Committee on toxicity of chemicals in food, Consumer products and the environment

Statement on the potential risk(s) of combined exposure to mycotoxins

Background

1. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has identified the potential risk(s) from combined exposure to mycotoxins as an additional possible concern during their review of mycotoxins in the diet of infants and young children, not only to these age groups but in other age groups as well.

2. Advances in analytical techniques have allowed the simultaneous detection and quantification of multiple mycotoxins in both food and feed commodities (Krska *et al*., 2007; De Santis *et al*., 2017; Flores-Flores & González-Peñas, 2017; Bessaire *et al*., 2019; Singh & Mehta, 2020; Agriopoulou *et al*., 2020), indicating that exposure to multiple mycotoxins *via* the diet is possible.

3. Furthermore, climate change could have a significant impact on the life cycle stages[1] and rates of development of toxicogenic fungi (*i.e.* mycotoxins), resulting in possible changes in host-resistance and host-pathogen interactions. Changes in the climate are expected to affect levels of rainfall,

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[1] There are four main stages for the fungi life cycle: hyphal growth, spore formation, spore dispersal and spore germination.

humidity, temperature *etc.* which in turn, will influence the conditions for mycotoxin production that varies for each individual pathogen species and/or strain.

4. Current government and industry regulations are usually based on assessing the risks from individual mycotoxins and, at most, group metabolites of mycotoxins with the parent compound, but take no account of the varied dynamics and potential interactions between co-occurring groups of mycotoxins.

5. In light of this, new combinations of factors (mycotoxins/host plants and geographical location) will need to be considered when assessing the potential risk(s) of from dietary exposure to mycotoxins.

Introduction

6. Mycotoxins are toxic secondary metabolites produced by plant fungi and can cause adverse health effects in both humans and animals. Cereals (for example, wheat, oats, rice, corn (maize), barley, sorghum, rye, and millet) are often the crops most severely affected; however, some nuts, fruits and spices can also be affected. Mycotoxins are stable, low-molecular weight chemicals and are often not affected by food processing (for example, cooking).

7. The mycotoxins of greatest concern to human health and livestock are those produced by several genera of filamentous fungi, namely *Aspergillus*, *Fusarium* and *Penicillium*, which produce aflatoxins (AFs), ochratoxin A (OTA), patulin (PAT), fumonisins (FBs), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), citrinin (CIT), T-2 and HT-2 toxins (WHO, 2018).

8. Exposure to dietary mycotoxins can lead to various adverse health outcomes in humans, which include carcinogenic, teratogenic, hepatotoxic, nephrotoxic, cytotoxic, immunological and haematological effects.

9. In addition to primary mycotoxins, modified mycotoxins can also be produced by fungi or generated as part of the defence mechanism of the infected plant, some of which are also directly toxic. They are described as metabolites and normally are not detected during the testing for parent mycotoxins (for example, deoxynivalenol-3-glucoside when testing for DON). It has been reported that some modified mycotoxins, whilst not very toxic themselves, can be converted into the parent mycotoxin during digestion in humans and animals, and thus have the potential to result in higher exposure to the parent mycotoxin and consequently increase the likelihood of adverse health effects. These have been termed masked mycotoxins. However, the toxicological data for these is scarce (Freire & Sant'Ana, 2018).

Prevalence and co-occurrence

10. DON, FBs, and ZEN are the most prevalent mycotoxins in the world, detected in 66%, 56%, and 53%, respectively of cereals and cereal based products (Smith, 2016).

11. The production of mycotoxins can occur pre-, during or post-harvest. Several factors can influence the production of mycotoxins pre-harvest, such as the sowing time, plant density, soil conditions, irrigation, presence of weeds and pests. During harvest, the influencing factors include drying, cleaning and sorting of the crops, whereas the post-harvest factors are mainly associated with storage and processing. Mycotoxin colonisation also depends on the temperature, relative humidity, rainfall and water activity[2] at each of these stages. The presence of the fungi spores in crops does not always result in the production of mycotoxins, since optimal growth conditions are required for biosynthesis (Battilani *et al*., 2020).

12. The natural co-occurrence of mycotoxins in food and feed is quite common and occurs for three main reasons; (i) some fungi can produce more than one mycotoxin (particularly *Fusarium* spp.), (ii) food commodities can be

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[2] Water activity is a measure of the availability in a substrate of water for microbial growth.

contaminated by several fungi and (iii) animal and human diets usually consist of multiple commodities. Co-occurrence can also occur during the processing and manufacturing stages. For example, during commercial mixing, where multiple batches of raw material assumed to each contain a mycotoxin are mixed together to produce a larger final batch.

13. At present, there is limited availability of data with regards to co-occurrence of mycotoxins in food commodities especially in Europe. In contrast, worldwide surveys for mycotoxins in animal feed are more common, of which the BIOMIN Mycotoxin Survey[3] is one of the most comprehensive.

14. Updated results for January – March 2021 on the occurrence of mycotoxins in ~5,000 finished animal feed and raw commodity samples from 58 countries based on ~23,500 analyses were published in April 2021. It was observed that mycotoxins are rarely found alone; multiple mycotoxins co-contaminated feed materials (51% of all European animal feed samples contained more than one mycotoxin). Results specific for Europe shows high contamination of DON at an average of 986 ppb in corn, detectable in 69% of corn samples (BIOMIN, 2021).

15. Table 1 provides a mycotoxin prevalence breakdown for Central, Eastern, Northern and Southern Europe in animal feed.

16. Some publications note that there is still limited knowledge on the presence and co-occurrence of multiple mycotoxins, both for primary mycotoxins and their modified forms, in food and feed (Palumbo *et al.*, 2020). Furthermore, it is difficult to infer trends or recent developments regarding mycotoxin contamination in European animal feed from available data due to the influence of the respective cropping season's climate on the

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[3] The BIOMIN Mycotoxin Survey constitutes the longest running and most comprehensive survey of its kind. The survey results provide insights on the incidence of the six major mycotoxins (AFs, ZEN, DON, FBs, T-2 and OTA) in the agricultural commodities used for livestock feed in order to identify the potential risk posed to livestock animal production. Further information available on the BIOMIN website.

contamination levels, which causes high year to year variation of results. In addition, there are differences in the applied analytical methods used to detect the contaminants (Streit *et al*., 2012).

Table 1 - the BIOMIN Mycotoxin Survey Central, Eastern, Northern and Southern Europe results on prevalence of mycotoxins in animal feed (%) for January to March 2021 (reproduced from BIOMIN, 2021).

Mycotoxin	**AF**	**ZEN**	**DON**	**T-2**	**FBs**	**OTA**
Central Europe	11	54	67	38	62	10
Eastern Europe	1	48	31	32	24	19
Northern Europe	2	33	62	12	21	8
Southern Europe	42	33	59	3	83	24

Abbreviations: AF - Aflatoxins; ZEN - Zearalenone; DON - Deoxynivalenol; T-2 - T-2 toxin; FBs - Fumonisins; OTA - Ochratoxin-A.

Methods for sampling and measuring mixtures of mycotoxins in food matrices

17. The main analytical methods to measure mycotoxins are enzyme-linked immunosorbent assay (ELISA), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Currently, LC based methods are the most frequently used (Serrano *et al*., 2012; Malachová *et al*., 2018), with several mass spectrometric detectors such as single-quadrupole mass spectrometer, time-of-flight, triple-quadrupole, ion trap and orbital ion trap mass analysers, as well as hybrid systems that combine two types of analysers (for example, fluorescence detector with LC systems).

18. An important and critical step is sample preparation and clean-up, with techniques including solid phase extraction, matrix solid-phase dispersion, liquid–liquid and solid–liquid partitioning, accelerated solvent extraction, multifunctional columns and immunoaffinity columns (Serrano *et al*., 2012; Agriopoulou *et al*., 2020).

19. The main analytical method used for detecting and measuring co-occurrence of very low concentrations of mycotoxins is liquid chromatography tandem mass spectrometry (LC–MS/MS) (Serrano *et al*., 2012; Gambacorta *et al*., 2018; Malachová *et al*., 2018; Shi *et al*., 2019; Battliani *et al*., 2020; Palumbo *et al*., 2020); however, multi-mycotoxins analyses are not widely performed due to their associated high cost, reduced sensitivity resulting in higher limits of quantification, and limited availability of reference materials for uncommon/emerging mycotoxins.

Current state of authoritative assessment and research

20. The COT have reviewed the European Food Safety Authority (EFSA) external report on mycotoxin mixtures in food and feed (Battiliani *et al*., 2020), several opinions by authoritative groups on some mycotoxin combinations, the work produced by the Mycotoxin mixtures (MYCOMIX) project led by Paula Alvito and her colleagues, as well as being aware of the Mycotoxin and Toxigenic Moulds (MYTOX) research group co-ordinated by Sarah De Saeger; as presented in TOX/2020/34. Brief summaries are provided below.

European Food Safety Authority (EFSA) external scientific report on mycotoxin mixtures

21. In this EFSA report, titled "*Mycotoxin Mixtures in Food and Feed: Holistic, Innovative, Flexible Risk Assessment Modelling Approach*" (MYCHIF), Battilani *et al*., (2020) performed an extensive literature review across four topics relating to the investigation of mycotoxin mixtures present in food and feed. These topics were:

i). Ecology and interaction with host plants of mycotoxin producing fungi, mycotoxin production, recent developments in mitigation actions of mycotoxins in crop chains.

ii). Analytical methods for primary, modified and co-occurring mycotoxins.

iii). Toxicity, toxicokinetics (TK), toxicodynamics (TD) and biomarkers relevant to humans and animals.

iv). Modelling approaches, and key reference values for exposure, hazard and risk modelling.

22. The data collected from these were then stored in the MYCHIF platform hosted by EFSA. The main objective of this was to develop an integrated method supported by modelling, for the risk assessment of mycotoxin mixtures in food and feed. Each topic will be summarised in the following paragraphs.

23. It was observed that *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., were the most prevalent mycotoxins worldwide, but also *Alternaria* spp. and *Clavicleps* spp. to a minor extent. The production of mycotoxigenic fungi is not commonly host specific, but their occurrence is associated mainly with a specific crop, depending on its region of growth and meteorological conditions. A fungus can produce different types of mycotoxins (for example, sterigmatocystin which is a precursor for AFs). As such, contamination of food and feed stuffs can occur concurrently. Modified mycotoxins may also co-occur with parent forms as a result of fungi-host plant interaction or during processing.

24. The MYCHIF report deemed that temperature, relative humidity, rainfall, and water activity are the most important ecological factors that influence fungal colonisation of substrates. Additionally, each species has its own ecological needs and requirements. In general, mycotoxins are stable compounds and can accumulate in crops over time (both during crop growth and post-harvest). Therefore, mitigation of contamination requires good practices at all production stages.

25. In terms of the methodologies used for mycotoxin analysis, these are split into two categories. Firstly, screening tests provide qualitative or semi-quantitative results. They are generally based on antibody recognition and

these methods are often relatively straightforward to carry out (for example, ELISA methodologies).

26. The other category is confirmatory analysis which provides confirmation of mycotoxin identity and quantitative results. The most widely used quantification method is High Performance Liquid Chromatography (HPLC). LC-MS is also used to identify and quantify mycotoxins. A number of high- or ultra-performance chromatography coupled to mass spectrometry systems have the ability to measure both regulated mycotoxins and other lesser tested for mycotoxins with analytical standards available (for example, CIT, sterigmatocystin) together in different feed/food commodities; however, there are some limitations in these systems including: cost, reduced sensitivity resulting in higher limits of quantification and reduced detection of *in vivo* metabolites. There is also a need for the development of harmonised guidance on how to measure multiple mycotoxins for regulatory purposes.

27. Toxicity, toxicokinetic (TK) and toxicodynamic (TD) parameters for humans and animals were collected from the literature to build three databases in the MYCHIF platform. These are: *in vitro* toxicokinetic data, *in vivo* toxicokinetic data, and *in vivo* toxicity data. A limited number of articles on mixtures were identified in comparison to those exploring effects of only single compounds. The information available covers only a limited number of combinations of mycotoxins. TK data were mainly reported in pigs and chickens, and in rats. TD modelling of mycotoxin mixtures could not be performed using the currently available data.

28. The mycotoxin dose, exposure pathway, interspecies and intraspecies differences were identified to be the most important parameters that may influence toxicokinetic parameters.

29. As part of the MYCHIF project, it was highlighted that testing of all mycotoxin mixture combinations was not feasible, and as such, focus should be given to: the prioritisation of mycotoxin mixtures, the creation of harmonised methods for generating *in vitro* and, if required, additional *in vivo*

toxicokinetic data, as well as utilising predictive kinetic modelling (that includes uncertainty, and inter- and intraspecies variability analysis).

30. For the exposure assessment, human biomonitoring data was collected from the literature; 66/176 articles that focused on biomarkers of exposure in humans had multi-biomarker measurements (Battilani *et al*., 2020)[4]. A multi-biomarker study was one where more than one parent mycotoxin were measured. Of the 27 studies that reported detection of multiple parent mycotoxins, different combinations were observed (see Table 2).

31. Regarding biomonitoring in humans, AFs are the most widely studied mycotoxin followed by OTA, DON, FBs, ZEN and other emerging mycotoxins such as alternaria (ALT), tenuazonic acid, fusarenon-X (Fus-X,) neosolaniol, CIT, NIV, T-2, 4,15-diacetoxyscirpenol (4,15-DAS), and enniatins (ENNs) in a very few studies. The most common sample matrix was urine, followed by serum, plasma, blood, breast milk, colostrum and amniotic fluid.

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[4] The search was limited to papers published in English between 2010 and 2017. Patents, editorials and letters were excluded from the searches.

Table 2 - Summary of the detected mycotoxin combinations in the multi-mycotoxin studies reviewed by Battilani et al., (2020).

Number of mycotoxins	**Combinations**				
Two	OTA, CIT	AFM_1, OTA	CIT, OTA	AFs, DON	AFs, FBs
Three	AFM1, FBs, OTA	DON, FBs, ZEN	AFs, FBs, OTA	ALT, DON, ZEN	AFM_1, OTA, DON
Four	CIT, DON, OTA, ZEN	-	-	-	-
Five	AFs, DON, FBs, OTA, ZEN	CIT, DON, ENNs, T2, ZEN	DON, FBs, NIV, OTA, ZEN	-	-
Six	AFs, DON, FBs, NIV, OTA, ZEN	AFs, DON, FBs, OTA, T2, ZEN	-	-	-
Seven	AFs, CIT, DON, ENNs, FBs, OTA, ZEN	DAS, DON, FUS-X, NEO, NIV, T2, ZEN	DAS, DON, FUS-X, NEO, NIV, T2, ZEN	--	-

Abbreviations: AFM_1 - Aflatoxin M_1; AFs - Aflatoxins; ALT - Alterneria; CIT - Citrinin; DON - Deoxynivalenol; ENNs - Enniatins; FBs - Fumonisins; FUS-X - Fusarenon-X; NEO - Neosolaniol; NIV - Nivalenol; OTA - Ochratoxin A; T2 - T2 toxin; ZEN - Zearelonone.

32. The simultaneous determination of more than one mycotoxin in human biological fluids presents a new challenge in mycotoxin biomonitoring. There are several constraints; in an analytical context, there is a lack of method standardisation and the unavailability of commercial reference standards (especially glucuronides). Their use in exposure assessments may be premature and cannot be fully exploited since there is a lack of: knowledge of effects of different combinations on the bioavailability of each individual component, the excretion rate, and consensus on a validated biomarker to be used in the context of a multi-mycotoxin analysis.

33. To paraphrase, there is still a lack of harmonisation in the experimental settings (for example, for the use and validation of analytical methods) and design of biomonitoring studies (for example, the selection of a candidate biomarker), in the data collection and in the definition of performance of fit for purpose analytical methods. These highlighted issues meant that it was not possible for Battilani *et al*., (2020) to exploit the biomonitoring dataset for exposure assessment goals. They recommend that international study guidelines should be prepared to support the production of data suitable for this purpose.

34. A human case study was presented to assess the risk of two mycotoxin mixtures, which can occur and co-occur in cereal based food products (DON, FBs and ZEN, and T2/HT-2 toxin, DON and NIV) using the component-based approach (CBA)[5] and provisional daily intake modelling methodologies (further detailed in the Exposure assessment section). A problem formulation, exposure assessment, hazard assessment and risk characterisation were completed. A Margin of Exposure (MOE)[6] value of 100 was chosen as a reference/cut off since the considered mycotoxins were neither genotoxic nor

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[5] Component based approaches are used to estimate the hazard or risk of combined exposures based on information on exposure and hazard for each individual component.

[6] The margin of exposure (MOE) is the ratio of the point of departure (typically the benchmark dose – lower confidence limit for a toxicological response in experimental animals), to the estimated human exposure for a compound. MOE values that are ≥100 are considered to be of low concern.

carcinogenic. In brief, due to data gaps and limitations a robust risk assessment could not be performed, however, the CBA modelling approach resulted in MOE values <100 for both mixtures, indicating that there is a potential health risk, which could be addressed by refining the risk assessment.

35. Hierarchy maps (based on EU Member states) were created when considering exposure to T2/HT-2 toxin, DON and NIV for adults. The maps provided a visual representation of higher risk exposure groups. With reference to the co-occurrence and occurrence data collected for the United Kingdom (UK); T2/HT-2 toxin, DON and NIV adult exposure levels based on the mean co-occurrence data and mean consumption values were >0.8 ,0.6-1.2 and 0.24-0.3 µg/kg bw/day, respectively.

36. To put into context the above exposure values, the health-based guidance values (HBGV) are provided. The acute reference dose (ARfD) for T2/HT-2 toxin is 0.3 µg/kg bw (EFSA, 2017a). DON and its acetylated forms (3- and 15-acetyl-DON) have an ARfD of 8 µg/kg bw and a tolerable daily intake (TDI) of 1 µg/kg bw (EFSA, 2017b). Whilst, NIV has a TDI of 1.2 µg/kg bw (EFSA, 2013). Therefore, the estimated exposure levels for UK adults of T2/HT-2 toxin is above the HBGV, whilst exposure levels for DON and NIV are below their respective HBGVs.

37. To conclude, the following data gaps and research recommendations were observed and/or suggested by Battilani *et al*., (2020). There is limited knowledge on the presence and co-occurrence of multiple mycotoxins, both for primary mycotoxins and their modified forms, in food and feed.

38. Available analytical methods have limitations for the routine monitoring of modified and multi-mycotoxins in food and feed. In the context of multi-mycotoxin analysis and the use of LC-MS methodologies; there remains an urgent need for the following: lower costs, fit for purpose methods characterised by the ability to measure multiple mycotoxins (with lower limits of quantification for all co-occurring mycotoxins, including their metabolites

investigated *in vivo*), and availability of commercial reference materials for providing reliable quantitative results.

39. In terms of toxicity data, a limited number of articles on mixtures were identified in comparison to those exploring effects of only single compounds. The available studies cover only a very limited number of combinations of mycotoxins and the available TK data is mainly in livestock species (for example, pigs and chickens), as well as in rats. The modelling of TD features of mycotoxin mixtures could not be performed based on the limited data available.

40. The development of prioritisation criteria for mycotoxin mixtures to be tested was suggested as a further research priority. In addition to this, consistent methodologies and harmonised guidance for generating *in vitro* and *in vivo* TK and TD data are needed to provide consistent data for pharmacologically based toxicokinetics and benchmark dose modelling of mycotoxin mixtures. Current analytical methods should have the capability to detect and analyse real world samples. Finally, the utilisation of TK and TD modelling should be further considered and explored.

41. With reference to the use of biomarkers for exposure assessment purposes; there is a need to derive qualitative and quantitative correlations between the mycotoxin intake from food and from other possible routes of exposure like dermal or inhalation (which may be important to consider as part of occupational hazard assessments; however, it is unclear how realistic it would be to achieve this level of granularity from such a population-based study).

Opinions by authoritative groups on some mycotoxin combinations

42. Assessments of some binary mycotoxin combinations have been carried out by EFSA, the Joint Food and Agriculture Organization and World Health Organisation Expert Committee on Food Additives (JECFA) and the

Scientific Committee on Food (SCF). These are summarised in the following paragraphs.

EFSA reviews

43. During their review of 4,15-DAS in 2018, the EFSA Panel on Contaminants in the Food Chain (CONTAM) considered the combined effects and interactions of 4,15-DAS with T-2 and HT-2 toxins, AFs, OTA and FBs. Following the analysis of the available database describing possible effects of combined exposure to 4,15-DAS and other mycotoxins, the EFSA CONTAM Panel concluded that “the data was weak and inconclusive” (EFSA, 2018).

44. In 2012, the EFSA CONTAM Panel also examined available publications addressing interactions of CIT with PAT, AFs and OTA, particularly on the subject of synergism. They concluded that “the available evidence indicated that CIT at low doses does not exacerbate the toxic effects of other mycotoxins and that the combined effect of CIT and OTA is, at most, additive” (EFSA, 2012a).

JECFA reviews

45. JECFA first reviewed the toxicology associated with concurrent exposure to FBs and other mycotoxin agents in 2011. The *in vitro* and *in vivo* studies reviewed were found to “show inconclusive and sometimes contradictory results. The effects of simultaneous exposure tend to be at most additive. In some *in vitro* and *in vivo* studies, the authors suggested that synergism or antagonism may occur, but often only single doses of each individual mycotoxins were used”, and as such JECFA concluded that “these study designs were inadequate to detect synergism”, and “overall, the available data on co-exposure were inadequate for their use in its evaluation” (JECFA, 2011).

46. However, studies by Carlson *et al*., (2001) and Gelderbloom *et al*., (2002) were noted; these documented the ability of FB1 to promote AFB1 initiated hepatocarcinogenicity in trout and orally dosed pure FB1 in rats induced precancerous lesions, respectively. The treatment regime and dose

at which these effects were observed in trout was through exposure to 100 ppb of AFB1 and then exposure to ≥ 23 FB1 ppm for 42 weeks (Carlson *et al*., 2001); whilst male Fisher rats (n = 5-8/group) were dosed with AFB1 at 17 µg/kg bw per day (*via* oral gavage) for 14 days, followed by FB1 at 250 mg/kg diet for 3 weeks (with a recovery period of 21 days between treatments) (Gelderbloom *et al*., 2002). JECFA noted that "co-exposures to AFB1, a compound with known genotoxic properties, and FBs, which have the potential to induce regenerative proliferation, would be of concern" (JECFA, 2011).

47. The topic of co-exposures to AFB1 and FBs was revisited in the JECFA 2018 evaluation (Riley *et al*., 2018) when new evidence became available. Synergistic effects were reported by Qian *et al*., (2016) for the development of preneoplastic lesions[7], where male F344 rats (n=13) were exposed to pure AFB1 (equivalent to 15 µg/kg bw per day for 14 days) followed by pure FB1 (equivalent to 25 mg/kg bw per day for 21 days), with a recovery period of 21 days in between treatments.

48. JECFA concluded that even though there are additive or synergistic effects observed from FB1 and AFB1 co-exposure in laboratory animals in inducing the development of preneoplastic lesions and hepatocellular carcinoma (as discussed above), there was currently no data available on such effects in humans. Furthermore, two prospective epidemiological studies (Magoha *et al*., 2016; Shirima *et al*., 2015), do not support the hypothesis of an interaction between AFB1 and FB1 in childhood stunting. JECFA concluded that there were few data available to support co-exposure as a contributory factor in human disease. However, the interaction between AFB1 (genotoxic), and FBs, which have the potential to induce regenerative cell proliferation (particularly at exposures above the provisional maximum tolerable daily intake), remained a concern.

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[7] A preneoplastic lesion is an identifiable local abnormality associated with an identifiable risk of a tumour developing at a site.

49. It was recommended that exposures to both compounds (*i.e.* FB1 and AFB1) should be reduced and that emphasis on human studies should be on biomarker-based approaches.

50. In 2018, JECFA further assessed the combined toxicity of pure FBs and pure DON in Swiss mice (Kouadio *et al*., 2013). JECFA concluded that “co-exposure suggested additivity, and the effects on growth, clinical chemistry and biochemical parameters were possibly more than additive… the reduced weight gain was seen at a very low dose of AFB1 compared to other studies” (JECFA, 2018).

SCF review

51. In 2002, the SCF published an opinion to evaluate whether the establishment of a group TDI for four trichothecenes (T-2 toxin, HT-2 toxin, DON and NIV) was appropriate and if so, the feasibility of doing this (SCF, 2002).

52. The SCF were in favour of establishing a group TDI for several compounds when they shared a common mode of action (MOA)[8] and there was frequent co-exposure. At the time of review, there were few studies found that addressed the effects of combined exposure to trichothecenes.

53. Although different types of trichothecenes appear to cause similar toxic effects at the biochemical (strong inhibitory effect on protein synthesis by binding to ribosomes, inhibitory effect on RNA and DNA synthesis) and cellular (toxic effect on cell membranes) level, the SCF noted that “it is not clear whether toxins work *via* identical mechanisms at the biochemical and cellular level.” Additionally, “there are also considerable differences in the spectrum of toxic effects *in vivo*. Large, non-systematic potency differences between these toxins were seen when different endpoints are being considered”.

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[8] A mode of action (MOA) describes a series of key events in biochemical, cellular and tissue processes necessary for an adverse health outcome, resulting from the exposure of a living organism to a substance.

54. In *in vitro* studies, dose additivity as well as antagonism have been observed for T-2 toxin, DON and NIV, whilst in *in vivo* studies only antagonism was observed for the combination of T-2 toxin and DON, and no dose additivity was observed. The SCF were not aware of any other combinations of NIV with other trichothecenes examined *in vivo*. As such with only *in vitro* studies suggesting dose additivity, the establishment of the nature of combined effects or relative potencies of trichothecenes was not further explored. In conjunction with this, the SCF considered “the available data, while limited, did not support the establishment of a group TDI for all trichothecenes evaluated, as synergism was not observed”.

MYCOMIX

55. The MYCOMIX project (funded by the Portuguese Foundation for Science and Technology) carried out in 2013-2015 aimed to contribute and fill the gap concerning the risk assessment of children exposed to multiple mycotoxins in infant foods (Alvito *et al*., 2015). The study involved 75 children in total; 18 males and 20 females (13-24 months), 9 males and 9 females (25-36 months), 7 males and 12 females (36-47 months). Three questions were posed. Firstly, are children exposed to one or several mycotoxins *via* the daily diet. Secondly, can this co-exposure affect children’s health, and lastly are there interactive effects in toxicity of mixtures of mycotoxins.

56. An overview of the risk assessment under the MYCOMIX project was published in 2018 (Assunçao *et al*., 2018). Analysis of 52 different cereal-based products revealed a co-occurrence of mycotoxins in 75% of the analysed samples (cereal breakfasts n= 26; infant cereals (flour) n= 20 and biscuits n=6), with two or more mycotoxins occurring simultaneously. The highest number of mycotoxins detected simultaneously was seven (n=2 in breakfast cereals). The combinations of two (OTA and DON; OTA and FBs) and four (AFs; AFB_1 and AFB_2, OTA and ZEN) mycotoxins were the most commonly detected, in breakfast cereals (n=3), infant cereals (n=3) and biscuits (n=3), respectively. Note that all analysed samples had mycotoxin

levels below the limits specified in the legislation (Regulation (EC) No 1881/2006)[9] and as such were compliant for marketing.

57. Food diary analysis revealed that ~92% of the children consumed one or more cereal-based products, and at least once in three days. 42%, 65% and 65% consumed breakfast cereals, infant cereals and biscuits, respectively. The mean daily consumption of these food groups, for all children (both non-consumers and consumers), were 5.6 g (breakfast cereals), 25.3 g (infant cereals) and 8.7 g (biscuits). For the only consumers group, the values increase to 15.4 g, 38.7 g, and 13.4 g, for the same food groups respectively.

58. Worst-case exposure for the summed daily intake of mycotoxins present in cereal-based products (breakfast cereal, infant cereal and biscuits) are presented in Table 3.

59. Population-based exposure includes the estimation of intake for all survey respondents (including non-consumers) and allows the identification of food groups that have made the highest contribution to overall intakes. Breakfast cereals were the highest contributor for the estimated daily intake of mycotoxins by Portuguese children under three years old, revealing the highest values for FBs, trichothecenes, ZEN and AFB1. On the other hand, processed cereal-based foods (flours) presented the highest contribution for the estimated daily intakes of AFM1, AFB2, AFG1, and OTA.

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[9] Regulation (EC) No 1881/2006 concerning setting maximum levels for certain contaminants in foodstuffs, which is available on the EUR-Lex website.

Table 3 - Sum of worst-case children's daily intake of mycotoxins present in cereal-based products (breakfast cereal, infant cereal (flour) and biscuits) based on a deterministic approach (reproduced from Assunçao *et al.*, 2018).

Toxins	Consumers and non-consumers Sum of daily intake (ng/kg bw/day)	Only consumers Sum of daily intake (ng/kg bw/day)
AFM_1	**0.069**	**0.116**
AFB_1	**0.012**	**0.028**
AFB_2	**0.003**	**0.006**
AFG_1	**0.016**	**0.028**
OTA	**0.131**	**0.227**
FB1	**6.4**	**14.0**
FB2	**1.0**	**2.6**
DON	**57.22**	**112.78**
NIV	**2.68**	**6.60**
ZEN	**0.86**	**1.64**

Abbreviations: AFM_1 - Aflatoxin M1; AFB_1 - Aflatoxin B1; AFB_2 - Aflatoxin B2; AFG_1 - Aflatoxin G1; OTA - Ochratoxin A; FB_1 - Fumonisin B1; FB_2 - Fumonisin B2; DON – Deoxynivalenol; NIV – Nivalenol; ZEN - Zearalenone.

60. The risk characterisation for AFs was performed by calculating the MOEs, whilst for the remaining mycotoxins, hazard exposure quotients (HQ; individual mycotoxins) and hazard exposure indexes[10] (HI; combined mycotoxins) were used. MOE values for AFB_1 and AFG_1 for the 90th, 95th and 99th percentiles were below 10,000 suggesting a potential health concern for compounds that were both genotoxic and carcinogenic. The highest HQ and HI was for DON and the simultaneous exposure to trichothecenes, respectively. However, these values were <1 indicating no cause for concern.

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[10] A hazard exposure index is a risk-assessment tool, which can indicate whether further investigations are required for mixtures. It is based on dose addition assumptions; it is the sum of the hazard quotients of the chemicals in the mixture.

61. The authors noted that interpretation of the results should take into account the identified uncertainties including; the number of analysed samples, the number of children in the cohort, and the available toxicological data. Additionally, the exposure was assessed using an indirect approach (*i.e.* combining data of mycotoxin occurrence in food and food consumption which has its own associated limitations[11]).

62. From the obtained results, the authors suggest that actions should be taken in order to protect this population group, recommending a national monitoring program to be carried out with the aim of establishing protective values in legislation. Additionally, further research should be conducted to obtain toxicological data, including health consequences resulting from early-life exposures to multiple mycotoxins. Good farming and food production processes should aim to reduce the generation of mycotoxins in crops, and greater consideration should be given to decontamination of plant-based foods destined for children consumption.

MYTOX

63. MYTOX is a multi-disciplinary research group, which deals with issues involving toxigenic moulds, mycotoxins, their effects on human health, and on animal health.

64. The mycotoxins and human health research unit includes all research projects in relation to the occurrence of mycotoxins and their effects on human health including epidemiological studies, risk assessment studies and scenario analyses. The Secretariat has confirmed with the MYTOX coordinator (De Saeger, personal communication, 2021) that the research group has several ongoing/or about to start projects to evaluate the impact of

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[11] These limitations include the assumption of heterogenous distribution of mycotoxins in food, exposure from other routes of exposure excluding oral ingestion, the potential presence of masked mycotoxins, the influence of food processing, inter-individual variation in toxicokinetic profiles, and the under- and overestimation of food consumption data.

multi-mycotoxin exposure, and that publications on this are expected in the future.

Toxicokinetics

65. The COT previously reviewed the available toxicokinetic data relating to combined exposures to mycotoxins (Warth *et al*., 2013; Battilani *et al*., 2020), and concluded that there are only a limited number of studies on the toxicokinetic profiles of different mycotoxin mixtures *in vivo* in humans.

66. Battilani *et al*., (2020) further highlighted the complexity of studying the toxicokinetics of mycotoxin mixtures, suggesting that these need to be addressed using a case-by-case approach. Mycotoxin exposure level, exposure pathway, interspecies and intraspecies differences were identified among the most important parameters that may influence the toxicokinetics of mixtures.

Toxicology

67. This section is presented in three parts. Firstly, the Committee's review on the toxicity of individual mycotoxins. Secondly, the review of the available data on relative potencies for mycotoxin groups, and lastly, the review of the toxicity of common binary mixtures found in the literature.

68. The Committee has previously reviewed the toxicity of individual mycotoxins; especially in the diet of infants and children aged 0-12 months and 1-5 years, respectively (TOX/2017/30)[12]. Annex B of TOX/2020/44[13], presented an overview of all mycotoxins previously covered in the scope of TOX/2017/30. The collated information includes: the reviewed mycotoxins, the species of fungus that produces them, their MOA, key toxicological endpoints. Also included are their recommended HBGVs as established by authoritative bodies such as EFSA, JECFA, SCF.

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[12] The first scoping paper TOX/2017/30 and the resulting addendum to the 0-5 years Overarching Statement are available on the COT website.

[13] Annex B of TOX/2020/44 is available on the COT website.

69. In mixtures toxicology, there are three main categories of possible interactions (SCHER, SCCS, SCENIHR, 2012; EFSA, 2019) between mycotoxins. These are:

i). Similar action (dose/concentration addition) occurs if chemicals in a mixture act by the same mechanism/MOA, and differ only in their potencies;

ii). Dissimilar action (independent action) occurs if chemicals act independently from each other, usually through different MOAs that do not influence each other, and;

iii). Interactions describes the combined effect of two or more chemicals as stronger (synergistic, potentiating, supra-additive) – or weaker (antagonistic, inhibitive, sub-additive, infra-additive) – than would be expected on the basis of dose/concentration addition or response addition. The observed interactions are therefore dependent on the relative dose levels, the exposure route(s), timing and duration of exposure, and the biological target(s).

70. A paper by Speijers & Speijers (2004) on combined toxic effects was identified to be one of the first reviews to assess the topic. It concluded that, at the time, tools were not fully developed to establish the type of interaction or whether there is any interaction at all (with regards to trichothecenes). More recent reviews have also been published by Grenier and Oswald (2011), De Ruyck *et al*., (2015), Alassane-Kpembi *et al*., (2017), Lee & Ryu (2017), and Battilani *et al*., (2020).

71. Authoritative groups such as EFSA, JECFA, and SCF have provided opinions on some binary mycotoxin mixtures (refer to paragraphs 21-54).

Summary of toxicology in the literature

72. The COT reviewed literature on binary mixtures, as the co-occurrence of two mycotoxins in food commodities is the most commonly reported situation. Summaries of OTA, AFB1 and *Fusarium* spp. mycotoxins and their interactions with other mycotoxins *in vitro* and for AFB1 *in vivo*, are presented in Tables 4, 5, 6 and 7, respectively. As these experiments were conducted for a range of different durations and concentrations (as detailed in TOX/2020/34), it was not possible to draw specific conclusions and so the details are not included in this statement.

73. *In vitro* studies testing for the combined effects of mycotoxins, with cell viability endpoints (for example, DNA damage, oxidative damage and immunotoxicity) are the most commonly reported in the literature. Available *in vivo* data (on animal models) reported potential adverse effects on the liver, kidneys and teratogenicity.

74. Other considerations, on the potential adverse effects on the intestinal microbiota (Baines *et al*., 2013; Liew & Mohf-Redzwan, 2018) and on the endocrine system (Demaegdt *et al*., 2016) were also reviewed by the COT.

75. The COT observed that there were a number of mycotoxins with MOAs involving ribosomal protein synthesis inhibition; however, there was a lack of information on possible additive toxicity. Additionally, there is a large amount of variability in the methodology utilised, since there is currently no harmonisation on combinative testing strategies for each toxicological endpoint for each plausible mycotoxin combination.

Table 4 - Summary of the observed combinative effects of ochratoxin A with fumonisin B1, zearalenone, and citrinin in different *in vitro* models.

Mycotoxin mixture	**Method**	**Cellular model**	**Endpoint**	**Combination effect**	**Reference**
OTA + FB_1	*In vitro*	Rat C6 glioma, Vero monkey and human Caco-2 cells	Cytotoxicity	Synergistic	Creppy *et al*., (2004)
OTA + FB_1	*In vitro*	PK-15 cells	Cytotoxicity	Additive	Šegvić Klarić *et al*., (2007)
OTA + FB_1	*In vitro*	human and pig lymphocytes	Cytotoxicity	Synergistic	Mwanza *et al*., (2009)
OTA + FB_1	*In vitro*	Male Wistar rats	Genotoxicity	Synergistic	Domijan *et al*., (2006)
OTA + ZEN	*In vitro*	hHepG2 cells	Cytotoxicity	Antagonistic	Wang *et al*., (2014)
OTA + ZEN	*In vitro*	hHepG2 and KK-1 cells	Cytotoxicity	Additive	Li *et al*., (2014)
OTA + CIT	*In vitro*	Piglets lymphocytes	Immunotoxicity	Synergistic	Bernhoft *et al*., (2004)
OTA + CIT	*In vitro*	Monkey kidney vero cells	Cytotoxicity	Synergistic	Bouslimi *et al*., (2008b)
OTA + CIT	*In vitro*	PK-15 cells	Cytotoxicity	Antagonistic	Šegvić Klarić *et al*., (2012)

Abbreviations: OTA = Ochratoxin A; FB1 = Fumonisin B1; ZEN = Zearalenone; CIT = Citrinin; PK-15 = Porcine kidney 15 epithelial cells; hHepG2 = human hepatoma cells G2; KK-1 = murine ovarian granular cells.

Table 5 - Summary of the observed combinative effects of aflatoxin B1 with aflatoxin B2, fumonisin B1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A in different in vitro models.

Mycotoxin mixture	**Method**	**Cellular model**	**Toxicity end point**	**Combination effect**	**Reference**
AFB_1 + AFB_2	*In vitro*	hUVEC	Cytotoxicity	Synergistic	Braicu *et* al., (2010)
AFB_1 + AFB_2	*In vitro*	hLF and A 2780	Cytotoxicity	Additive	Braicu *et* al., (2010)
AFB_1 + AFB_2	*In vitro*	Rat liver slices	Toxicity	Antagonistic	Friedman *et al*., (1997)
AFB_1 + FB_1	*In vitro*	Spleen mononuclear cells	Cytotoxicity	Synergistic	Mary *et al*., (2012)
AFB_1 + FB_1	*In vitro*	hHep-G2; hBEAS-2B cells	Cytotoxicity	Antagonistic; Additive	McKean *et al*., (2006a)
AFB_1 + DON	*In vitro*	PK-15 cells	Cytotoxicity	Synergistic	Lei *et al*., (2013)
AFB_1 + DON	*In vitro*	BRL 3A cells	Cytotoxicity	Synergistic	Sun *et al*., (2015)
AFB_1 + DON	*In vitro*	Ames test	Mutagenicity	Synergistic	Šmerák *et al*., (2001)
AFB_1 + ZEN	*In vitro*	PK-15 cells	Cytotoxicity	Synergistic	Lei *et al*., (2013)
AFB_1 + ZEN	*In vitro*	BRL 3A cells	Cytotoxicity	Synergistic	Sun *et al*., (2015)
AFB_1 + T-2	*In vitro*	Ames test	Mutagenicity	Synergistic	Šmerák *et al*., (2001)
AFB_1 + T-2	*In vitro*	hBEAS-2B cells	Cytotoxicity	Additive and synergistic	McKean *et al*., (2006b)
AFB_1 + T-2	*In vivo*	Fischer 344 rats	Acute	Additive	McKean *et al*., (2006b)
AFB_1 + OTA	*In vitro*	Ames test	Mutagenicity	Synergistic	Sedmíková *et al*., (2001)

AFB$_1$ + OTA	*In vitro*	Monkey kidney vero cells	Cyto and genotoxicity	Additive	Golli-Bennour *et al*., (2010)
AFB$_1$ + OTA	*In vitro*	hHep-G2 cells	Cytotoxicity	Additive	Corcuera *et al*., (2011)
AFB$_1$ + OTA	*In vitro*	hHep-G2 cells	Genotoxicity	Antagonistic	Corcuera *et al*., (2011)

Abbreviations: AFB$_1$ = Aflatoxin B$_1$; AFB$_2$ = Aflatoxin B$_2$; FB1 = Fumonisin B$_1$; DON = Deoxynivalenol; ZEN = Zearalenone; T-2 = T-2 toxin; OTA = Ochratoxin A; HUVEC = Human umbilical vein endothelial cells; HLF= Human lung fibroblasts; hHep-G2 = Human hepatoma G2 cells; hBEAS-2B = Human bronchial epithelial cells; PK-15 = Porcine kidney 15 epithelial cells; BRL 3A = Buffalo rat liver cells.

Table 6 - Summary of the observed combinative effects of various combined Fusarium mycotoxins including: zearalenone and fumonisin B1; zearalenone and T-2 toxin; deoxynivalenol and zearalenone; deoxynivalenol and T-2 toxin; deoxynivalenol and 15-acetlydeoxynivalenol; deoxynivalenol and nivalenol; and deoxynivalenol and fumonisin B1 in different in vitro models.

Mycotoxin mixture	**Method**	**Cellular model**	**Endpoint**	**Combination effect**	**Reference**
ZEN + FB_1	*In vitro*	Human Caco-2 cells	Cytotoxicity	Antagonistic	Kouadio *et al*., (2007)
ZEN + FB_1	*In vitro*	Human Caco-2 cells	Lipid peroxidation	Synergistic	Kouadio *et al*., (2007)
ZEN + FB_1	*In vitro*	Human Caco-2 cells	Inhibition of DNA synthesis	Antagonistic	Kouadio *et al*., (2007)
ZEN + FB_1	*In vitro*	Human Caco-2 cells	DNA fragmentation	Synergistic	Kouadio *et al*., (2007)
ZEN + T-2	*In vitro*	hCFU-GM cells	Myelotoxicity	Additive	Ficheux *et al*., (2012)
DON + ZEN	*In vitro*	hCFU-GM cells	Myelotoxicity	Additive	Ficheux *et al*., (2012)
DON + ZEN	*In vitro*	HCT116 cells	Cytotoxicity	Antagonistic	Bensassi *et al*., (2014)
DON + T-2	*In vitro*	hCFU-GM cells	Myelotoxicity	Additive or synergistic	Ficheux *et al*., (2012)
DON + 15-AcDON	*In vitro*	hGES-1 cells	Cytotoxicity	Synergistic	Yang *et al*., (2017)

Mycotoxin mixture	**Method**	**Cellular model**	**Endpoint**	**Combination effect**	**Reference**
DON + NIV	*In vitro*	Human Caco-2 cells	Cytotoxicity	Synergistic and additive	Alassane-Kpembi *et al*., (2013)
DON + NIV	*In vitro*	hGES-1 cells	Cytotoxicity	Synergistic	Yang *et al*., (2017)
DON + FB_1	*In vitro*	Human Caco-2 cells	Cytotoxicity	Synergistic	Kouadio *et al*., (2007)
DON + FB_1	*In vitro*	hCFU-GM cells	Myelotoxicity	Antagonistic	Ficheux *et al*., (2012)

Abbreviations: ZEN = Zearalenone; T-2 = T-2 toxin; DON = Deoxynivalenol; 15-AcDON = 15-acetyldeoxynivalenol; NIV = Nivalenol; FB_1 = Fumonisin B_1; hCFU-GM = Human colony forming unit-granulocyte and macrophage cells; HCT116 = human colon carcinoma cell line; GES-1 = Human gastric epithelial cells.

Table 7 - Summary of the observed combinative effects of aflatoxin B1 with aflatoxin B2, fumonisin B1, deoxynivalenol, zearalenone, T-2 toxin, and ochratoxin A in different in vivo models.

Mycotoxin mixture	**Method**	**Animal model**	**Toxicity endpoint**	**Combination effect**	**Reference**
$AFB_1 + FB_1$	*In vivo*	Fischer 344 rats	Acute	Synergistic	McKean *et al*., (2006a)
$AFB_1 + FB_1$	*In vivo*	Male Fischer rats	Hepatotoxicity	Synergistic	Gelderblom *et al*., (2002)
$AFB_1 + FB_1$	*In vivo*	White rabbits	Hepatotoxicity	Synergistic	Orsi *et al*., (2007)
$AFB_1 + FB_1$	*In vivo*	Male Wistar rats	Hepatotoxicity	Synergistic	Theumer *et al*., (2008)
$AFB_1 + FB_1$	*In vivo*	Maler F344 rats	Hepatotoxicity	Synergistic	Qian *et al*., (2016)
AFB_1 + T-2	*In vivo*	Fischer 344 rats	Acute	Additive	McKean *et al*., (2006b)
AFB_1 + OTA	*In vivo*	Wistar rat dams	Teratogenicity	Antagonistic	Wangikar *et al.,* (2004)
AFB_1 + OTA	*In vivo*	Male Sprague Dawley rats	Hepatotoxicity and nephrotoxicity	Synergistic	Abdel-Wahhab *et al*., (2015)
DON + FB_1	*In vivo*	Male crossbred castrated piglets	Morphological changes	Antagonistic	Bracarense *et al*., (2012)
DON + FB_1	*In vivo*	Male crossbred castrated piglets	Immunological changes	Synergistic-Antagonistic	Bracarense *et al*., (2012)

Abbreviations: AFB_1 = Fumonisin B_1; FB_1 = Fumonisin B1; T-2 = T-2 toxin; OTA = Ochratoxin A; DON = Deoxynivalenol.

Exposure assessment

76. As stated previously, the co-occurrence of mycotoxins in food and feed is possible since some fungi species are able to produce more than one mycotoxin (for example the *Fusarium* spp.), food commodities can also be contaminated by several fungi species. A combined exposure is therefore highly likely in humans due to varied diets and/or one food commodity may be contaminated with more than one mycotoxin.

77. The completion of an exposure assessment is challenging when limited information is available. Little to no UK relevant data could be obtained from the literature when all age groups were considered. It would be necessary to assess all age groups to determine those who would be at greatest risk. Additionally, different methodologies have been reported in the literature to assess the levels of exposure (for example, food diaries, biomarker analyses), and hence data comparison across studies may not be accurate. The development and application of multi-analyte methods has been advancing as detailed earlier; however, this has not yet been internationally applied as a gold standard for assessing the presence of multiple mycotoxins in food commodities. The use of current methodologies for mycotoxin analysis (for example, HPLC) still presents an issue in terms of management of left-censored data[14].

Stepwise approach

78. A stepwise approach to the exposure assessment was considered by the Committee (summarised below).

79. Firstly, mycotoxins should be categorised based on toxicological similarities where an endpoint is defined. This will then determine how occurrence data for the considered mycotoxins should be grouped together to calculate total residues for each mycotoxin group by summation in the

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[14] Data below a limit of detection for which the true value is unknown are often referred to as “left-censored”.

exposure assessment (either in one food or multiple foods). An opportunity to note any missing data can be recorded throughout this step.

80. The exposure should be then calculated deterministically, and if major exceedances are observed in relation to the toxicological endpoint a probabilistic calculation should be considered. The estimated exposure can then be compared against the point of departure to determine the MOE. Depending on the endpoint, an MOE value that is <100 (for non-genotoxic and non-carcinogenic compounds) or <10,000 (for genotoxic carcinogens) would indicate a potential health concern whilst values that are ≥100 or ≥10,000, respectively indicate no appreciable cause for concern (EFSA, 2012b). The MOE value aids in putting exceedances into perspective.

81. Lastly, if probabilistic modelling was carried out; a sensitivity analysis[15] should be considered for assessing the impact of different variables.

Potential data sources

82. Three potential data sources to use in the exposure assessment were proposed and summarised in the following paragraphs.

UK Food Standards Agency (FSA) – Mycotoxin Total Diet Study

83. The UK FSA has previously carried out a Total Diet Study (TDS), in 2011, which included mycotoxin analysis (FS102081), where a total of 3, 312 food samples were analysed for the presence of mycotoxins. The main aim of the study was to calculate background exposure to various mycotoxins from the whole diet and to compare exposure to those calculated by other sources (Stratton *et al*., 2017).

84. Co-occurrences were observed in the TDS dataset. For example, sample S14-042859 (a wholemeal bread) contained DON, some ergot alkaloids and also a low level of OTA.

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[15] Sensitivity analysis is used during probabilistic modelling to analyse how different values of an independent variable affect a particular dependent variable under a certain set of assumptions.

85. The following limitations were observed with the TDS dataset including; it was limited to a small number of food groups, some recovery rates were poor, food samples were collected from 2009 and as such may not be reflective of the current levels of mycotoxins present in foods. Most results were at or below the limit of quantification (LOQ), meaning that much of the data is left-censored as the values could not be accurately determined. Therefore, lower- and upper- bound estimates were derived from the occurrence data to reflect the uncertainty in the exposure assessment. This approach has been discussed by EFSA in their Scientific Report on the Management of left-censored data in dietary exposure assessment of chemical substances (EFSA, 2010). Here, EFSA comment that the robustness of this approach is limited by the computational difficulties of calculating percentiles and basic statistics, if the percentage of left censorship is large, and in the application of statistical techniques like regression methods (EFSA, 2010). Finally, as mentioned, multi-mycotoxin analysis was not consistently used for each food sample.

86. Further information received from the project manager (MacDonald, personal communication, 2020) has confirmed that a method for multi-mycotoxin analysis (*i.e.* different classes/families) was not performed for the TDS. This was due to the different chemistries and properties of the mycotoxins themselves rather than the different food matrices. Therefore, samples were analysed by several methods to obtain the full suite of analyte results with the lowest reporting limits achievable. Although not strictly a multi-analyte method, mycotoxins from the same family (for example, ergot alkaloids and trichothecenes) were detected using one methodology. The possibility and availability of a multi-mycotoxin method were also discussed with the project manager. It was confirmed that a methodology is both possible and available although this means that compromises have to be made in order to make it suitable for all tested mycotoxins. The compromises include the lack of dedicated extraction techniques, sample clean-up and analyte enrichment. This results in higher reporting limits, which in turn can affect the estimate of intake (*i.e*. overestimation), as well as potentially

requiring additional resources in terms of sample re-analyses to avoid false positives.

EFSA – MYCHIF Platform

87. It was noted that UK co-occurrence data was presented in the scientific report for MYCHIF, therefore the MYCHIF platform (Battilani *et al*., 2020) was included as a potential data source.

88. A human case study was presented as part of the MYCHIF report (Chapter 3.8.2, pp. 88) where a cumulative chronic exposure assessment for two mycotoxin mixtures in cereal food sources (1: DON, FBs and ZEN and 2: T-2/HT-2 toxin, DON and NIV) was carried out using two modelling methodologies: CBA and provisional daily intake approach.

89. In the CBA, the co-occurrence of mycotoxins and consumption data of cereal-food based products (as an example) for each single mycotoxin were combined to obtain an individual mycotoxin exposure, this was then summed up to obtain the total exposure, under the dose addition assumption. The main uncertainty identified was the adopted deterministic approach of the input modelling data for mycotoxin concentrations. Due to the scarcity of concentration data for many countries, a probabilistic approach was applied at an EU level only. It was assumed that the maximum exposure limits (*i.e.* the lower and upper bound highest 95th percentile chronic exposure) were the most conservative values. The risk decision was based on the calculated MOE values. Based on the methodology and assumptions in the MYCHIF case study the MOE values were <100 for all age groups (adolescent, adult and elderly).

90. The provisional daily intake (expressed in µg/kg bw/day) models the internal dose using the available human biomarker data, to derive exposure to the mixture. This was estimated by combining the mycotoxin concentration in the urine, the available excretion rate for each of the mycotoxins in the mixture, the human body weight and the daily urine excretion volume (µg/L mycotoxin, L urine in 24 h, % excretion rate, kg bw, respectively). Values were

calculated for single mycotoxins present in the mixture and for the mixture. A hazard index was used to estimate the risk; if the value is ≤1 the combined risk is deemed acceptable, whereas when it is >1 there is a potential health concern. The identified uncertainties included the default body weight of 70 kg, the excretion rate where values were derived from a single study or from correlation approximations, urine volumes where the urine was not corrected for dilution factors, and data representativeness. Overall, a hazard index could not be calculated due to the uncertainties for single mycotoxins described above, since these would also need to be integrated into the analysis of mixtures where additional variables should be considered for unknown toxicokinetics and toxicodynamics, as well as unknown synergistic/additive effects.

91. The MYCHIF data was located and the file downloaded. Extraction of relevant UK data was attempted; however, the datasets are extremely complex and were not accompanied with straightforward guidance. The Secretariat has made the relevant contact in order to gain further guidance. The following benefits to using this data have been observed; the use of multi-mycotoxin analysis, the more recent data collection of mycotoxin co-occurrences in cereal commodities, and the integration of singular and multi-biomarker mycotoxin analysis data.

92. Nevertheless, at present it has been difficult to determine potential limitations due to the complexity of the datasets. Uncertainties for both modelling methodologies were also identified.

Exposure data derived from the literature

Non-UK data

93. Co-exposures have been reported in the literature for various age groups such as those observed from the MYCOMIX Portuguese studies in children as seen in paragraph 55.

94. For the exposure assessment, non-UK co-occurrence data and UK consumption data for each individual mycotoxin could be used to obtain an exposure estimate for each mycotoxin. These could then be totalled for each co-occurrence type and also for each mycotoxin family to obtain the total exposure within each food or food group.

95. Obvious limitations include the use of non-UK data, which may not be applicable to consumers in the UK. However, the use of non-UK data may expedite an exposure assessment to reveal common mycotoxin combinations as well as the most affected food groups in Europe.

Human biomonitoring data

96. The use of human biomonitoring (HBM) data was explored as part of TOX/2020/44, and outputs from this exercise are summarised below.

97. Biological monitoring utilises biomarkers[16] to represent or estimate the internal exposure as a result of inhalation, ingestion or dermal exposure to a chemical. In addition to exposure, appropriate biomarkers can provide an indication of effect and/or susceptibility. Exposure assessments to any dietary contaminant is based on intakes from food (or feed), otherwise known as the external exposure or oral dose. However, the bioaccessibility[17] and bioavailability[18] of the contaminant determine the internal exposure.

98. Mycotoxins can be classified as short-lived chemicals that can be effectively measured only if the individual is undergoing continuous or frequent exposures or if the timing of exposure(s) is known. Mycotoxin biomarkers have been defined as the compounds themselves (for example,

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[16] A biomarker of exposure is a chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell, which indicates that exposure to the chemical has occurred

[17] Bioaccessibility describes events that take place during food digestion for transformation into potentially bioavailable material.

[18] Bioavailability describes the fraction of bio-accessible material that reaches the systemic circulation through epithelial tissue and avoiding pre-systemic metabolism.

parent compounds and/or a metabolite) or as a result of interaction with target molecules (for example, DNA or protein adducts) (Marín *et al*., 2018). Urinary excretion mainly represents recent mycotoxin intake, whereas measurements in plasma/serum are more likely to represent longer-term exposure.

99. The main analytical methods employed to perform biomarker analyses are based on either chromatography (for example, LC) or immunochemistry (for example, ELISA).

100. European human biomonitoring initiatives such as the Consortium to Perform Human Biomonitoring on a European Scale (COPHES)[19], Human Early-Life Exposome (HELIX)[20], and European Union Human Biomonitoring (HBM4U)[21], as well as typical literature databases (PubMed, Science Direct, Google Scholar, Scopus and Zenodo) and the Information Platform for Chemical Monitoring (IPCheM) platform – were mined for any relevant UK biomonitoring data on combined exposures to mycotoxins.

101. Both COPHES and HELIX did not include exposure to mycotoxins in the scope of their work, however, in the HBM4EU initiative it was. In brief, Alvito *et al*., (2019)[22] conclude that there are numerous factors that need to be considered when attempting to integrate biomarker data for exposure assessment – and thus the following risk assessment. These factors include: the validation and harmonisation of analytical methods to assess mycotoxin exposure biomarkers, a greater understanding of the current exposure levels of the European population to multiple mycotoxins and whether this differs for each Member State.

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[19] The COPHES final report is available on the EU HBM website, a brief technical report is also available.

[20] Further information on the HELIX project is available on the CORDIS EUROPA website.

[21] A pdf file for a brief informative guide for HBM4EU is available on the HBM4EU website.

[22] The HBM4EU mycotoxin paper by Alvito *et al*., (2019) is available on the HBM4EU website.

102. It was found that there are no UK government led HBM initiatives relating to mycotoxins, however, scientific interest for this has and continues to grow, which has led to several publications. These publications were previously reviewed by the COT. It was observed that the available literature focused on estimating DON exposures from using total DON (free DON and DON-glucuronides) in urinary samples as biomarkers in the UK population. Only one other study, for OTA exposure, was reported, by Gilbert *et al*., (2001).

103. DOM-1, deepoxy-DON, a metabolite of DON that can be produced by gut microbiota metabolism, has been suggested as a possible biomarker of DON exposure. However, the presence of DOM-1 in urine is rarely reported suggesting that it is unlikely to be suitable as an exposure biomarker for DON (Turner *et al*., 2008a, 2008b, 2009, 2010, 2011; Hepworth *et al*., 2012; Wells *et al*., 2016, 2017; Papageorgiou *et al*., 2018a, 2018b). From the reviewed studies, a proportion of the UK population (adults, children, adolescents, pregnant women, elderly and vegetarians) exceeded the group-TDI for DON of 1 µg/kg bw for chronic dietary consumptions, often of the 95th percentile exposure groups. Regular exceedance of the group TDI indicates a potential health concern. However, the EFSA CONTAM Panel noted the uncertainty associated with exposure estimates due to a high fraction of left-censored data (EFSA, 2017b).

104. At the time of review, only one report by Gratz *et al*., (2020) had performed multi-mycotoxin biomarker analyses. In this pilot study, UK children (n=21) were estimated to frequently exceed the TDI, for 52% of DON and 95% of OTA cases.

Summary of exposure assessment

105. As discussed, there is little to no relevant UK co-occurrence data for mycotoxins. Available data from food surveys, total diet studies or other databases have their own associated limitations.

106. The advancement and availability of biomarker detection techniques and equipment has progressed. However, these are mainly limited to AFs, OTA, DON, FBs, ZEN and, to a lesser extent, emerging mycotoxins such as Fus-X, CIT, NIV, T-2 toxin, 4,15-DAS, ENNs, ALT and tenuazonic acid.

107. Understanding the toxicokinetics of mycotoxin metabolites and their availability in different biological samples (for example, OTA has the potential to be transferred to breastmilk) and how they may correlate with exposure still needs further investigation. Additionally, there is a lack of harmonisation in experimental conduct and design, with particular reference to data collection and in the definition of performance criteria of fit for purpose analytical methods. Deficiencies in sampling strategy were also noted, for example the lack of knowledge regarding the stability of the biomarker, defined time of sampling, and detailed information regarding the way samples were collected and stored. Furthermore, there is a need for the development of biomarkers of exposure for the detection of masked mycotoxins.

108. As such, it was concluded by the COT that further research is required to enable the inclusion of HBM data for a robust exposure assessment.

Risk assessment

109. The potential effect(s) of exposure to a combination of mycotoxins in humans is not yet known and the relevance of the *in vitro* and *in vivo* data presented in Tables 4, 5 , 6 and 7 are unclear. In these models, positive (synergistic, potentiating, supra-additive) or negative (antagonistic, inhibitive, sub-additive, infra-additive) interactions have been reported; however, these interactions are dependent on the relative dose level(s) of each mycotoxin in the mixture, the exposure route(s), timing and duration of exposure, and the biological target(s).

110. In the published literature, the combined toxic effects that are observed will greatly depend on the experimental design, as evidenced in Tables 4, 5 6 and 7. Factors such as the type of experimental cells or animal models, the duration of exposure, the dosage and relationship between mycotoxins (i.e.

the ratio of each mycotoxin in the mixture), the tested endpoint and methodology used, including any statistical aspects used for modelling scenarios, can all affect the outcome.

111. Potential uncertainties arise when comparing between toxicity studies utilising "*natural*" contaminated test samples and purified extracts. For example, in livestock studies where feed is naturally contaminated with DON, a higher toxicity was observed when compared to exposure groups treated with purified DON. This result was attributed to the presence of additional fungal metabolites, where low concentrations of ZEN or the 3- or 15-AcDON precursors were found in some cases.

112. The main challenges in assessment of multiple mycotoxins include: the lack of accurate information regarding the toxicokinetic profiles of mycotoxin mixtures and their bioaccessibility (*i.e*. the actual percentage of mycotoxins that can be absorbed in the small intestine) that would enable a more accurate exposure and thus subsequent risk assessment. Additionally, the variability within mycotoxin bioaccessibility values depends on the compound, food product, contamination level and the nature of contamination (spiked or naturally contaminated). Furthermore, the breadth of *in vitro* digestion models used to assess the bioaccessibility of mycotoxins constitutes another important challenge. The suitability and reliability of *in vitro* models for *in vivo* extrapolation also need to be considered.

113. Mycotoxin absorption constitutes another challenge, considering that toxins could reach the intestine as the parent compound or as metabolites formed during digestion; the available methods for mycotoxin metabolites are also still in their infant stages. Studies on combined genotoxic effects of mycotoxin mixtures should also be further developed.

114. As for the exposure assessment, there is little to no relevant UK co-occurrence or HBM data for mycotoxins. Available data from food surveys, total diet studies or other databases have their own associated limitations and at this time an exposure assessment could not be performed.

115. In terms of risk assessment, one of the main challenges posed to risk characterisation is the absence of toxicological data. A deeper understanding of the interactions between multiple mycotoxins at a molecular level will assist in drawing real life conclusions on the possible health impact of human exposure to mycotoxin mixtures.

116. Based on the limitations discussed above and the inability to obtain an adequate estimate of exposure, a full risk assessment on the potential adverse effects of combined dietary exposure to mycotoxins could not be performed by the COT.

Further considerations

117. Concerns over the effect of climate change on fungi and host specific interactions were mentioned previously in this document. Moretti *et al*., (2019) provided a narrative review on the potential emerging mycotoxin risks under a climate change scenario in Europe. It was hypothesised that the contamination risk of aflatoxin (produced by *Aspergillus flavus*) in maize in South and Central-Europe will extend to new regions in the next 30 years. The *Fusarium* spp. profile on wheat is also hypothesised to change in Northern, Central and Southern-Europe. It is unknown whether these changes will be similar across these regions. As a result, new combinations of mycotoxins/host plants/geographical areas may arise. It was recommended by Moretti *et al*., (2019) that developments of new diagnostic tools, and a deeper knowledge of both biology, and genetics of toxigenic fungi may be required.

118. Possible co-exposures to mycotoxins from breast milk and infant formula also need to be considered in infants and young children. Several publications have already considered this as a potential risk. For example, Ortiz *et al*., (2018) have investigated the multiple mycotoxin exposure of infants and young children (0-23 months) *via* breastfeeding and complementary/weaning foods consumption in Ecuadorian highlands and

Braun *et al*., (2020) performed a longitudinal assessment of mycotoxin co-exposures in exclusively breastfed infants in Austria.

Summary

119. Mycotoxins are toxic secondary metabolites produced by fungi and are capable of causing adverse health effects in both humans and animals. Those of greatest concern to human health are produced by several fungal genera of filamentous species, namely *Aspergillus*, *Fusarium* and *Penicillium* spp.

120. DON, FBs, and ZEN are the most prevalent mycotoxins in the world with regards to cereals and cereal based products, with a prevalence of 66%, 56%, and 53%, respectively. Regulatory limits for these compounds are based on considerations of the toxicity of individual mycotoxins. There are several reports where co-exposures to multiple mycotoxins are observed in both humans and animals. As such, the literature was reviewed to investigate the potential risks of combined dietary exposure from mycotoxins.

121. The co-occurrence of mycotoxins in food and feed is quite common since some fungi can produce more than one mycotoxin (particularly *Fusarium* spp.), food commodities can be contaminated by several fungi, and animal and human diets usually consist of multiple commodities.

122. An external EFSA report by Battilani *et al*., (2020) was published; the group carried out an extensive literature review whereby a platform was built named MYCHIF. The database comprises four components: the ecological background of mycotoxins and their interactions with host plants, the available analytical methods to detect the co-occurrence of mycotoxins, the toxicological and biomarkers data relevant to humans and animals, and modelling approaches in order to perform risk modelling.

123. Using the gathered information, a case study was carried out for two mycotoxin mixtures (1: DON, FBs and ZEN and 2: T-2/HT-2 toxin, DON and NIV). Biomarker data was utilised as the basis for the exposure assessment, which was carried out probabilistically either with a component-based or provisional daily intake approach.

124. For the CBA an MOE of <100 for all age groups (adolescent, adult and elderly) was calculated, whereas a hazard index could not be calculated for the provisional daily intake approach. A hierarchy map (based on EU member states) for adults was compiled to provide a visual representation of higher risked exposure groups to T2/HT-2 toxin, DON and NIV.

125. With reference to the co-occurrence and occurrence data collected for the United Kingdom (UK); T2/HT-2 toxin, DON and NIV adult exposure levels based on the mean co-occurrence data and mean consumption value were >0.8 ,0.6-1.2 and 0.24-0.3 µg/kg bw/day, respectively.

126. To put this into context, these exposure values were compared with the respective HBGV for the individual mycotoxins (see paragraph 36 for values) The estimated exposure levels for UK adults of T2/HT-2 toxin are above the HBGV, whilst exposure levels for DON and NIV are below their respective HBGVs.

127. In terms of data gaps, there is still only limited knowledge on the presence and co-occurrence of multiple mycotoxins, both for primary mycotoxins and their modified forms, in food and feed, since current analytical methods have limitations. Furthermore, there is a limited number of toxicity data and there is a lack of consensus on methodologies and guidance for generating *in vitro* and *in vivo* TK and TD. Model definitions are also required for the utilisation of biomarkers for exposure assessments.

128. Opinions by other authoritative groups on some mycotoxin mixtures were also summarised. The EFSA CONTAM Panel concluded that the available data for interactions between 4,15-DAS and other mycotoxins (T-2 and HT-2 toxins, AFs, OTA and FBs) is weak and inconclusive (EFSA, 2018).

129. In contrast, the EFSA CONTAM Panel concluded that the combined effect of CIT and OTA is, at most, additive (EFSA, 2012). JECFA concluded that even though there are additive or synergistic effects observed from FB1

and AFB1 co-exposure in laboratory animals in inducing the development of preneoplastic lesions or hepatocellular carcinoma (Torres *et al.*, 2015; Carlson *et al.*, 2001; Gelderbloom *et al.*, 2002), there was no data available on such effects in humans. The combined toxicity of FBs and DON was suggestive of being additive or more than additive; however, the observed effect is dependent on the endpoints measured (JECFA, 2018).

130. The main analytical methods to measure mycotoxins in food commodities are: ELISA, GC and LC-MS, whilst LC–MS/MS is the main analytical method for detecting and measuring co-occurrence of very low concentrations of mycotoxins; however, these advanced multi-mycotoxin techniques are not yet commonly applied in routine screening analyses due to their associated high cost.

131. In terms of toxicokinetic data, only one human study was identified to have investigated and analysed the combined exposure to DON and ZEN (Warth *et al*., 2013). This study, however, had its limitations which was mainly due to the number of volunteers (n=1 male) and in effect, does not cover inter-individual variations. The toxicokinetics of mycotoxin mixtures may need to be addressed on a case-by-case basis; however, it is recognised that the mycotoxin dose, exposure pathway, interspecies and intraspecies differences have been identified as the most influential parameters that may affect observations.

132. The toxic effects of some binary combinations of mycotoxins were discussed in this paper (for example, AFB1 and FB1, OTA and DON, see tables presented in the Toxicology section). The availability of *in vivo* data directly relevant for humans is scarce, with most studies covering only a limited number of mycotoxin combinations and more generally focused on animal models of agricultural importance *i.e*. pigs and chickens.

133. The toxicity of the combinations could not be predicted based on the toxicity of the individual mycotoxins. Furthermore, the comparison of studies was challenging as there was a large amount of variability between each

methodology used, due to the lack of harmonisation on combinative testing strategies for each toxicological endpoint.

134. In terms of exposure assessments, the use of biomarker data was explored in the MYCHIF report by Battilani *et al*., (2020) (see paragraphs 30-35 and 88-93). It was concluded that the use of biomarkers may be premature, since there is a lack of: knowledge on the human bioavailability of the toxin combination, and the excretion rates. There are also several limitations associated with multi-biomarker monitoring. These include:

i). Biological fluids contain extremely low analyte concentrations following dietary exposure, as such sample preparation is crucial to obtain an acceptable limit of detection.

ii). There is considerable chemical diversity among analytes and this makes clean-up methodologies challenging (for example, polar compounds like glucuronides).

iii). Careful optimisation needs to be carried out to overcome matrix effects and interfering matrix peaks, eluents, the chromatographic gradient, and the dilution factor.

iv). The co-elution of matrix components is said to have a negative influence on the accuracy of quantitative methods through ion suppression or enhancement in the ion source.

v). In general, there is a lack of authentic reference standards and certified reference materials and consensus on a validated biomarker to be used in context with multi-mycotoxin analyses.

135. A major knowledge gap was the potential concurrent exposure to mycotoxins and other environmental chemicals that may exhibit some interactive activity and/or exert a biological function converging on the same molecular pathways.

136. A stepwise approach for the exposure assessment was detailed (see paragraphs 79-82) in this paper, based on the use of deterministic and if necessary, probabilistic approaches. Three data sources were also identified to use in the exposure assessment, these were the FSA TDS Mycotoxin Study (Stratton *et al*., 2017), the MYCHIF platform (Battilani *et al*., 2020), and exposure data derived from literature.

137. Based on the limitations presented throughout this document and the inability to obtain a reliable estimate of exposure, due to data limitations, a full risk assessment on the potential adverse effects of combined dietary exposure to mycotoxins could not be performed by the COT.

COT conclusions and recommended research

138. As noted above, the COT was unable to complete a risk assessment on the potential risk(s) from combined exposure to mycotoxins for several reasons. There is a lack of harmonisation of approaches/methodologies and data analysis/modelling for toxicological investigations. Additionally, the underlying mechanisms of interactions between individual mycotoxins in different combination have yet to be fully elucidated and understood. Further knowledge gaps preventing a full risk assessment include the potential toxic effects of mycotoxin mixtures on the gut microbiota.

139. Possible co-exposures from breastmilk and weaning foods also need to be considered for infants and young children.

140. Furthermore, the availability of co-occurrence data in food is scarce, and multi-analyte methods are still not being fully applied as standard. Lastly, the management of left-censored data (*i.e.* data for which the true values are below the LOD and could not be accurately determined), the use of probabilistic models and a multi-biomarker approach should be consistent and well-defined.

141. The COT noted that there was a lack of UK data, particularly in biomonitoring; however, there were a number of studies ongoing and additional information will be available in the future. The Public Health England Secretariat informed COT Members that the UK will not be collecting new data for mycotoxins under the HBM4EU initiative. However, in the future, more data could be obtained through Health Protection Research Units.

142. The results of such research would be of potential value in the risk assessment of co-exposures to mycotoxins; however, COT Members recommended as a pragmatic first step that a review should be carried out of the compounds that appeared to show a common effect on protein synthesis, assuming dose additivity, and that frequently co-occur in food commodities – an exposure estimate (based on the Stepwise approach discussed in the Exposure assessment section) could be performed and the estimates

compared with the PODs (to calculate the MOEs) or the HI utilised, to determine whether there is any potential concern from co-exposure to these mycotoxins in UK consumers.

143. Depending on the outcome of this screening risk assessment, research may be needed on those mycotoxins affecting ribosomal protein synthesis, to determine whether they do exhibit dose additivity in their effects, to help develop a reliable basis for their cumulative risk assessment.

COT Statement 2021/04
October 2021

Abbreviations

15-AcDON	15-acetyldeoxynivalenol
3-AcDON	3-acetyldeoxynivalenol
4,15-DAS	4,15-diacetoxyscirpenol
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFM1	Aflatoxin M1
AFs	Aflatoxins
ARfD	Acute reference dose
CBA	Component-based approach
CIT	Citrinin
CONTAM	Panel on Contaminants in the Food Chain
COPHES	Consortium to Perform Human Biomonitoring on a European Scale
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DNA	Deoxyribonucleic acid
DOM-1	De-epoxy deoxynivalenol
DON	Deoxynivalenol
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ENNs	Enniatins
FB1	Fumonisin B1
FBs	Fumonisins
Fus-X	Fusarenon-X
GC-MS	Gas chromatography-mass spectroscopy
HBGV	Health based guidance value
HBM	Human biomonitoring
HBM4EU	European Union Human Biomonitoring
HELIX	Human Early-Life Exposome
HPLC	High-performance liquid chromatography

JECFA	Joint Food and Agriculture Organization and World Health Organisation Expert Committee on Food Additives
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectroscopy
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MOA	Mode of action
MOE	Margin of exposure
MYCHIF	Mycotoxin Mixtures in Food and Feed: Holistic, Innovative, Flexible Risk Assessment Modelling Approach
MYCOMIX	Mycotoxin mixtures
MYTOX	Mycotoxin and Toxigenic Moulds
NIV	Nivalenol
OTA	Ochratoxin A
PAT	Patulin
SCF	Scientific Committee on Food
TD	Toxicodynamic
TDI	Tolerable daily intake
TDS	Total diet study
TK	Toxicokinetic
UK	United Kingdom
ZEN	Zearalenone

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