1 Prioritised substance group: Mycotoxins- UPDATED

Responsible author	Paula Alvito, Susana Viegas, Maria João Silva
Short name of institution	INSA ESTeSL
Co-authors	Ricardo Assunção Carla Martins

1.1 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 harmonised analytical methods to assess the target mycotoxins exposure?
2. What is the current exposure levels of the European population to DON and FB1? Are there exposure data for other mycotoxins?
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, overtime)?
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. Is the risk associated to human exposure to these mycotoxins characterised? Are there health impact assessment studies?
6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations?
7. Is it possible to set a HBM guidance value for mycotoxins?
8. Does the aggregated exposure to mycotoxins/other food contaminants contribute to combined effects? What are the knowledge gaps for risk assessment?
9. Which are the most reliable and informative effect biomarkers for single and combined effects of mycotoxins?
10. Which research needs and gaps on target mycotoxins HBM?

1.2 Research activities to be undertaken

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

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
1. Are there validated and harmonised analytical methods to assess the target mycotoxins exposure?	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, harmonised methods, reference materials, proficiency tests, expert laboratories A. • Identify across Europe the analytical capacity for determination of multiple biomarkers of exposure, availability of reference materials and standards, best biomarkers, matrices and methods (task 9.1; Y3, Y4). • Promote training and harmonisation on analysis of mycotoxin biomarkers. Identify quality assurance requirements including the performance of an interlaboratory comparison exercise (ICI). Start chemical analysis as part of the aligned studies (task 9.2 and task 9.4; Y3, Y4, Y5).

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
2. What is 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. Collect, compare and evaluate the available data on mycotoxins exposure (DON and its metabolites, FB1 and other if possible) (WP 7,10, Y3, Y4, Y5) and integrate into IPCheM (WP10; Y4, Y5)
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 localisation, overtime)	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. • Identify risk groups, including highly exposed, vulnerable and hotspots in Europe; evaluate significant differences between analysed groups (WP10; Y4, Y5)
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. • Identify possible temporal and geographic trends in mycotoxin exposure and possible reasons for the differences found (WP10; Y4, Y5)

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
5. Is the risk associated to human exposure to these mycotoxins characterised? Are there health impact assessment studies? 6. Are there exposure models and toxicokinetics data for mycotoxins and which are their limitations? 7. Is it possible to set a HBM-GV for mycotoxins?	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). DON and its metabolite DON-3-glucoside were absorbed, distributed, metabolised 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: Risk characterisation and health impact assessment (HIA), exposure models and toxicokinetics in humans A • Estimate exposure levels to mycotoxins from HBM data and establish exposure distribution and/or European reference values for mycotoxins if possible (WP5, 12; Y4, Y5).

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
8. 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. • Identify the main mycotoxin mixtures from available HBM data and available reports on toxicological/cocktail effects. Look for significant differences and trends (WP15; Y3, Y4, Y5)
9. Which are the most reliable and informative effect biomarkers for single and combined effects of mycotoxins?	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. 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. Several health effects known and mechanistic data available but AOP for DON and FB1 lacking; limited information on available biomarkers of effects A. • Linking the targeted and untargeted biomarkers of effect identified for mycotoxins and its mixtures with mechanistic data to develop AOPs for the target mycotoxins (WP13/14; Y3, Y4, Y5).

Policy question	Substance	Available knowledge	Knowledge gaps (G) and activities needed (A)
10. Which are research needs and gaps on target mycotoxins HBM?	mycotoxins		G. Research need and gaps A. • Identify research needs and gaps (analytical data, toxicokinetics, exposure studies via biomarkers, mixtures risk assessment, etc.) (WP9, 10, 12, 15; Y4, Y5)