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MycoBoost
Partnering Grants - GP/EFSA/ENCO/2020/03

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Abstract

The MYCOBOOST project was aimed to develop an integrated collaborative model for knowledge exchange and methodology harmonization in mycotoxin risk assessment. A specific focus was given to the identification of methodological data gaps in the mycotoxins field and to promote data quality and methodological harmonization among the community. More specifically, the activities covered two main aspects, the need for a methodological harmonisation and the lack of extensive data quality procedures in the field. Specific needs were identified through online survey and interviews with experts, and further analysed within the consortium. The gap analysis was complemented by literature review and assessment of the current available data resources. As for methodological harmonisation, the tasks covered molecular methods for fungal identification, the inclusion of emerging mycotoxins in occurrence data, the use of computational methods in mycotoxins risk assessment and the inclusion of alternative/innovative food items in monitoring plans. For all these aspects, relevant gaps were highlighted and recommendations have been drawn. Concerning the improvement of data quality, the focus was on the revision of resources and database available online to support mycotoxins analysis and on the impact of left-censored data on mycotoxins exposure assessment. Moving from the collected evidence, a fit-for-purpose training was outlined and delivered through two physical workshops. The training materials (e.g. slides, video, exercises, handbooks) were later uploaded to ZENODO repository and made available to the scientific community.

Key words: mycotoxins; toxigenic fungi; data quality; occurrence; analytical methods; molecular methods

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Summary

Mycotoxins are toxic compounds produced by pathogenic fungi, primarily belonging to the genera Aspergillus, Penicillium and Fusarium. Although mycotoxin occurrence in food and feed has been regulated on the EU market since decades, their presence in crops and along the production chain is a very complex issue in food safety, especially due to their co-occurrence in multiple crops and due to the effect of climate change.

The MYCOBOOST project was focused on the identification of gaps and specific needs in methodological harmonisation and data quality, with the final aim to set up a dedicated training program for young scientists in the field of mycotoxins analysis and risk assessment.

As a first step, the consortium identified and discussed internally through virtual meetings a range of potential data gaps and limitations affecting the sector. This internal discussion, together with an analysis of ongoing initiatives in the field to avoid work duplication, led to the design of an online survey to capture the needs of the «mycotoxins community». The online survey and the subsequent round table with selected experts, addressed the following topics: a) for harmonisation of molecular and chemical methods and data analysis; b) future needs for data quality in risk assessment.

The results of the online survey and the experts round table pinpointed the lack of standardisation, harmonisation and transparency as a major issue when it comes to the risk assessment of mycotoxins. The lack in analytical standards, reference materials, as well as the insufficient staff training, was identified as major hurdles, while little awareness and lack of available low-cost resources for data collection and management have been reported as the main limitation to the implementation of data management and data sharing. Finally, a still fragmented «mycotoxins community» and difficulties in finding proper funding schemes were identified as major drawbacks for the adoption of best practices.

Following the survey, the consortium defined the state of the art through systematic reviews covering the following areas: a) harmonisation of molecular method for fungal identification; b) inclusion of emerging mycotoxins in occurrence data; c) uptake of computational methodologies in mycotoxins risk assessment. Reviews will be submitted to peer review journals, while data set will be made available through Zenodo repository (10.5281/zenodo.8161526).

The very limited exploration of alternative/innovative food for mycotoxins occurrence has led to the design and submission of fit-for-purpose collaborative projects, two of them already funded under the Marie Skłodowska Curie Actions (MSCA) scheme.

As far as best practice in data quality and management, a screening of the online available databases to support mycotoxins research and risk assessment was performed and the effect of left censored data on mycotoxins exposure assessment evaluated. Although the project activities were focused on mycotoxins, identified best practices could be extended and adapted to other hazards.

Finally, the collected knowledge was used for the production of fit-for-purpose training materials, tested during two hands-on workshops held in Bari (October 2022) and in Parma (May 2023). The training materials encompass slides, handbooks, exercises, spreadsheets and tutorials, as well as a video-tutorial for the development of analytical methods following the official DG SANTE guidelines. The materials will be made available through open source repositories, and distributed through the FoodSafety4EU project platform.

The work carried out during the project, with specific regard to the analysis of knowledge gaps, allows to draw up some recommendations for future initiatives. There is a need to raise awareness within the scientific community about the data quality level required for risk assessment. Furthermore, the adoption of harmonised workflows for fungal identification and molecular analysis has to be promoted, especially through the use of (certified) reference materials. This asks for a continuous training and dissemination among stakeholders from academia, control laboratories, and the private sector. The consortium acknowledges the need for a consensus definition and the identification of a priority list of "emerging mycotoxins", to favour their inclusion in monitoring plans and the subsequent population of occurrence databases.

Overall, the MYCOBOOST project acted as an accelerator of synergies for the creation of new collaborations, the stabilisation of the existing ones, and the presentation of common proposals in response to competitive tenders. Some fundings have been already secured by consortium partners to continue the activities initiated within MYCOBOOST. The Partnering Grant scheme allowed the partners a. to exchange knowledge on different areas of the mycotoxin field, thus increasing their own scientific capacity; b. to identify relevant methodological gaps for the scientific community, and to analyse limitations and specific needs; c. to define a collaborative framework which will be further exploited in future research and training initiatives.

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1 Introduction

Mycotoxins are toxic compounds mainly produced by fungi of the genera Aspergillus, Penicillium and Fusarium. They are present in many food commodities including cereals, fruit and vegetables (Wan et al., 2020). Their ubiquitous presence and the large diversity in toxicological effects, represent a threat to the health of humans, animals and the environment (Marin et al., 2013). Although mycotoxin occurrence in food and feed has been regulated on the EU market since decades, their presence in crops and along the production chain is regarded as a very complex issue in food safety inherently related to climate change, changes in agronomic practices and food/feed processing, demographic changes, consumption habits and global trade (Wild and Gong, 2010), Eskola et al., 2020b). Various types of mycotoxins usually co-occur in crops, and can each be further modified in the field by plants and microorganisms, or along the food production chain. Almost all food and feed categories can be affected by mycotoxin contamination. In spite of the regulation framework and continuous monitoring, humans and animals are thus exposed to multiple mycotoxins within a single meal, as biomonitoring studies have demonstrated (Eskola et al., 2020a), (Turner et al., 2012). Current drivers such as new dietary patterns (e.g., increase of alternative protein consumption or plant-based diets) may affect overall exposure, especially in sensitive population groups (Pereira et al., 2022) (i.e., infants, children, pregnant women, people with specific dietary needs).

The mycotoxin scientific community is vibrant and proactive, with very well interconnected multidisciplinary expertise and long-lasting collaborations. Despite this, there are still knowledge gaps and mismatches especially with regards to risk assessment needs. Bridging these gaps could therefore lead to a significant advance in knowledge and could help deliver to the sector best practices for mycotoxin data collection.

The MYCOBOOST consortium, formed by Article 36 institutions, has taken advantage of the EFSA Partnering Grant scheme to team up and address with a systematic approach some of the main gaps and limitations of the sector.

1.1 Background and terms of reference as provided by the requestor

This grant was awarded by EFSA to:

Beneficiary: University of Parma

Partners: University of Gent, National Research Council of Italy, French National Institute for Agriculture, Food and Environment

Grant title: Boosting knowledge and harmonisation in the mycotoxin field through sustainable scientific alliances – MYCOBOOST

Grant number: GP/EFSA/ENCO/2020/03 – Partnering Grant

1.2 Interpretation of the Terms of Reference

The consortium is formed by well-renown institutions in the field of mycotoxins research from Belgium, France and Italy. Based on their previous expertise, the partner decided to focus on the identification of gaps in methodological harmonisation as well as in data collection, curation and sharing. Well aware that such difficulties need time and efforts to be overcome, the main goal of the project was to take advantage of the partnering scheme and the range of scientific alliances put in place during the project lifespan, to implement a common ground for data quality and to prepare for training for young scientists in the field. The outcome of the project has been and will be disseminated within the scientific community to increase awareness and best practices.

Project planning and objectives

MYCOBOOST timeline was planned over 24 months, starting from June 2021, in the following 3 stages:

Stage 1 – definition of all technical aspects; definition and analysis of gaps and needs. Preparation and distribution of an online survey to collect expert knowledge on different tasks.

Stage 2 – all partners to actively contributing to the harmonisation and standardisation activities on data and methodologies. Organisation of virtual round tables with invited external experts for discussing and reviewing best practices under an Expert Knowledge Elicitation (EKE) approach (European Food Safety Authority, 2014). Preparation of thematic scoping reviews on relevant topics. Experts were selected according to their relevant expertise and are representatives from the academia, the public institutions, as well as the stakeholder associations.

Stage 3 – all partners transferred the gathered knowledge into the MYCOBOOST e-leaning material, laying the basis for the Mycotoxin Knowledge Hub through the organisation of the training hands-on workshops.

2 Data and Methodologies

In order to prioritiseprioritise the actions to be undertaken for increasing knowledge in the field, gaps and need in methodological harmonisation and in the adoption of data quality best practices were collected and analysed through expert elicitation activities. To avoid work replication, the MYCOBOOST team identified a range of ongoing initiatives with similar goals. The consortium has then initiated an intensive dialogue with selected consortia, in order to set up a strategic partnership to increase the project outreach.

Following meeting and negotiation, the following agreements were signed:

- FoodSafety4EU https://foodsafety4.eu/ (Month 12)

FoodSafety4EU is a collaborative action funded under the H2020 framework program to support the European Commission in aligning research, policy and innovation with the societal needs and perspectives and improving food safety across Europe. More specifically, the consortium has released a multi-stakeholder platform to facilitate interactions among stakeholders and citizens in the field of food safety. The partnership with MYCOBOOST allowed the consortium to increase the outreach efforts, delivering its activities to a larger community.

- MycoTwin https://www.mycotwin.eu/ (Month 12)

MycoTwin is a H2020 Twinning Action project, aimed at creating a partnership among TUBITAK institute in Turkey with ISPA-CNR in Italy and University of Valencia in Spain, in the field of toxinogenic fungi and mycotoxin research. The alliance between MycoTwin and MYCOBOOST ensured a larger attendance to the Training Workshop 1 in Bari, allowing for the involvement of International experts outside the consortia.

- FunShield4Med https://funshield4med.eu/ (Month 20)

FunShield4Med is an Horizon Europe Twinning Action project, aimed at supporting the knowledge and expertise transfer on mycotoxigenic spoilage fungi and mycotoxins in relation to one main EU entry point for imports (Greece) from the South and East (African and Asian countries). FunShield4Med has started on December 2022, and will therefore take on the Mycoboost legacy with regards to training activities dedicated to EU pre-accession and widening countries.

While FoodSafety4EU and MycoTwin are both part of the H2020 funding scheme, and therefore they will be closed by the end of 2023, the HorizonEurope FunShield4Med project has been launched in December 2022 and will run until November 2026. This ensures sustainability for training activities started within MYCOBOOST.

In addition, to maximise the outreach and the impact of the project, a further alliance was formed with the International Society for Mycotoxicology (ISM; http://www.mycotox-society.org/). More specifically, ISM associates were involved in the online survey. In addition, ISM funded 5 additional scholarships for young researchers to attend the MYCOBOOST training workshop 1 in Bari.

2.1 Definition of needs

At a very first stage and taking advantage of the knowledge gained by the partners within previous projects, the consortium identified and discussed internally a range of potential gaps

and limitations affecting the sector. Dedicated virtual meetings were organised over the first 6 months of the project to discuss and analyse the scenario. The following issues were identified:

i. Heterogeneous quality of the occurrence data, with relevant differences between the data in the EFSA occurrence database and those reported in the open literature. Data in the EFSA database mainly cover the regulated mycotoxins, co-occurrence data collected for the same sample batch are scarce, occurrence data on modified mycotoxins are lacking or left-censored. On the other hand, co-occurrence data in the open literature are abundant, with a good coverage of non-regulated mycotoxins and modified forms. However, due to the inherent nature of the scientific works, scientific papers are often biased in terms of sampling and representativeness of the batches.

ii. Spreading of non-harmonised multi-analyte methods. In the last decade, multi-toxin methods have become the routine in the academic environment, with hundreds of mycotoxins analysed in the same run and allowing retrospective datamining. However, methods performance, validation and set up are still very diverse, with difficulties in comparing data. Standardisation of protocols and workflows is therefore urgent to develop a robust database for co-occurrence data to be used in risk assessment.

iii. Toxicological grouping is still in its very first stage and often is not related to the analytical grouping. Despite the large availability of multi-toxin methods, the choice of mycotoxins to be included in the analytical protocols is often driven by the availability of analytical standards, instead of similarity in toxicological mechanisms. Available co-occurrence data are not tailored for group Health Based Guidance Values (HBGV). Co-occurrence ratios between parent and modified forms are often poor, and the derivation of relative potency factors is still difficult.

iv. The use of New Approach Methodologies (NAMs) for understanding the toxicological mechanisms, is still uncommon with very few exceptions in toxicodynamics and toxicokinetics. A widespread knowledge of NAMs would enable a better risk assessment of mycotoxin mixtures, especially supporting toxicological grouping and the identification of species-specific sensitivity.

v. Lack of harmonisation in the omics data. Genomics, transcriptomics and metabolomics are largely applied in mycotoxin research, especially for studying the plant-pathogen interaction and to support breeding of resistant crops. Bioinformatic methods have developed alongside the so-called omics techniques (i.e. genomics, transcriptomics, proteomics, metabolomics) but the application of these methods is still far from being consistent. As a consequence, the application of different bioinformatics methods to the same data set can lead to quite different conclusions. Standardised workflows are therefore urgent to unlock the great potential of the system biology approach in risk assessment and to support an effective uptake of untargeted methodologies in the field.

2.2 Project limitations

The MYCOBOOST project was presented in July 2020 and selected for fundings in December 2020. The project was then kicked off in June 2021. Although the proposal preparation was conducted during the first COVID-19 wave, the consortium was not able at that time to properly foreseen the impact of the pandemic for the coming months.

More specifically, the pandemic situation and the emergency measures put in place at a global level strongly affected the expected training activities. Physical meetings became fully operational only from Autumn 2022. To overcome these difficulties, the consortium prioritised the activities to be carried out according to the available budget and put in place a series of partnership activities to enable the achievement of the objectives. Moreover, in order to allow sustainability after the end of the project, the consortium defined and is participating in number competitive tenders/calls to further strengthen the partnership and carry on further work to fill in the scientific gaps identified within MYCOBOOST project.

3 Defining gaps and needs in the mycotoxins field through elicitation of experts

Being aware that facing all the open issues in the mycotoxin field within a single project was not realistic, the MYCOBOOST consortium decided to prioritise the needs of the scientific community through public consultation.

An online survey was prepared and distributed to collect gaps and needs in molecular methods for fungal pathogens, multi-toxin analysis and human biomonitoring. Specific attention was given to workflow harmonisation, data quality and adoption of best practices. The online survey was distributed to relevant stakeholders using the ISM network plus the professional network of all the participants.

The list of questions submitted for the online survey together with the accompanying letter is reported in Annex A. The analysis of the survey output is discussed in paragraph 3.1.

Starting from the survey results, the most relevant gaps and limitations in data quality and methodological harmonisation were discussed in a round table organised in Parma on 18th May 2022.

The overall results of the survey and the round table will be used for a scientific paper, currently under preparation.

3.1 Online survey preparation and distribution

The survey aimed to collect information to identify gaps and current practices regarding the risk assessment approaches and data management strategies for toxigenic fungi and mycotoxins.

The survey reached more than 60 participants, including experts and stakeholders affiliated with the general scientific community, universities, industries, research centres, reference laboratories and public health agencies. Most respondents were academics and experts from institutions.

Four areas were identified:

- Mycotoxins occurrence in food
- Human biomonitoring
- Molecular approaches in the study of toxigenic fungi
- Data management

For each area, the questionnaire focussed on:

- Standardisation and harmonisation
- Data sharing and access
- State-of-the-art for the methods

The complete list of questions submitted to the participants, is reported as Annex A.

The outcome of the online survey are summarised below and further used for expert knowledge elicitation in Round Table 1 (see section 3.2)
The lack of standardisation, harmonisation and transparency appears to be a major issue when it comes to the risk assessment of mycotoxins. This is one of the most popular answers among participants when asked about the biggest obstacles to the risk assessment. Most of the answers to the online survey questions support this statement.
Only 25% of the respondents use any standardised sampling plan regardless of the field of application. The main reasons for this trend seem to be the limited availability of standardised plans and the difficulties involved in their application. The lack of standardised and harmonised analytical and sampling methods represents a problem especially in the field of mycotoxins occurrence in food, while ethical constraints and the difficulty of volunteer recruitment are the most complained obstacles to human biomonitoring.
More than 50% of the respondents do not share their data. Mainly, data are not shared because of confidentiality restraints, while other participants report a lack of knowledge and organisation. This limited flow of information is likely to hinder the harmonisation of methods, also considering that only 19% of participants use a Data Managing Plan (DMP) to guide their data collection, curation, preservation and sharing. Furthermore, most of

the participants implement their own models and do not perform archiving. It means that even if the data were shared, it could be difficult to use it.

Moving onto the current analytical practices and methods, while regulated mycotoxins are widely covered in the analytical methods adopted by the respondents, only a limited number of emerging mycotoxins are included in the protocols of analysis. The emerging mycotoxins of major interest resulted to be Fusarium metabolites (Enniatin, Beauvercin, modified forms of DON) and Alternaria toxins. The lack of standards is the main reason why participants do not include emerging mycotoxin in their studies. The research of toxigenic fungi in food prioritises the genus Aspergillus, Fusarium and Penicillium.

Only 53% of the participants reported using reference strains (or sequences) for species identification/quantification. This reference material often comes from their own collections. Participants frequently indicated the inadequacy and excessive costs of equipment, when asked about the biggest obstacle to the identification and study of toxigenic fungi. Insufficient staff training and limited access to certified reference material (CRM) were also mentioned.

Food matrices related to alternative dietary lifestyles are still partially excluded from the studies on mycotoxin occurrence in food: only 25% of the participants report including these matrices within their studies. This gap is likely to cause the underestimation of the real exposure to certain contaminants, preventing a correct risk assessment. The partial exclusion of alternative matrices also applies to human biomonitoring studies.

Finally, looking at the future perspectives, almost all the participants agreed that data sharing and harmonisation would help the application of predictive modelling. However, less than half of the participants consider their current data management compatible with the potential application of predictive modelling.

To conclude, the results of this survey suggest that the scientific community needs more harmonisation and transparency to work more efficiently and produce reliable data. On the other hand, the transition to a more open and harmonised scientific framework requires the implementation of data sharing and standardisation policies, which make data easily accessible and usable without harming the interest of those who produced it. The new generation of risk assessors should be aware of the importance of data management as regards transparency and harmonisation. For this reason, they should be properly trained. Lastly, shifting to this new concept of scientific community would also facilitate the implementation of predictive modelling, a fundamental tool for the future of risk assessment and management in the field of mycotoxins.

3.2 Expert Knowledge Elicitation Round Table

Following the survey, a round table was organised to discuss the outcome and collect further opinions as regards to gaps in data quality and methods harmonisation. An expert knowledge elicitation (EKE) approach was adopted, following the EFSA guidance (European Food Safety Authority, 2014) Taking advantage to the World Mycotoxin Conference in Parma, 30 participants were selected for the round table from academia, research institutions and the private sector. The round table was conducted jointly with the H2020 Twinning project MycoTwin. A signed list of participants is available upon request.

The discussion was focused on the following subtopics:

- ☐ Subtopic 1: Needs for harmonisation of molecular and chemical methods and data analysis
- ☐ Subtopic 2: Future needs for data quality in risk assessment

The participants were not introduced to the survey outcome, but just informed about the overall scope of round table. A short introduction to each subtopic was prepared and delivered. Only after the first round of discussion, the results of the survey were disclosed and jointly discussed.

As a first step, the participants were asked to assign a score to each subtopic statement (via Mentimeter.com) for priority and uncertainty, according to the following definitions:

- High level of priority of action = something given or meriting attention before competing alternatives (since represents a pre-requisite - or a key requisite - to undertake the other issues in the list)
- High level of uncertainty = not fully investigated and researched; not based on well-established knowledge; values are unknown; possible outcomes are incomplete and ambiguous; unstable; hard to predict; difficult to estimate; unobvious impact.

Results were then critically discussed by the MYCOBOOST expert group.

To favour and focus the discussion, two sessions were planned based on the online survey results: a live poll session, and a collective discussion session.

Results from the online survey were not provided in advanced to the experts taking part to the EKE round table, to avoid any bias in discussion. To provide a framework, a summary was given during the EKE launch, while a critical discussion of the online poll outcome was provided at the end of the live poll session.

Table 1: Subtopics discussed at the round table

Round Table subtopics and questions
Subtopic 1: Needs for harmonisation of molecular and chemical methods and data analysis
Statement: *Method harmonisation calls for fit-for-purpose tools and approaches. Identify current constraints and enablers for method harmonisation. Please, rank in terms of priority.* A) Availability of certified reference materials (CRMs), standards (STDs), quality control materials (QCMs), Blanks/control samples B) Availability of Inter-laboratory ring trials (IRTs) and/or proficiency testing programs (PTPs) C) Fit-for-purpose validation programs and guidelines for official control and rapid testing (auto-control) D) Measurement uncertainty protocols E) Simplified standard data format F) Guidelines and Acceptance criteria for matrix effects G) Criteria for calculating and evaluating LOQ (Limit of Quantification, scientific, not legislative) H) Standardised sampling protocols
Subtopic 2: Gaps in data quality for risk assessment
Statement: Identify the main requirements in data collection/curation/sharing and propose a roadmap to increase data quality in risk assessment. Please, rank in terms of priority. A) Simplified standard data format B) Increased knowledge of data management plans C) Harmonised protocols and guidelines for sample metadata collections D) Availability of affordable and user friendly data management software E) Availability of open source platform for data storage and sharing F) Policies to support metadata and data sharing

For each subtopic, the participants were provided with a list of propositions, and asked to rate the perceived level of priority of action (rating scale: 1 – 10, with 10 as the highest priority). The outcome of the live poll is reported in Figure 1 and briefly summarized below.

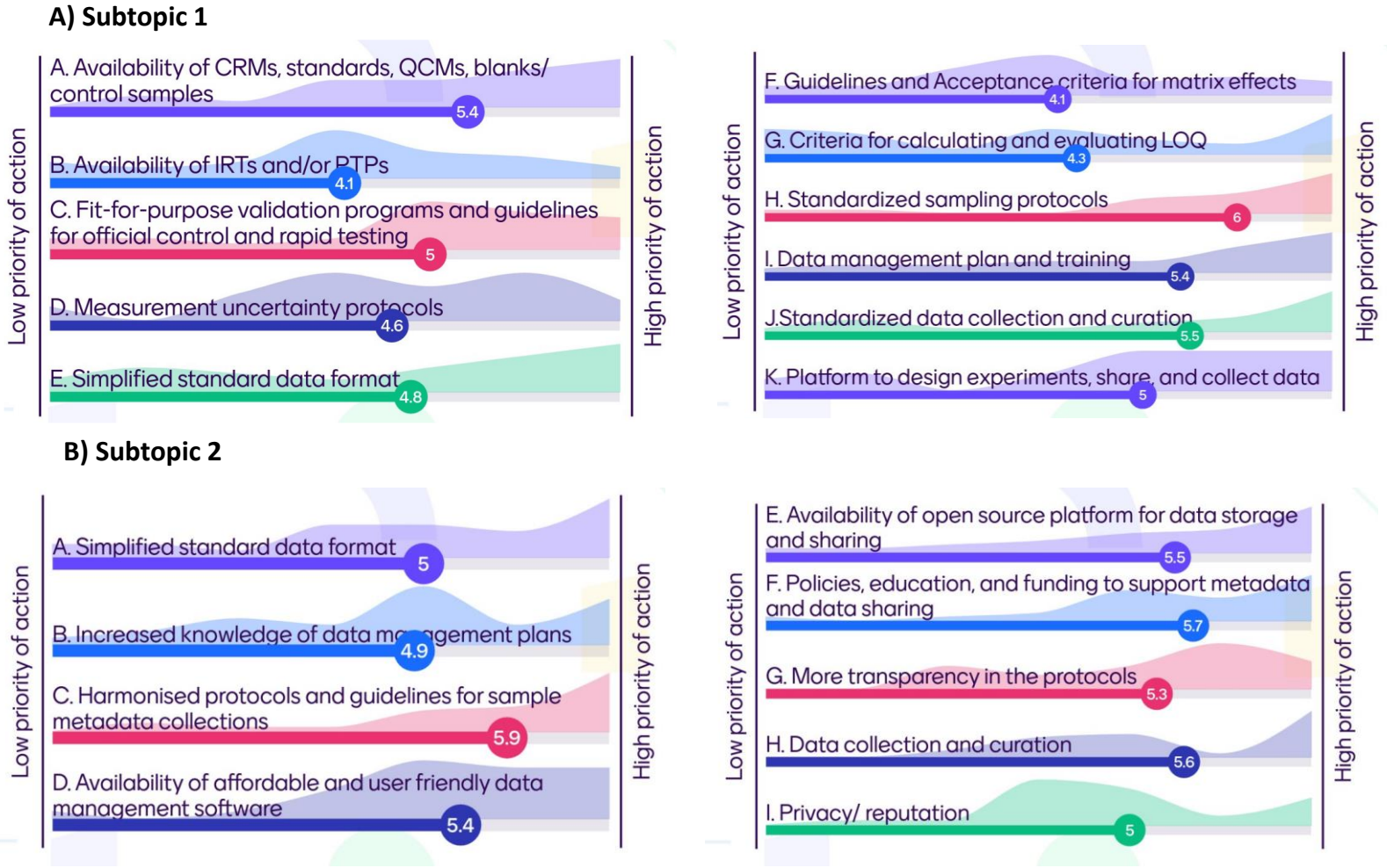

Figure 1 – Results of the first live poll, Mycoboost Round Table – Plots from mentimeter.com

For subtopic 1, the experts identified the following priorities:

1. Standardised sampling protocols
2. Standardised data collection and curation
3. Data management plan and training

For subtopic 2, the experts identified the following priorities:

1. Harmonised protocols and guidelines for sample metadata collection
2. Policies, education and funding to support metadata collection and curation
3. Create awareness/information/communication
4. Availability of open source platforms for data storage and sharing

Following this first step, for each subtopic the experts were asked to rank the same propositions previously assessed based on their level of uncertainty, i.e. the need for further exploration/actions.

The outcome of the live poll is reported in Figure 2, and briefly summarised below.

For subtopic 1, the experts identified the main gaps and needs for further research for the following priorities

- Availability of CRMs, STDs, QCMs, blanks and control samples
- Standardised data collection and curation

For subtopic 2, the experts identified the following priorities:

- Harmonised protocols and guidelines for sample metadata collection
- Policies, education and funding to support metadata collection and curation

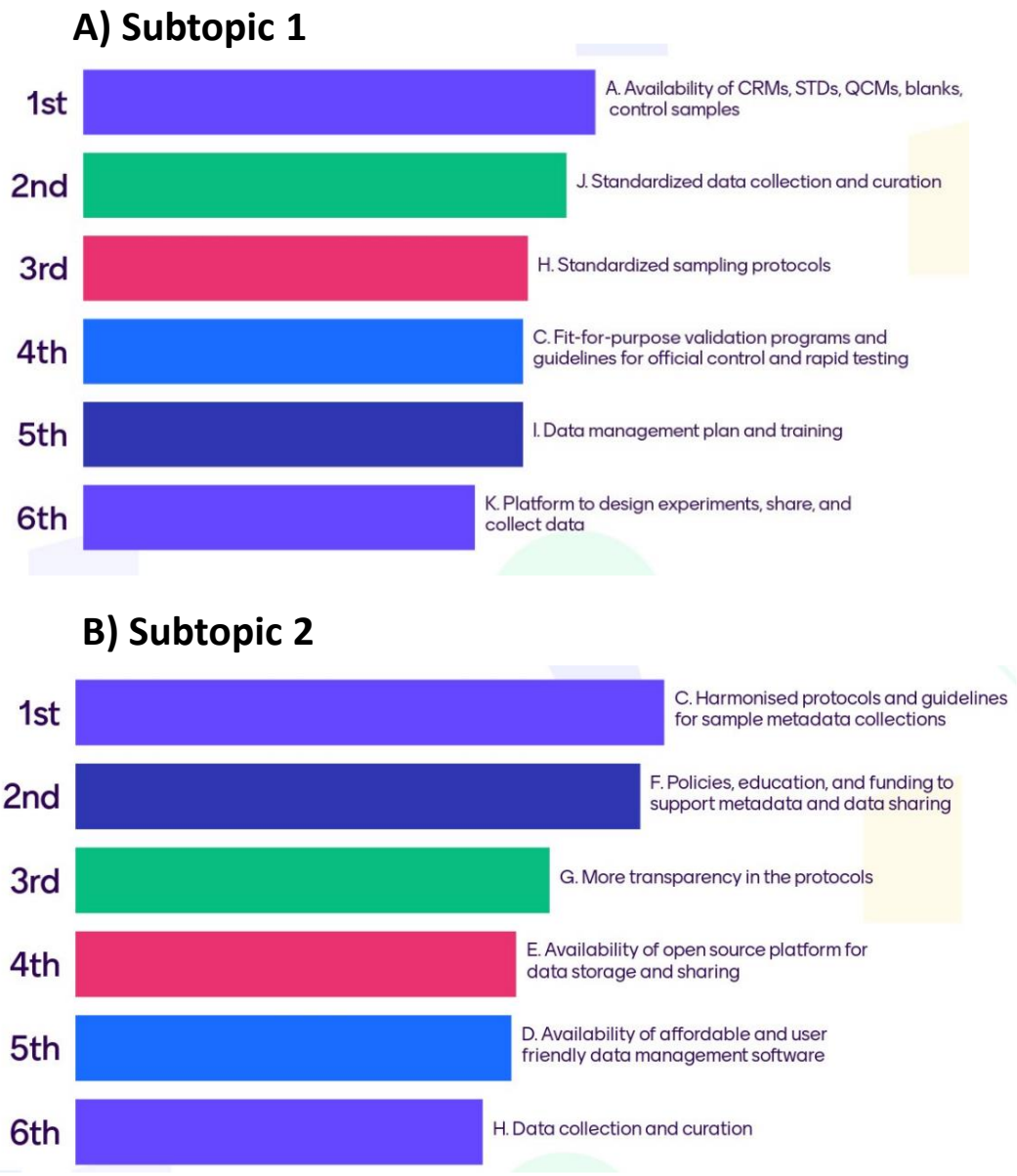

Figure 2 – Results of the second live poll, Mycoboost Round Table – Plots from mentimeter.com

Live poll results were critically discussed with experts during the EKE round table. As the main outcome, the harmonisation of protocols and guidelines for sample metadata collection is felt as the point with the highest priority of action. At the same time, the current knowledge is perceived as insufficient to ensure an effective methodological harmonisation, as well as the stakeholders' community is still highly fragmented. Among the other topics proposed, two additional high priority points were selected: the need to implement policies, education and funding to support metadata and data sharing, and the need to enhance data collection and curation. The experts underlined the urgency of common platforms and metadata format for data sharing. Experts from the private sector reported difficulties in sharing data due to data protection reasons and recommended the implementation of dedicated policies. One of the major obstacles reported by all experts

for data collection and curation is the lack of dedicated budget. More specifically, the experts underlined the efforts required to implement and maintain a proper data management system and the need for specific staff training. This is particularly important for actors from the official laboratories and from the private sector.

4 Methodological Harmonisation

Based on the needs collected in the online survey and discussed in Round Table 1, the MYCOBOOST team started collecting the most used molecular methods for the identification of fungal pathogens. Particular attention was given to the most advanced protocols to be used for the early identification of possible emerging pests.

In consideration of the identified gaps in data and metadata sharing, the main objective is a *clear ontology definition*, to create a common ground favouring information exchange, i.e. in the case of outbreaks.

When it comes to the *analytical protocols and occurrence data*, based on the online survey and expert elicitation exercise, the MYCOBOOST team identified two main gaps related to 1) the inclusion of emerging mycotoxins in the analytical protocols and the collection of proper occurrence data; 2) the adoption of common parameters and practices for analytical quality assurance.

More in details, the EKE highlighted that only a few emerging Fusarium mycotoxins are commonly monitored in food and only regulated food categories are commonly included in the monitoring plans, while a wide range of alternative/innovative food items are often overlooked. This is reflected in a lack of occurrence data for risk assessment.

In order to better frame this gap, two actions were undertaken by the consortium and more specifically:

1. A scoping review about the inclusion of emerging mycotoxins in occurrence data (section 5.2)
2. A preliminary literature review about the occurrence of mycotoxins in alternative/innovative food items (section 5.3)

4.1 Molecular Methods for fungal identification

Starting from the EKE exercise outcome, the consortium identified 2 mainly areas of intervention: a. the lack of harmonised protocols in fungal identification; b. the lack of adaption of a common ontology. These gaps represent a significant limitation for the field of research and affect the quality of data reported in the literature, generating inaccurate fungal identification/classification.

With the aim of better understanding the context and contributing to generate best practices, the consortium decided to undertake two actions:

1. To prepare an opinion paper reviewing the importance of the challenge of accurately detecting and identifying mycotoxigenic phytopathogens using molecular methods including current practices and recommendations (in preparation).
2. To internally discuss and define strategies for promoting the adoption of a correct ontology in fungal identification.

Preparation of the opinion paper

Accurate data is vital in molecular methods for the identification of toxicogenic fungi, especially in view of the changes in the geographical distribution and prevalence of pathogenic fungi driven by climatic changes. Although identification of fungi is still often based on isolation techniques, molecular methods may offer superior reliability and accuracy. Having a comprehensive knowledge of the mycotoxin biosynthetic capabilities of different species is essential when evaluating the toxicological hazards linked to fungal pathogens. Numerous records exist regarding mycotoxin production by various species and several endeavours have been made to consolidate this information. However, this task has been challenging due to the presence of conflicting classification systems that rely on factors such as morphology, sexual compatibility, and phylogenetic relationships (Munkvold et al., 2021). In this regard, the field of fungal identification has advanced significantly with the development of various molecular methods that offer rapid and accurate identification of fungal species, i.e. DNA sequences, PCR-based advanced techniques, DNA barcoding, whole genome sequencing, etc. However, due to the large amount of data generated by molecular methodologies, the need for properly standardised protocols and reference sequences obtained starting from official strains libraries is of upmost importance.

Based on the gap analysis performed following the online survey and EKE exercise, it has emerged that the use of reference materials and harmonised workflows is poor and suffer from a lack of harmonisation, whereas many researchers still rely on in-house collection and protocols. More than a technical barrier, this reflects a lack of data quality culture among the researchers and invites for proper actions to raise awareness.
The accurate identification of fungal species highly depends on the availability, quality, and complexity (in terms of numbers of represented species) of reference sequence data.

The availability of curated fungal collections is growing, but the use of standard material is still based on collaboration and exchange of material among peers. Protocols from the literature often suffer from low reproducibility, due to an incomplete description. This leads to little harmonisation and may generate false data reporting.
The consortium identified several public libraries containing reference sequence data that can be used to ensure superior quality standards in fungal identification. Among them, GenBank is a generalist but high quality repository, while more specific databases for fungal identification are FUSARIUM-ID (http://isolate.fusariumdb.org) and CBS-KNAW Fungal Biodiversity Center (http://www.cbs.knaw.nl databases). In particular, these databases contain a variety of sequences assigned to corresponding taxa, which are useful for comparative analysis of sequence variations. Their use should therefore be included in fungal identification workflows and best practices.

Databases for DNA Barcoding-based identification of fungi particularly need to have high taxonomic coverage and reliable sequences, for species-reference or species-type strains, to ensure users can accurately match sample sequences to the correct reference sequences. Therefore, high numbers of curated sequences from different strains for each species should be represented, so that the accuracy of the identification can be increased, but also in order to reflect more realistically the existing species variation.

The manuscript in preparation will include a comprehensive discussion of best practises for fungal identification as well as a critical description of available open access libraries and databases. INRAE-MycSA and ISPA-CNR have been responsible for setting up a preliminary outline of the work, later discussed with the other partners during online meeting. The exposition of UNIPR and UGENT partners, whose main expertise is in analytical chemistry, to INRAE-MycSA and ISPA-CNR knowledge, has been highly beneficial especially for early-stage researchers.

Adoption of correct ontology approach

As highlighted by the EKE exercise, one of the main issues referred to standardisation and harmonisation is the lack of a common ontology. By utilising correct ontologies, fungal researchers can ensure consistent and standardised representation of fungal species, traits and associated data. This facilitates data integration, comparative analysis and knowledge sharing, advancing our understanding of fungal biology, evolution and pathogenicity. It also enables the development of sophisticated tools and resources for fungal research and enhances the reproducibility and reusability of fungal data. With the only exception of specific activities carried out by single groups, a common framework for the definition of a consensus ontology is lacking, as well as dedicated fundings. Dedicated actions for the promotion of correct ontology are therefore recommended, also involving strategies to raise awareness, encourage adoption, and facilitate community engagement.

While both INRAE-MycSA and ISPA-CNR partner shared good knowledge of the topic and were already aware of the lack of adoption of a correct ontology among the scientific community, UNIPR and UGENT did not have internal expertise on this topic.

The partnering project offered them the opportunity to acquire a basic knowledge, which will be incorporated in the teaching programs and later transferred to undergraduate and pre-doctoral scientists.

At a general level, the MYCOBOOST consortium noticed the relevance of the adoption of correct ontology in the field, and has therefore identified potential actions to promote its development and adoption within the mycotoxin community. Among them:

- *Education and training*: Offer practical guidance on ontology selection, annotation, and integration to help users understand how ontologies can improve data quality, interoperability and analysis.
- *Documentation and Guidelines*: Develop comprehensive documentation and guidelines that explain the purpose, scope, and usage of specific ontologies relevant to a particular research domain. These materials should include clear instructions on ontology integration, term usage, and best practices. Make these resources easily accessible and regularly update them to reflect the evolving needs of the research community.
- *Ontology Integration in Tools and Platforms*: Integrate ontologies into data analysis and management tools, databases, and bioinformatics platforms commonly used in the research community. By incorporating ontology support into these tools, researchers are encouraged to use ontologies during data annotation and analysis. This integration can simplify ontology adoption and make it more accessible to a wider user base.
- *Community-driven Ontology Curation:* Encourage community-driven ontology curation and expansion by establishing guidelines and processes for ontology contributions and updates. Provide mechanisms for researchers to propose new terms, suggest modifications and report errors or inconsistencies. Develop transparent review and approval procedures to ensure the quality and integrity of ontology updates

At this regard, all partners are committed to adopt and to promote among peers the adoption of correct ontologies, leading to improved data quality, standardisation and interoperability. In terms of *education and training*, the MYCOBOOST consortium has already introduced and discussed the importance of correct ontology in training workshop 1 and 2, and is committed to further promote best practices in fungal identification in the coming events, also thanks to the ISM support.

In order to put in practice the best practice discussed above, and to provide real examples for current and future training, the consortium addressed two selected case-studies:

1. consolidated protocols were applied for the identification of new genome sequences of one strain of *Fusarium nodosum*, one strain of *F. sporotrochioides*, three strains of *F. culmorum* and two strains of *F. graminearum* including one of the 3-ADON chemotype, and will be made available together with the generated data sequences in open access repositories (in progress);

2. a protocol on how to quantify fungal abundance in a mixed sample by qPCR was prepared following best practices and will be later made available in open access repositories (in preparation).

4.2 Inclusion of emerging mycotoxins in occurrence data

One of the major open issues returned by the EKE exercise is the lack of consensus on the term "emerging mycotoxins". Although this definition is widely used and accepted within the scientific community, there's no clear idea of which mycotoxins are considered as emerging and why. What is largely accepted, the term "emerging mycotoxins" is commonly used to define non regulated mycotoxins which are frequently found in food, in spite of their toxicity or contribution to the overall exposure. No clear link with the concept of "emerging risk" is drawn, and therefore the term is often confusing or even misleading.

The different disciplinary expertise of MYCOBOOST partners, allowed them to address this issue from different angles, and therefore to promote internal discussion and confrontation. The collaborative work allowed the group to further elaborate on the gaps of knowledge about emerging mycotoxins, and more specifically on the need of a consensus definition and of a set of inclusion criteria to define which compounds can be listed among "emerging mycotoxins". The consortium acknowledged the need to enlarge such discussion to more actors in the field, i.e. including risk assessors and managers, and committed to promote further debate among the community.

To define the state of the art, a scoping review was prepared during the project lifespan with the contribution of all partners, following the scheme reported below:

- During a first online meeting, partners carried on a preliminary discussion on the definition of "emerging mycotoxins". A preliminary list of compounds to be included in such definition was agreed.
- The outline of the review was collaboratively discussed during the physical meeting held in Parma during the World Mycotoxin Forum (May 2022), and further set up by email and short video calls among partners. All partners took equally part to the discussion and to the definition of the outline. ELS keywords were defined and agreed among all partners.
- UNIPR was in charge of the literature search and of the preparation of the first draft, which was later circulated among partners for final revision.

The review has been published on Toxins journals, and available at https://doi.org/10.3390/toxins15090583.

Preparation of the scoping review: background

Emerging mycotoxins are a group of fungal metabolites that have recently been identified, whose presence, toxicological properties and potential health risks are not yet fully

understood. These mycotoxins are typically produced by various fungal species under certain growth conditions and can contaminate a wide range of agricultural commodities, posing a potential threat to human and animal health.

The emergence of these mycotoxins has necessitated ongoing research to elucidate their occurrence, toxicological mechanisms, and appropriate regulatory measures for their control and mitigation. There are several emerging mycotoxins that have been identified, such as:

1. Alternaria toxins (Crudo et al., 2019),(Fraeyman et al., 2017): Alternaria species are common fungal contaminants of various crops, including grains, fruits, and vegetables. Alternaria toxins, such as alternariol (AOH) and alternariol monomethyl ether (AME), have been identified as emerging mycotoxins with potential health risks. In addition, minor Alternaria mycotoxins such as those presenting a perylene quinone structure (e.g. Altertoxin II - ATX II) are suspected of genotoxic activity.

2. Enniatins and Beauvericin(Fraeyman et al., 2017; Luz et al., 2017): a group of cyclic hexadepsipeptide mycotoxins produced by Fusarium species. They are commonly found in cereals, maize, and other grains. Enniatins have been associated with cytotoxic, neurotoxic, immunotoxic, and estrogenic effects, although their relevance for human health is still debated.

3. Emerging trichothecenes(Gruber-Dorninger et al., 2017; Luz et al., 2017): Non-regulated, such as DAS, NIV or FUS-X, or less prevalent trichothecene mycotoxins (like NX-2 toxin and T2 triol) are commonly identified as emerging mycotoxins.

4. Moniliformin(Frisvad, 2018): Produced by Fusarium species, moniliformin has been detected in maize, rice, and wheat. It has been linked to nephrotoxicity and hepatotoxicity in animals.

It is important to note that the identification and characterisation of emerging mycotoxins are ongoing processes, and new compounds continue to be discovered as analytical techniques advance and research progresses in this field. It is therefore difficult to give a consensus definition of emerging mycotoxins, as well as to define a consolidated list of compounds. It is noteworthy that emerging mycotoxins are currently not routinely determined nor commonly included in monitoring plans. Therefore, a significantly lower number of entries are present in the official occurrence database compared to regulated mycotoxins.

To the scope of assessing the occurrence data for emerging mycotoxins in the scientific literature, a scoping review was conducted for selected emerging mycotoxins, following the process reported below.

Preparation of the scoping review: methodology

The selection of emerging mycotoxins included in the abovementioned scoping review was therefore based on two criteria: 1. Not yet regulated; 2. Already addressed by EFSA for a preliminary risk assessment due to their widespread occurrence in food commodities.

Recently the European regulation for mycotoxins has been updated for AOH, AME and TeA in eight food products such as processed tomato products, paprika powder, sesame and sunflower seeds, sunflower oil, etc. (EC, 2022). However, we decided to include them in this

review due to the fact that they are known to occur in other foods not included in the current legislation and due to their "emerging" nature.

The primary search was performed on 3 databases: Scopus, PubMed and Web of Science, within the timeframe of January 2018 – December 2022 (5 years). The following search keywords were used: ("Alternaria toxin*" OR "Alternariol*" OR "Alternariol monomethyl ether" OR "Tenuazonic acid" OR "Tentoxin*" OR "Enniatins" OR "Beauvericin" OR "Moniliformin" OR "Diacetoxyscirpenol" OR "Nivalenol" OR "Sterigmatocystin" OR "Neosolaniol" OR "Fusarenon X*) AND ("food*" OR "cereal*" OR "oilseed*" OR "nut*" OR "fruit*" OR "vegetable*" OR "bread*" OR "wine*" OR "sauce*" OR "spicy*" OR "coffee*" OR "legume*" OR "bean*"OR "potato*" OR "starchy*") AND "analytical method*".

Screening of studies was carried out according to Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA)(Moher et al., 2015, 2009) statement protocol, to assure the scientific quality of this review and to minimise the risk of bias.

Initially, the titles and abstracts of the retrieved papers were scrutinised to determine their relevance to the study. Subsequently, the full text of the selected papers were evaluated for eligibility. Due to data scarcity, the search was extended from peer-review articles to the Rapid Alert System of Food and Feed (RASFF), Food and Standards Agency (FSA), and the European Food Safety Authority reports.

The literature review was performed by two reviewers in order to minimise bias. Any discrepancies between the reviewers were noted and resolved through discussion. The reviewers met in person before the screening process to discuss study eligibility through defining inclusion and exclusion criteria.

The inclusion of studies in the systematic review was based on predetermined criteria: (a) Full-text of paper in the English language published; (b) samples of products intended for human consumption; (c) accurate analytical methods used; (d) sample size, prevalence of positive samples, mean concentration and(or) contamination range or original data were provided. Studies other than in the field of food, full-text not available, concentration values or range of contamination not mentioned and reviews were excluded.

Using the key words mentioned above, the literature search returned 1,537 articles published from January 2018-December 2022. Among them, 886 articles were retrieved from Scopus, 450 articles were retrieved from Web of Science (WoS) and 201 articles were retrieved from PubMed. After removing 194 duplicate articles, 1,237 articles that did not meet the requirements of this review were excluded after reviewing their titles and abstracts. The remaining 106 articles were read in full. In addition, 7 EFSA reports, 3 RASFF notifications, and 2 reports from Food Standards Agency Committee on Toxicity (FSA COT).

The data collected from each publication encompassed: type of food, country, detection method, type of mycotoxin, number of the sample, incidence of positive sample and mean value or range of contamination. According to the exposure hierarchy of FoodEx2, the samples from the collected articles were classified into 19 food categories, as reported in Table 2. Articles providing mean contamination value was used to calculate the overall mean contamination value. For samples with the contamination value below LOD and below LOQ, a lower bound approach was used.

Table 2: Total number of articles for each analysed food

Analysed food	Number of the articles (each category)
Grains and grain-based products	61
Legumes, nuts, oilseeds and spices	21
Alcoholic beverages	13
Food products for young population	13
Vegetables and vegetable products	12
Fruit and fruit products	9
Animal and vegetable fats and oils and primary derivatives thereof	8
Meat and meat products	8
Fish, seafood, amphibians, reptiles and invertebrates	7
Milk and dairy products	7
Products for non-standard diets, food imitates and food supplements	7
Coffee, cocoa, tea and infusions	6
Starchy roots or tubers and products thereof, sugar plants	6
Eggs and egg products	5
Water and water-based beverages	5
Seasoning, sauces, and condiments	4
Sugar and similar, confectionery and water-based sweet desserts	4
Composite dishes	3
Fruit and vegetable juices and nectars (including concentrates)	2
Major isolated ingredients, additives, flavours, baking and processing aids	2

Preparation of the systematic review: outcome of the review process

The work done has been arranged in a systematic review manuscript submitted to the Toxins Journal Special Issue (Toxins: 15th Anniversary) and available at https://doi.org/10.3390/toxins15090583. Therefore, to avoid any potential plagiarism and duplication, only a summary of the collected data will be reported in the present report.

The manuscript covered the worldwide occurrence of emerging mycotoxins in the past five years. Fifteen non-regulated mycotoxins were found in 19 food categories worldwide. On top of that, 38 different combinations of non-regulated mycotoxins were found with mixtures varying from binary combinations up to 12 mycotoxins. Most of the interactions between these mycotoxins have additive/synergistic effects, therefore the co-occurrence of emerging mycotoxins, and especially their co-occurrence with regulated mycotoxins sharing similar toxicological endpoints, might cause a concern for human health.

The most analysed food category was grains and grain-based products while the least analysed food category consisted of major isolated ingredients, additives, flavours, baking and processing aids. Overall, fifteen non-regulated mycotoxins were found in 19 food categories worldwide, as reported in Figure 3.

Enniatins and mycotoxins from the Alternaria family are the most studied emerging mycotoxins with the focus on occurrence in grains and grain-based products, while neosolaniol and moniliformin are the least studied emerging mycotoxins.

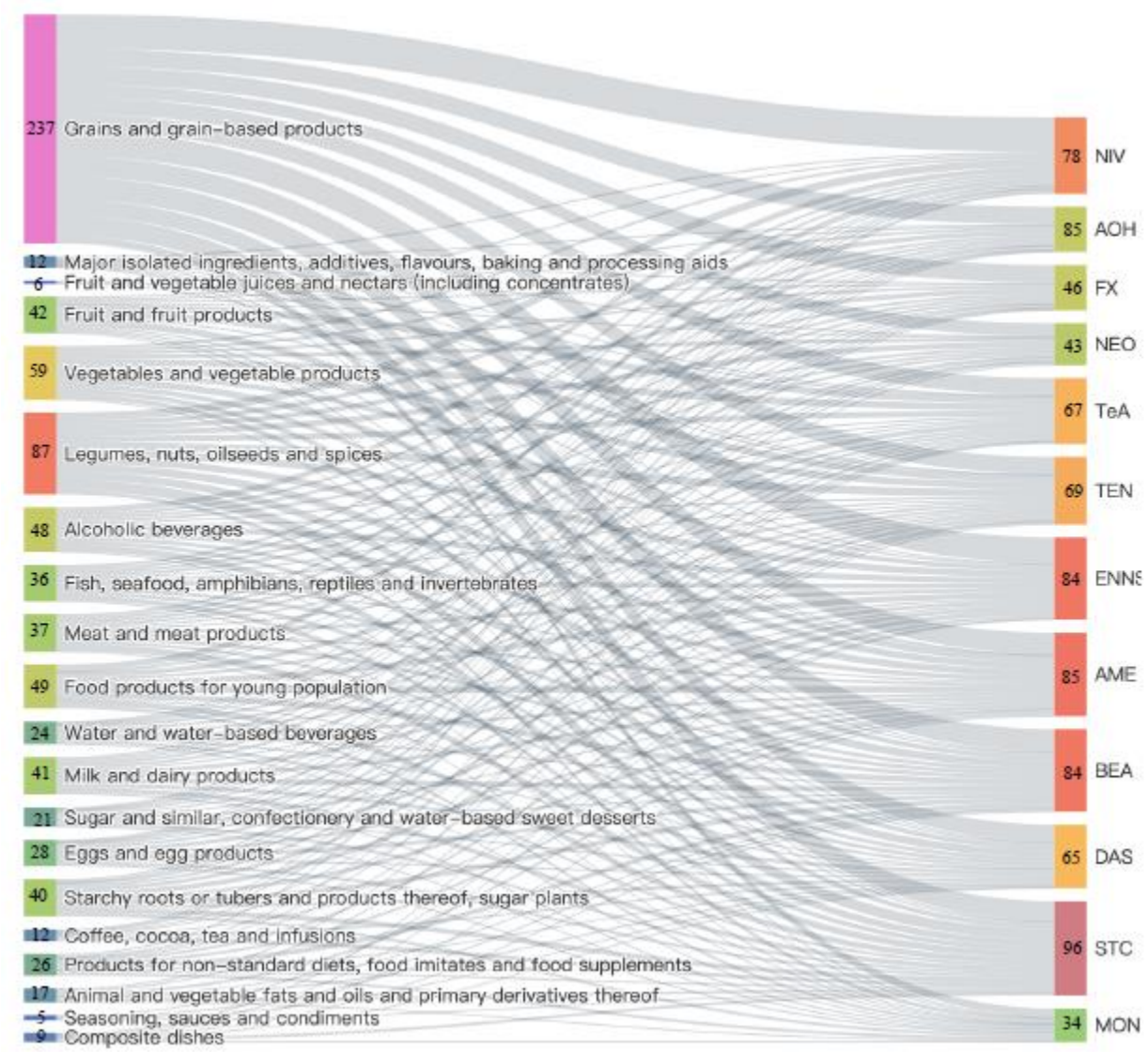

Figure 3: Mycotoxins analysed in each food category from the 106 articles included in the review

To give an example of emerging mycotoxins and in which foods they were detected, Figure 4 shows more in detail the occurrence of mycotoxins from the Alternaria family in food. Alternariol, alternariol methyl ether, tenuazonic acid and tentoxin were mainly detected in grains and grain-based products, vegetables and vegetable-based products, and food for young population (children). Fruit and vegetable juices, coffee and cocoa-based products, food imitates and supplements have the lowest occurrence rate.

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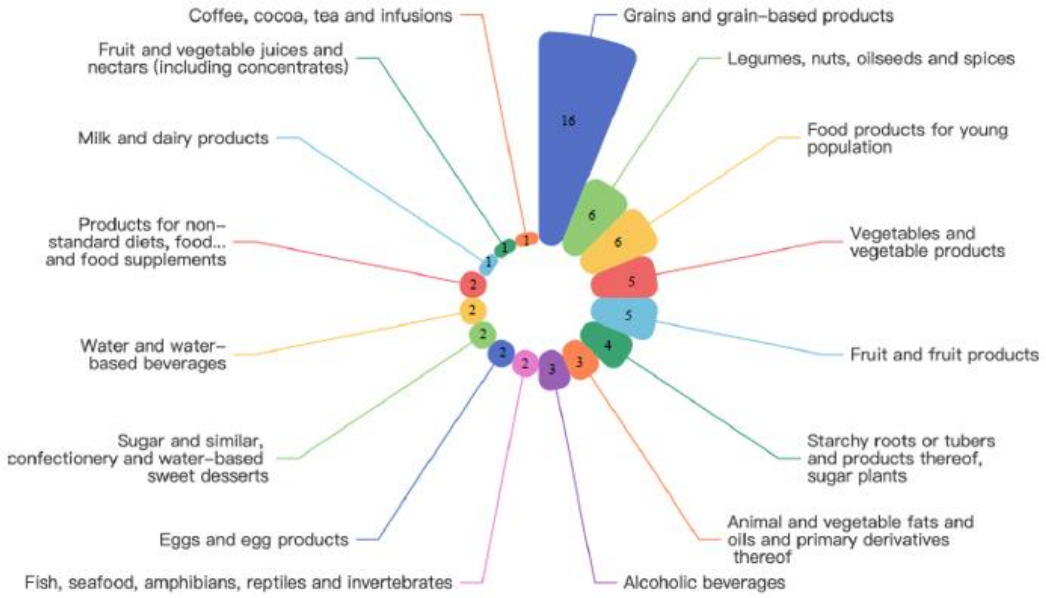
A)



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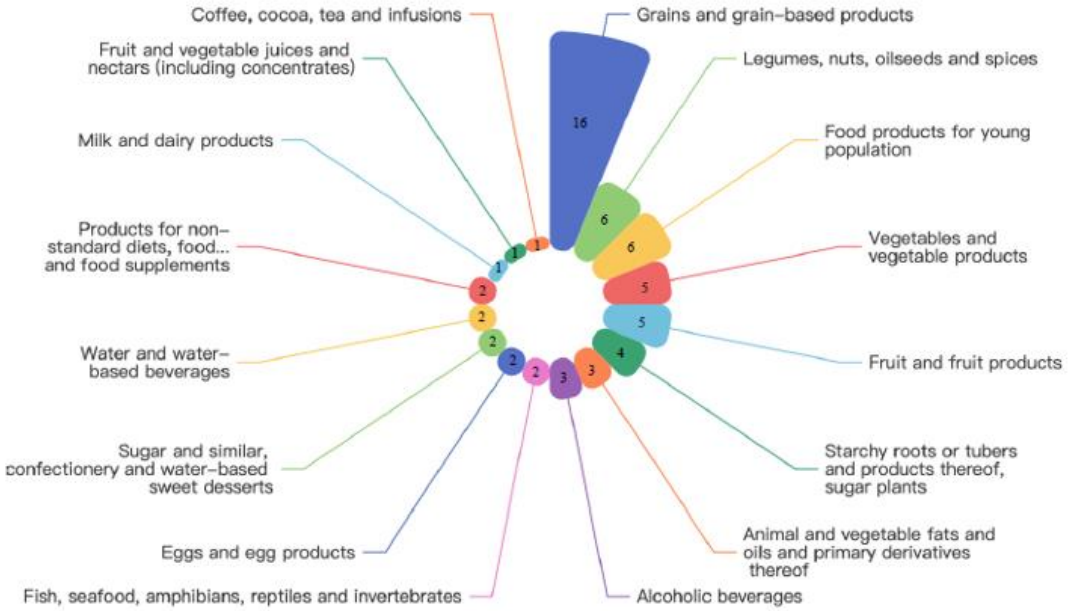


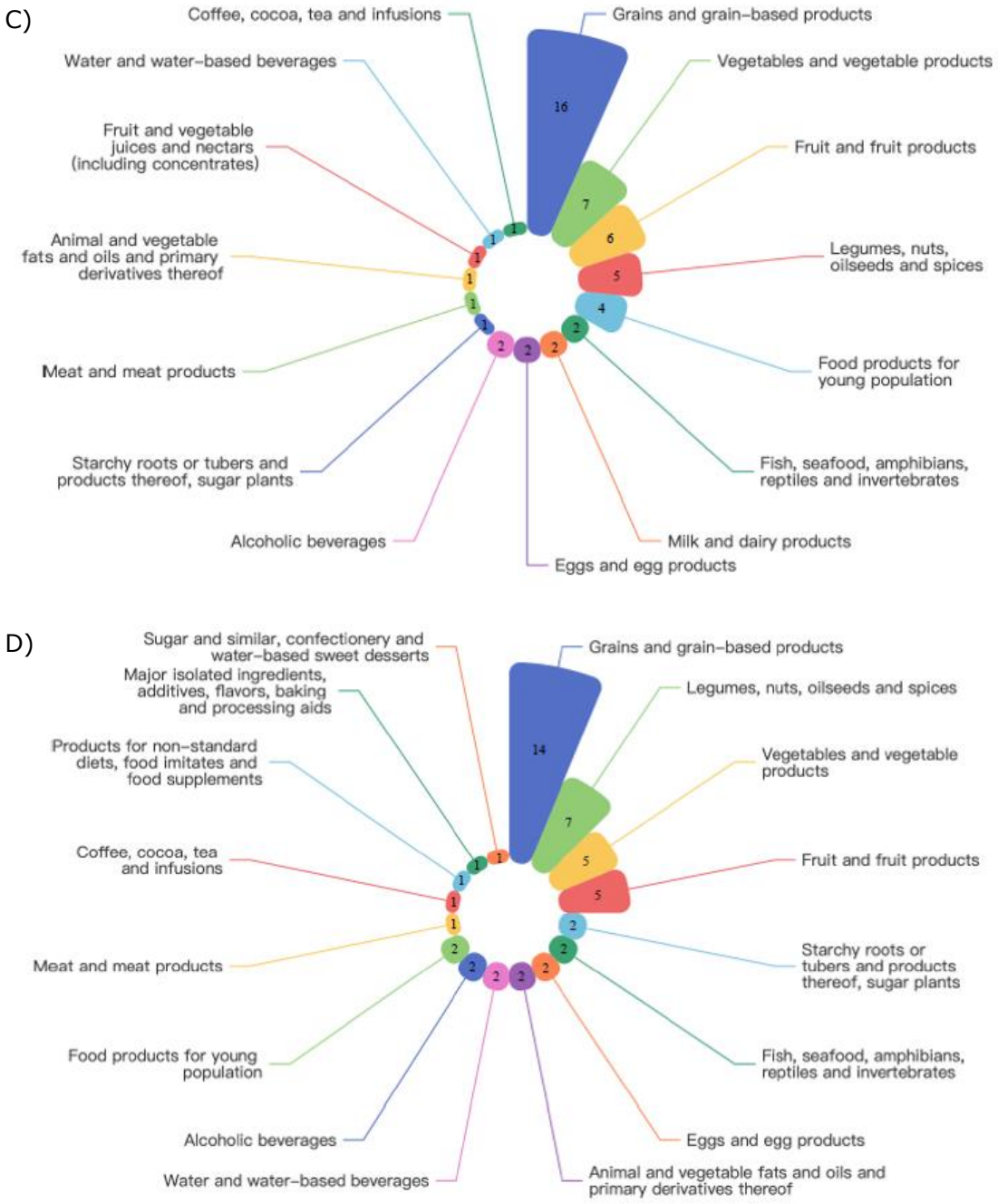


Figure 4: Mycotoxins from the Alternaria family occurrence in foods; a) Alternariol, b) Alternariol methyl ether, c) Tenuazonic acid, d) Tentoxin

The results were further discussed in the review paper, also considering the current knowledge about potential combined toxicological effects.

1. Uptake of computational methods in mycotoxins risk assessment

New Approach Methods (NAMs) in risk assessment (Magurany et al., 2023; Nikolopoulou et al., 2023) encompass a range of novel and alternative approaches utilised for evaluating the safety and potential risks posed by chemicals, drugs, or other substances. These methods, often based on *in vitro* and *in silico* techniques, aim to provide robust and predictive data, while minimising or eliminating the reliance on animal testing. NAMs includes diverse methodologies (Cattaneo et al., 2023), such as computational modelling, high-throughput screening, omics technologies, and mechanistic toxicology, which collectively contribute to improved hazard identification, dose-response assessment, and exposure estimation, thereby enhancing the overall efficiency, ethical considerations, and scientific basis of risk assessment practices.

Among NAMs, computational methods in toxicology refer to the application of computational approaches, such as mathematical modelling, data analysis and computer simulations, to understand and predict the toxicological properties and potential risks of chemicals, drugs or other substances. These methods leverage various algorithms, databases and software tools to analyse and interpret toxicological data, predict toxicity endpoints, assess chemical interactions and support risk assessment processes.

Among the MYCOBOOST consortium, UNIPR has been involved in the use of computational methods in toxicology since a decade, being one of the first group to introduce this approach to the mycotoxins field. Although the consortium was aware that, with the exception of some groups that have already adopted for several years the use of an integrated in silico/in vitro workflows (Dellafiora et al., 2018b; Ehrlich, 2015) to support hazard identification and characterization, computational methods are not widespread in the mycotoxin community. As one of the EKE objectives, the consortium aimed to know if the scientific community was aware of the opportunities offered by the integration of such methods into mycotoxins risk assessment. Moreover, the consortium was interested in understanding the reasons of such a limited uptake of computational methodologies in the field.

Based on the EKE outcome, the community seems to be still unprepared to uptake computational methodologies, and appears to be unfamiliar with the different approaches that can be adopted. The greatest limitation perhaps lies in the lack of specific training courses which allow to approach computational techniques and to transfer skills often borrowed from Drug Discovery in the context of Food Safety.

The MYCOBOOST consortium therefore chose to follow 3 paths to promote the diffusion of computational methods for the risk assessment of mycotoxins:

1. collecting a brief description about the potential applications of the most common computational approaches (presented below)

2. drafting a systematic review on the use of computational techniques in mycotoxins risk assessment;

3. presenting the methodology and giving an introductory training during the Training Workshop 2 in Parma.

Furthermore, with the aim of promoting the uptake and dissemination of the methodologies in the reference community, it was decided to present the methodology through some case studies in reference conferences (e.g., World Mycotoxin Forum 2022, World Mycotoxin Forum 2023, Mycotoxin Workshop 2022, Mycotoxin Workshop 2023).

Definition of computational approaches and their potential application to the field

Among the most important methods, the following can be applied to the mycotoxins field:

Read-across is a computational approach that involves extrapolating toxicity information from similar compounds to the target compound. It relies on the assumption that structurally similar compounds are likely to exhibit similar toxicological properties. By utilising read-across methodologies, researchers can make informed predictions about the toxicity of compounds for which limited or no experimental data are available (Benfenati et al. 2019). This methodology can be applied to the mycotoxin research in order to prioritise testing resources and reduce animal testing.

QSAR models are utilized to predict and quantify the relationship between the structural features of target compounds and their toxicological properties. It is based on a comprehensive dataset consisting on experimental toxicity data (i.e. acute toxicity, genotoxicity, carcinogenicity, and other relevant endpoints). A second dataset containing molecular descriptors (i.e. physicochemical and structural parameters) for all the compounds of interest, is compiled. Finally, a statistical model is built up based on the available dataset, using algorithms such as multiple linear regression or support vector machine to analyse the structure-activity relationship. The model should be then validated to assess its predictive performance, and later applied to new or untested compounds based on their chemical structures (Benfenati et al. 2019; Hemmerich & Ecker, 2020).

In Silico Toxicokinetics models simulate the ADME processes of a target compound in the body using computational techniques. These models help predict the bioavailability, distribution, metabolism, and elimination kinetics. In silico toxicokinetics methodologies could represent a reliable tool for better understanding the toxicokinetic properties, potential accumulation, and elimination of mycotoxins, also in support of biomonitoring methodologies (Grech et al. 2017; Sarigiannis et al. 2019; Lootens et al. 2023)

Computational methods and modelling techniques are employed ***for*** understanding ***toxicodynamics*** of target compounds, focusing on their interactions with biological targets, signalling pathways, and the resulting toxic effects. These approaches provide insights into the mechanisms of toxicity and aid in risk assessment. More specifically, *Molecular Docking* techniques are used to simulate and predict the interactions between mycotoxins and specific biological targets, such as enzymes or receptors (Dellafiora et al. 2018a). These simulations help identify the binding sites, binding affinities, and potential mechanisms of action of mycotoxins. In addition, *Molecular Dynamics Simulations* are employed to study the dynamic behaviour of mycotoxins and their interactions with biological systems at an atomic level. These simulations provide insights into the conformational changes, stability, and flexibility of mycotoxin-protein complexes,

aiding in understanding the toxicodynamic processes (Dellafiora et al. 2018b; Dorne et al. 2022).

In silico methodologies are often integrated with Adverse Outcome Pathways models to provide a more complete understanding of the toxicological processes. This integration helps link the exposure and internal concentrations of a target compounds with their toxic effects on biological systems (Hemmerick & Ecker, 2020).

These computational methods in toxicology contribute to more efficient and cost-effective screening of chemicals, prioritisation of testing, and risk assessment processes, while reducing the need for extensive animal testing. Especially when these strategies are combined into integrated tested workflows, they could play an important role in supporting the comprehensive assessment of mycotoxins toxicity. By integrating data from different sources, such as in vitro assays, in silico predictions, and structure-activity relationships, integrated testing strategies aim to provide a more holistic view of toxicological risks.

Preparation of the systematic review: methodology

With regard to the ***systematic review***, the data were analysed using a scientometric approach and the results were used for the preparation of a scientific work being finalised. Therefore, results are reported herein only in brief to avoid any duplication.

The primary search was performed on 3 databases: Scopus, PubMed and Web of Science, within the timeframe of January 2002 – December 2022 (20 years). The following search keywords were used: ("New Approach Methods" OR "computational methods" OR "QSAR" OR "SAR" OR "read across" OR "read-across" OR "toxicokinetic modeling" OR "molecular docking" OR "molecular dynamics") AND "mycotox*"

Screening of studies was carried out according to Preferred Reporting Item for Systematic Reviews and Meta-analysis (PRISMA) statement protocol, to assure the scientific quality of this review and to minimize the risk of bias. Initially, the titles and abstracts of the retrieved papers were scrutinized to determine their relevance to the study. Subsequently, the full texts of the selected papers were evaluated for eligibility. The review process was performed by two reviewers in order to minimize bias. Any discrepancies between the reviewers were noted and resolved through discussion. The reviewers met in person before the screening process to discuss study eligibility through defining inclusion and exclusion criteria. The inclusion of studies in the current systematic review was based on predetermined criteria: (a) Full-text of paper in the English language published; (b) application of computational methods intended for food toxicology and not for drug discovery. Studies other than in the abovementioned field, full-text not available, and reviews were excluded.

ELS data were than elaborated under a scientometric approach, using the open software VosViewer (van Eck and Waltman, 2010) (https://www.vosviewer.com/) and CiteSpace (Chen and Song, 2019) (https://citespace.podia.com/).

Preparation of the systematic review: results

Using the key words mentioned above, a search was conducted in three databases - Scopus, PubMed, and Web of Science. After the application of filtering criteria and removal of duplicates, 427 papers were retrieved in the period January 2002 – December 2022. As reported in Figure 5, the large majority of studies were published in the last 5 years (54%), although there was a gradual uptake of the methodologies within the field of research in the last decade.

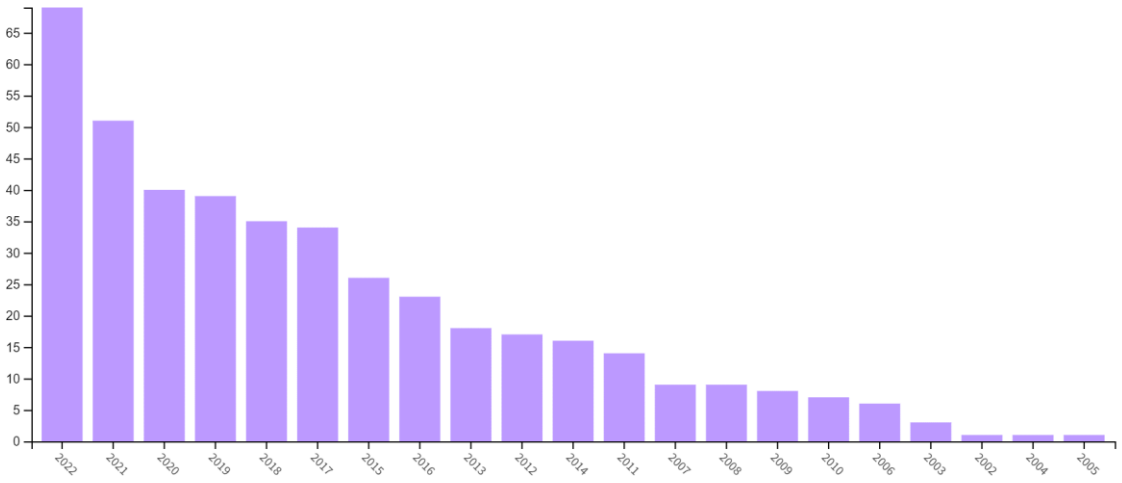


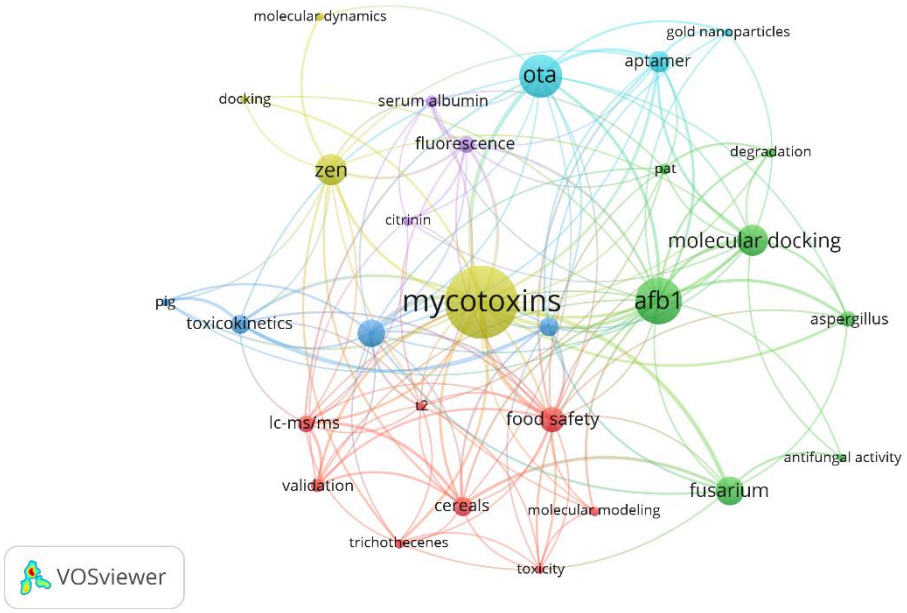
Figure 5: Number of studies from the systematic review and the corresponding years of publication (source: Web of Science, access on June 2023)

The clusterization of articles based on the use of authors keywords (A) and their distribution over time (B) are reported in Figure 6. Data have been analysed using the open source software Vosviewer (https://www.vosviewer.com/).

The data clearly showed that computational methods have been prevalently applied to AFB1, OTA and ZEN for four different purposes: 1. Support to the analysis; 2. Development of antibodies, aptamers, and immunoassays; 3. Studies on protein-ligand interactions; 4. Identification of antifungal properties.

In particular, a number of studies have been focused on the interaction among OTA and albumin. More specifically, the words "molecular docking" and "toxicokinetics" appear only recently as keywords, attesting the very recent uptake of such methodologies in the specific field of research.

A)



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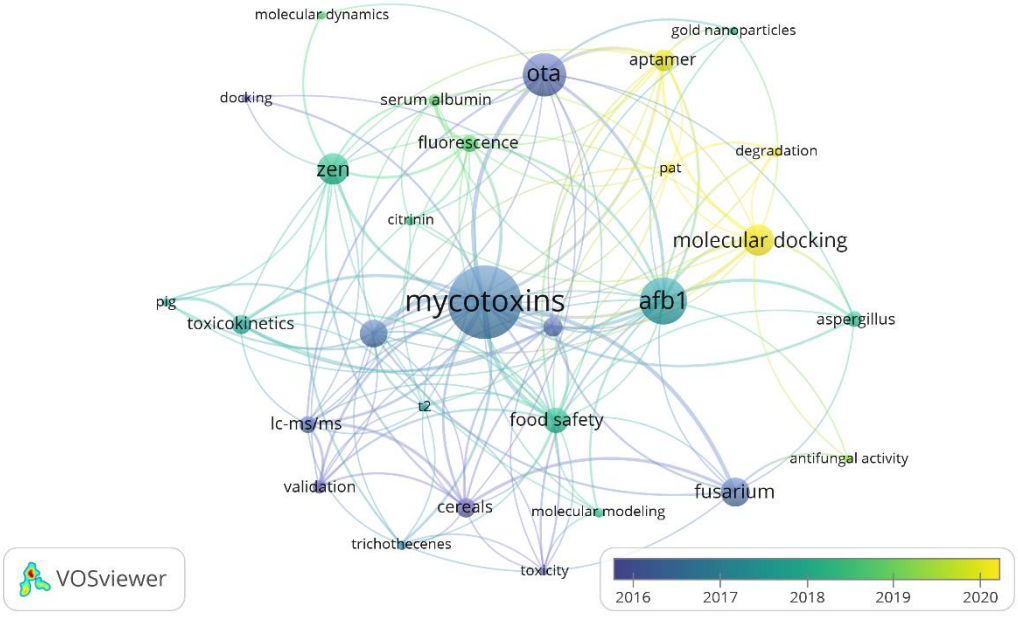

Figure 6: Article clusterization based on: A) the use of keywords and B) their distribution over time

Although the selected papers were co-authored by 2237 authors, only 28 appeared on at least 4 papers and most of them were affiliated to the same institutions. In addition, very scarce interactions are highlighted, with groups mostly remaining as isolated clusters. This observation again demonstrates how the application of computational methods is still a niche in the field of research and initiatives to foster collaboration are needed (Figure 8A).

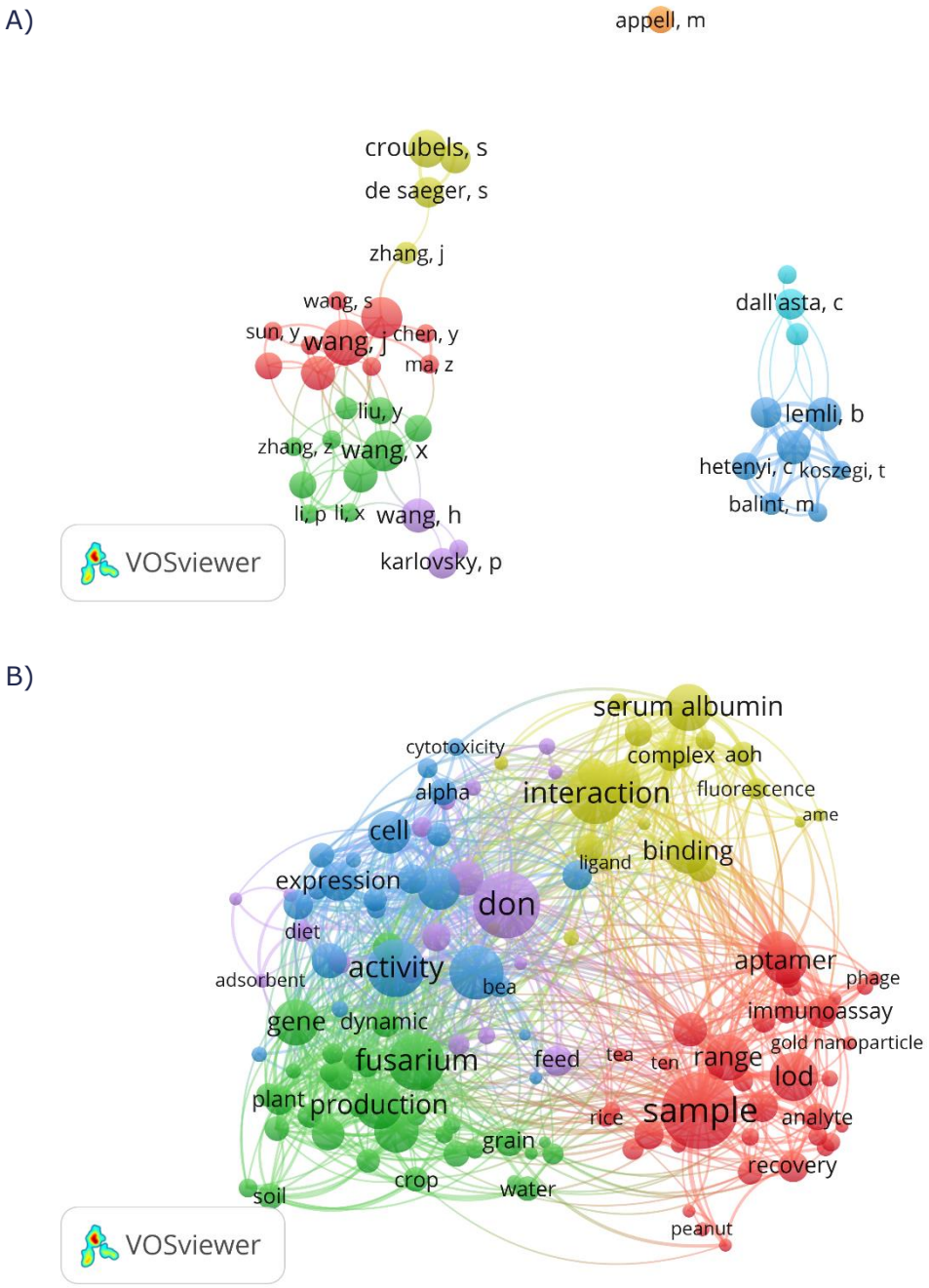

Figure 8: A) Clusterization of authors related to articles on computational methods. B) Clusterization of terms occurring at least 10 times; three main clusters were detected: activity (blue), detection (red), interaction (yellow), and production (green).

To better understand the field of research, recurring words have been extracted from titles and abstracts of the retrieved papers. Only terms occurring at least 10 times were extracted. Of 12,982 terms, 322 met the threshold. An in-house thesaurus was applied for resolve synonyms.

The resulting plot is reported in Figure 8B. Four main clusters, have been identified, around the terms "activity", "detection", "production" and "interaction", while DON is the most recurrent mycotoxin. These terms clearly identify the current research applications for computational methodologies in mycotoxin research.

Overall, the literature analysis indicated a relatively low uptake of computational methods in the field of research, and a need for further collaboration among active groups to promote the introduction of NAMs in the mycotoxins field.

Preparation and delivery of training activities

The use of molecular modeling in the context of mycotoxin risk assessment has been lined up a two-module seminar. The first module introduced the use of molecular modeling in the frame of computer sciences with historical nods to understand the origin of computer sciences applied to risk assessment. A selection of case studies were described to better understand how this kind of methods can support the hazard identification and characterization of mycotoxins allowing to investigate the toxicodynamics and toxicokinetics from a molecular viewing angle. The second module provided practical examples of a typical workflow of analysis, from data retrieval to software set up and data analysis, providing the audience with a direct contact with the modeling analysis.

Due to the interest arose among the attendees and the positive feedback collected during the Mycoboost Training Workshop 2, and also moving from the request of introductory events on the topic posed during EKE exercise, both seminar and training were repeated during the first FunShield4Med summer school, held in Parma on July 3-7 2023 (https://funshield4med.eu/event/summer-school-1/). This has allowed to disseminate the topic among 24 attendees from Italy, Greece and the UK.

1.4. Alternative/Innovative food items

Previous work conducted by UNIPR within the European Food Risk Assessment Fellowship Programme (EFSA-EUFORA) highlighted the risks and benefits of shifting from an omnivore diet to a vegan diet with a specific focus on meat substitution (O.A. Mihalache et al., 2022). Over the past few years reducing the consumption of animal-based foods has been considered a key element for healthy and sustainable diets. For this reason, plant-based alternatives, such as dairy and meat substitutes have been developed and are continuously expanding in the food. The European market of plant-based meat alternatives (PBMA) was valued at €4.4 billion in 2019 and is expected to grow by 70% in the next 6 years up to €7.5 billion by 2025. Although the urgency to decrease meat consumption has been largely debated in the literature discussing the impact in terms of sustainability and health, little to nothing has been done so far to assess the risk related to an extensive consumption of plant-based meat analogues.

Within the EFSA EUFORA project, a multi-mycotoxin determination method was developed for plant-based meat alternatives. PBMAs from the local markets from Parma were found to be contaminated with up to seven co-occurring mycotoxins (Mihalache et al., 2023). While wheat/wheat-based food are commonly carefully monitored for mycotoxin occurrence, there is a gap regarding data for semifinished materials used for seitan, and nothing is known about the fate of mycotoxins along its production chain. Of utmost relevance, the most common PBMAs such as soybeans, chickpeas, peas, lentils and seitan are understudied or not studied at all regarding mycotoxin occurrence.

Moving from this observation, the potential monitoring of non conventional food commodities was investigated through online survey and EKE exercise. Data and knowledge gaps highlighted in Section 3 clearly pinpointed that innovative food matrices – mainly those based on alternative proteins - are not included in current monitoring plans, neither they are covered by research schemes nor food consumption surveys. This has brought the evidence that such matrices deserve more exploration to support risk assessment.

Taking advantage on the previous experience gained within the EU-FORA scheme, and making use of the data quality approach developed within MYCOBOOST, UNIPR presented and obtained the MSCA-PostDoc Fellowship project PRISMA which has launched on June 1st. The project is fully dedicated to explore the presence of mycotoxins in plant-based meat alternatives, and to evaluate the human exposure through a biomonitoring pilot project. A strong interaction with EFSA has been already planned, through the activation of short visiting periods. This will be an important follow up of previous funded projects, and will hopefully provide occurrence data for further risk assessment and policy-making. The evidence about mycotoxins and plant-based food collected through the EU-FORA and MYCOBOOST projects leading to the MSCA PRISMA project will be presented at the World Mycotoxin Forum 2023 in Antwerp in October 2023.

As a complementation of the PRISMA project, UNIPR has been awarded as coordinator of the MSCA-Staff Exchange project MYCOBEANS, which will be launched at the end of 2023, addressing the potential occurrence and further mitigation of mycotoxins in protein fractions obtained from legumes and beans. Moreover, UGENT is coordinating the MYCOPROF project on "Mycotoxins in vegetarian protein-rich food and fibre-rich food" (March 1, 2022 - August 31, 2023 financed by FOD Public Health, Belgium). All occurrence results are in the process of being transferred to the EFSA occurrence database.

UNIPR and UGENT are committed to promote protocols exchange and data discussion within projects, in a way to ensure a quick closure of data gaps.

5 Data Stewardship

The online survey and the EKE exercise clearly highlighted the urgency of criteria for generation, collection and curation of molecular and occurrence data. Harmonised criteria for metadata collection are also urgently needed.

Due to the technical and cultural difficulties highlighted by the stakeholders, strategies for the adoption of data management plans and data sharing should be put in place. Among main difficulties in data sharing, the lack of a simple but effective protocol for meta-data collection and sharing was often reported together with a lack of proper training. The MYCOBOOST consortium noticed that the simplification of meta-data collection for occurrence data was already listed among activities carried on within the H2020 project FoodSafety4EU. Therefore, to avoid work duplication and dispersion of information, the MYCOBOOST team decided to offer external support to FoodSafety4EU consortium, and to address their efforts to the implementation of a proper training scheme (see Section 6). Moreover, considering the advances in mycotoxins analysis and the adoption of untargeted High Resolution Mass Spectrometry approaches for (retrospective) data collection, the MYCOBOOST team has focus their efforts on the identification and review of dedicated open access libraries and repositories.

More specifically, with regards to data quality, the actions carried out within the MycoBoost projects are the following:

1. to identify and revise mycotoxins database available online
2. to assess the impact of left censored data (LCD) on the exposure assessment are under evaluation through a range of case studies.
3. to develop proper data quality training for young scientists

5.1 Revision of mycotoxins database available online

While mycotoxins quantification still largely relies on LC-MS/MS analysis, which offers excellent accuracy and reliability, the diffusion of High-Resolution MS methods has opened the possibility for retrospective data analysis and identification of unknown metabolites and minor mycotoxins. While bringing a large potential for improvement in knowledge, the large amount of data generated in each experiment requires relevant efforts in data mining.

When it comes to advanced technologies, the consortium noticed that untargeted HR-MS studies are largely adopted among the scientific community, but consolidated open access repositories to assist an unique compound annotation are still limited. While topic-related workflows and repositories are available for other compounds, no specific libraries are available as open access for mycotoxins so far and the lack of common workflows is evident.

Each research group often follows its own protocols, data analysis methods, and reporting criteria, making it difficult to compare and reproduce results across studies. Collaborative efforts among researchers, institutions, and industry stakeholders are crucial to establishing standardised workflows and guidelines. Several initiatives are currently ongoing in this field, at a European as well as at an international level. However, the scene appears to be still immature to set common criteria, and more research and discussion is needed.

Within MYCOBOOST, a specific focus was set on the collection of chemical data for mycotoxins, that are important for the preparation of inclusion list for HR-MS analysis and retrospective data mining. Such data are usually available in large chemicals collections (e.g. PubChem, ChemSpider, Metlin) and in more focused libraries of natural compounds (e.g. NP Atlas).

Generalist comprehensive database such as PubChem are surely easy to handle and very common among users, and they provide a comprehensive collection of information on natural compounds, including their chemical structures, properties, and associated analytical data (i.e., formula, exact mass, mol files, smiles, chemico-physical parameters, CCS data, MS fragmentation spectra). However, due to the large number of compounds, it could be difficult for a not experienced scientist to correctly identify the origin of the compound. In addition, the growing pressure to publish on scientists has generated a very large number of unmaintained database. While data for mycotoxins and other fungal metabolites may be often retrieved from generalist database, the use of more focused collections could be of help by bringing together also information related to formation, prevalence, and toxicity. However, no collection dedicated to mycotoxins and metabolites from toxinogenic fungi has been identified so far. Nonetheless, mycotoxins and related fungal metabolites are classified as natural products (NP) and therefore are listed in NP database.

In spite of the growing interest into natural products from both the pharmaceutical and food sectors, it must be noted that there is no globally accepted initiative for NPs, with a dedicated platform where NP structures and annotations can be submitted, edited and queried by the community, like UniProt for proteins (The UniProt Consortium, 2017) and NCBI Taxonomy for living organisms (Federhen, 2012). As a consequence, chemical information for NP compounds, among them mycotoxins, are spread over a large number of open and commercial database/repositories as well as vendor catalogues. Very recently Sorokina & Steibeck (Sorokina and Steinbeck, 2020) have reviewed the resources for natural products developed since 2000, listing a total of 123 resources for natural products, among which 92 are open. Globally, the authors noted that the physicochemical properties of all datasets are comparable, although mismatching for stereochemistry information have been noted. Moreover, especially for minor mycotoxins and fungal metabolites for which standards are lacking, the univocal identification may be hindered by the presence of multiple entries for the same molecule with differences in stereochemistry and isotopes.

Among the most comprehensive and well-maintained database for natural products including fungal metabolites and mycotoxins, we could list the following:

- NPASS(Zeng et al., 2018) - https://bidd.group/NPASS/

The NPASS database in the current version (V2.0) provides 94,413 unique natural products isolated from 32,287 source organisms and together with 958,866 activity records on 7,753

targets. Besides chemical structures and physico-chemical parameters, also information about the source organism and the potential biological activity is given.

- NPAtlas(van Santen et al., 2022) – www.npatlas.org

NPAtlas encompasses secondary metabolites from bacteria, fungi and cyanobacteria, among them mycotoxins and toxinogenic fungi metabolites. The online platform requires registration and subscription for some functions, but the metabolite DB can be downloaded and used for local data mining.

- Toxic Exposome Database T3DB(Wishart et al., 2015) – www.t3db.ca

T3DB is the largest toxin database. It houses 3,678 toxins described by 41,602 synonyms, including pollutants, pesticides, drugs, and food toxins, which are linked to 2,073 corresponding toxin target records, for a total of 42,374 toxin-toxin target associations (data on June 2023). The main target applications of T3DB include toxin metabolism prediction, toxin/drug interaction prediction, and general risk assessment.

In addition, it is important to mention Exposome Explorer (http://exposome-explorer.iarc.fr/), hosted by the International Agency for Research on Cancer, the most comprehensive database for biomarkers of exposure to environmental risk factors for diseases. The database is specifically meant to provide support to scientists involved in exposomics and risk assessment.

When it comes to mycotoxins, the only open database exclusively dedicated to these compounds is MycotoxinDB (http://www.mycotoxin-db.com/). Unfortunately, no reference paper and little information are provided to check for accuracy of data reported.

In general, available resources are well-designed and curated, and therefore there is no need for further repositories dedicated to mycotoxins. However, an informed use of such database, especially when it comes to data interpretation, calls for proper training which is often missing among scientists, especially outside the academia.

5.2 Impact of left-censored data on the mycotoxins exposure assessment

The assessment of consumers' exposure to chemical contaminants in food relies on accurate occurrence and consumption data. The handling of occurrence data below LOD or LOQ is crucial, as these non-detects form LCD. However, data collection protocols lack harmonisation, leading to variations in analytical workflows and uncertainty levels. Adhering to established guidelines, such as those by WHO, FAO, and EFSA, regarding fit-for-purpose analytical methods, proficiency testing, and matrix effects evaluation is essential for generating robust occurrence data. Analytical criteria must consider both regulated and unregulated contaminants at trace levels. Challenges arise when dealing with unregulated contaminants, including unknown exposure sources, inadequate characterisation, and lack of reference materials. The use of different analytical techniques and sensitivities results in varying LODs and LOQs within datasets, affecting risk assessment. Mycotoxins show a high

percentage of LCD and play a significant role in exposure assessment, risk characterization, and regulatory implications. Given the widespread presence of mycotoxins and climate change's potential impact, understanding the effect of LCD on risk assessment is crucial for future regulations and associated economic implications.

Non-detects are part of the everyday life in environmental science, affecting the quality of analytical outcomes in many different fields. However, when data are used for deriving an exposure assessment, the impact of LCDs on the uncertainty is high and may lead to economical and legislative implications. It is worth noting that the understanding and discussion of the implications of LCD in risk assessment are not widely spread among scientists, especially those who are not directly involved in risk assessment. Increasing awareness and promoting discussions on the handling of LCD can help improve the accuracy and reliability of risk assessments in various fields.

Within MYCOBOOST a scientific paper was published (Mihalache and Dall'Asta, 2023), highlighting the economical and legislative implications of LCD. We emphasize that there is a clear need for the harmonisation of protocols in use for occurrence data collection and a comprehensive understanding between analysts and risk assessors. Harmonisation between analysts and risk assessors could potentially improve data quality which in this case translates to a reduction in the percentage of LCD, offer more accurate occurrence data, and subsequently adequate dietary exposures for consumers which have an impact at the regulatory level (establishing lower/higher maximum limits).

Case-studies arising from the study were used during the final Training Workshop on Data Quality hosted by UNIPR (May 2023).

More specifically, the impact of data quality in mycotoxin dietary exposure and chemical risk assessment was presented in a three-hour seminar. Overall, the seminar provided an overview of the steps of chemical risk assessment, types of consumers, exposure, occurrence, and consumption data. Then, case studies were presented to provide the audience with a better understanding on how data quality is important throughout the steps of risk assessment. Based on the good feedback received during the training, the same case studies were replicated during the first FunShield4Med summer school, held in Parma on July 3-7 2023 (https://funshield4med.eu/event/summer-school-1/). This has allowed to disseminate the topic among 24 attendees from Italy, Greece and the UK.

The paper will be further presented at the upcoming World Mycotoxins Conference 2023 in Antwerp. This will help in rising further awareness about the impact of LCDs in mycotoxins risk assessment within the scientific community.

6 Generating fit-for-purpose e-learning and training materials

Starting from the collected gaps of knowledge, the MYCOBOOST team prepared a fit-for-purpose range of activities to be delivered in blended modes. The overall goal was to generate a fit-for-purpose learning material to train young scientists in the field of mycotoxins and

beyond. With the awareness of not being able to cover all topics of interest in the field of mycotoxins, the partners decided to address the priorities identified during the round table.

As planned in the submitted proposal, a creation of a dedicated e-learning platform was originally planned, under the name of Mycotoxin Knowledge Hub.

However, already at the first stage of the project, the consortium noted several ongoing initiatives in the field aimed at the creation of a knowledge centre. One of the main limitations listed by the participants to the online survey and to the round table, is the fragmentation of resources through the multiplication of portals and database which do not survive beyond project lifespan. The lack of maintenance and curation over time contribute indeed to the disintegration of knowledge, the creation of false practices and the spread of incomplete or outdated protocols. Therefore, with the highest priority of avoiding work replication and promoting cooperation, the consortium has identified FoodSafety4EU as a major partner for the creation of a capacity building platform. The materials produced within the MYCOBOOST project will be therefore made available through the ZENODO repository (10.5281/zenodo.8161526) and hosted on the FoodSafety4EU platform. The large community around FoodSafety4EU will indeed ensure a large outreach and a long-term sustainability.

Two training courses were delivered within the project lifespan.

First Training Workshop (Bari, October 17 – 21 2022)

The first course has been organised in Bari, involving experts from Mycoboost and MycoTwin consortia as well as external experts. The training program, entitled "Rapid and official methods for mycotoxin detection and toxigenic fungi identification (morphological and molecular approaches) in wheat" was aimed to provide general foundations to young researchers interested in the topic of mycotoxins. The program is provided as Annex C.
Six travel grants (500 euro each) for participation of young students have been funded by the International Society for Mycotoxicology. Grants have been advertised through ISM and ISPA-CNR websites. Selection of applicants was done based on the scientific profile.
The joint MYCOBOOST-MycoTwin organization allowed to invite international experts outside the consorti as an additional opportunity for engagement and networking.
The course brought together frontal lessons and hands-on laboratories training to introduce students to current protocols of analysis. With the aim of laying the grounds in the mycotoxin field, fundamentals about a. fungi and fungal infection; b. mycotoxins; c. pre- and post-harvest strategies; d. protocols for fungal identification; e. rapid and confirmatory methods of analysis for mycotoxins. Each theoretical part wa complemented with practical laboratories. Overall, the course was attended by 20 international students, and recorded an highly positive feedback about the organization and quality of training.

Second Training Workshop (Parma, May 25 – 27 2023)

A second training course has been organized in Parma, with the support of the UNIPR School of Advanced Studies in Food and Nutrition. The course, entitled "Data Quality in Mycotoxins Risk Assessment" was completely devoted to data stewardship, and experts from EFSA were invited to take part (see Annex D for the program). Differently from th first Training Workshop, the course was more specialistic and therefore it was offered to students with a

preliminary background in mycotoxins research. In addition, it was opened to EFSA trainees and to selected undergraduate students from the International Master Degree in Food Safety and Food Risk Management, hosted in Parma by the 5 universities of the Emilia-Romagna region. In addition, 4 post-doctoral students and young professional from Rwanda participated, through the Erasmus Plus project EnRHEd (Enhancement of Rwandan higher education in strategic fields for sustainable growth) coordinated by the University of Parma. Due to the high prevalence of mycotoxins in the African area and the need for a strong EU-Africa partnership for food safety, the participation of attendees from Rwanda was particularly welcomed. A total of 30 students attended the course over the three days.

The program was developed over three days, each of them focused on a specific topic (day 1- toxinogenic data and molecular analysis; day 2 – mycotoxins occurrence and analysis; day 3 – computational methods and exposure assessment). The lectures alternated with sessions of exercises and discussion of case studies, specifically designed for the course.

The consortium worked on the development of the following dedicated teaching material:

- frontal lessons (all partners)
- a handbook for mycotoxins analysis and method development (UGENT - not sharable)
- a tutorial for the use of the open source software Galaxy for molecular data analysis and fungal identification (ISPA-CNR and INRAE)
- exercises for the correct identification of fungi through the analysis of sequences and the use of standards and references (ISPA-CNR and INRAE)
- exercises for evaluating the quality of analytical data (UGENT)
- exercises for calculating the exposure and evaluating the impact of left-censored data (UNIPR)
- video on LC-MS/MS identification criteria (UGENT)

Screenshots from the training materials are reported in Annex E. The material was made available to the students, and has been uploaded to the Zenodo repository (10.5281/zenodo.8161526). It will be further disseminated within the mycotoxin community thanks to the partnership with the International Society for Mycotoxicology.

The consortium has also prepared a video tutorial for the correct identification of mycotoxins with LC-MS/MS following the SANTE guidelines. The video tutorial was presented and launched during the training workshop in Parma and will be made openly available to the scientific community. Indeed, correct use of the LC-MS/MS identification criteria is of utmost important and is an important quality control parameter for analysis of mycotoxins in food, feed, and other biological samples including human biospecimen such as blood and urine. Next to the video, UGENT designed an Excel template for automated calculation of the identification criteria. This template design will be further developed towards a scientific publication. It can be found at the following link: https://vimeo.com/834024841/44a33eaa55

As already specified, all the abovementioned materials will be further disseminated through the FoodSafety4EU platform and through the International Society for Mycotoxicology platform and newsletter.

Moreover, considering the positive feedback obtained from participants, School for Advanced Studies in Food and Nutrition of the University of Parma has decided to host in 2024 a second edition of the Training Course on Data Quality in Risk Assessment.

7 Conclusion

Overall, the MYCOBOOST project acted as an accelerator of synergies for the creation of new collaborations, the stabilisation of the existing ones, and the presentation of common proposals in response to competitive tenders. Some fundings have been already secured by consortium partners to bring on the activities initiated within MYCOBOOST.

The participation to the Partnering Grant scheme to the MYCOBOOST partners, has been a significant opportunity for all partners to strengthen their collaboration in the field of mycotoxins and to define strategies for future research.

More specifically, the online survey and EKE exercise has allowed the consortium to become aware of the current gaps and limitation in the field, and to identify relevant needs. This will help partners in focusing their research efforts, avoiding duplication and addressing relevant issues for the overall advancement of the field. The possibility to share expertise and to discuss and debate methodological issues within the consortium, has acted as a cross-fertilizer for new ideas and concepts, which will be further developed through collaboration and short mobility stays among partners (i.e. undergraduate student exchange through Erasmus+ SMT program, joint PhD positions). The strong connection among Mycoboost partners and the International Society for Mycotoxicology as well as the involvement of several partners in the scientific committees of the major conferences in the field (i.e. World Mycotoxin Forum, Fusarium Seminar, Mycotoxin Workshop) will allow to bring the discussion initiated within the consortium to an higher level, and therefore to translate ideas and concepts into practice.

The flexibility of the Partnering Grant scheme, compared to other funding schemes focused on specific activities/tasks, has provided the consortium with a unique opportunity to explore topics usually not considered within TRL-oriented actions. As an example, the effect of left-censored data on the exposure assessment or the importance of adopting a correct ontology in fungal identification would have unlikely find a place in any EU RIA/IA project. However, the impact of such issues on risk assessment is enormous, and the lack of awareness among stakeholders is surely of great concern.

The development of a dedicated training program is worth of mention. Besides having provided in-depth training to selected young researchers (>40 students, considering both events), it has offered the opportunity to test new collaborative teaching schemes, and may act as a pilot for future initiatives, some of them already in the pipeline. Furthermore, the materials developed within MYCOBOOST will be included in the BSc and MSc courses as well as in the post-graduate programs offered by UNIPR and UGENT.

In terms of further collaborations, partners had the opportunity to discuss ongoing funding schemes and to define common strategies to apply for competitive grants. This will ensure a long-lasting collaboration among the MYCOBOOST team well beyond the project lifespan.

8 Recommendations

The work carried out during the project, with specific regard to the analysis of knowledge gaps, allows to draw up some recommendations for future initiatives.

In particular, there is a need to raise awareness within the scientific community about the data quality level required for risk assessment. This will ask for a continuous training and dissemination among stakeholders from academia, control laboratories, and the private sector. This could be complemented with the adoption by stakeholders of good practices for data collection and curation. At this purpose, the identification of proper nudging techniques is highly recommended.

Furthermore, the adoption of harmonised workflows for fungal identification and molecular analysis has to be promoted, especially through the use of (certified) reference materials. Finally, the consortium acknowledges that the adoption of a consensus definition of emerging mycotoxins and the identification of a priority list of "emerging mycotoxins", will favour the inclusion of non-regulated mycotoxins in the monitoring plans and the subsequent collection of occurrence data for risk assessment.

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Abbreviations

ADME	Adsorption, Distribution, Metabolism, Excretion
AME	Alternariol monomethyl ether
AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
AOH	Alternariol
ATX-II	Altertoxin II
CCS	Cross-collision section
CRM	Certified reference materials
DAS	Diacetoxyscirpenol
DB	Database
DON	Deaoxynivalenol
EKE	Expert Knowledge Elicitation
FAO	Food and Agriculture Organization
FSA	Food and Standards Agency
FSA-COT	Food Standards Agency Committee on Toxicity
FUS-X	Fusarenon X
HR-MS	High resolution mass spectrometry
IARC	International Agency on Research for Cancer
IRT	Inter-laboratory ring trials
ISM	International Society for Mycotoxicology
LCD	Left censored data
LC-MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass Spectrometry
MSCA	Marie Sklodowska Curie Actions
NAM	New Approach Methodologies
NIV	Nivalenol
NP	Natural product
NX-2	NX-2 toxin
OTA	Ochratoxin A
PBMA	Plant-based meat alternatives
PRISMA	Preferred Reporting Item for Systematic Reviews and Meta-analysis
PTP	Proficiency testing programs
QCM	Quality control materials
QSAR	Quantitative Structure Activity Relationship
RASFF	Rapid Alert System of Food and Feed
SAR	Structure-Activity Relationship
STD	Standards
TeA	Tenuazonic Acid
TEN	Tentoxin
WHO	World Health Organization

List of Annex

ANNEX A – Mycoboost Online Survey and Cover Letter

ANNEX B – Program of the first Mycoboost Workshop (Bari, October 2022)

ANNEX C - Program of the second Mycoboost Workshop (Parma, May 2023)

ANNEX D – Screenshots from the Handbook for Analysis