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SCOPING DOCUMENT

(2nd round of prioritization)

Prioritized substance group: Mycotoxins

Chemical Group Leader: Paula Alvito,
Maria João Silva & Susana Viegas

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1. Introduction

Human Biomonitoring for Europe (HBM4EU) has established a strategy for deriving prioritized substance groups that HBM4EU will work on in 2019 and 2020. This stepwise strategy included input from national and EU policy makers and from stakeholders. The substances were nominated and prioritised according to a transparent procedure that is described in Deliverable 4.3 on the Prioritisation strategy and criteria, produced by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). The detailed description of how this prioritisation strategy was implemented in practice, the inputs received and the methodology applied for selecting substances to include in the second list of prioritised substances is the subject of the Deliverable D4.4 (lead European Environment Agency, EEA).

First, a survey was launched to understand the demands of the National Hubs, EU policy makers and members of the HBM4EU Stakeholder Forum. Subsequently an online survey requested the nomination of substances for research under HBM4EU. A long list of new nominated single substances and substance groups was produced. Substances on the long list were ranked according to the number of nominations received, enabling to reduce the list down to a short list of approximately 25 substances Background documents on the substances on the short list were produced. An expert group of HBM4EU scientists scored and ranked the substances according to their hazardous properties, exposure characteristics; and public concern. The ranked list was discussed at a joint meeting of the HBM4EU Management Board and the EU Policy Board in March 2018, where agreement was reached on the draft 2nd list of HBM4EU priority substances. The Governing Board approved the final list. The Governing Board members were asked to identify a list of candidate institutions and experts for the positions of Chemical Substance Group Leaders for the new substances/groups of substances. The substance group leaders were approved and were asked to produce the scoping documents for the new list of prioritised substances. The process is documented in D4.5 Second list of HBM4EU priority substances and Chemical Substance Group Leaders for 2019-2021.

2. Background information

Following a prioritization strategy described in Deliverable 4.3, mycotoxins were selected as a prioritized substance group for 2019-2020. Given the wide variety of compounds comprised in the group of mycotoxins and following the view of the EU Policy Board, EFSA and DG SANTE, the focus was set on deoxynivalenol (DON) and fumonisin B1 (FB1).

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2.1 Hazardous properties

Mycotoxins are secondary fungal metabolites often found as natural contaminants in agricultural commodities all over the world and their occurrence pose a risk for human and animal health (Bennett and Klich, 2003; Wu et al., 2014). Generally, mycotoxins are chemically and thermally stable compounds, surviving storage and most production process (Koppen et al, 2010). Currently, the main human and animal health burdens of mycotoxin exposure are related to chronic toxicity, such as carcinogenic, teratogenic, immunotoxic, nephrotoxic, and endocrine disrupting effects. Chronic or even acute exposure to mycotoxins remains a daily fact, and therefore it is crucial that the mycotoxins' metabolism is unravelled so more knowledge on biomarkers in humans and animals is required.

The major foodborne mycotoxins of public health concern are the aflatoxins (e.g. aflatoxin B1, AFB1), fumonisins (e.g. fumonisin B1, FB1), trichothecene mycotoxins (e.g. deoxynivalenol, DON), and ochratoxin A (OTA) (Wu et al, 2014). These are produced primarily by fungi of the genera Aspergillus, Fusarium, and Penicillium, which commonly infect food crops. The International Agency for Research on Cancer (IARC) classified some mycotoxins from carcinogenic to humans (e.g. aflatoxin B1, group 1) to not classifiable regarding its carcinogenicity to humans (e.g. deoxynivalenol, group 3) (IARC 1993, 2002, 2012). In the coming decades climate change is expected to impact fungal growth and agricultural practices (Battilani et al, 2016; Sundheim et al, 2017) and, consequently, mycotoxins' concentrations and incidence in crops leading to an increase in human dietary exposure; (WHO, 2018; Assunção et al, 2018).

DON and FB1 were prioritized in the 2nd round of substance priorisation under HBM4EU and therefore, a more detailed review will be performed related to these mycotoxins.

Although there are structural alerts for DON as a suspected mutagen and carcinogen (Toolbox profiler Carcinogenicity by ISS) EFSA considers that DON is devoid of genotoxic potential (EFSA, 2014). Accordingly, IARC considers that there is inadequate evidence in experimental animals for its carcinogenicity (group 3, IARC, 1993).

Its hepatotoxicity has been shown (Peng et al., 2017) although has not been consensual and thereby a systematic discussion of the hepatic toxicity of DON is needed. DON is suspected to be toxic for reproduction and it is able to cross the human placenta (Nielsen et al., 2011). In addition, its teratogenic potential has been shown in animals (Yu et al., 2017) and deserves to be further studied. DON (and other trichothecenes), is immunotoxic, acting as a potent inhibitor of protein synthesis and stimulating the pro-inflammatory response (Sundheim et al., 2017). EFSA CONTAM Panel established a group TDI of 1 µg/kg bw per day for the sum of DON and itsacetylated and modified forms (3-Ac-DON, 15-Ac-DON and DON-3-glucoside) based on reduced body weight gain in mice.

In order to assess the acute human health risk, epidemiological data from mycotoxicoses were assessed and a group-ARfD of 8 µg/kg bw per eating occasion was calculated (EFSA, 2017).

FB1 is a suspected carcinogen according to the CLP classification and it is classified by IARC as possibly carcinogenic to humans (Group 2B, IARC, 2002). In vivo studies have shown that the repeated exposure to this toxin leads to liver and kidney toxicity (EFSA, 2018) and it is able to induce the formation of liver and kidney tumours (IARC, 2002). FB1 is not mutagenic in bacteria but it induces oxidative stress, being clastogenic to mammalian cells (EFSA, 2018). FB1 adverse effects are mainly mediated by the inhibition of ceramide synthases, which are key enzymes in sphingolipid metabolism. Based on the results of animal studies, JEFCA considered FB1 as a potential immunotoxic substance (WHO, 2011). It also causes developmental toxicity in several animal species (IARC, 2002). To derive HBGV for FB1, megalocytic hepatocytes in male mice were considered as the most appropriate outcome and a benchmark dose lower confidence limit 10% (BMDL10) of 165 µg/kg bw per day for FB1 was established (EFSA, 2018). The CONTAM Panel used the BMDL10 of 0.1 mg/kg bw per day and an uncertainty factor of 100 for intra and interspecies variability resulting in a TDI of 1.0 µg FB1/kg bw per day. Based on structural similarity and the limited data available indicating similar MoA and similar toxic potencies, the Panel decided that FB2, FB3 and FB4 should be included in a group TDI with FB1 (EFSA, 2018).

Recent surveys have highlighted the fact that humans are more frequently exposed to multiple than to single mycotoxins (Alvito et al, 2010; Solfrizzo et al., 2014; Alassane-Kpembi et al, 2016; Assunçao et al, 2016), raising a concern about their potential combined effect on human health. The presence of DON, FB1 and other mycotoxins was reported in foods (Sirot et al, 2013; De Boevre et al, 2013; Garcia-Moraleja et al, 2015; Assunção et al, 2016; Martins et al, 2018), in biological samples from general population (Heyndrickx, 2015; Vidal et al, 2016; Brera et al, 2015) and in occupational settings (Fromme et al, 2016; Viegas et al, 2018, 2018a). Besides the regulated mycotoxins, an increasing number of studies are paying attention to mixtures involving the “emerging” ones (beauvericin, enniantins, Alternaria toxins, etc.) (Alassane-Kpembi et al, 2017; Gruber-Dorninger et al, 2017; Puntscher et al, 2018). Other authors also refer the possible interactions between environment and food contaminants, cadmium and deoxynivalenol, in different target organs (Le TH et al, 2018).

2.2 Exposure characteristics

Mycotoxins are commonly detected in cereal-based foods, cereals or fruit-based beverages, and several animal products (Bennett and Klich, 2003) and the general population is currently exposed by the oral route, via the ingestion of contaminated foods. Additional exposure routes include inhalation and dermal absorption, which can be particularly relevant for occupational exposure (Fromme et al, 2016; Viegas et al, 2015; Viegas et al, 2018).

Results of the BIOMIN Mycotoxin Survey conducted from January to March 2018 indicate that deoxynivalenol (DON) and fumonisins (FUM) are the most common mycotoxins found in food commodities and feedstuffs (https://www.biomin.net/en/biomin-mycotoxin-survey/).

DON is the most prevalent Fusarium toxin in European grains and its occurrence is frequently reported in cereals and cereal-based products such as bread, pasta, or beer (Marin et al., 2013), thereby main exposure is by oral route. A total of 72,011 results of DON and its metabolites in food were obtained from 27 reporting countries and were related to samples collected between 2007 and 2014 (EFSA, 2017).

According to EFSA (2017), the estimated chronic dietary exposure 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, thus indicating a potential health

concern. The EFSA CONTAM Panel noted that the overall human dietary exposure to the sum of DON and its metabolites, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside was mainly driven by DON (EFSA, 2017). DON and DON-3-glucoside were absorbed, distributed, metabolized and rapidly excreted through urine as shown recently by a human intervention study after exposure to DON and DON-3-glucoside (Vidal et al, 2018b). The analysis of 24h urine samples revealed that DON-15-glucuronide was the most prominent urinary biomarker followed by free DON and DON-3-glucuronide. Other studies have reported the detection of DON (total DON) in the urine of the general population in UK (Turner et al, 2010a), France (Turner et al, 2010b), Sweden (Turner et al, 2010), Italy (Solfrizzo et al, 2014), Croatia (Sarkanj et al, 2013), Austria (Warth et al, 2012b), Belgium (Huybrechts et al, 2014) and Germany (Gerding et al, 2014).

Females and males show different patterns of exposure levels, and human exposure to DON also shows some geographical differences (Cheng et al, 2017; Vidal et al, 2018b). Additional exposure by inhalation in occupational settings were also reported (Fromme et al, 2016; Viegas et al, 2018).

The occurrence of FB1–3 is well documented in maize and products thereof and the main exposure route is the oral route (EFSA, 2018). Animal studies indicate that FB1 is poorly absorbed from the gastrointestinal tract and rapidly cleared from the blood by the biliary route, and preferentially excreted with the faeces (EFSA, 2018). In human biomonitoring studies FB1 has been detected in urine of the general population in Sweden (Wallin et al, 2012), Austria (Warth et al, 2012), Belgium (Ediage et al, 2012) and Germany (Gerding et al, 2014). Despite the low excretion rates for FB1 (0.93-2.6%) it has been proposed as biomarker of exposure. (Shephard et al. 1994; Dilkin et al. 2010; Gambacorta et al. 2013; Souto et al. 2017).

2.3 Policy relevance

In Europe, the European Commission (EU) has introduced comprehensive mycotoxin regulations for food to facilitate world trade and protect consumer´s health (Cheli et al., 2014). The EU Regulation (EC) No 1881/2006 established the maximum permissible limits for aflatoxins (AFB1, sum of AFB1, AFB2, AFG1 and AFG2, AFM1), ochratoxin A (OTA), patulin (PAT), DON, zearalenone (ZEN), FBs (sum of FB1 and FB2), sum of T-2 and HT-2 toxins, and citrinine in specific food products (EC, 2006a and its amendments). This regulation also includes much lower regulatory limits for food for infants and young children due to their particular vulnerability and different consumption pattern. In addition to mycotoxin maximum levels, EU Regulation (EC) No 401/2006 provides sampling plans according to nine different groups of food commodities taking into account the heterogeneous distribution of mycotoxins in agricultural commodities (EC, 2006b).The issue of effects resulting from exposure to multiple toxins (combined effects) and from different routes (aggregated exposure) had particularly concerned policy makers because combined effects can differ from individual effects of each chemical contaminant (Bouaziz et al, 2008). Government and industry regulations are usually based on individual mycotoxin toxicities and do not take into account the complex dynamics associated with interactions between co-occurring groups of mycotoxins (Assunção et al., 2016; Kienzler et al., 2016).

Farmers need to continuously assess the risk from mycotoxins to both crops and animals. These good practices together with harmonized international legislation on permitted maximum levels will ensure that highly contaminated cereals do not enter the food chain. From growers to retailers, all food business operators following the rules set by Codex Alimentarius Committee are able to ensure that food is safe in every home (Codex Committee on Contaminants in Food).

Concerning inhalation, the absence of exposure limits makes it difficult to interpret the exposure values and to determine acceptable values for occupational settings, in order to ensure workers' health (Viegas et al., 2018; Viegas et al., 2018a).

2.4 Technical aspects

Mycotoxin exposure assessment throughout biomonitoring studies based on the analysis of mycotoxin themselves, protein or DNA adducts, and/or major phase I or phase II metabolites (e.g. glucuronide conjugates), in human biological samples such as urine, serum and breast milk, have provided useful information over recent years. Fast advances in LC–MS technology have allowed multiple mycotoxins to be analysed simultaneously (Ediage et al, 2012; Warth et al, 2013; Solfrizzo et al, 2014; EFSA, 2017; Sarkanj et al., 2018).

Recent progress in biomarker research has allowed the determination of DON and its metabolites in urine, primarily as DON-glucuronides, by using single or multiple biomarker methods. DON-15-glucuronide, the sum of DON-glucuronides, or total DON (sum of free DON + DON-glucuronides after deconjugation) are considered suitable DON-biomarkers of exposure in urine. DON-3-glucoside, a modified form of DON, has a similar excretion profile as DON with DON-15-glucuronide being the most abundant metabolite (Vidal et al, 2018b). To determine the urinary glucuronides, a preliminary approach was developed based on the enzymatic hydrolysis of deoxynivalenol-glucuronides, and subsequent determination of the “total DON” (sum of free and released mycotoxins by hydrolysis) (Solfrizzo et al, 2014; Turner et al, 2010). Afterwards, a direct method for quantification of glucuronides such as DON-3-glucuronide and DON-15-glucuronide was developed using in-house synthesized mycotoxin-standards (Ediage et al, 2012; Warth et al, 2013). These analytical developments permitted the scientific community to find strong correlations between the sum of urinary DON and its glucuronides (Vidal et al, 2018b). Most of the reported analytical methods for DON biomarker analysis in urine were sensitive enough to differentiate exposure levels. However, commercial sources for DON glucuronide standards are scarce and no certified reference materials are available for urinary DON biomarkers (EFSA, 2017). New trends in high-resolution MS for untargeted metabolic profiling and metabolomics may unravel and identify novel metabolites, biotransformation products and/or modified DON forms (EFSA, 2017; Vidal et al, 2018b; Sarkanj et al, 2018). Recently, DON-3-sulfate, a novel human metabolite and potential new biomarker of DON exposure was also reported in urine samples obtained from pregnant women in Croatia (Warth et al, 2016). Exposure to fumonisins can be assessed using urinary biomarkers. FB1–3 and hydrolysed form of FB1, HFB1, have been suggested as direct biomarkers of exposure by several authors (Shephard et al., 2007; Ediage et al, 2012; Heyndrickx et al., 2015). However, because of the poor urinary excretion of fumonisins and the consequent need for high sensitivity analytical procedures, the sample protocol requires an extensive clean-up and concentration step, based on SPE C18 cartridge or immunoaffinity purification. (EFSA, 2018).

2.5 Societal concern

It has been well recognized for many years that large economical losses occur worldwide owing to the mycotoxin contamination in agricultural products as recently summarized by Pitt and Miller (2016). Climate change is expected, in the upcoming decades, to impact fungal growth and agricultural practices and, consequently, to shift mycotoxins incidence, concentration and geographical spread.

The changing climate conditions will also lead to higher human and animal dietary exposure and, consequently, to increased human health risks (Wu and Mitchell, 2016). A recent report from WHO (2018) also refers to the effects that climate change could have on mycotoxins occurrence in Europe and their impact on human health.

In this context, health effects resulting from exposure to multiple mycotoxins (combined effects) and from different routes (aggregated exposure) constitutes a rising concern, especially because health effects resulting from multiple mycotoxins exposure could lead to different output toxicity

and carcinogenicity than exposure to single mycotoxins (Bouaziz et al., 2008). A multidisciplinary effort should be developed to perform the human health risk assessment of multiple mycotoxins present in food, considering that the information obtained from the risk assessment process will be used by risk managers to prioritise possible public health concerns and to develop risk management options towards disease prevention (Assunçao et al, 2016).

3. Categorization of Substances

Table 3.1: Substances included in the mycotoxins group, DON and FB1, listed according to availability of toxicology and human biomarker data, in category C substances*

**HBM scarcely exists, efforts to develop an anlytical method to obtain relevant HBM results need to be done, hazardous proprieties of the substances are identified, yet greater knowledge on toxicological characteristics and effects on human health is needed, interpretation of HBM data is not possible, due to the lack of HBM guidance values.*

Category	Designation (Abbreviation/ Acronym)	Systematic name (IUPAC name?)	CAS No.	Regulation
C	Deoxynivalenol (DON)	trichothec-9-en-8-one, 12, 13-epoxy-3,7,15-trihydroxy-, (3α,7α)	51481-10-8	Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
C	Fumonisin B1 (FB1)	(2R)-2-[2-[(5R,6R,7S,9S,11R,16R,18S,19S)-19-amino-6-[(3R)-3,4-dicarboxybutanoyl]oxy-11,16,18-trihydroxy-5,9-dimethylicosan-7-yl]oxy-2-oxoethyl]butanedioic acid	116355-83-0	Sum B1+B2 Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

4. Policy-related questions

The following questions are mandatory for deoxynivalenol (DON) and its acetylated and modified forms and fumonisin B1 (FB1). Data on other mycotoxins could be added, if possible.

1. Are there validated and harmonized analytical methods to assess the selected mycotoxin exposure biomarkers?
2. What are the current exposure levels of the European population to the selected mycotoxins? Are there exposure data for other mycotoxins?
3. Does the exposure to mycotoxins differ among countries/EU geographical regions and different population groups? Which are the main factors related with these differences (age, gender, occupational settings, geographic localisation, season/year)?
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)?
5. Are there exposure models and toxicokinetics data for mycotoxins? Which are their limitations?
6. Is the risk associated with human exposure to these mycotoxins characterized? Are there health impact assessment studies? Is it possible to set a HBGV for mycotoxins in biological samples?
7. Does the aggregate exposure to mycotoxins/other food contaminants contribute to combined effects? What are the knowledge gaps for risk assessment?
8. Which are the key-events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins? Which are the health effects associated with high short-term exposure by inhalation (occupational exposure)?
9. Which are the most reliable and meaningful effect biomarkers for single and combined effects?
10. Are there mycotoxins (including metabolites masked and/or other modified forms) besides those covered by the current risk assessment, which could be potentially relevant concerning their (co-) occurrence and toxicological properties?

5. Research Activities to be undertaken

Table 5.1: Listing of research activities to be carried out to answer the policy questions

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
1. Are there validated and harmonized analytical methods to assess mycotoxin exposure biomarkers?	mycotoxins	Analytical methods for DON and its glucuronides as well as FB1–4 are mainly based on MS. However, commercial sources for DON glucuronide standards are scarce and no certified reference materials are available for urinary DON biomarkers Only FB1–3 are available on the market as calibrant solutions, while FB4 can be purchased as purified powder. Except for HFB1, analytical standards for modified forms are not commercially available.	G: Current analytical methods, harmonized methods, reference materials, proficiency tests, expert laboratories A: 1. Identify across Europe the analytical capacity for determination of multiple biomarkers of exposure, availability of reference materials and standards; best biomarkers, matrices and methods (Y3) WP9 2. Promote training and harmonization on analysis of selected mycotoxins biomarkers including an inter-laboratorial assay (Y3, 4) WP2, WP9 3. Identify expert laboratories to conduct the inter-laboratorial trial (Y3) WP9 4. Elaboration of SOP for trial assay (Y3) WP9 5. Extension of qualified laboratories by introduction of HBM specialised laboratories (Y3,4) 6. Identify quality assurance requirements (Y3, 4) WP9 7. Identify needs and gaps.
2. What are the current exposure levels of the European population to DON and FB1? Are there exposure data for other mycotoxins?	Mycotoxins DON and FB1 are mandatory but HBM data on other mycotoxins are also welcome	Wide exposure to mycotoxins have been reported mainly through food commodities. Additional studies also report exposure by inhalation in occupational settings. DON (total DON) and FB1 were detected in the urine of the general population in United Kingdom, France, Sweden, Italy, Croatia, Austria, Belgium, Germany as well as in occupational settings (although in a lower extent).	G: Current data on mycotoxin exposure from EU countries for general population (different population groups including vulnerable populations as children, special diet, pregnant women) and workers. A: 1. Perform an inventory survey on FB1 data before intiation of a large scale QA and monitoring activities to evaluate the percentage of left-censored date available. 2. Create a database for mycotoxin exposure using HBM data from different EU countries (gathered by national hubs) including mycotoxin identification, population group and ages, routes of exposure and HBM data. Collect, harmonize, compare data from different population groups available and evaluate (Y3, Y4). WP7, WP8, WP10 3. Integrate into IPChem (Y4). WP10 4. Identify needs (WP4) and gaps.WP7

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
3. Does the exposure to mycotoxins differ among countries and different population groups? Which are the main factors related with these differences (age, gender, settings, geographic localization, season/year)	mycotoxins	Females and males show different excretion patterns, and human exposure to DON also shows some geographical differences. Occupational exposure revealed exposure associated with professional activity.	G: Current risk groups related to age, gender, diet, occupational setting, location, in EU A: 1.Identify risk groups, including highly exposed, vulnerable and hotspots in Europe (Y3,Y4) WP10 2. Statistical analysis (Y3). 3. Identify significant differences between analysed groups (Y4). WP10 4. Identify needs and gaps
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)?	mycotoxins	More than half of all worldwide agricultural samples contain DON and FUM (Biomin Mycotoxin Survey). A total of 72,011 results of DON and its metabolites in food were obtained from 27 reporting countries and were related to samples collected between 2007 and 2014 (EFSA, 2017).	G: Analysis of trends on HBM mycotoxin exposure A: 1.Identify possible temporal and geographic trends related to HBM mycotoxin exposure taking seasonal variation into account (Y4) WP10 2. Evaluate significant differences (Y4) WP10 3. Identify possible reasons for the differences founded (Y4) 4.Identify needs and gaps.
5. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?	mycotoxins	DON and its metabolite DON-3-glucoside were absorbed, distributed, metabolized and rapidly excreted through urine as shown recently by a 1st human intervention study after exposure to DON and DON-3-glucoside. Animal studies indicate that FB1 is poorly absorbed from the gastrointestinal tract (less than 4% of the dose), rapidly cleared from the blood (with half-lives of less than 4 h) by the biliary route, and preferentially excreted with the faeces (usually more than 90% of the dose).	G: Exposure models and toxicokinetics in humans A: 1. Explore the possibility of applying the previously developed toxicokinetic models to DON and FB1 (Y3) 2. Determine exposure levels from HBM databases and available literature through reverse dosimetry models (Y4) WP12 3. Identify needs and gaps

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
6. Is the risk associated with human exposure to these mycotoxins characterized? Are there health impact assessment studies? Is it possible to set a HBGV for mycotoxins in biological samples?	mycotoxins	The estimated mean chronic dietary exposure was above the group-TDI in infants, toddlers and other children, and at high exposure also in adolescents and adults, indicating a potential health concern. Little if any work has been done in estimating the burden of human disease caused by exposure to the dietary mycotoxins. The only studies available are related to aflatoxin B1 (Wu et al, 2014; Assunçao et al, 2018b).	G: Risk characterisation and health impact assessment (HIA) A: 1. Identify available estimates of human exposure via biomarkers (Y3,4); 2. collect toxicological data (Y3,4) 3. If possible, establish HBGV values for mycotoxins in biological samples (Y4) (WP5) 4. From risk assessment to health impact assessment: trying to derive the consequences of human exposure to mycotoxins using epidemiological data (e.g. incidence of disease, age of onset of disease and its evolution) and data gathered on human exposure studies (e.g. DALYs) (Y5) WP5, WP11 5. Identify needs and gaps.
7. Does the aggregate exposure to mycotoxins/other food contaminants contribute to combined effects? What are the knowledge gaps for risk assessment?	mycotoxins	Co-occurrence of DON or FB1 and other mycotoxins has been widely reported and human aggregated exposure to mycotoxins and other food contaminants is likely to occur	G: Lack of an inventory of exposure to DON or FB1 and other mycotoxins/other food contaminants in EU and potential interactive effects A 1. Identify main mycotoxin/other food contaminants mixtures from available HBM data (biomarkers and routes of exposure); (Y3) WP15 2. Compare available HBM mixtures data over EU countries, look for significant differences and trends (Y4) WP15, WP10? 3. Assess common endpoints, determine whether the additive model is adequate to describe mycotoxins/other food contaminants combined effects; assess if this is dependent of mode of action or the target organ toxicity (Y4) WP15 4. Identify needs and gaps
8. Which are the key events that determine the long-term health effects from low-dose continuous exposure to the target mycotoxins?Which are the health effects associated with short-term high exposure by inhalation (occupational exposure)?	mycotoxins	DON is considered as immunotoxic, reprotoxic and a probable endocrine disruptor. There is limited evidence on its potential genotoxicity and carcinogenicity. It is a potent inhibitor of protein synthesis and stimulates the pro-inflammatory response leading to oxidative stress. FB1 is a liver and kidney toxicant and it is immunotoxic. It is a probable carcinogen but there are data gaps on its mutagenicity. Its adverse effects are mainly mediated by the inhibition of ceramide synthases, which are key enzymes in sphingolipid metabolism	G: Several health effects known and mechanistic data available but AOP for DON and FB1 lacking A. 1. Identify for DON and FB1 the health effect for which a AOP might be developed, e.g. immunotoxicity for DON and liver toxicity for FB1 (Y3) 2. Disclose the key-events for the effects referred in 1. in order to contribute to AOPs development (Y3, Y4) WP13 3. Identify needs and gaps

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
9. Which are the most reliable and meaningful effect biomarkers for single and combined effects?	mycotoxins	Some biomarkers of early biological effects have been pointed for DON (e.g., pro-inflammatory cytokines) and FB1 (e.g., sphinganine-to-sphingosine ratio in blood) but further knowledge is needed	G: Limited information on available biomarkers of effects A:1. Identify available targeted and untargeted biomarkers of effect for the selected mycotoxins (Y3, Y4) WP14 2. Identify biomarkers of effect related to interactive effects of mixtures (Y3, Y4) 3. Identify needs and gaps
10. Are there mycotoxins beside those currently covered by the risk assessment, which could be potentially relevant concerning their (co-)occurrence and toxicological properties?	mycotoxins	An increasing number of studies are paying attention to mixtures involving the “emerging” toxins (enniantins, beauvericin, Alternaria toxin, etc.).	G: Co-occurring forms (emergent mycotoxins) with potential toxicity and health impact that are not covered in risk assessment A1. Bibliography search (Y3,4) WP16 2. Identify most relevant co-occurring forms other than those already covered, to refine human risk assessmen (Y3,4,).WP16, WP5 3. Identify needs and gaps.

6. References

1. Alassane-Kpembi I, Schatzmayr G, Taranu I, Marin D, Puel O, and Oswald IP. (2017). Mycotoxins co-contamination: Methodological aspects and biological relevance of combined toxicity studies. Crit Rev Food Sci Nutr. 2017 Nov 2; 57(16):3489-3507.
2. Alvito PC, Sizoo EA, Almeida CM, and van Egmond HP (2010). Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. Food Analytical Methods, 3(1), 22-30.
3. Assunção R, Silva MJ, Alvito P (2016). Challenges in risk assessment of multiple mycotoxins in food. World Mycotoxin J. 9:791–811.
4. Assunção R, Martins C, Vasco E, Jager A, Oliveira C, Cunha SC, Fernandes JO, Nunes B, Loureiro S, and Alvito P (2018a). Portuguese children dietary exposure to multiple mycotoxins – An overview of risk assessment under MYCOMIX project. Food and Chemical Toxicology, 118, 399–408.
5. Assunção R, Martins C, Viegas S, Viegas C, Jakobsen L, Pires S and Alvito P (2018b). Climate change and the health impact of aflatoxins exposure in Portugal – an overview. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2018 Mar 8:1-12
6. Battilani P, Toscano P, Van Der Fels-Klerx HJ, Moretti A, Camardo Leggieri M, Brera C, Rortais A, Goumperis T, Robinson T (2016). Aflatoxin B1 contamination in maize in Europe increases due to climate change. Sci Rep. 6:24328.
7. Bennett JW, Klich M (2003) Mycotoxins. Clin Microbiol Rev 16:497–516.
8. Brera, C., de Santis, B., Debegnach, F., Miano, B., Moretti, G., Lanzone, A., … Sathyapalan, T. (2015). Experimental study of deoxynivalenol biomarkers in urine. EFSA supporting publication. Parma. Retrieved from www.efsa.europa.eu/publications.
9. Bouaziz, C., Sharaf El Dein, O., El Golli, E., Abid-Essefi, S., Brenner, C., Lemaire, C., Bacha, H. (2008). Different apoptotic pathways induced by zearalenone, T-2 toxin and ochratoxin A in human hepatoma cells. Toxicology 254, 19–28. http://dx.doi.org/10. 1016/j.tox.2008.08.020.
10. De Boevre M, Jacxsens L, Lachat C, Eeckhout M, Di Mavungu J., Audenaert K, Maene P, Haesaert G, Kolsteren P, De Meulenaer B, De Saeger, S. (2013). Human exposure to mycotoxins and their masked forms through cereal-based foods in Belgium. ToxicologyLetters, 218: 281-292.
11. Dilkin P, Direito G, Simas MMS, et al (2010) Toxicokinetics and toxicological effects of single oral dose of fumonisin B1 containing Fusarium verticillioides culture material in weaned piglets. Chem Biol Interact 185:157–162. doi: 10.1016/j.cbi.2010.03.025
12. Ediage, Emmanuel Njumbe, Jose Diana Di Mavungu, Suquan Song, Aibo Wu, Carlos Van Peteghem, and Sarah De Saeger.(2012). “A Direct Assessment of Mycotoxin Biomarkers in Human Urine Samples by Liquid Chromatography Tandem Mass Spectrometry.” Analytica Chimica Acta 741 (September):58–69. https://doi.org/10.1016/j.aca.2012.06.038
13. EFSA Panel on Contaminants in the Food Chain (CONTAM), (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 2014;12(12):3916, 107 pp. doi:10.2903/j.efsa.2014.3916
14. EFSA Panel on Contaminants in the Food Chain (CONTAM), (2017). Scientific opinion on the risk to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA Journal, 15(9), 345.
15. EFSA Panel on Contaminants in the Food Chain (CONTAM), (2018). Scientific Commission on the Appropriateness to set a group health-based guidance value for fumonisins and their modified forms EFSA Journal 2018;16(2):5172
16. Escrivá L, Font G, Manyes L and Berrada H (2017). Studies on the Presence of Mycotoxins in Biological Samples: An Overview, Toxins 2017, 9, 251.
17. Eskola M, Altieri A, and Galobart J (2018). Overview of the activities of the European Food Safety Authority on mycotoxins in food and feed. World Mycotoxin Journal, 2018; 11 (2): 277-289.
18. European Commission (2006a). Commission regulation EC 1881/2006, setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Communities L364, 5e24.

19. European Commission (2006b). Commission regulation EC 401/2006, laying down the methods of sampling and analysis for the official control of the levels, of mycotoxins in foodstuffs. Off. J. Eur. Communities L70, 12e34.
20. Fromme, H.; Gareis, M.; Völkel, W.; Gottschalk, C. (2016). Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings—An overview. Int. J. Hyg. Environ. Health, 219, 143–165.
21. Gambacorta S, Solfrizzo H, Visconti A, et al (2013). Validation study on urinary biomarkers of exposure for aflatoxin B 1 , ochratoxin A, fumonisin B 1 , deoxynivalenol and zearalenone in piglets. World Mycotoxin J 6:299–308. doi: 10.3920/WMJ2013.1549
22. García-Moraleja A, Font G, Mañes J, and Ferrer E. (2015). Analysis of mycotoxins in coffee and risk assessment in Spanish adolescents and adults. Food and Chemical Toxicology 86: 225-233.
23. Gerding, Johannes, Benedikt Cramer, and Hans-Ulrich Humpf. (2014). "Determination of Mycotoxin Exposure in Germany Using an LC-MS/MS Multibiomarker Approach." Molecular Nutrition & Food Research 58 (12):2358–68. https://doi.org/10.1002/mnfr.201400406
24. Grenier B and Oswald I (2011). Mycotoxin co-contamination of food and feed: meta-analysis of publications describing toxicological interactions. World Mycotoxin Journal, August 2011; 4 (3): 285-313.
25. Gruber-Dorninger C, Novak B, Nagl V, Berthiller F.(2017), Emerging Mycotoxins: Beyond Traditionally Determined Food Contaminants. J Agric Food Chem. 2017 Aug 23;65(33):7052-7070. doi: 10.1021/acs
26. Heyndrickx E, Sioen I, Huybrechts B, Callebaut A, De Henauw, and De Saeger S (2015). Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. Environment International, 84, 82–89.
27. Huybrechts, B., Martins, J.C., Debongnie, P., Uhlig, S., Callebaut, A., (2014). Fast andsensitive LC–MS/MS method measuring human mycotoxin exposure usingbiomarkers in urine. Arch. Toxicol., p11.
28. IARC (1993). http://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Evaluation-Of-Carcinogenic-Risks-To-Humans/Some-Naturally-Occurring-Substances-Food-Items-And-Constituents-Heterocyclic-Aromatic-Amines-And-Mycotoxins-1993
29. IARC (2002). http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf
30. IARC (2012). Monographs. http://monographs.iarc.fr/ENG/Monographs/vol100F/index.php
31. Aude Kienzler, Stephanie K. Bopp*, Sander van der Linden, Elisabet Berggren, Andrew Worth - Regulatory assessment of chemical mixtures: Requirements, currentapproaches and future perspectives. Regulatory Toxicology and Pharmacology 80 (2016) 321e334
32. Komsky-Elbaz A, Saktsier M, Roth Z. (2018). Aflatoxin B1 impairs sperm quality and fertilization competence. Toxicology 393:42-50.
33. Köppen R, Koch M, Siegel D, Merkel S, Maul R, Nehls I (2010). Determination of mycotoxins in foods: current state of analytical methods and limitations. Appl. Microbiol. Biotechnol., 86, 1595–1612. doi:10.1007/s00253-010-2535-1.
34. Martins C, Assunção R, Cunha SC, Fernandes JO, Jager A, Petta T, Oliveira CO, Alvito P (2018). Assessment of multiple mycotoxins in breakfast cereals available in the Portuguese market. Food Chemistry 239:132-140.
35. McCoy, L. F., Scholl, P. F., Sutcliffe, A. E., Kieszak, S. M., Powers, C. D., Rogers, H. S., Gong, Y. Y., Groopman, J. D., Wild, C. P., and Schleicher, R. L. (2008) Human aflatoxin albumin adducts quantitatively compared by ELISA, HPLC with fluorescence detection, and HPLC with isotope dilution mass spectrometry. Cancer Epidemiol Biomarkers Prev 17: 1653-1657.
36. Mehrzad, J., Devriendt, B., Baert, K., and Cox, E. (2014). Aflatoxin B1 interferes with the antigen-presenting capacity of porcine dendritic cells. Toxicol. Vitro 28: 531–537.
37. Nielsen JK, Vikstrom AC, Turner P, Knudsen LE (2011). Deoxynivalenol transport across the human placental barrier. Food Chem. Toxicol., 49:2046-2052.
38. Ostry V, Malir F, Toman J and Grosse Y (2017). Mycotoxins as human carcinogens—the IARC Monographs classification. Mycotoxin Res 33:65–73.
39. Paterson RM, Lima N (2011). Further mycotoxin effects from climate change. Food Research International 44:2555–25.

40. Pitt JI and Miller JD, (2016). A concise history of mycotoxin research. Journal of Agricultural and Food Chemistry 65(33): 7021-7033.
41. Peng Z, Chen L, Nüssler AK, Liu L, Yang W, (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.
42. Sirot V, Fremy J.M., and Leblanc, J.C.,(2013). Dietary exposure tomycotoxins and health risk assessment in the second French totaldiet study. Food and Chemical Toxicology 52: 1-11.
43. Puntscher H, Kütt ML, Skrinjar P, Mikula H, Podlech J, Fröhlich J, Marko D, Warth B.(2018). Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified Alternaria toxins. Anal Bioanal Chem. 2018 Jul;410(18):4481-4494. doi: 10.1007/s00216-018-1105-8. Epub 2018 May 16.
44. Sarkanj B, Warth B, Uhlig S, Abi WA, Sulyok M, Klapec T, Krska R, Banjari I, (2013). Urinary analysis reveals high deoxynivalenol exposure in pregnantwomen from Croatia. Food Chem. Toxicol. 62: 231–237.
45. Sarkanj B, Ezekiel C N, Turner P C, Abia W A, Rychlik M, Krska R, Sulyok M, Warth B (2018). Ultra-sensitive, stable isotope assisted quantification of multiple urinary mycotoxin exposure biomarkers. Analytica Chimica Acta 1019: 84-92.
46. Shephard G, G Thiel P, W Sydenham E, et al (1994) Distribution and excretion of a single dose of the mycotoxin fumonisin B1 in a non-human primate. Toxicon 32:735–741
47. Shephard GS, Van Der Westhuizen L & Sewram V, (2007). Biomarkers of exposure to fumonisin mycotoxins: A review. Food Additives & Contaminants Vol. 24, Iss. 10.
48. Solfrizzo M, Gambacorta L, Visconti A (2014). Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. Toxins (Basel). 2014 Jan 28;6(2):523-38.
49. Souto PCMC, Jager A V., Tonin FG, et al (2017) Determination of fumonisin B1levels in body fluids and hair from piglets fed fumonisin B1-contaminated diets. Food Chem Toxicol 108:1–9. doi: 10.1016/j.fct.2017.07.036
50. Sundheim L, Lillegaard I T, Fæste C K, Brantsæter A-L, Brodal G, & Eriksen GS (2017). Deoxynivalenol Exposure in Norway, Risk Assessments for Different Human Age Groups. Toxins, 9(2), 46.
51. Thanh-Huong L, Alassane-Kpembi I, Oswall, I, Pinton P (2017). Analysis of the interactions between environmental and food contaminants, cadmium and deoxynivalenol, in different target organs. Science of The Total Environment 622-623:841-848
52. Turner PC, (2010). Deoxynivalenol and nivalenol occurrence and exposureassessment. World Mycotoxin J. 3, 315–321.
53. Turner PC, White KLM., Burley V, Hopton RP, Rajendram A., Fisher, J., Cade,J.E., Wild, C.P., (2010a). A comparison of deoxynivalenol intake and urinarydeoxynivalenol in UK adults. Biomarkers 15, 553–562.
54. Turner C, Hopton RP, Lecluse Y, White KLM, Fisher J, Lebailly P, (2010b).Determinants of urinary deoxynivalenol and de-epoxy deoxynivalenol in malefarmers from Normandy, France. J. Agric. Food Chem. 58, 5206–5212.
55. Vidal A , Mengelers M, Yang S, De Saeger S, and De Boevre M (2018a). Mycotoxin Biomarkers of Exposure: A Comprehensive Review. Comprehensive Reviews in Food Science and Food Safety
56. Vidal A, Claeys L, Mengelers M, Vanhoorne V, Vervaet C, Huybrechts B, De Saeger S & De Boevre, M (2018b). Humans significantly metabolize and excrete the mycotoxin deoxynivalenol and its modified form deoxynivalenol-3-glucoside within 24 hours. Scientific Reports, 8(1).
57. Viegas S, Veiga L, Almeida A, dos Santos M, Carolino E and Viegas C (2015) Occupational Exposure to Aflatoxin B1 in a Portuguese Poultry Slaughterhouse. Ann Occup Hyg. doi: 10.1093/annhyg/mev077.
58. Viegas S, Viegas C and Oppliger A.(2018). Occupational Exposure to Mycotoxins: Current Knowledge and Prospects. Annals of Work Exposures and Health, 1–19. doi: 10.1093/annweh/wxy070.
59. Viegas S, Assunção R, Nunes C, Osteresch B, Twarużek M, Kosick R, Grajewski J, Martins C, Alvito P, Almeida A, Viegas C (2018a). Exposure Assessment to Mycotoxins in a Portuguese Fresh Bread Dough Company by Using a Multi-Biomarker Approach. Toxins, 10, 342; doi:10.3390/toxins10090342a.

60. Wallin S, Hardie, L. J., Kotova, N., Warensjo Lemming, E., Nälsén, C., Ridefelt, P., … Olsen, M. (2013). Biomonitoring study of deoxynivalenol exposure and association with typical cereal consumption in Swedish adults. World Mycotoxin Journal, 6(4), 439–448. http://doi.org/10.3920/WMJ2013.1581 439
61. Warth B, Braun D, Ezekiel C, Turner P, Degen G, and Marko D (2016). Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. Chem. Res. Toxicol. 29, 1087−1097.
62. Warth B, Del Favero G, Wiesenberger G, Puntscher H, Woelflingseder L, Fruhmann P, Sarkanj B, Krska R, Schuhmacher R, Adam G & Marko D (2016). Identification of a novel human deoxynivalenol metabolite enhancing proliferation of intestinal and urinary bladder cells. Scientific Reports, 6:33854.
63. WHO (2011). 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.
64. WHO (2017). WHO Technical report series; no. 1002 Evaluation of certain contaminants in food: eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives.
65. Wild C P, & Turner P C (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis, 17, 471-481.
66. Wogan, G. N., Kensler, T. W., and Groopman, J. D. (2012) Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29, 249-257.
67. Wu F, Groopman, JD and Pestka, JJ (2014). Public health impacts of foodborne mycotoxins. Annual Review of Food Science and Technology 5: 351-372.
68. Wu F, and Mitchell NJ (2016). How climate change and regulations can affect the economics of mycotoxins. World Mycotoxin Journal 9: 653-663.
69. Yu M, Chen L, Peng Z, Nüssler AK, Wu Q, Liu L, Yang W, (2017). Mechanism of deoxynivalenol effects on the reproductive system and fetus malformation: Current status and future challenges. Toxicology in Vitro, 41, pp. 150-158