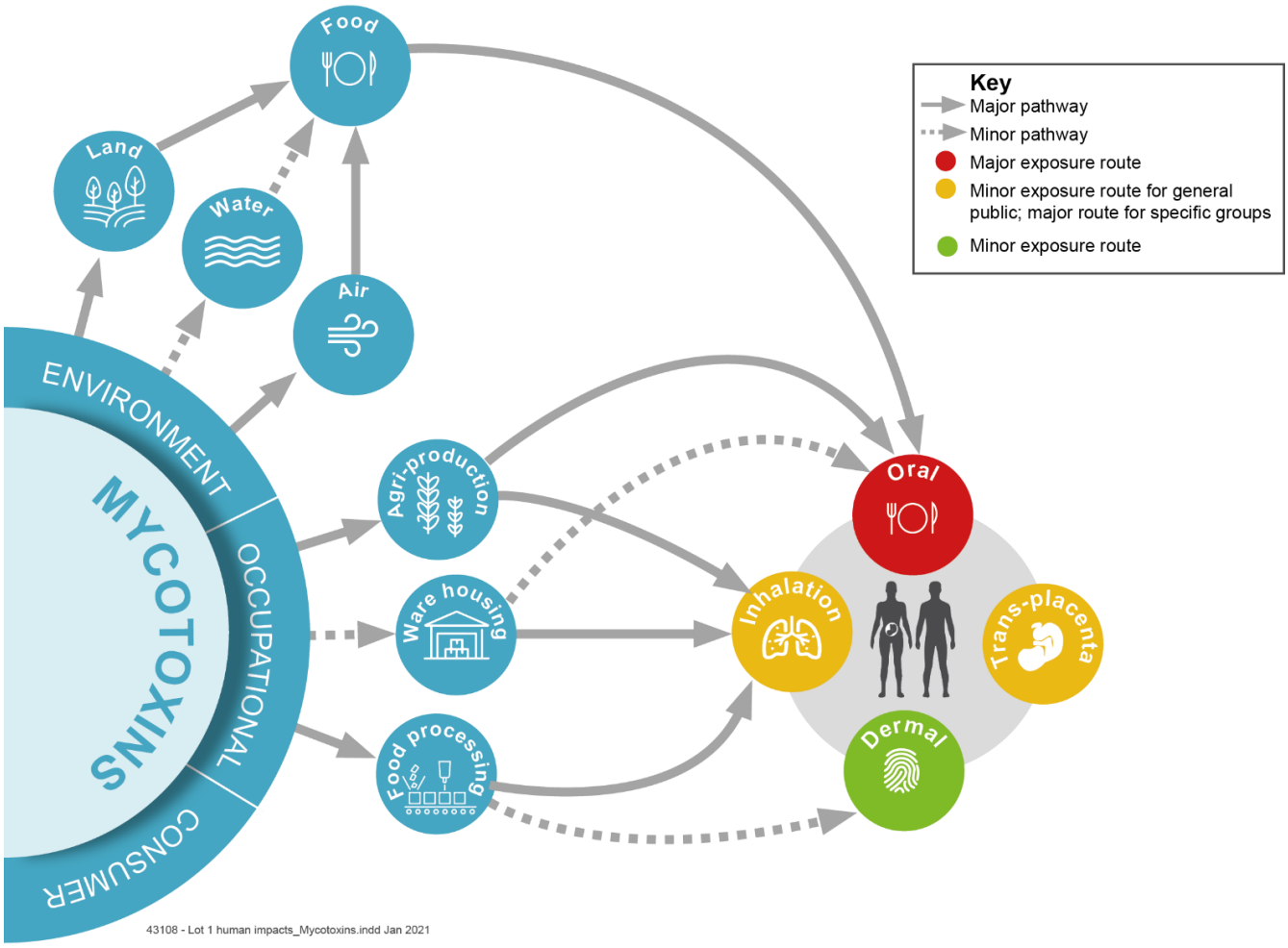
In particular FB_1 has been associated with “mouldy corn poisoning” in horses, mules and donkeys, a disease of the central nervous system (brain), with symptoms including blindness, discoordination of the muscles and muscle spasms, leading ultimately to death. In pigs, FB_1 has been shown to cause pulmonary oedema (excess fluid in the lungs). Further details on the health effects of FB1 are provided here.

3 Human exposure to mycotoxins

An overview of the main sources of exposure (environmental, occupational, consumer) and exposure pathways (oral, inhalation, dermal) is provided in Figure 1.1 below.

Additional information on these sources and pathways is provided in Appendix 1.

Figure 1.1 Overview of exposure routes and pathways for mycotoxins



3.1 Environmental exposure

As indicated in the introduction, mycotoxins are globally produced by fungi within all agricultural settings, particularly where the environmental conditions (warm and humid) favour mould and fungi growth.

Mycotoxins are formed as unintentional by-products, with no commercial use, and thereby unintentional human exposure may occur.

3.2 Occupational exposure

For occupational exposure, the main settings include:

- Agriculture – involving the handling of crops during treating, harvesting, feeding of animals or livestock production.
- Storage of crops and food stuffs prior to use; and
- Within food processing and production.

For occupational settings exposure may occur through inhalation or dermal contact (as well as oral). This can be important for fine dusts created during the harvesting, storage, or processing of

cereals and other dried crops (e.g., fruits, nuts, and spices) within more enclosed settings (Viegas et al., 2018).

DON is the most prevalent *Fusarium* mycotoxin in European grains, frequently reported in feed, cereals, and cereal-based products such as bread, pasta, or beer (Marin et al, 2013). This explains the presence of this mycotoxin in several workplace environments such as bakeries and swineries (Viegas et al., 2020).

3.3 Consumer exposure

Human exposure occurs primarily through the consumption of contaminated foodstuffs, with the oral route as the dominant exposure pathway. Additional secondary pathways are inhalation or dermal exposure, but this is more restricted to occupational exposure.

According to EFSA (2017), the estimated chronic dietary exposure for DON was above the TDI of 1 µg/kg bw/day for infants, toddlers, and other children regarding the mean exposure scenario, and for adolescents and adults regarding the high exposure scenario, which indicates a potential health concern.

4 Health impacts of mycotoxins

4.1 Overview of key health impacts from mycotoxins

An overview of current EU (ECHA C&L Inventory) and/or IARC classification of mycotoxins is provided in Table 4.1. Additionally, Figure 4.1 (on the subsequent page) provides a further overview of health effects for mycotoxins.[2]

Some mycotoxins have been shown to exhibit carcinogenic and/or mutagenic potential in human and animals (rats, ferrets, ducks, trout, dogs, turkeys, cattle, and pigs). In particular, repeat dose testing of FB_1 highlighted the development of tumours in both kidneys and liver of rodents, with additional evidence also highlighting risks for the digestive system. Both DON and FB_1 have specific target organ toxicity affecting the liver (hepatoxicity) and central nervous system (Bryden, 2019; Omotayo, 2019).

Studies have shown that mycotoxins (including DON) can pass over the placenta with concerns for health impacts on unborn children (Sobrova et al., 2010; Yu et al., 2017).

[2] An explanation of the categorisation of the strength of evidence for the health effects presented in Figure 1.1 is provided in Appendix 2.

Table 1.1 Overview of CLP classifications for mycotoxins

Substance	Properties of concern				Category according to CLP criteria								ECHA info card
	Carcinogenicity	Mutagenic	Skin sensitising (SS)	Reproductive Toxicity	Carcinogenicity	Acute Toxicity	Specific target organ tox (repeated exposure)	Reproductive Toxicity	Mutagenic	Eye Damage/ Eye Irritation	Skin Sensitivity	Skin Corrosion/ Irritation	
DON					3								Link
FB_1					2B								Link

* Harmonised classification under the CLP Regulation. (Other classifications are those notified to the CLP inventory but without harmonised EU classification.); ** Based on IARC classification

Confirmed

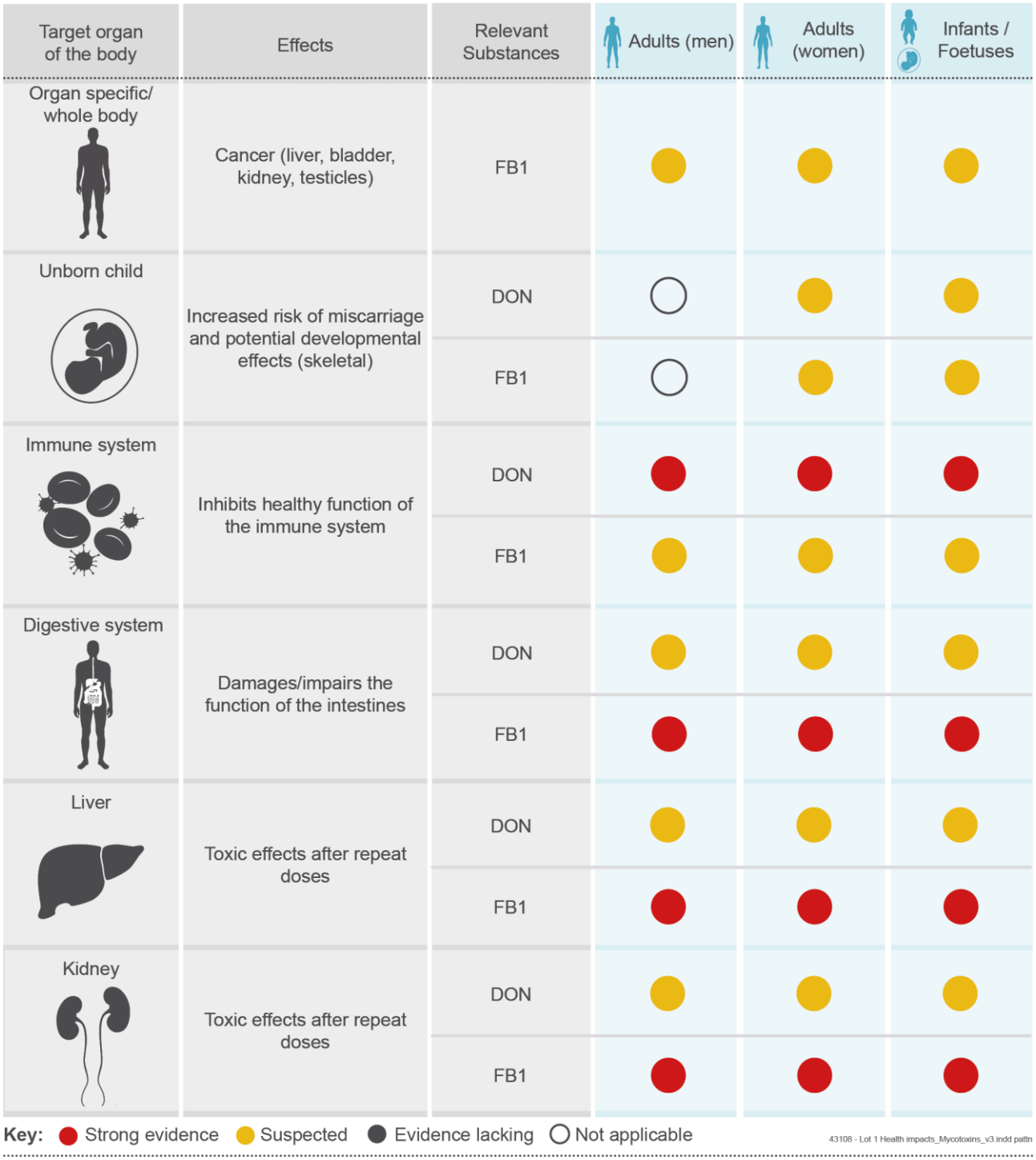
Suspected

Some data

Most hazardous — Least hazardous

1 2 3 4

Figure 2.1 Overview of health effects associated with exposure to the studied mycotoxins



4.2 Vulnerable target groups

The analysis of data highlights two key vulnerable target groups. Firstly, EFSA (2017) highlighted concerns for babies and young children (including an exceedance of the TDI threshold). Target organ toxicity in the very young, where renal and hepatic systems (liver) are developing is a concern. Likewise, exposure to unborn children is a concern where mycotoxins have been shown to pass over the placenta and can furthermore pass over the blood brain barrier (Kyei et al., 2020; Sarron et al., 2020).

The second key group relates to occupational exposure of farm workers and personnel in the food processing industries. In this case, the generation of contaminated dusts and the repeated exposure that can occur via inhalation, dermal and oral routes are a concern since it can result in exposures during short periods of time.

4.3 Societal concerns

The FAO estimates that up to 25 % of all foodstuffs may have some form of contamination with mycotoxins which is a concern. Furthermore, changes in climate and agricultural practices may mean that the prevalence or increased prevalence of mycotoxins may be of a significant concern in future years. In utero, babies and young children exposure is also of concern due potential associated effects on health on the long term.

5 EU policies on mycotoxins

Several policy measures have already been introduced in the EU to address human exposure to mycotoxins and managing risks. The policy briefs under HBM4EU have presented the legislative context as four categories i) regulations on chemicals; ii) the environment; iii) consumer products and iv) occupational exposure. An overview of these regulatory measures at EU level is provided in Table 2.1.

Table 2.1 Overview of EU policies

Chemicals	No specific legislation. Note that DON and FB_1 are not registered under REACH and formally have no known commercial use. Some voluntary classifications have been provided under CLP.	*Food* • Regulation EU 1881/2006 establishes maximum permissible limits for AFB_1, OTA, DON, FB_1 etc. in specific food products. This includes lower thresholds for products intended for infants and young children. • Under the Codex Committee on contaminants in food, farmers need to continuously assess the risk from mycotoxins to both crops and animals. • Commission recommendation 2012/154/EU requires the monitoring of presence for ergot alkaloids in feed and food. • Regulation EC 401/2006 provides sampling plans according to nine different groups of commodities considering mycotoxins.	**Consumer**
Environmental	Commission recommendation 2006/576/EC Sets out recommendations for advisory levels of mycotoxins in products intended for animal feed.	Mycotoxins are covered indirectly by existent legislation. Commonly, mycotoxins are not recognised as risk factor present in the workplaces due to lack of information. No occupational exposure limit available.	**Occupational**

6 Policy questions for mycotoxins

6.1 Introduction

Embedded in the substance prioritisation, stakeholders were asked to identify policy related questions that HBM4EU should address in order to contribute to the strengthening of policy ambitions on mycotoxins. Further background details on mycotoxins and how the policy questions were selected is available in the scoping document and the report on stakeholder consultation and mapping of needs.

6.2 Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?

There is a validated method for total DON (after deconjugation) in urine, that has been chosen as biomarker for exposure to DON and its derivatives (3/15-acetyl-DON, DON-3G).

The fact that FB_1 is mainly excreted in faeces and not in urine has impaired the development of reliable analytical methods to measure FB_1 exposure, e.g., in urine.

6.3 What are the current exposure levels of the European population to DON and FB_1? Are there exposure data for other mycotoxins?

New data for tDON is currently available from a HBM4EU Aligned Study focused on adults. The data was collected between 2014-2021 across 6 sampling sites in Europe (Iceland, Poland, France, Germany, Portugal and Luxembourg) representing 1270 individuals. P50 and P95 of urinary tDON concentrations are in the range of 0.39 - 9.05 µg/g crt and 2.38 - 39.18 µg/g crt respectively. Share of individuals with exposure levels exceeding the BE-value of 23 µg/L range is from 0.0% - 20.73%.

Concerning FB_1, new data was not obtained due the limitations related to the selection of a reliable exposure biomarker.

In conclusion, urinary HBM data on total DON obtained from a literature search and from the aligned studies developed under HBM4EU confirm that the European population is exposed to DON and that a fraction of this population is, to some extent, exposed to levels that represent a potential health concern.

6.4 Does the exposure to mycotoxins differ among different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localisation)?

According to literature search, children and pregnant women, who are traditionally considered vulnerable population groups, were the groups with the highest risk. The Eastern region was not presented in the current assessment. Additionally, and based only on the data from the literature search, it is important to emphasize that some studies reported a statistically significant difference between workers and control groups, confirming that the occupational environment can have an important role in increasing the exposure to DON.

6.5 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)?

The risk assessment performed using the exposure data obtained from the HBM4EU aligned studies indicates that a part of the adult population from Poland and, to some extent, from Luxembourg, France and Portugal is at high risk, which raises a potential health concern.

The current HBM data from aligned studies set a baseline to allow future data comparison and time trend analysis.

6.6 Is the risk associated to human exposure to these mycotoxins characterised?

The HBM-GV derived for DON allowed, for the first time, to assess the risk of DON based on exposure biomarkers data.

The risk assessment performed using published data and a mass balance approach showed that exposure to DON in the European population is generalized, affecting different age groups of the population. Children and pregnant women, presented the highest risk groups according to data from literature survey. The children group deserves particular attention considering the associated vulnerability and the potential long-term consequences that are difficult to predict.

The risk assessment performed using results from the aligned studies conducted in the adult population, as referred above, showed that the highest percentiles of exposure (P90 and P95) represented a potential health concern for the population from Poland and, to a certain extent from Luxembourg, France and Portugal, since the hazard quotient determined is above one. However, the mean and median levels of exposure were considered as not representing a concern for health. The risk is low for populations from Iceland and Germany and thereby does not represent a health concern

6.7 Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?

Kinetic models for DON in humans are available but not for FB_1. ARegarding FB_1, future efforts should be undertaken to increase the knowledge on their toxicokinetics and, consequently, contribute to a better human risk assessment. Other mycotoxins PBPK model available include zearalenone and its metabolites.

6.8 Is it possible to set HBM guidance values for mycotoxins?

A provisional HBM-GVGenPop (general population) was derived for DON that should be considered as an orientation value. The value was set as 23 µg DON/L urine for 24 h sample (CI: 5-33 µg/L) corresponding to an intake of 1 ug total DON/kg bw/total 24h.

6.9 Which are the key events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?

Neural tube defects in the foetuses of pregnant women after chronic exposure to FB_1 may result from the inhibition of ceramide synthase, which affects sphingolipid metabolism in the cell.

6.10 Which are the most reliable and informative AOP- based effect biomarkers for prioritised mycotoxins?

Combining toxicological information on FB_1 with AOPs (in vitro) and data from human studies allowed to identify the measurement of sphinganine (Sa) and sphingosine (So) and Sa/So ratio in urine as potential effect biomarkers associated with fumonisin FB_1) exposure, which can be linked also to a health outcome, the neural tube defects.

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.

No human relevant long-term health effects were yet identified after chronic exposure to DON. Likewise, no informative effect biomarker was identified for DON.

7 HBM4EU outputs to date

7.1 Categorisation

Substances under HBM4EU have been categorised depending on availability of HBM data. The categorisation indicates the information gaps hence allowing to develop targeted activities to fill the knowledge gaps. Substances will pass from Category E over D, C, B towards Category A as more information becomes available. Fully characterised substances should end up as category A substances.

Table 3.1 Categorisation of mycotoxins

Category		Priority substance(s)	Details
A	HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible.		
B	HBM data exists, but not sufficiently to have a clear picture across Europe.	DON	
C	HBM data scarcely or doesn't exists. Efforts to develop an analytical method	FB_1	While a good body of evidence exists on the types of health effects, limited data

	to obtain relevant HBM results need to be done		exists on pathways and population level exposure and human health impacts.
D	A toxicological concern exists but HBM data are not available.		
E	Not yet identified as of toxicological concern and for which no HBM data are available		

7.2 Key outputs

Methods

Under HBM4EU, an interlaboratory comparison study comprising three rounds, dedicated to urinary total DON (sum of free DON + DON-glucuronides after deconjugation), was conducted for the 1st time, and several European laboratories were identified that are capable of providing comparable HBM data for determination of this biomarker of exposure. Samples from the aligned studies of six European countries were analysed for urinary total DON by LC-MS/MS or LC-HR-MS/MS, in the four qualified HBM4EU laboratories.

Exposure differences and trends

Differences on mycotoxins exposure profiles as well as the geographic variations of FB_1 and DON exposure were analysed either through the literature survey or using the new data obtained in the aligned studies.

An attempt was made under WP10 to address the time trends of mycotoxins exposure; however, the data available were limited and not sufficient to perform this analysis.

Risks of human exposure

A risk assessment of DON was developed in the scope of task 5.3. The main objectives were to assess the risk associated to human exposure to DON in populations from different European countries or regions, based both on published human biomonitoring data and on the new data generated for the adult population in the context of the aligned studies developed across Europe. FB_1 was not included because there is very limited human biomonitoring data to support its exposure assessment therefore, only DON risk assessment was assessed.

The establishment of an HBM-GV for DON was also performed under task 5.2 and it is considered of major importance for risk assessment, contributing to a significant decrease in uncertainty. The human biomonitoring guidance value for DON derived under HBM4EU allowed for the first time, to assess the risk of DON based on exposure biomarkers and an HBM-GV. This accomplishment was possible for DON due to the available kinetic models in humans. The same was not possible for FB_1 due to the lack of models.

Limitations and guidelines

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.

As biological half-life values of mycotoxins DON and FB_1 in humans were not found, data from half-life values in different biological media (plasma, liver, kidney, muscles and brain) of animals were reported.

After consulting experts from WP 5, task 5.2 and the CGLs, identified the possibility of deriving an HBM guidance value for DON and a value was set for the first time, based on published data. The critical endpoint was “reduced body weight gain” from a chronic feeding study in mice (Iberson et al ,1995). This study had been previously identified by EFSA (2017) as the critical study for this

purpose. The provisional HBM-GV was based on a BMDL05 as point of departure, using a reverse dosimetry factor and assessment factor of 100.

Exposure effects

A working group addressed the effect biomarkers reported in HBM studies for the selected mycotoxins, FB_1 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 FB_1 in relation to hepatotoxicity, nephrotoxicity or carcinogenicity and central nervous system alterations. For DON, the health outcomes considered were immunotoxicy, reprotoxicity or endocrine disruption. These liaised with the work performed in WP13 on an AOP development for FB_1 and neural tube defect. Regarding DON, a single study referring the use off effect biomarkers related to its immunotoxicity and autism was retrieved.

Research needs and gaps

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.

7.3 Key data gaps

HBM4EU is a five-year project, that kicked off in 2017. HBM4EU has helped to identify a number of specific data gaps that are needed to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. However, some gaps and needs for action will remain after the end of HBM4EU which should be addressed in the future:

- Current analytical methods, harmonized methods, reference materials, proficiency tests, expert laboratories – further work needed.
- Current data on FB_1 and other mycotoxins exposure from EU countries for general population (different population groups) and, if possible, workers.
- Current risk groups related to age, gender, occupational setting, location, in EU- further work needed.
- Analysis of trends on HBM-based mycotoxin exposure – further work needed.
- Risk assessment (RA) and risk characterisation - further work needed.
- Exposure models and toxicokinetics in humans, especially for FB_1.
- Development of a HBM guidance value for DON in occupational settings.
- Development of a HBM guidance value for FB_1 for the general population and workers.
- Adverse output pathway (AOP) for DON based on adverse health effects known (in animals) and mechanistic data available.
- Limited number of biomarkers of effects available - further work needed.

8 Future recommendations

Building on the legacy of HBM4EU, including the harmonized study design and methods, and considering the scenario of climate change, new biomonitoring campaignes should be organized to follow up on the European population exposure to DON. Other mycotoxins should be also included

in those field studies. Given the adverse effects from (some) mycotoxins exposure to the unborned and young children, recommendations on the diet of pregnant women, babies and toddlers should deserve further attention. More studies on occupational exposure are encouraged.

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Appendix 1: Additional information on exposure

Source of exposure	References
Environmental •	• Manthey et al, 2004, 'Microbial loads, mycotoxins, and quality of durum wheat from the 2001 harvest of the Northern Plains region of the United States. J Food Prot. 2004;67:772–780 • Sugita-Konishi, 2006 'Differential upregulation of TNF-alpha, IL-6, and IL-8 production by deoxynivalenol (vomitoxin) and other 8-ketotrichothecenes in a human macrophage model. J Toxicol Env Health Pt A. 2001;64:619–636. • Visconti et al, 2004 'Reduction of deoxynivalenol during durum wheat processing and spaghetti cooking. Toxicol Lett. 2004;153:181–189 • EFSA, 2014, 'Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. EFSA Journal; 12(12):3916, 107 pp. doi:10.2903/j.efsa.2014.3916.
Occupational •	• Viegas, S et al. 2018, ,Occupational exposure to mycotoxins: current knowledge and prospects', Annals of work exposure and health, vol 62 pp 923-941 • Marin et al, 2013, 'Mycotoxins: Occurrence, toxicology, and exposure assessment, Food and Chemical Toxicology 60, 218–237. • Viegas, S et al, 2020,Occupational exposure to mycotoxins – different sampling strategies telling a common story regarding occupational studies performed in Portugal (2012-2020)', Toxins (Basel) doi: 10.3390/toxins12080513
Consumer •	• See exposure through food under the environmental compartment

Route of exposure	Reference
Oral Primary exposure for both workers and the general public is through the diet. Mycotoxins are generated by specific types of fungi with a range of crops (primarily cereals and fruits/fruit-based beverages) and certain types of meat product.	EFSA, 2017, 'Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed', scientific opinion of CONTAM. Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16:497–516. Marin S, Ramos AJ, Cano-Sancho G, Sanchis V (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment, Food and Chemical Toxicology 60, 218–237.
Dermal Dermal exposure is a secondary pathway, which is more closely limited to workers. This is partly where mycotoxins can form both before and harvesting of	Fromme, H et al., (2016). Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings—An overview. Int. J. Hyg. Environ. Health, 219, 143–165

crops. Particularly if stored in warm and humid conditions. Risk of dermal exposure for the general population is lower and could be considered a minor pathway.	Viegas S, et al., (2015) Occupational Exposure to Aflatoxin B1 in a Portuguese Poultry Slaughterhouse. Ann Occup Hyg. doi: 10.1093/annhyg/mev077 Viegas et al. (2018), Occupational exposure to mycotoxins: current knowledge and prospects, Annals of work exposure and health, v62: 923-941.
Inhalation Inhalation exposure is a secondary pathway, which is more closely related to occupational exposure. This is particularly where mycotoxins can be formed both both and after harvesting, particularly in warm and humid conditions. Exposure can also occur through inhalation of dusts in animal settings (e.g., poultry) Risk of dermal exposure for the general population is lower and could be considered a minor pathway.	Fromme, H et al., (2016). Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings—An overview. Int. J. Hyg. Environ. Health, 219, 143–165 Viegas S, et al., (2015) Occupational Exposure to Aflatoxin B1 in a Portuguese Poultry Slaughterhouse. Ann Occup Hyg. doi: 10.1093/annhyg/mev077 Viegas et al. (2018), Occupational exposure to mycotoxins: current knowledge and prospects, Annals of work exposure and health, v62: 923-941.
Trans-placenta DON has been shown to be able to cross the placenta to the unborn child and is suspected to be toxic for reproduction. FB_1 is classified as possibly carcinogenic (2A), with data showing that it causes developmental effects in the offspring of several animal species. Effects on human unborn children have not been confirmed. The exposure pathway is likely to be via the mother over the placenta, with primary exposure to the mother via the diet, or inhalation if under occupational settings.	IARC, 2002, http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf Sobrova, P et al, 2010, 'Deoxynivalenol and its toxicity', Interdisciplenary toxicology vol 3 pp 94-99. Yu, M et al, 2017, 'Mechanism of deoxynivalenol effects on the reproductive system and fetus malformation: Current status and future challenges',Toxicology in Vitro, 41, pp. 150-158. Kyei et al, 2020,'Maternal mycotoxin exposure and adverse pregnancy outcomes: a systematic review', Mycotoxin research vol 36 pp 243-255

Appendix 2: Additional information on health effects

DON and FB_1 do not currently have harmonised classifictaions under CLP. Categories (strong, suspected, lack of evidence, or not applicable) have been awarded based on IARC classifications and evidence presented in the scoping document.

Human health effect	Category	Justification for category	References
Carcinogen – suspected to cause cancer (particularly, stomach, liver, and kidneys)	FB_1 (Suspected)	IARC concluded that there was sufficient evidence based on animal experiments to determine that FB1 causes cancer in other animals. IARC have classified FB1 as a Group 2B (possibly carcinogenic to humans) substance.	IARC, 2002, http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf
Unborn child – Increased risk of miscarriage and potential developmental effects (skeletal)	DON (Suspected) FB_1 (Suspected)	DON: The scoping document comments that evidence exists to show that DON is toxic for reproduction and is able to pass over the placenta. However, further systematic studies are required. FB1: IARC commented that a number of studies have indicated that FB1 causes developmental effects in several animal species.	IARC, 2002, http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf Sobrova, P et al, 2010, 'Deoxynivalenol and its toxicity', Interdisciplenary toxicology vol 3 pp 94-99. Yu, M et al, 2017, 'Mechanism of deoxynivalenol effects on the reproductive system and fetus malformation: Current status and future challenges',Toxicology in Vitro, 41, pp. 150-158. Kyei et al, 2020,'Maternal mycotoxin exposure and adverse pregnancy outcomes: a systematic review', Mycotoxin research vol 36 pp 243-255
Immune system – Inhibits healthy function of the immune system	DON (strong evidence) FB_1 (suspected)	DON: The scoping document concludes that studies have shown that DON is immunotoxic and a protein inhibitor. FB1: Based on review of available studies, JEFCA concluded that FB1 was potentially an immunotoxic substance.	WHO, 2011,' Joint FAO/WHO Expert Committee on Food Additives (JEFCA)', WHO food additives series; 65 "Fumonisins." In Safety Evaluation of Certain Additives and Contaminants, 65:325–794. Rome, WHO. http://www.inchem.org/documents/jecfa/jecmono/v65je01.pdf Sundheim, et al, 2017, 'Deoxynivalenol Exposure in Norway, Risk Assessments for Different Human Age Groups. Toxins, 9(2), 46'

Digestive system – damages/impairs the function of intestines	DON (strong evidence) FB_1 (suspected)	DON: Studies have shown that DON impairs the function of the digestive system within animals based on the conclusions of the EFSA CONTAM panel. FB_1: Studies reviewed as part of the JEFCA (2011) assessment, highlight reduced body weight gains in mice and rats. However, further research is needed to make a formal conclusion.	EFSA, 2017, 'Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed', scientific opinion of CONTAM. EFSA, 2004, 'Opinion of CONTAM on a request from the Commission related to DON as undesirable substance in animal feed', Adopted 2 June 2004. Pastemak, JA et al, 2018,' Molecular and Physiological Effects on the Small Intestine of Weaner Pigs Following Feeding with Deoxynivalenol-Contaminated Feed', Toxins (Basel) Vol 10 issue 1. WHO, 2011,' Joint FAO/WHO Expert Committee on Food Additives (JEFCA)', WHO food additives series; 65 "Fumonisins." In Safety Evaluation of Certain Additives and Contaminants, 65:325–794. Rome, WHO. http://www.inchem.org/documents/jecfa/jecmono/v65je01.pdf
Liver – toxic effects after repeat doses	DON (Suspected) FB_1 (strong evidence)	DON: The scoping documents state that studies have shown toxic effects for DON upon the liver and kidneys, but results are not consensual and further research is needed. FB_1: EFSA concluded that based on analysis of a wide range of studies that repeated exposure to FB_1 leads to toxic effects in both the liver and kidneys.	EFSA, 2018, 'CONTAM opinion on a request from the Commission on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms' EFSA Journal; 16(2):5172. Peng et al, 2017, Current sights for mechanisms of deoxynivalenol induced hepatotoxicity and prospective views for future scientific research: A mini review. Journal of Applied Toxicology, 37 (5): 518-529
Kidneys – Toxic effects after repeat doses	DON (Suspected) FB_1 (strong evidence)	DON: The scoping documents state that studies have shown toxic effects for DON upon the liver and kidneys, but results are not consensual and further research is needed. FB_1: EFSA concluded that based on analysis of a wide range of studies that repeated	EFSA, 2018, 'CONTAM opinion on a request from the Commission on the appropriateness to set a group health-based guidance value for fumonisins and their modified forms' EFSA Journal; 16(2):5172. Peng et al, 2017, Current sights for mechanisms of deoxynivalenol induced hepatotoxicity and prospective

		exposure to FB_1 leads to toxic effects in both the liver and kidneys.	views for future scientific research: A mini review. Journal of Applied Toxicology, 37 (5): 518-529

For the categorisation of the strength of evidence for human health effects, the following criteria has been used:

- **Strong –** where the health effect is confirmed by either a harmonised classification indicating that there is a known effect (e.g. 1A or 1B for CMRs) (see Table 4.1), or where there is no applicable C&L classification, a statement in the Scoping Document that concludes there is strong evidence (or where a significant body of evidence is presented in the scoping document).
- **Suspected –** where there is either (a) a harmonised classification indicating that there is a suspected effect (e.g. category 2 CMRs or similar); (b) notified classification for that effect, or (c) where there is no applicable C&L classification, a statement in the Scoping Document (or other references presented in the Table above) that there is a suspected health impact.
- **Evidence lacking –** where a health effect is noted in the Scoping Document (or other evidence sources referenced in the Table above), but it is stated that evidence is currently lacking or there are uncertainties or inconsistencies in the available evidence.
- **Not applicable –** where a health effect does not apply to a specific group/gender