



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

**An overview of mycotoxins relevant for
the food and feed supply chain: using a
novel literature screening method**

RIVM letter report 2019-0223
A.D. van den Brand | A.S. Bulder



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Colophon

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Synopsis

An overview of mycotoxins relevant for the food and feed supply chain: using a novel literature screening method

Certain types of fungi make substances, known as 'mycotoxins', that protect them from bacteria and other fungi. If agricultural crops are contaminated by fungi, these substances can end up in foods. The ingestion of mycotoxins by people or animals can be harmful to their health. The Netherlands Food and Consumer Product Safety Authority (NVWA) therefore checks whether there are mycotoxins in human foods and animal feeds.

There are many different types of mycotoxins. Dozens of types were assessed earlier to find out whether they form a risk to the public health. However, we do not yet know the foods in which the majority occur, whether they are harmful and, if so, in what quantities. RIVM has therefore drawn up an overview of all the available information on the mycotoxins that may be present in human food and animal feed chains. The organisation has developed a new method for collecting this information and has entered it into a database. The NVWA can use this overview to see, at a glance, what mycotoxins it has to look out for with regard to particular products.

Among other things, the overview contains information about the maximum permitted quantities of mycotoxins in products (maximum limits). The products in which specific mycotoxins occur and the harmful effects they may cause are also included in the overview.

The overview was drawn up using the results of the most recent evaluations carried out by international organisations involved in risk assessments, such as the European Food Safety Authority (EFSA). The scientific literature was, furthermore, researched for human and animal food products in which the assessed mycotoxins occur. The mycotoxins that occur in the highest concentrations were listed separately and their effects, where known, were looked up and included in the overview.

Keywords: mycotoxins, database, occurrence, toxicity, limits

Publiekssamenvatting

Een overzicht van schimmeltoxinen in de voedsel- en diervoederketens: een nieuwe aanpak om literatuur te screenen

Schimmels kunnen stoffen maken die hen beschermen tegen bacteriën en andere schimmels. Als schimmels landbouwgewassen besmetten, kunnen deze stoffen, die mycotoxinen heten, in voedsel terecht komen. Als mensen of dieren mycotoxinen binnenkrijgen, kan dat schadelijk voor hun gezondheid zijn. De Nederlandse Voedsel en Warenautoriteit (NVWA) controleert daarom of mycotoxinen in voedsel en diervoer zitten.

Er bestaan veel verschillende mycotoxinen. Van tientallen typen is eerder al beoordeeld of ze een risico vormen voor de gezondheid. Van een groter aantal is niet bekend in welk voedsel ze voorkomen en of ze schadelijk zijn en zo ja, bij welke hoeveelheid. Het RIVM heeft daarom een overzicht gemaakt met alle informatie die bekend is over de mogelijke mycotoxinen in de voedsel- en diervoederketen voor mens en dier. Het RIVM heeft een nieuwe methode ontwikkeld om deze informatie te verzamelen en deze in een database gezet. Met dit overzicht kan de NVWA per product snel inschatten op welke mycotoxinen ze moeten letten.

Het overzicht bevat onder andere informatie over de maximaal toegestane hoeveelheden van mycotoxine op producten (maximale limieten). Ook staat erin in welke producten een mycotoxine voorkomt en welke schadelijke effecten ze kunnen hebben.

Voor het overzicht is informatie gebruikt van de nieuwste evaluaties van internationale organisaties die risicobeoordelingen doen, zoals de Europese Autoriteit voor Voedselveiligheid (EFSA). Daarnaast is in de wetenschappelijke literatuur nagegaan in welke producten de beoordeelde mycotoxinen in voedsel en diervoeder voorkomen. De mycotoxinen die in de hoogste concentraties voorkomen, zijn apart op een rij gezet. Van deze mycotoxinen zijn ook de effecten opgezocht en opgenomen in de database, voor zover die bekend zijn.

Kernwoorden: mycotoxinen, database, voorkomen, toxiciteit, limieten

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Summary

An overview of mycotoxins relevant for the food and feed supply chain: using a novel literature screening method

Mycotoxins are secondary metabolites produced by fungi, that can have a variety of adverse effects on the health of humans and animals. Mycotoxins can enter the food and feed supply chain via contamination of agricultural products in the field or during storage or transport of such products. Many fungal metabolites have already been identified, however, only a fraction of these have been evaluated in international risk assessments.

To support the Dutch Food and Consumer Product Safety Authority (NVWA) in the identification of relevant mycotoxins in the food and feed supply chain, an overview of mycotoxins was created in the form of an Access database. To obtain this overview, first a list of approximately 455 evaluated and unevaluated mycotoxins was compiled. General information on the occurrence and toxicity of the evaluated mycotoxins was gathered, as well as existing international regulatory limits and health based guidance values. This information was obtained from international risk assessments (from international bodies as well as national agencies), scientific literature and publicly accessible sources on mycotoxins. Subsequently, an additional literature search was performed to obtain further information on the occurrence of these mycotoxins, since their last evaluation. A new method was used to compile the information, without detailed qualitative assessment of the studies. This facilitated the extraction of information from a large number of articles in a short period of time. Based on this search, also the occurrence of other, unevaluated mycotoxins was identified. For these mycotoxins, additional information on the published toxicological effects was obtained.

All this information on both evaluated and unevaluated mycotoxins was compiled in an Access database. The overview generated by this new method can be used as a first step to identify mycotoxins of interest in the food and feed supply chain.

1 Introduction

Mycotoxins are secondary metabolites produced by fungi that can evoke a toxic response in humans and animals after exposure e.g. via ingestion of contaminated food or feed (Bennett, 1987). Mycotoxins can enter the food supply chain via fungi that infect plants during their growth and development, and subsequently contaminate derived agricultural commodities with mycotoxins. In addition, cereals and other (agricultural) commodities can be infected by fungi and contaminated by mycotoxins during harvest, storage, processing and shipment (Ismail & Papenbrock, 2015). Fungi belonging to the genus *Fusarium* are most important with respect to contamination in the field. *Aspergillus* is an opportunistic fungus, infecting maize (and peanuts) during the growing season in climate zones with a more elevated temperature. Both *Aspergillus* and *Penicillium* fungi are commonly associated with contamination during storage (Norwegian Scientific Committee for Food Safety (VKM), 2013).

While some fungal metabolites are used as pharmaceuticals or industrial chemicals due to their anti-microbial effects (Butler, 2004), others can evoke carcinogenic, immunotoxic, hepatotoxic or other adverse health effects in humans or animals after intake of contaminated food or feed (Ostry et al., 2018). Generally, pigs are particularly sensitive to the effects of mycotoxins, whereas ruminants have a lower sensitivity as a result of an extensive rumen metabolism and microbiota (Gallo et al., 2015; VKM, 2013). To ensure food and feed safety, maximum and guidance limits for mycotoxins in specific food and feed products have been established internationally by e.g. the European Union and the Codex Alimentarius Commission. The European Food Safety Authority (EFSA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) issued risk assessments on the most common mycotoxins found in food and feed to support the development of effective measures for risk management.

The exact number of all secondary metabolites produced by fungi is unknown and only a fraction of these metabolites have been subjected to a risk assessment or have been regulated in food or feed. Due to the development of novel analytical methods, the total list of fungal metabolites is ever expanding and has been estimated to be over 3000 metabolites (Klitgaard et al., 2014). The toxic potential of many of these fungal metabolites is unknown. As such, the fungal metabolites may not all qualify as mycotoxins. In this report, we will further refer to all fungal metabolites as mycotoxins.

State-of-the-art methods greatly aid to the discovery of new mycotoxins, as well as of modified forms of known mycotoxins. In this respect, modified forms of mycotoxins comprise all conjugates of the parent molecule which are formed in the infested plant and mammalian organism (Rychlik et al., 2014). For example, plants can metabolize the mycotoxins that they are contaminated with by forming glucoside or sulfate conjugates. Most of these modified mycotoxins are not regulated or monitored in food or feed. This can result in an underestimated (risk

of the) exposure to the basic compound, as the modified mycotoxins occurring in the plants can be deconjugated to the parent mycotoxin during metabolism in humans and animals (EFSA 2017a; EFSA 2018a; EFSA 2018b). Modified mycotoxins can thus add to the exposure and effects of the parent mycotoxin. The occurrence and toxicological effects of newly discovered or modified metabolites are however largely unknown.

In addition to the unknown occurrence and effects of new mycotoxins, it appears that the occurrence of 'traditional' mycotoxins sometimes shifts to untypical products/matrices, or unusual geographical regions possibly partially as a result of global warming (Gruber-Dorninger et al., 2016). As the demand of plant based food and feed increases (Molina et al., 2019), it is of importance to have an overview of the mycotoxins that can occur in the food and feed supply chain.

An overview that includes previously evaluated mycotoxins and newly identified, unevaluated mycotoxins can help to identify possible relevant mycotoxins in the food and feed supply chain. Upon request of the Dutch Food and Consumer Product Safety Authority (NVWA), such an overview of the general information (e.g. maximum limits, health based guidance values and transfer information), the occurrence (e.g. type of products or country of origin) and the toxicological properties of the evaluated and unevaluated mycotoxins was compiled from available literature. A new method was used to gather large quantities of occurrence data, without detailed qualitative assessment of the studies. The gathered information was compiled in a database to be able to create various overviews. The overviews can e.g. be used to identify the possible presence of mycotoxins in specific food or feed products, the maximum limits that have been set for the respective mycotoxins or the health based guidance values and toxicological effects of mycotoxins. In this report, the methods that were used to obtain the information, as well as the main observations from this information are described.

2 Methods

As a starting point, a list of known mycotoxins in the food and feed supply chain was compiled. Of all these identified mycotoxins, several had been subjected to an evaluation by international risk assessment agencies. In this report, we will further refer to these mycotoxins as 'evaluated mycotoxins'. We will further refer to the other mycotoxins on the list as 'unevaluated mycotoxins'. Information on their occurrence in food and feed, general and toxicological properties and the available maximum limits was extracted from international risk assessments and other publicly available sources.

In addition, a literature search was performed for the evaluated mycotoxins to identify new information on the occurrence of mycotoxins in food and feed, as this information is most relevant to support the evaluation of mycotoxins in the food and feed supply chain.

In the literature studies that reported on the occurrence of the evaluated mycotoxins, many unevaluated mycotoxins were identified in food and feed products as well. A general overview of the occurrence of unevaluated mycotoxins was thereby also generated. An additional literature search for toxicological information was performed on the unevaluated mycotoxins that were frequently reported to occur in food and feed.

To generate this overview on mycotoxins, all the information was compiled in a database to facilitate the screening. Figure 1 describes the general approach taken to generate this mycotoxin overview. It must be noted that the absence of studies reporting on toxicological effects of unevaluated mycotoxins does not imply that there is no toxicological risk for these mycotoxins.

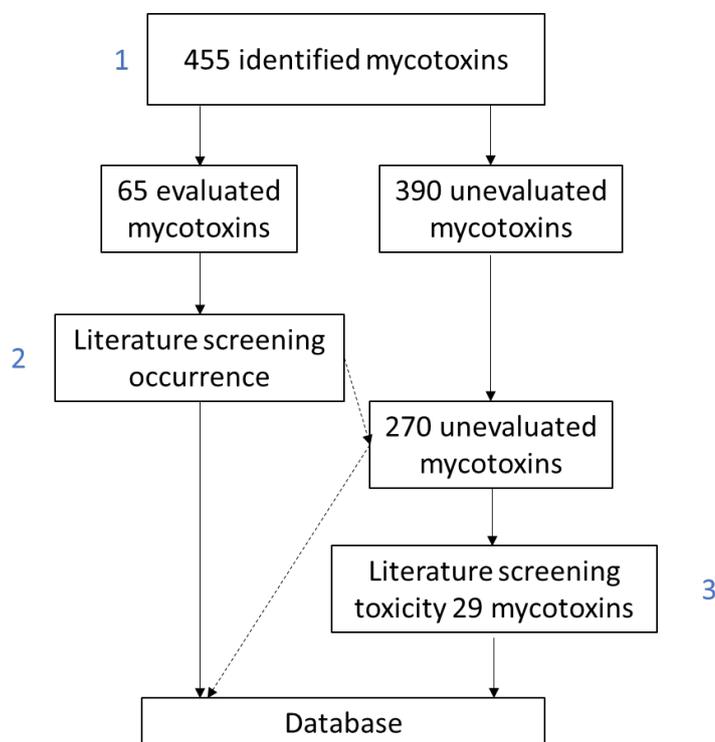


Figure 1. Schematic overview of the steps taken to generate the information included in the database. A list of mycotoxins that were relevant for the food and feed supply chain was compiled (step 1). For the evaluated mycotoxins, additional (to the evaluation) literature was screened for the reported occurrence in food and feed (step 2). During this screening, also information on the occurrence of the unevaluated mycotoxins was extracted. The literature was subsequently screened for the toxicological effects of selected unevaluated mycotoxins in humans and animals (step 3). All information was gathered in a database to facilitate a screening of the information.

2.1 List of mycotoxins

The first step to generate the overview on known mycotoxins was to identify the mycotoxins that may be of importance in the food and feed supply chain. To identify the mycotoxins (and their metabolites) that had already been considered in food and feed, the archives of several international risk assessment agencies were searched (Table 1). Of the evaluated mycotoxins, all were evaluated by the European Food Safety Authority (EFSA). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also evaluated many of the mycotoxins that have been evaluated by EFSA. Other agencies included, US-FDA, Food Safety Committee in Japan, Health Canada, Nordic Council of Ministers amongst others (see Table 1 for a complete overview). The most recent risk assessments of evaluated mycotoxins were however by either EFSA or JECFA and therefore these mycotoxins were included in a first list. To identify relevant other mycotoxins the search terms "new or emerging and/or mycotoxin, secondary metabolites, masked mycotoxins, fungal toxin, mold toxin" were used in Google and literature databases as Embase, Pubmed and Scencedirect. Articles and books were scanned and other identified mycotoxins were listed. In addition, NVWA reports were screened for results on mycotoxins. Several articles were identified in

which many (100+) mycotoxins were analysed in food and feed products. The article by Malachova et al. (2014) reporting on 288 mycotoxins was used to supplement the first list. Other articles and reports were used to supplement this list. Due to the development of analytical techniques, especially LC-MS equipment, up to 3000 fungal metabolites were identified under experimental conditions (Klitgaard et al., 2014). It was decided to include only those mycotoxins in the list that were detected in food and feed matrices in published research, as these are considered to give a more representative overview of the relevant mycotoxins in the food and feed supply chain. This resulted in a list of approximately 455 identified mycotoxins, both evaluated and unevaluated. This list is likely to expand in the coming years due to further development of the analytical methods in feed and food matrices.

Table 1. Overview of international agencies used to identify evaluated mycotoxins feed and food.

Organization	Abbreviation
European Food Safety Authority	EFSA
Joint FAO/WHO Expert Committee on Food Additives	JECFA
Scientific Committee on Food	SCF
World Health Organization	WHO
United States Environmental Protection Agency	US-EPA
Dutch Food and Consumer Product Safety Authority	NVWA
National Institute for Public Health and the Environment	RIVM
Nordic Council of Ministers	
Norwegian Scientific Committee on Food Safety	VKM
Health Canada	
Danish Environmental Protection Agency	Danish EPA
Food Standards Australia New Zealand	FSANZ
The Australia New Zealand Food Authority	ANZFA
New Zealand Environmental Protection Agency	NZ-EPA
French Agency for Food, Environmental and Occupational Health & Safety	ANSES
United States Food and Drug Administration	US-FDA
Austrian Agency for Health and Food Safety	AGES
German Federal Institute for Risk Assessment	BfR
Australian Government Department of Health	DOHA
Food Standards Agency UK	FSA
UK Department for Environment, Food & Rural Affairs	DEFRA
Food Safety Committee of Japan	FSCJ
US Department of Agriculture	USDA
USA National Toxicology Program	NTP
International Life Sciences Institute	ILSI
International Agency for Research on Cancer	IARC
European Union Law	EUR LEX
Codex Alimentarius Commission	CODEX

As a next step, information on the occurrence, toxicological effects and HBGVs of the evaluated mycotoxins on the list was extracted from

international risk assessments and the other publicly available sources on mycotoxins. Information on other, but unevaluated mycotoxins, that was also included in the risk assessments and other sources was also compiled in the database. In addition, international regulatory limits for mycotoxins in food and feed were identified and compiled in the database.

2.2 Literature search

After gathering information from international evaluations, more information with respect to the reported occurrence and toxicological effects of these mycotoxins was gathered by literature searches. A different approach was used for evaluated and unevaluated mycotoxins.

2.2.1 *Evaluated mycotoxins*

The literature search for evaluated mycotoxins was aimed at identifying new developments on toxicological effects and reported occurrence in literature since their most recent risk assessment (i.e. by EFSA or JECFA) (Table 2). The aflatoxins and ochratoxin A were under re-evaluation by EFSA at the time of the search and were therefore excluded.

Diacetoxyscirpenol was also excluded from the search as it had been evaluated by EFSA within a year previous to the search.

In consultation with the information specialist from RIVM, different search strings were developed for three different literature databases: Embase, Pubmed and Scopus. This approach was taken to reduce the possibility that important articles were missed, as the scope of the journals in the databases differs. The search strings were developed to specifically identify mycotoxin occurrence in food or feed, toxicological effects and transfer to edible animal tissue. During the literature search, the search terms were adapted for each mycotoxin in consultation with the information specialist. Annex I describes the general approach that was taken to adapt the search strings for the mycotoxins. Annex I also describes an example search string for the mycotoxin patulin.

Table 2. Overview of evaluated mycotoxins, their most recent evaluated risk assessment.

Mycotoxin	Organisation + year
Aflatoxins	JECFA 2016, EFSA pending
<i>Alternaria</i> toxins	EFSA 2011
Beauvericin	EFSA 2014
Citrinin	EFSA 2012
Diacetoxyscirpenol	EFSA 2018
Deoxynivalenol + modified forms	EFSA 2017
Enniatins	EFSA 2014
Ergot alkaloids	EFSA 2012
Fumonisin	EFSA 2018
Moniliformin	EFSA 2018
Nivalenol + modified forms	EFSA 2017
Ochratoxin A	JECFA 2007, EFSA pending
Patulin	SCF 2000
Phomopsins	EFSA 2012
Sterigmatocystin	JECFA 2016
T-2 + HT-2 toxin	JECFA 2016
Zearalenone + modified forms	EFSA 2016*

* Risks for animal health reported separately in 2017.

Clean-up of search results

To select the relevant literature, all references that were obtained using the search strings were imported into an Endnote database and duplicate entries were removed. References that were considered not relevant for further screening were excluded based on titles and abstracts. General exclusion criteria were: journal abstracts and book sections, retracted articles, articles that did not actually analyze the occurrence of mycotoxins (i.e. review articles on occurrence of mycotoxins), studies with only *in vitro* experiments, studies with only mixtures of mycotoxins as observed effects can then not be allocated to a specific mycotoxin (unless control groups with single mycotoxin treatment were included), studies with zebrafish experiments, studies with *ex vivo* experiments, studies on the phytotoxic effects of mycotoxins and studies on biomonitoring as these cannot show a specific effect of one mycotoxin. In addition, as the database will be used to assess mycotoxins in the food and feed supply chain, only recent information on the occurrence of mycotoxins was considered relevant. Therefore, studies with information on the occurrence of mycotoxins before 2009 were also excluded (as agreed upon with the NVWA). These selection criteria resulted in a collection of relevant articles containing information on both the reported occurrence and the toxicological effects of the evaluated mycotoxins. PDF files of these articles were then retrieved.

Selection

The selected references were combined in one Endnote database with a total of 665 references. It was decided to focus on articles that report on occurrence of mycotoxins in food and feed, instead of articles on the possible toxic effects (as agreed upon with the NVWA). As many of these mycotoxins were already evaluated by e.g. EFSA and/or JECFA, the toxicological properties on these mycotoxins were already largely identified in the database. New studies would therefore not add much new toxicological information to the database. The large number of 665 references were therefore grouped into articles reporting on information on occurrence and on toxicological effects of the mycotoxins based on screening of title and abstract. Approximately half of all articles were classified as 'occurrence' articles and the other half as 'toxicological' articles. The references with information on the occurrence of mycotoxins in food and feed were subsequently used to extract the relevant information which was then entered in the database. Without detailed qualitative analysis of the studies, information regarding the analyzed product, the range of the mycotoxin level that was reported, the methods of analysis and the origin of the samples was extracted and entered in the database (for more detailed information see 2.3.2). This information was considered relevant to support an analysis of the food supply chain.

During the classification of the literature in occurrence articles and toxicological articles, the screening of the literature that reported on the occurrence of mycotoxins in food and feed was not always deemed representative for the realistic situation. For example, some studies aim to develop new methods to analyze mycotoxins and report the presence of mycotoxins in certain products after spiking them with the mycotoxins. Therefore, not all occurrence articles were considered

suitable for use and additional exclusion criteria were applied to studies that inoculated/spiked/fortified products with fungi or mycotoxins, studies that specifically selected moldy products (so not real/commercial/market/retail samples), and studies solely on method development were not used for this project.

It should be noted that the literature search was performed using the publication year of the most recent evaluation of the respective mycotoxins. Therefore, studies reporting information on the occurrence of mycotoxins between 2009 and the evaluation year were also not included in the overview. It was assumed that the international evaluations on the mycotoxins also reported on the known mycotoxin occurrence in products. Therefore, publications before the most recent evaluation date will likely not reveal new commodities in which that respective mycotoxin was found.

2.2.2 *Unevaluated mycotoxins*

For the approximately 390 unevaluated mycotoxins, an overview of information is also warranted.

Selection

Information on the occurrence of unevaluated mycotoxins was also identified during the literature search on the occurrence of the evaluated mycotoxins. New analytical methods are continuously developed or improved for many food or feed matrices. Liquid chromatography-mass spectrometry (LC-MS) also allows the determination or analysis of multiple mycotoxins in a sample (multi-method). Recently, the development of analytical methods has evolved to methods which allow the determination of 100+ mycotoxins in one analytical run, which often reveal the occurrence of many fungal metabolites, here referred to as unevaluated mycotoxins.

It was assumed that, as a result of the use of these multi-methods, a general overview of the occurrence of unevaluated mycotoxins was also generated while searching for the occurrence of evaluated mycotoxins. It was further assumed, after consultation with the contact person of the Division Tactical Direction & Expertise of NVWA who is responsible for the monitoring and surveillance program of the NVWA regarding mycotoxins, that it is unusual to specifically analyse the occurrence of unevaluated mycotoxins, without analysing the occurrence of 'well-known' evaluated (and regulated) mycotoxins.

The additional literature search for relevant information on the unevaluated mycotoxins was therefore limited to the toxicological effects of a selection of mycotoxins. Firstly, these included those mycotoxins that were frequently reported to occur at high levels in food and feed, i.e. where the maximum mycotoxin levels were reported at high (above 1 mg/kg) or medium high (above 0.5 mg/kg) levels in at least 2 references during the literature search for evaluated mycotoxins (Table 3). These values were chosen in consultation with the contact person of the Division Tactical Direction & Expertise of NVWA, and based on the occurrence data included in the database. It should be noted that a different dataset may warrant a different choice of these selected values (e.g. other contaminants which may occur in different concentrations).

In addition, this selection was complemented with unevaluated mycotoxins that were found in surveys by the NVWA in 2016 or 2017 as well as with unevaluated mycotoxins that were listed as posing the greatest potential risk to human and animal health by CAST in 2003, if their occurrence was reported in the developed database (which was only the case for fusarin C). This resulted in a selection of 29 unevaluated mycotoxins for a literature search on toxicological effects (Table 3).

Table 3. Overview of the selected unevaluated mycotoxins based on the literature search on evaluated mycotoxins, surveys of the Dutch Food and Consumer Product Safety Authority (NVWA) and the Council for Agricultural Science and Technology (CAST).

Source			
Literature search evaluated mycotoxins 2009 to now or from year of publication		NVWA studies 2016-2017	CAST report 2003
15-hydroxyculmorin	3-nitropropionic acid	Cyclopiazonic acid	Cyclopiazonic acid
5-hydroxyculmorin	Asperglaucide	Fusarenone-x	Fusaric acid
Aurofusarin	Butenolide	Gliotoxin	Fusarin C
Culmorin	Curvularin	Mevinolin	Gliotoxin
Emodin	Equisetin	Mycophenolic acid	Isofumigaclavines A and B
Fusaproliferin	Fusaric acid	Penicillic acid	Mycophenolic acid
Kojic acid	Mycophenolic acid		Penicillic acid
Phycion	Roquefortine C		<i>Penitrems*</i>
Rubrofusarin	Rugulosin		<i>PR toxin*</i>
Skyrin	Tryptophol		Roquefortine
Citreoresein	Cyclo (L-Pro-L-Val)		
Cyclopiazonic acid	Viomellein		

* These mycotoxins were mentioned by CAST as posing a threat to human or animal health, but were not found at high levels in the literature search for the occurrence of evaluated mycotoxins and therefore excluded from the selected unevaluated mycotoxins.

To check for possible missed occurrence information on the unevaluated mycotoxins by following the approach as described above, a test literature search was performed with three random unevaluated mycotoxins that were not reported in the literature search on the occurrence of evaluated mycotoxins i.e. chetoseminudin, normidulin and sclerotigenin. This search yielded only a few references, primarily on studies that reported the synthesis of these mycotoxins and one *in vitro* study. No studies on occurrence or toxicological effects in animals were found. Based on this test, it was concluded that no major information was missed using the abovementioned approach.

It is important to note that, since the literature search on evaluated mycotoxins was performed on the period after the publication of the evaluation, some studies on unevaluated mycotoxins in the period 2009-

to publication year, are not included in the database (see 2.2.1.). In addition, diacetoxyscirpenol, aflatoxins and ochratoxin A were not included in the literature search for evaluated mycotoxins. These mycotoxins were recently evaluated or a new evaluation is pending at the time of this report. Studies that targeted these mycotoxins as well as possible unevaluated mycotoxins might not be included in the database. The occurrence of unevaluated mycotoxins as well as the mycotoxins under evaluation by EFSA can therefore not be considered a complete overview.

Toxicological literature search and screening

The additional literature search for relevant toxicological information on each of the 29 selected unevaluated mycotoxins was conducted similar as described earlier for evaluated mycotoxins, but without a restriction regarding the search period. However, for some unevaluated mycotoxins, no references were found using the regular search terms. In that case, the search string was replaced with the unevaluated mycotoxin name or searched for in the title and abstract.

To select relevant articles, all references, including the references used in the literature search on evaluated mycotoxins, were imported in an Endnote database and duplicate entries were removed. Conference abstracts and book sections were excluded. This resulted in a list of 3705 new references for the 29 unevaluated mycotoxins. As some (unevaluated) mycotoxins are e.g. used as a pharmaceutical, the literature search generated occasionally more than 500 references per mycotoxin.

To prioritize the most relevant references, it was decided to filter the Endnote library by specifically searching for reviews on toxicological effects of the unevaluated mycotoxins. It is important to note that the consequence of this approach is that the overview of the toxicological effects of the selected unevaluated mycotoxins may not be complete. All fields were searched for containing the words 'safety assessment', 'risk assessment' and 'review'. In addition, reviews were searched by selecting reviews in the 'type of work' category in Endnote. The reviews on the toxicological effects of the respective mycotoxins were screened for information which was subsequently compiled in the database. Reviews on the effects of the mycotoxin on plant health, the efficacy as a therapeutic, the biosynthesis or as mixtures were not considered relevant. When no review articles were identified for a mycotoxin, all initially identified references were screened manually. This was the case for cyclo (L-Pro-L-Val) and viomellein.

2.3 Database

To support searching the overview of mycotoxins, a database was developed in which all information was compiled. This database was created using Access, a Windows Office 365 desktop application. In this database, data was compiled on general information on the mycotoxins, information on the occurrence of mycotoxins, toxicological data of mycotoxins and on maximum (or advisory) limits and health based guidance values of mycotoxins. These data were clustered in separate tables to allow an easy search within and between the information in the

tables, by using queries. All data were added as single entry for every information type and information source. All references were listed with their respective URL or DOI.

2.3.1 *General data*

In this table, general information on the mycotoxins was compiled. This information included e.g. CAS numbers and alternative names. To allow an easy search between the parameters, the information on the fungal genus that produce the mycotoxins was separated from the general data table. The general data comprises data that was not specifically searched for in literature, but was mentioned in e.g. the risk assessments of the respective mycotoxins or the introductions of articles on the mycotoxins.

2.3.2 *Occurrence data*

The information on occurrence of the mycotoxins in different food and feed products in the database is based on review data from the respective evaluations (e.g. by EFSA or JECFA) and information on the reported occurrence of mycotoxins in literature since their most recent evaluation. The information on occurrence of mycotoxins in different food and feed matrices was extracted from literature and the reported concentration ranges were entered in the database. The only quality check that was performed on the studies was to only extract data from studies on occurrence of mycotoxins when the respective analytical reference standard was used. It is important to note that the quality of the study and the used analytical methods were not evaluated. Instead, the methods (e.g. ELISA, LC-MS/MS) were included in the database as an indication of the appropriateness and quality of the analytical methods and the related concentrations of mycotoxins.

To facilitate the identification of relationships or trends in mycotoxin occurrence, food and feed products were categorized in raw agricultural commodities and processed products. The type of the products were specified when reported (e.g. wheat bran and not simply feed). However, different types of beer were aggregated at a higher level, i.e. as beer, and not as lager/pale ale/stout to facilitate the search within the database. The concentration ranges are the reported upper and lower values. When (one of) these values was not reported, it was replaced with the median or mean concentration. Lowest concentrations were rounded to 0.001 mg/kg.

To be able to screen products on their mycotoxin concentration, the extracted information on the occurrence levels of mycotoxins was allocated in 3 different categories: 'high' occurrence levels, 'medium high' occurrence levels and occurrence levels below the limit of detection (LOD) or limit of quantification (LOQ). High occurrence levels were defined as levels being higher than 1 mg/kg product. An exception was made for aflatoxins and ochratoxin A, where occurrence levels above 0.1 mg/kg product are considered high due to their toxicity. Medium high occurrence levels are defined as half of the high occurrence levels, i.e. 0.5 mg/kg product. Again, an exception was made for the medium high occurrence threshold levels for aflatoxins and ochratoxin A which are lower, i.e. 0.05 mg/kg product. These definitions were made in accordance with NVWA.

In addition, the region/country of origin of the samples was recorded. In some references, the product origin was not specified, or not clear (often for ready to eat food products or products from supermarkets) which was then indicated under remarks.

2.3.3 *Toxicological data*

The reported adverse effects of the mycotoxins were also compiled in the database. Toxicity categories were made for carcinogenicity or other types of toxicity (liver-, kidney-, immune-, repro- and neuro-toxicity). Activity of the respective mycotoxins on these effects was checked in the respective toxicity categories. When available, more detailed information on the *in vivo* effects of mycotoxins were also compiled. Species in which the effects occurred, including humans, were also recorded, to facilitate a screening for the known effects of different mycotoxins in a specific species. In addition, information on the transfer of mycotoxins to edible tissues or milk of animals was identified and entered in the database.

Data on *in vitro* effects of the mycotoxins were not included in the database. An exception was made for the unevaluated mycotoxins when *in vivo* data was scarce.

Reported effects of mycotoxins used as a model compound in *in vivo* disease models or pharmaceutical effects were not included, but only shortly summarized. The effects of mycotoxins on plant health, or kinetics and metabolism were not included in the database. As noted before, the quality of the obtained studies was not verified, other than the use of a reference standard in analyses. For example, when it was reported that a mycotoxin exerts hepatotoxic effects, this was noted in the database without verifying the original source of that respective statement.

2.3.4 *Queries*

Several queries were prepared as an example to facilitate a search within the Access database. For example, queries were created where the mycotoxin occurrence in products was reported high (above 1 mg/kg), medium high (above 0.5 mg/kg) or below LOD/LOQ. Using these queries, products where high mycotoxin levels or products where no mycotoxins were detected/quantified can be selected. In addition, queries were created where mycotoxins with no maximum limits but with high reported mycotoxin levels in food and feed can be selected. Additional queries can be created as desired.

3 Analysis of the present data

To support the evaluation of mycotoxins in the food and feed supply chain, an overview of general, toxicological and occurrence information on mycotoxins was compiled in a database. With this overview, a first screening for signals on the unexpected occurrence of mycotoxins in certain food or feed products can be performed.

The overview was obtained by compiling information from international risk assessments and literature searches in a database. For the mycotoxins that had been evaluated by an international risk assessment organisation, the screening of literature focused on the reported occurrence of mycotoxins in food and feed products. As a result of the simultaneously reported occurrence of unevaluated mycotoxins, an indication of the occurrence of relevant unevaluated mycotoxins was also obtained. For these unevaluated mycotoxins that were reported to occur at high concentrations, an additional literature search was performed that focused on the toxicological effects of these mycotoxins. The screening of the literature was general and the quality of the reported studies was not assessed in detail, which allowed for processing a large quantity of literature in a short period of time.

In this chapter, observations from the gathered information are described.

3.1 Evaluated mycotoxins

A literature search was performed for the evaluated mycotoxins since their most recent published risk assessment (Table 2, Methods). For all mycotoxins, their most recent risk assessment was performed by EFSA or JECFA. To identify new information since the most recent risk assessments, a literature search was performed. The main focus of this search was on the occurrence of the evaluated mycotoxins in food and feed products or raw agricultural products (see Methods), as the information in this overview is intended to be used to support an analysis of the food and feed supply chain. The reported level of contamination of mycotoxins varies greatly between products. This can also be a consequence of reported surveillance data that sometimes analyse samples with a relative high LOQ and state-of-the-art methods that can analyse samples with lower LOQs. Lower LOQs reduce the incidence of left-censored data and thereby reduce uncertainty in exposure assessments by e.g. EFSA. Here, the main observations of this literature search are described for the evaluated mycotoxins.

3.1.1 *Unexpected mycotoxin/product combinations*

The occurrence of well-known food/feed/mycotoxin combinations were reported in the evaluations by EFSA and JECFA and are gathered in the database. In addition, Table 4 describes a table from a recent report on the occurrence of the most relevant evaluated mycotoxins for each group of food products (ILSI, 2019). In the paragraphs below, these well-known food/feed/mycotoxin combinations as reported by EFSA, JECFA and Table 4 were compared with the observations from the

literature screening on the occurrence of evaluated mycotoxins. The mycotoxins that were reported to occur in *other* than the well-known food and feed products or were reported to occur at high levels are mentioned per mycotoxin/product category.

Table 4. Combination of the occurrence of the most relevant evaluated mycotoxins in some food categories (ILSI, 2019).

	AF	EA	OTA	FBs	PAT	Tri	ZEN	Alt
Cereals	x	x	x	x		x	x	x
Apple juice and cider	x				x			x
Cocoa	x		x					
Milk and dairy	x		x					
Vegetable oils	x			x		x	x	x
Dried fruits and nuts	x		x			x	x	x
Spices	x		x	x			x	x
Coffee	x		x	x		x		
Beers			x	x		x	x	x

AF: aflatoxins; EA: ergot alkaloids; OTA: ochratoxin A; FBs: fumonisins; PAT: patulin; Tri: trichothecenes type A and B; ZEN: zearalenone; Alt: *Alternaria* toxins.

Cereals

Multiple mycotoxins are commonly detected in cereal grains (Table 4) and high levels of many mycotoxins have been reported in various cereal grains as well as cereal-based products in the database¹. Maximum limits are in place for aflatoxins, ochratoxin A, deoxynivalenol, ergot alkaloids, zearalenone and fumonisins in food and feed (Regulation (EC) No 1881/2006; Directive 2002/32/EG; Recommendation 2006/576/EC; CXS 193-1995). In addition, maximum limits for the sum of T-2 and HT-2 in cereals are under discussion (European Commission working group, 2019).

In addition to the mycotoxins that are regulated in cereals, high levels of other evaluated mycotoxins such as *Alternaria* toxins, beauvericin, enniatins, moniliformin, nivalenol, sterigmatocystin and modified forms of deoxynivalenol have been reported in cereal grains and also in cereal-derived products. For a detailed analysis with respect to the reported occurrence of mycotoxins in specific types of cereals (e.g. oats, maize, wheat etc.), a query can be created in the database.

Apple-based products

Patulin is well known for its global contamination of fruits, and in particular apples and apple-based products (Table 4). Although the reported concentrations of patulin in apple-based products occasionally exceed the maximum limits for patulin (Regulation (EC) No 1881/2006; Codex 193-1995), there were also other mycotoxins reported to occur in apple-based products. The presence of aflatoxins and *Alternaria* toxins in apples and apple-based products has already been known (EFSA, 2011; Table 4), but also enniatin B and T-2 toxin were reported to occur in apple-based products yet at concentrations not higher than 0.015 mg/kg.

¹ In the database, multiple queries were prepared to screen the mycotoxins that are reported to occur in various types of products. Additional queries can be created as desired.

Cocoa products

Ochratoxin A and aflatoxins are known for their possible presence in cocoa beans and derived chocolate products (EFSA 2006; JECFA 2016; Table 4). In addition to these mycotoxins, the presence of citrinin and zearalenone was reported in chocolate and cocoa derived products, although the maximum reported levels of these mycotoxins were not higher than 0.01 mg/kg (Dinckelacker et al., 2017).

Milk and dairy products

In addition to aflatoxin and ochratoxin A (Table 4), other mycotoxins have been reported to occur in milk as a result of transfer of the mycotoxins in animals fed contaminated feed, e.g. T-2 toxin, patulin, fumonisins, zearalenone and deoxynivalenol (Fink-Gremmels, 2008). The occurrence of beauvericin, enniatin B and tenuazonic acid has now also been reported in milk (Ojuri et al 2018; Sun et al., 2019). Although the reported levels are considered low (not higher than 0.003 mg/kg), it could be of importance to consider this matrix for these mycotoxins.

Vegetable oils

A variety of mycotoxins can be detected in vegetable oils, for example *Alternaria* toxins (e.g. sunflower and rapeseed oil), aflatoxins (e.g. in peanut, maize, soy beans and sun flower oils), beauvericin (e.g. oilseeds), zearalenone (e.g. in maize oils), deoxynivalenol (e.g. in maize oils), fumonisins (e.g. in maize oils) and ochratoxin A (e.g. in maize and peanut oils) (EFSA, 2011; EFSA 2014; EFSA 2017a; EFSA, 2018a; ILSI, 2019). In the EU, legal limits in oil seeds and products thereof (crude vegetable oils) are only set for aflatoxins and for zearalenone in refined maize oil (Regulation (EC) No 1881/2006).

In addition to these mycotoxins, T-2 toxin and sterigmatocystin have also been reported to occur in vegetable oil mixtures (palm oil and peanut oil, palm oil and other vegetable oil, respectively), yet at maximum reported concentrations below 0.01 mg/kg. T-2 toxin has been reported in a dietary exposure assessment by EFSA to occur in sunflower oil and olive oil (EFSA, 2017b).

Dried fruits and nuts

Dried fruits and nuts can be contaminated with aflatoxins and ochratoxin A, for which EU maximum limits are in place (Regulation (EC) No 1881/2006). It is known that *Alternaria* toxins can also be present in sundried tomatoes, dried fruits and nuts as well as beauvericin in dried fruits and nuts (EFSA, 2014; EFSA, 2016; ILSI 2019). Citrinin, enniatins, sterigmatocystin and T-2 and HT-2 toxins have also been reported in nuts (EFSA 2012; EFSA 2013; EFSA 2014; EFSA 2017b). In addition to these mycotoxins, patulin has been reported to occur in dried apricots and fumonisin B2 in dried date palm fruit. Fumonisins, nivalenol, moniliformin and zearalenone have been reported in peanut cake (an animal feed ingredient). Fumonisin B1 has even been reported at medium high (above 0.5 mg/kg) levels in peanut cake.

Spices

EU legal limits apply to the presence of aflatoxins and ochratoxin A in some spices (Regulation (EC) No 1881/2006). EFSA reported also the occurrence of citrinin, fumonisin B1 and sterigmatocystin in spices. In addition to these mycotoxins, the occurrence of *Alternaria* toxins,

beauvericin, enniatins and zearalenone was reported in the literature. Of the latter mycotoxins, especially tenuazonic acid has been reported to occur at high (above 1 mg/kg) levels in some spices (Asam et al., 2012).

Coffee

It is known that some mycotoxins like aflatoxins, ochratoxin A, fumonisins, sterigmatocystin and trichothecenes can occur in coffee (EFSA 2004; EFSA 2013; ILSI, 2019; JECFA 2016). For ochratoxin A, EU maximum limits exist for instant coffee, roasted coffee beans and ground roasted coffee (Regulation (EC) No 1881/2006). In the literature, enniatins A, A1, B and B1 were reported to occur at high (above 1 mg/kg) levels in retail coffee samples sold in Spain (Garcia-Moraleja et al., 2015). 15-acetyldeoxynivalenol, aflatoxin G1, and deoxynivalenol were reported at medium high levels (above 0.5 mg/kg or 0.05 mg/kg for aflatoxin G1). Although not reported by other risk assessment agencies, fumonisin B1 and B2, T-2 toxin and HT-2 toxin, neosolaniol, and nivalenol were also reported to occur in coffee samples (Garcia-Moraleja et al., 2015).

Beer

A variety of mycotoxins have been reported to occur in beer (EFSA 2018b; EFSA, 2017a; ILSI, 2019). Barley, malt and maize are the largest contributors to the mycotoxin load in beer (ILSI, 2019). Barley (and sometimes other grains) and malt prepared from barley are frequently used in the process of beer making. Maize is also frequently used as an adjunct in the brewing of beer. Mostly, the maximum levels of these mycotoxins are not exceeding the legal limits for cereals and processed cereal products for direct human consumption or use as an ingredient in food. However, high (above 1 mg/kg) levels of fumonisin B1 have been reported in beer from Brazil, possibly due to applying maize as an ingredient. In addition, aflatoxins, *Alternaria* toxins altenuene, alternariol, alternariol monomethyl ether and tentoxin, enniatin B, fusarenone-x, T-2 and HT-2 toxin, neosolaniol, nivalenol and sterigmatocystin have been reported to occur in beer, for which no legal limits apply.

Additional products

Liquorice

For ochratoxin A, maximum limits are set in the EU for the occurrence in liquorice root or extract, for the use in food (Regulation (EC) No 1881/2006). In addition to ochratoxin A, the mycotoxins alternariol, citrinin, deoxynivalenol, fumonisin B1, mycophenolic acid and zearalenone have been reported to occur in liquorice. Although most of the mycotoxins were not reported to occur at high levels, in one study 10 out of 17 liquorice samples were positive for alternariol in China with a reported medium high (above 0.5 mg/kg) maximum concentration (Huang et al., 2018).

Fish

Multiple mycotoxins have been reported to be present in (edible) tissues of (aqua-cultured) fish, which could be explained by the reported presence of mycotoxins in fish feed (ingredients). Mycotoxin occurrence in fish feed increases because animal protein sources are increasingly

being substituted by plant-based proteins (Molina et al., 2019; Tolosa et al., 2019). The mycotoxins present in fish feed have also been detected in the edible tissues of aqua-cultured fish, such as salmon, brass and bream (Table 5).

Table 5. Mycotoxins reported in fish tissue/fish based food and fish feed.

Fish tissue	Fish feed
3-&15-Acetyldeoxynivalenol	3-&15-Acetyldeoxynivalenol
Aflatoxins (total)	Aflatoxins (total), B1
Diacetoxyscirpenol	Alternariol
Enniatin A, A1, B, B1	Beauvericin
Fusarenone-X	Deoxynivalenol
Neosolaniol	Diacetoxyscirpenol
Ochratoxin A	Enniatin A, B, B1
Zearalenone	Fumonisin B1, B2, B3
α -zearalenol	T-2 toxin, HT-2 toxin
β -zearalenol	Nivalenol
	Ochratoxin A
	Zearalenone

Kelp

One study reported the occurrence of mycotoxins in kelp. 43 out of 50 samples were found positive for 3- and 15-acetyldeoxynivalenol. Despite this high contamination rate in kelp, the maximum reported concentration of these mycotoxins was only 0.16 mg/kg (Li et al., 2018). Nevertheless, the contamination of kelp with mycotoxins is an interesting finding, although the origin of the mycotoxins in this product is not clear.

3.1.2 *Occurrence of mycotoxins in products from unexpected geographical regions*

Due to changes in the global climate, fungal species may increasingly thrive in new geographical regions. The origin of the samples containing the reported mycotoxins in food and feed commodities has been compiled in the database, when available. However, based on this overview, it was not possible to conclude on the possible shift of mycotoxin occurrence to other geographical regions. In part because the reported origin of the samples may not always correspond to the agricultural origin of the samples, but the processing or analytical site. However, vegetable food originally grown in the South of Europe may be cultivated in the (near) future in more Northern regions and may become infested with fungi. In addition, (un)intended mixing of ingredients can also result in an apparent shift of the occurrence of mycotoxins to other products (oral communication, the Director European reference laboratory (EURL) mycotoxins & plant toxins, Wageningen University and Research).

3.1.3 *Mycotoxins with "unexpected" high occurrence, without maximum limit in EU or Codex*

Alternaria toxins

Alternaria toxins have been reported to occur in many different food and feed products. In particular alternariol and tenuazonic acid have been found at relative high levels.

Alternariol has been reported to occur at high (above 1 mg/kg) or medium high (above 0.5 mg/kg) levels in approximately 5 different types of products: sorghum, cabbage, legumes, liquorice and tomato ketchup.

Tenuazonic acid has also been frequently analyzed in a wide variety of food and feed products. In approximately 25 individual references, maximum concentrations higher than 0.5 mg/kg have been reported. EFSA concluded that this compound is likely of no concern to human health, due to its low average occurrence (EFSA, 2011; Gruber-Dorninger et al., 2016). A monitoring recommendation for *Alternaria* toxins is in preparation in combination with guideline levels for specified foods (European Commission working group, 2019).

Enniatins

The presence of enniatins in food and feed was reported in many studies. High or medium high levels were reported in 19% of the reports on enniatin A, in 5% on enniatin A1, in 21% on enniatin B and in 17% on enniatin B1.

Although wide occurrence of enniatins in food was found, no risk assessment could be performed for enniatins by EFSA due to an overall lack of data on toxicity (EFSA, 2014). When an HBGV is established by a risk assessment agency based on newly identified *in vivo* toxicity studies, it can be assessed if this exposure poses a concern for human and animal health.

Modified forms of deoxynivalenol

In addition to high levels of deoxynivalenol, high levels of its modified forms 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and deoxynivalenol-3-glucoside have been reported to occur. These mycotoxins are generally analyzed in cereal grains and products thereof, often in feed, which is similar for deoxynivalenol. Interestingly, Arroyo-Manzanares et al. (2018) reported that for the maize samples contaminated with deoxynivalenol, 75% was also contaminated with its derivatives while for the wheat samples, this was only 13%. Maximum limits in these products are under discussion in the EU (European Commission working group, 2019).

Moniliformin

This mycotoxin was reported to occur at high (above 1 mg/kg) or medium high (above 0.5 mg/kg) levels in approximately 20 of the 56 reported occurrences of moniliformin in food or feed products. Most of the processed products were, or intended for, animal feed. No maximum limits have been established for this mycotoxin in the EU or Codex. In addition, no HBGV could be established for this mycotoxin by EFSA as the toxicological information was not sufficient (EFSA 2018c).

Nivalenol

High (above 1 mg/kg) or medium high (above 0.5 mg/kg) levels of nivalenol have been reported in several food and feed products such as oats, wheat, maize and barley. While its HBGV is only slightly higher than that of deoxynivalenol, no maximum limits have been established for nivalenol in the EU or Codex.

α -Zearalenol

α -Zearalenol has been reported to occur in a variety of different food and feed products. In fish, cassava and maize silage, it was even reported at high (above 1 mg/kg) or medium high (above 0.5 mg/kg) levels. The relative estrogenic potency of α -zearalenol is approximately 60 times higher than that of zearalenone (EFSA 2016b). This indicates that this mycotoxin is of interest in food and feed products, in addition to zearalenone.

3.2 Unevaluated mycotoxins

Approximately 455 mycotoxins were identified that may be relevant to the food and feed supply chain. Of these, approximately 65 have been evaluated in an international risk assessment. Of the 390 unevaluated mycotoxins, about 270 were reported to occur in one or more food or feed products, in addition to the evaluated mycotoxins and their modified forms.

3.2.1 *Unevaluated mycotoxins with high reported occurrence*

24 of the unevaluated mycotoxins were reported in relative high concentrations in at least 2 individual reports, i.e. above 0.5 mg/kg or even 1 mg/kg (see Table 3 in Methods).

Some of these mycotoxins were found more frequently than others. The high concentration of kojic acid has been reported in sorghum, bush mango seeds, cassava, melon seeds, milled rice, maize, fermented maize drink, maize-doughdish (fufu), dried date palm fruit, cassava-based products and feed. The maximum reported concentration of kojic acid was even higher than 500 mg/kg.

In addition, the levels of 3-nitropropionic acid were high in many different products. Relative high concentrations have been reported in cassava, groundnuts, maize, milled rice, melon seed, fermented melon seed products (ogiri), locus bean seed and feed.

High levels of fusaproliferin and fusaric acid have been reported in less than 5 different products. Although their presence appears not alarmingly frequent or high, these *Fusarium* mycotoxins may be of some concern with respect to co-occurrence with other *Fusarium* mycotoxins.

3.2.2 *Highly contaminated products*

With the development of the multi-methods many mycotoxins can be detected simultaneously, without specifically targeting them. This results in the increased detection of unevaluated mycotoxins as well as the detection of many mycotoxins in one specific product. This may be the reason why e.g. fusarenone-X and roquefortine C have been reported in beer in addition to the evaluated mycotoxins. Many of those unevaluated mycotoxins are not frequently prevalent. However, there have been products in which the reported presence of unevaluated mycotoxins is high.

For example, in dried date palm fruit samples from Egypt, the presence of over 25 unevaluated mycotoxins has been reported with levels up to 90 mg/kg (kojic acid) (Abdallah et al., 2018). In bush mango seeds from

Nigeria, the presence of over 40 unevaluated mycotoxins has been reported with levels up to 11 mg/kg (asperglaucide) (Ezekiel et al., 2016).

Of 110 mycotoxins reported to occur in maize, maize silage and corn, approximately 38 were evaluated mycotoxins. Of the 72 unevaluated mycotoxins, 3-nitropropionic acid, aurofusarin, culmorin, dihydrocitrinone, fusaproliferin, fusarin C, kojic acid, marcfortine A, mycophenolic acid, roquefortine C, rugulovasine A, viomellein were reported to occur at high levels (above 1 mg/kg).

In barley and barley malt, 28 different unevaluated mycotoxins have been reported in addition to the 49 evaluated mycotoxins and their metabolites. Aurofusarin was the only unevaluated mycotoxin reported to occur at high or medium high levels.

- 3.2.3 *Toxicological relevance of unevaluated mycotoxins with high occurrence*
 Of the mycotoxins that were reported to occur in high concentrations, the occurrence of kojic acid and 3-nitropropionic acid was reported most frequently. These mycotoxins were the only unevaluated mycotoxins that were reported at high concentrations in more than 10 studies. Below, the toxicological information found on kojic acid and 3-nitropropionic acid mycotoxins is summarized.

Kojic acid

Kojic acid is currently in use as food additive and whitening agent in skin cosmetic products in Asia (Blumenthal et al., 2004). The Cosmetic Ingredient Review Expert Panel considered kojic acid not a toxicant in acute, chronic and reproductive studies after oral administration (Burnett et al., 2010). However, these authors also reported that some animal data suggested tumor promotion after initiation with e.g. diisopropanolnitrosamine and weak carcinogenicity after oral exposure to kojic acid. Acute or subchronic toxicity after oral administration of kojic acid were not reported, but convulsions appeared after injection of the mycotoxin. Also, after chronic oral administration of kojic acid, decreases in body weight and effects on the thyroid were reported (Burdock et al., 2001a).

A NOAEL of 6 mg/kg bw/day for suppressed iodine uptake as well as changed numbers of colloid in thyroid follicles and follicular cell hypertrophy was identified by the Scientific Committee on Consumer Products (SCCP) from a study in which rats were orally exposed to kojic acid for 28 days (SCCP, 2008). A NOAEL of 15.5 mg/kg bw/day for thyroid tumor promoting effects was identified by Burdock et al. (2001a) from a study in which mice were orally exposed to kojic acid in a chronic study (Burdock et al., 2001a).

In addition, poultry appears to be sensitive to kojic acid, as the LD50 for acute toxicity in chickens is 125-fold lower than that of mice. However, the route of administration was not specified.

Burdock et al. (2001a) claim that the intake of kojic acid, at levels normally found in food, is safe. Yet, it is not indicated what these 'normal' levels of kojic acid in food are, and whether they have increased over the past decades. Furthermore, as kojic acid has been reported to occur at high levels in feed and poultry is particularly

sensitive to this mycotoxin, special attention might be needed for the assessment of risk for animal health.

3-Nitropropionic acid

Toxicity of 3-nitropropionic acid is manifested as pathological changes in the striatal areas of the brain. These changes result in clinical symptoms similar to those observed in Huntington's disease (Burdock et al., 2001b). 3-nitropropionic acid is therefore used to induce this disease model in experimental animals (Brouillet et al., 2005).

Burdock et al., (2001b) proposed an ADI of 25 µg/kg bw/day for 3-nitropropionic acid, based on a NOAEL of 2.5 mg/kg bw/day after oral administration in a 2 year carcinogenicity study in rats, which was the highest dose tested. Although, a slightly higher incidence of hepatocellular neoplasms was reported in male rats (NTP, 1978), which was considered as not significant and within the normal range for that specific rat strain. This ADI is relatively high compared to some of the TDIs of other mycotoxins. However, information regarding the exposure to this mycotoxin in the general population is lacking.

3.2.4 *Other unevaluated mycotoxins*

Several studies have indicated that other mycotoxins may be of concern to human and animal health. Gruber-Dorninger et al. (2017) reported that butenolide is possibly a mycotoxin of concern as it is toxic *in vivo* and causes oxidative stress to cells. Yet, the authors reported that is unclear whether this mycotoxin occurred in high concentrations. In the literature search, two studies reported the high (above 1 mg/kg) occurrence of butenolide in feed. Gruber-Dorninger et al. (2017) also indicate a lack of relevant toxicological data for fusaproliferin and fusaric acid.

Sulyok et al. (2010) state that roquefortine C and mycophenolic acid are among the most relevant mycotoxins in the context of mouldy food. They suggest that these mycotoxins are included in the list of target substances in multi-mycotoxin analyses.

4 Conclusions

An overview of general, toxicological and occurrence information on mycotoxins was created from literature, international risk assessment reports and other publicly available sources, and compiled in a database. This overview can be used as a first step to identify mycotoxins that can be of interest for risk assessment of the food and feed supply chain. The newly developed method, without detailed qualitative assessment of the gathered studies, that was used to compile this overview allows for screening of many references for data and providing a first identification of available information. The information gathered proves to be useful in identifying topics e.g. commodities or products that require attention or toxicological questions related to specific mycotoxins for further research on mycotoxins, in relation to possible risks in the food and feed supply chain. This literature screening approach may also be useful for compiling information on any issue for which a literature search results in a large number of references.

5 Discussion

Approach

To support the screening of relevant mycotoxins in the food and feed supply chain, an overview of general, toxicological and occurrence information of mycotoxins was compiled in a database. This overview can e.g. be used to do a first screening on the reported occurrence of mycotoxins in specific food or feed products, the maximum limits that have been set for the respective mycotoxins or the health based guidance values and toxicological effects of mycotoxins. This overview can also be used to specifically relate the presence of mycotoxins to certain food or feed products/groups, and vice versa. The information was gathered using a newly developed method, without a detailed qualitative assessment of the studies. When specific or more detailed information is desired, the respective references should be consulted. In addition, a user-friendly interface can be developed to facilitate the search within the database.

Update

It should be noted that the content of the database reflects the available information as per 2019. New fungal secondary metabolites are continuously being discovered in food or feed, as a result of advancing analytical methods. In addition, risk assessment authorities are continuously (re)evaluating 'well-known' as well as these so called 'new' mycotoxins. To support risk management decisions, the overview in this database will need to be updated regularly to keep it up to date.

Toxicological effects

Due to the relevance for supply chain analysis, it was decided to focus on studies that reported on occurrence of the evaluated mycotoxins in food and feed. This database does not include toxicological information on the evaluated mycotoxins that may have been released since their last evaluation. In addition, for some mycotoxins, toxicological data was lacking at the time of the evaluation. When this information is needed, it can be obtained by further analysis of the literature for the toxicological effects of the evaluated mycotoxins that has already been identified in this project.

Furthermore, for the unevaluated mycotoxins, toxicological effects were only compiled in the database for those that occur at high levels.

Reported occurrence

Aflatoxins, ochratoxin A and diacetoxyscirpenol were excluded from the literature search because they were recently evaluated or a new evaluation is pending. An additional search covering the past few years, may yield more studies that report on the occurrence of these mycotoxins, including possible unevaluated mycotoxins that were co-analyzed in these studies, due to improved analytical methods.

The information obtained from the studies reporting on the occurrence of mycotoxins can be refined. Refinements can be made by e.g. also including data regarding the co-occurrence with other mycotoxins, the

total number of samples in the study and the percentage of positive samples. In addition, the studies on mycotoxins of interest, e.g. that report on high occurrence of certain mycotoxins, could be subjected to a more detailed quality assessment.

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8 Annex I

In this Annex, the general approach that was taken to adapt the search strings is described. In addition, the developed search strings for patulin were given as an example.

General approach

Three databases were used to search for literature: Embase, Pubmed and Scopus. The general approach that was taken for the Embase database was to elaborate the search when less than 200 hits were found. The search strings were adapted by changing the mycotoxin as a major focus to a general focus and studies were screened on the presence in both title and abstract.

The general approach that was taken for the Pubmed database was to firstly consult the Pubmed MeSH finder for the presence of the respective mycotoxin. In case the mycotoxin was not registered as a MeSH term but as a supplementary concept, the search string was adapted by replacing the MeSH term by the supplementary concept term and removing the MeSH subheadings. When a total of 100+ hits were found, the search string was refined to search only for the mycotoxin in the article titles in addition to the MeSH or supplementary concept terms.

For each evaluated mycotoxin, the time period of the search string was adapted based on the year of their most recent evaluation (i.e. by EFSA or JECFA).

Example search strings for patulin

Embase

Query	Results	No.
#13	#12 AND (english:la OR dutch:la)	309
#12	#7 OR #11	353
#11	#3 AND #10	202
#10	#8 OR #9	1,362,737
#9	'expos*':ti OR ((food NEAR/3 contaminat*):ti) OR ((food NEAR/3 safe*):ti) OR 'risk assessment':ti OR occur*':ti OR 'carry over':ti,ab OR 'carryover':ti,ab	351,516
#8	'exposure'/exp OR 'food contamination'/exp OR 'fungal contamination'/exp OR 'food safety'/exp OR 'food control'/mj OR 'food quality'/exp OR 'risk assessment'/exp	1,158,610
#7	#3 AND #6	196
#6	#4 OR #5	1,127,943
#5	toxic*':ti OR intoxic*':ti OR carcinogen*':ti OR cytotox*':ti OR teratogen*':ti OR neurotox*':ti OR immunotox*':ti OR reprotox*':ti OR hepatotox*':ti OR haemotox*':ti OR hemotox*':ti OR mutagen*':ti OR genotox*':ti OR nephrotox*':ti	408,029

Query	Results	No.
#4	'toxicity testing'/exp OR 'toxicity'/exp OR 'carcinogenicity'/exp OR 'toxicological parameters'/exp OR 'cytotoxicity'/de	916,939
#3	#1 OR #2	914
#2	'patulin':ti OR 'clavacin':ti OR 'penicidin':ti	706
#1	'patulin'/exp/mj	795

Add an additional field to select a time period:

#13 AND [2000-2020]/py

PubMed

```
(((((("Patulin/adverse effects"[Mesh] OR "Patulin/poisoning"[Mesh] OR
"Patulin/toxicity"[Mesh]) OR ("Patulin"[Majr]) OR (patulin[ti])) AND
(("Toxicity Tests"[Mesh] OR "Toxicology"[Mesh:noexp] OR "Toxicological
Phenomena"[Mesh] OR "Toxicokinetics"[Mesh]) OR ("Noxae"[Mesh] OR
"Pathology"[Mesh]) OR (toxic[ti] OR toxicit*[ti] OR toxicol*[ti] OR
intoxic*[ti] OR carcinogen*[ti] OR cytotox*[ti] OR teratogen*[ti] OR
neurotox*[ti] OR immunotox*[ti] OR reprotox*[ti] OR hepatotox*[ti] OR
haemotox*[ti] OR hemotox*[ti] OR mutagen*[ti] OR genotox*[ti] OR
nephrotox*[ti]))) OR (((("Patulin/adverse effects"[Mesh] OR
"Patulin/poisoning"[Mesh] OR "Patulin/toxicity"[Mesh]) OR
("Patulin"[Majr]) OR (patulin[ti])) AND (("Dietary Exposure"[Mesh] OR
"Environmental Exposure"[Mesh:noexp] OR "Food
Contamination"[Mesh:noexp] OR "Food Quality"[Majr:noexp] OR "Food
Safety"[Majr:noexp] OR "Food Microbiology"[Mesh] OR "Risk
Assessment"[Mesh]) OR (expos*[ti] OR food contaminat*[ti] OR food
safe*[ti] OR "risk assessment"[ti] OR occur*[ti] OR "carry over"[tiab]
OR "carryover"[tiab]))) AND (english[Language] OR dutch[Language]))))
```

To select a time period, add to the search string:

AND 2000 : 2020[dp]

Scopus

```
( TITLE-ABS-KEY ( patulin ) ) AND ( ( TITLE ( *toxic* OR carcinogen*
OR teratogen* OR mutagen* ) ) OR ( TITLE ( expos* OR food W/3
contaminat* OR food W/3 safe* OR risk-assessment OR occur* )
OR TITLE-ABS-KEY ( carry-over OR carryover ) ) )
```

To select a time period, add to the search string:

AND PUBYEAR > 1999

