Framework for the Risk Analysis of Microorganisms In Microbial Based Cleaning Products

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and

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Table of Contents

Preface

This document provides a framework by which potential risks associated with microorganisms intentionally added to microbial-based cleaning products (MBCPs) can be considered as part of product development. This document is not a formal risk assessment and does not prescribe specific risk assessment procedures. However, this document does include strategies and recommendations which can be used to develop an appropriate risk management strategy for these microbial ingredients, helping to avoid unacceptable risks to MBCP users or other individuals.

The intended audiences for this document are those in industries that formulate cleaning products containing microorganisms including formulators, toxicologists, risk assessors and product safety professionals, each of which play a role in assessing the risks starting from performing an initial product feasibility assessment through to deciding on commercialization of a MBCP. A risk assessment should be considered at each stage of the project.

The information presented here may not be entirely applicable to all situations where microorganisms are used in a product formulation. Furthermore, certain conclusions are of limited certainty as detailed in the document. Product manufacturers should consult individuals with appropriate expertise and information from other sources to judge the applicability of this information.

For additional information on risk assessment and risk practices for microorganisms, contact your supplier, or:

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Introduction

Microbial-based cleaning products (MBCPs) contain intentionally added, viable microorganisms as an ingredient in the formulation. While MBCPs have a long history of use in drain cleaners and septic tank treatment products, there is also increasing interest in the use of MBCPs in household, professional, and industrial cleaning applications. This interest arises, in part, from the benefits microorganisms provide, such as the ability to break down organic matter, mitigate the matter-associated odors, facilitate cleaning in otherwise inaccessible places (e.g., cracks and crevices), and support cleaning activities over extended periods of time.

As is the case for chemical ingredients in conventional cleaning products, microbial ingredients in MBCPs need to be evaluated for potential risks to humans, other organisms, or the environment under their anticipated use conditions and per product labeling. While there is a long history of identifying and considering these risks for conventional chemical ingredients, the same level of historical knowledge does not yet exist for MBCPs as they are relatively new to the market. This reality, combined with the continued expansion and interest of the use of microorganisms in cleaning applications, highlights the need to establish a shared industry framework for how companies should analyze risks associated with microbial ingredients in MBCPs. Several recent publications have reiterated the need to consider these risks for microbial ingredients (e.g., Chokesajjawatee et al., 2020; Maestra et al., 2021, Todorov et al., 2021). It is important to improve consistency within these reviews in published literature and reduce the variability in the scope and procedures used for analysis.

Historic experience within the cleaning products industry demonstrates that the potential risk of adverse effects can be successfully managed by identifying the applicable hazards, carefully assessing exposure, characterizing the risk, and then applying appropriate risk management measures. Proper labeling and directions for use, as well as various other factors, can help to mitigate risk. The historic success of risk assessment and risk management practices in gauging and managing risk for conventional cleaning products suggests that a similar framework could be useful for MBCPs as well. The purpose of this document, therefore, is to describe a general framework by which risks associated with microbial ingredients in MBCPs can be analyzed so that they can be understood and mitigated as part of both MBCP product development and ongoing product stewardship (e.g., labeling). While this document is not intended to provide prescribed procedures for microbial risk assessment of such products, it is intended to help provide an organizing baseline for how microbial risks can be analyzed.

The proposed framework allows manufacturers to develop comprehensive programs based on sound science to identify, assess, and manage the risks of using microorganisms in consumer and professional MBCPs. The general guidance provided in this framework should be applied on a case-by-case basis to address the characteristics and use patterns of a given MBCP and its microbial ingredient(s). Key steps in this process include hazard identification, exposure assessment, hazard and risk characterization, risk management and risk communication. Good understanding of these steps and their underlying principals will support informed product development and stewardship decisions and facilitate the development of sound approaches to identifying and managing risks associated with microorganisms used in MBCPs.

This document focuses on understanding both the intrinsic properties of microorganisms used in MBCPs (hazards) as well as potential exposure to these microorganisms. This program is based upon previous experience with stewardship programs for cleaning products, which have been in place for more than 20 years.

Microorganisms are ubiquitous in the environment and, as such, the human body is routinely exposed to them. The goal of the stewardship program is to ensure that microorganisms used in MBCPs are well-characterized, have known hazard profiles which are adequately mitigated where necessary, and are generally suitable for use in the relevant consumer product(s). The commitment through an industry stewardship program as proposed in this document will help ensure safe use of this technology.

Chapter 1 – Microorganisms and Cleaning

What are Microbial-Based Cleaning Products?

MBCPs are cleaning products which contain one or more intentionally added, viable microorganism as part of the product formulation. The term "microorganism" can refer to a variety of different biological entities (e.g., bacteria and bacterial spores, fungi, yeast, and viruses); however, only the use of bacteria and bacterial spores are covered in this document. Bacteria in the form of bacterial spores are most commonly used in MBCPs because they are very robust and can survive for long periods of time in relatively harsh environments.

MBCPs have been on the market for many years in home- and professional-use products, such as drain cleaners and septic system additives. In recent years, consumer and professional surface cleaning products which contain microbial ingredients are becoming increasingly popular as well. . This document addresses MBCPs which are intended for both consumer use in and around the home and professional use in institution and workplace environments.

Why Are Microbial Ingredients Used?

Some microorganisms are capable of breaking down organic material and using it as a source of carbon, other nutrients, or energy as part of their normal metabolic activity. This can result in degradation of the organic matter itself and may make surfaces easier to clean via conventional means (e.g., wiping, laundering). The inclusion of microbial ingredients in cleaning products can therefore yield many benefits with respect to establishing and maintaining clean conditions and mitigating odors associated with organic soil. As described in further detail below, these benefits are often realized through the direct activity of the microbial ingredient itself.

Because the microorganisms in MBCPs are alive, they can facilitate these benefits for as long as they remain viable and/or metabolically active on a treated surface. In fact, many microbial ingredients are selected for use in MBCPs, in part, based upon their ability to emerge from a spore state, become active, and impart a cleaning benefit when conditions are favorable (e.g., when moisture, food source, etc. are present). When favorable conditions are not present, these microbial ingredients may then return to a spore state. Potential benefits of microbial ingredients therefore include, but are not limited to, the following:

- Extended cleaning and the maintenance of clean conditions across different matter types;
- Odor mitigation and control;
- Sustained cleaning activity when conditions are favorable; and,
- Ability to actively clean deep pores, cracks, and crevices inaccessible to standard mechanical cleaning techniques.

It is up to the product manufacturer to evaluate the risks associated with microbial ingredients used in MBCPs and clearly communicate those risks, if present.

How Do Microbial Ingredients Work?

MBCPs work through a myriad of mechanisms. Generally speaking, the cleaning action of MBCPs is largely based on the metabolic activity of the microbial ingredients present in these products. Through biochemical reactions, microbes can eliminate (degrade) organic material, mitigate associated

odors, facilitate cleaning in otherwise inaccessible places (e.g., cracks and crevices), and support cleaning activities over an extended period of time while the microbial ingredient remains viable.

Chapter 2 – Introduction to Risk Analysis

Risks from microbiological hazards are of concern to human health. In this document, the term "hazard" refers to the potential for a microbial ingredient to cause adverse effects, while the risk is the probability of occurrence and severity of a human health adverse effect as a consequence of exposure to the hazard. Minimizing the risk to the consumers and professional workers from MBCPs is an essential part of good stewardship for any company. Evaluating risk has three components: risk management, risk assessment, and risk communication. Risk analysis is an iterative process between risk assessors and risk managers whereby the results of the risk assessment are considered, and a strategy is developed to manage, control, or eliminate the exposures likely to cause health effects (Figure 1). This process could involve introducing appropriate risk-reducing procedures that control or eliminate sources of exposure. These components are shown below and expanded upon in subsequent chapters.

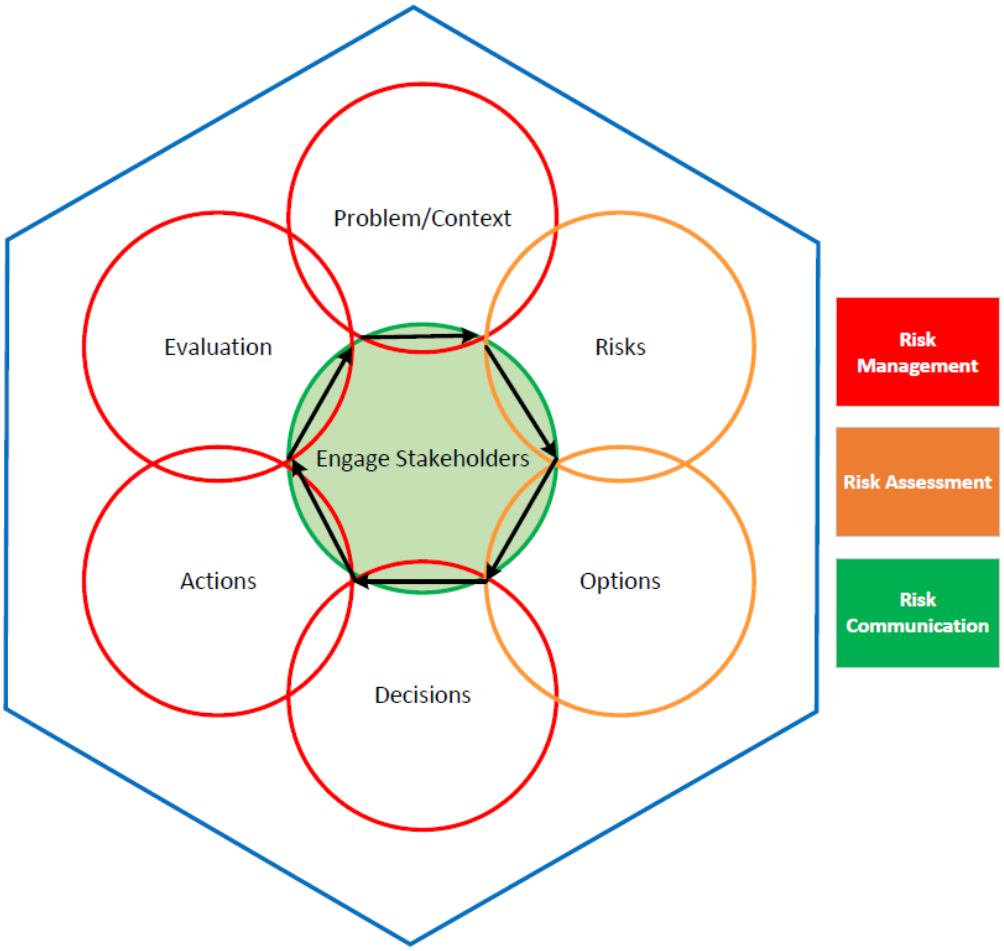

Figure 1: Framework for risk analysis

Risk Management

The process of developing a risk assessment should be initiated and controlled by those versed in risk management (i.e., risk managers). An organization may need to define more than one risk manager for a particular project. Risk managers are typically senior managers identified within an organization with broad perspective on the product and its uses, business customers, consumers, and other stakeholders.

Risk managers initially determine the need for a risk assessment and define its scope and objectives for evaluation. They will also have input in specifying the various scenarios that the risk assessment team will consider based on the nature and use pattern of the product. This can be an iterative process between risk managers and risk assessors as initial calculations intended to answer the risk managers' inquiries will generate further questions for the risk assessors to evaluate. For example:

- What are the metrics that a manufacturer or applicator can use to ensure that the public is protected?
- Which subpopulations are the most sensitive (e.g., immunocompromised, elderly, infants/children) to such products and need to be protected?
- What are the risk management options to minimize risks to the public?
- How can a manufacturer effectively communicate with suppliers, industrial customers, and consumers?
- What policies need to be implemented?

Risk managers also have a major role to evaluate the final risk assessment as described in Chapter 7.

Risk Assessment

Risk assessment is the process of identifying the hazard profile of a given material and gauging the likelihood of adverse effects that could potentially occur during or after handling and/or use. It is the process of assembling the relevant data, determining risks specified by the risk managers, calculating any scenarios or mitigations, and communicating the results in an appropriate manner to risk managers and other stakeholders. Risk assessment is important for microbial-containing consumer and professional products since it helps ensure the continued safe use of currently marketed products and is a basis for determining the safe use of potential new products undergoing commercial development.

Risk assessment is generally thought of as having four components: hazard identification, exposure assessment, hazard characterization, and risk characterization. These four components are shown in Figure 2 and their general description is as follows:

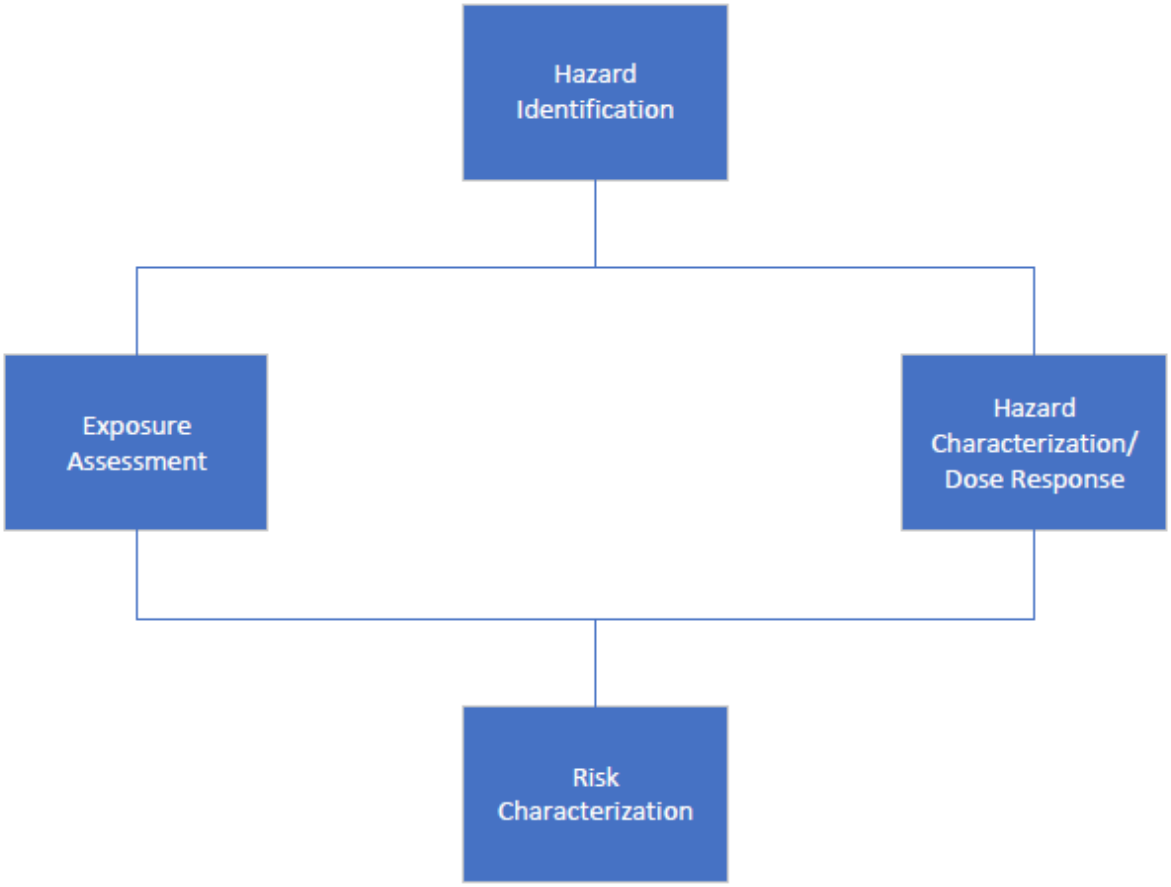

Figure 2: Risk assessment components

- **Hazard Identification.** For microbial ingredients in MBCPs, hazard identification encompasses the recognition of potential hazards that may be associated with the intentionally added microorganisms. This is largely a descriptive process that summarizes important information that will be in the risk assessment.
- **Exposure Assessment.** Exposure assessment describes the routes, as well as actual or estimated frequency, of exposure and exposure levels to the microorganism(s), toxin(s), or other relevant factors as described in the hazard identification section.
- **Hazard Characterization.** This is also termed the dose-response relationship and describes the nature and extent of adverse health effects for individuals exposed to a specified number of the microorganisms. This includes evaluating factors that may affect the immunological status of exposed human populations based on age (children, elderly), fertility/pregnancy conditions, and immune vulnerabilities. Understanding the presence of genes conducting virulence factors in the specific strains is important.
- **Risk Characterization.** The data gathered above can then be used in risk characterization to determine the likelihood and severity of humans experiencing adverse effects from using a microbial ingredient in a MBCP. This combines the exposure assessment with the specific dose-response relationship to calculate an estimate of the risk. Note that for microbial ingredients,

benchmark doses (see Chapter 3) instead of classic dose-response curves are typically used to define effect and no-effect thresholds. An estimate of risk characterization may be based on a specific scenario/individual (probability of a defined harm for a single use by a specific scenario of application/individual), or it may be a population risk (number of affected individuals per million products sold). The risks for different scenarios may be calculated and evaluated. Results may be expressed in numerical probabilities or by relative comparisons between different scenarios.

The following chapters describe the risk assessment steps in greater detail and their application to microorganisms being considered for use as ingredients in MBCPs.

Chapter 3 – Hazard Identification

For microorganisms used in MBCPs, hazard identification refers to the processes of helping identify potential physical, chemical, or biological effects of the microorganism in question on humans, other biological systems, and the environment. Hazard identification should focus on three areas, including (a) the microorganism's identity, (b) the host and any effects to the host caused by the microorganism, and (c) where the formulation will be used and potential for exposure (Razenberg et al., 2020). Broadly speaking, hazards of this nature may be related to the microorganism itself (e.g., infection), compounds produced by the microorganism (e.g., toxic secondary metabolites), and/or the interactions between the microorganism in question and other (micro)organisms (e.g., impacts on the microbiome), among other factors. To be as fulsome as possible, hazard identification is generally performed without regard for dose-response relationships and is inclusive of all potential exposure routes, regardless of the microorganism being evaluated or the type of cleaning product containing that microorganism. Hazard identification should include a description of the genes conducting virulence factors associated with the microorganism(s), as well as the host reaction, including infection, toxigenesis, or allergic reactions. Other factors, such as the conditions where a microorganism may grow or die, or those where toxins are synthesized, are also important to describe.

Data Sources

Data informing the hazard identification process can be obtained from multiple sources, including, but not limited to, (a) authoritative scientific documents such as peer-reviewed scientific publications, textbooks, etc., (b) publicly available medical case reports, (c) the results of testing conducted for relevant endpoints, and (d) information provided from microbial ingredient suppliers. This data may come from industry, academic, government, trade associations, suppliers, international organizations, or other stakeholders. Public-facing documents from regulatory authorities (e.g., regulatory assessments or guidance) can also be used for this purpose.

It is recommended that organizations develop internal written procedures describing the steps and resources used (e.g., scientific publication databases) to collect information for use in hazard identification. Such procedures can help ensure a sufficiently thorough review of the available hazard information and can be also leveraged to assist with traceability, quality assurance, ongoing reassessment frameworks, etc.

Example Intrinsic Factors

Intrinsic factors that should be evaluated during hazard identification may vary somewhat between microbial ingredients. That said, examples of general factors that should be considered during hazard identification include, but are not limited to, those listed below.

Microbial Identity. Establishing the identity of the microbial ingredient is a critical component of the hazard identification process as it underpins the gathering and evaluation of additional data from external sources. It is important to establish if any of the microbial ingredients are genetically modified, as there may be specific regulatory hurdles or public perception concerns for such ingredients. To the extent possible, it is critical for microbial identities to be established on a strain-by-strain basis because different hazards may be present for different strains of the same microbial species. Historically, microorganisms have been identified using a number of methodologies ranging from genetic analyses to a variety of different physiological or analytical methodologies. Genetic analyses are a common means of

identification in the modern era; however, nuances in this regard should be considered on a case-by-case basis. For example, while 16S ribosomal gene sequencing is commonly used for species identification, this sequencing alone may not be sufficient to distinguish between clades of genetically similar bacteria which have vastly different risk profiles. In these cases, whole-genome sequencing (WGS) or the sequencing of other distinguishing genetic markers (e.g., housekeeping genes such as those encoding for gyrase enzyme subunits) can be used to more firmly establish strain identity. The importance of this nuance is well known for certain groups of microorganisms (e.g., members of the *Bacillus* genera). Where appropriate, WGS information from multiple genetic elements (e.g., 16S, housekeeping genes, etc.), or other analyses, may help to make important distinctions between microorganisms which can, in turn, inform and improve the overall hazard identification process. In addition to the above, during literature reviews one should be aware of species identification limitations for older studies which rely solely on non-genetic data to ascribe genera and/or species identifications, as well as possible outdated taxonomy.

Once identity is established, a search should be conducted to determine if the microbial ingredient has a history of safe use. It is also important to understand characteristics about the life cycle of the strain such as germination, growth rate of vegetative cells and sporulation (if a spore former). Other strain specific life history details such as the intrinsic and extrinsic factors that influence growth, survival and reproduction are important to understand (generation time, optimum growth temperature range, pH, oxygen requirements, preferred energy and carbon sources).

Pathogenic Potential. Hazard identification should include information regarding the pathogenicity and virulence of the microbial ingredient in question. Both frank and opportunistic pathogenicity should be considered. Although hazard identification does not typically encompass a formal exposure assessment, an evaluation of pathogenic potential associated with different types of exposures, (e.g., dermal, ocular, oral/ingestion, inhalation), both direct and indirect, should be conducted. Likewise, the severity and scope of typical infections associated with the microbial species in question (e.g., local vs. systemic infection) should be identified and considered. Per Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work, microorganisms in Risk Group 1 are considered to demonstrate low pathogenic potential. In addition, there are regulatory documents, such as FDA General Recognized as Safe (GRAS) notifications and the Qualified Presumption of Safety (QPS) list issued by the European Food Safety Authority (EFSA), that can be helpful since in these cases the identification and evaluation of potential hazards in the context of food and agricultural applications has already been reviewed by authoritative bodies. For species and strains that are less known, it becomes important to conduct a thorough investigation of the strain and understand whether genes of concern are present and could be expressed during the intended use of the cleaning product.

Irritation and Sensitization. Irritation and/or sensitization properties may be associated with microbial ingredients themselves and compounds produced by those microorganisms. The potential for irritation and sensitization should be considered both in general and with respect to specific exposure patterns and tissues (in particular dermal, ocular, and inhalation exposures). The potential development of allergenicity should be considered, as well; notably, a number of publicly available bioinformatics resources exist which are intended to help predict allergenic potential based upon an organism's genome (see discussion on Example Tools for Evaluation of Intrinsic Factors, below). Use of such resources in conjunction with standard literature reviews, available test data, etc., can help to understand and mitigate associated hazards.

Toxin Production. Some microorganisms can produce toxins capable of adversely impacting human beings or other living organisms. Toxins include both exotoxins (which are secreted from the cell into the local environment) and endotoxins (which are associated with certain bacterial membrane components). Such toxins can be important virulence factors determinative of a microorganism's ability to establish infection and/or cause disease. Toxins can also impact the severity and symptoms of an infection, disease, or other adverse condition caused by microorganisms. The ability of a given microbial species to produce toxins may be strain-specific and does not necessarily track with species identification or common genetic sequences used for speciation (e.g., 16S gene sequence). It is therefore important to consider if there are genes present that could be involved in toxin production on a strain-by-strain basis. In addition to reviews of existing data, certain analyses can help determine the potential for toxin production by a given microorganism. For example, many laboratories can screen for production of certain toxins in growth cultures or in tissue cultures. A variety of genetic and bioinformatics tools are also available to screen for the presence of genes that could be involved in toxin production; and frameworks exist to help consider the genetic identity/similarity thresholds of unknown genetic sequences to known toxins (see discussion on Example Tools for Evaluation of Intrinsic Factors, below). All of these tools can help to ensure that hazards associated with toxins are adequately identified for a given microbial isolate. To mitigate risks of toxin production by a microbe during product manufacturing, while in the packaged product, and/or following application of the product in question, the potential for toxin production should include scenarios that encompass the lifecycle of the microorganism in the context of product formulation, manufacturing, and end use.

Antibiotic Resistance. The antibiotic resistance characteristics of a microbial active ingredient can impact treatability and overall hazards resulting from potential infections. Furthermore, the spread of antibiotic resistance in bacteria is a public health concern, and it is well understood that antibiotic resistance genes in one bacterium can be spread throughout a wider microbial population through various mechanisms. Therefore, it is important that hazard identification for microbial active ingredients includes an assessment of antibiotic resistance characteristics and whether a resistance phenotype is expressed. Antibiotic resistance can be assessed through susceptibility testing (against the minimum inhibitory concentration (MIC)). Antimicrobial resistance can also be evaluated by conducting a genetic analysis, such as following a WGS approach and conducting a bioinformatics analysis for the presence of antimicrobial-resistance genes. Should a given bacterium exhibit actual or potential antibiotic resistance, it is likewise important to consider the potential mechanism(s) of that resistance, their clinical relevance, and how that resistance may be encoded in the bacterium's genetic information. The risk level for antimicrobial resistance genes can then be evaluated based upon factors such as intrinsic nature, relevance, and mobility (genetic mobility is discussed in more detail below). A review of the nature of the resistance to specific antimicrobial compounds, and whether the resistance is intrinsic or transferrable, should be considered to evaluate risk of the resistance gene.

When identifying hazards associated with antibiotic resistance, it is important to draw a distinction between acquired and intrinsic antibiotic resistance, as the genetic basis of resistance has different implications. Acquired resistance occurs through gene exchange either within (intracellular, vertical) or between (intercellular, horizontal) bacteria. Vertical gene transfer (VGT) is often synonymous with mutation and the propagation of genetic information when bacterial cell division occurs, while horizontal gene transfer (HGT) occurs through the mechanisms of transduction, conjugation, and transformation. Transduction involves a virus of bacteria, otherwise known as a bacteriophage, while conjugation is

through direct contact between bacterium using pili. In contrast, transformation refers to the direct uptake of exogenous genetic material from the environment. In contrast, intrinsic resistance to antibiotics is typically chromosomally-encoded and is often due to structural or functional characteristics inherent to that microorganism. Such as: the physical structure of bacterial spores makes them more resistant to being killed. Other factors, such as reduced cell membrane permeability, can also contribute to intrinsic antibiotic resistance. Intrinsic resistance is essentially more predictable, given that it is characteristic to the bacterial species and not unique to a strain.

In addition to the above, is also important to consider the identity of the antibiotics against which resistance is observed or anticipated, and the roles of those specific antibiotics in typical clinical treatment.

Antibiotic Production. It is known that some microorganisms can produce antibiotics which should be considered during hazard identification because (a) some antibiotics can be allergens and (b) the production of antibiotics can impact beneficial microorganisms or microbial communities. Both genetic and physiological screening tools can be used to assess the potential for antibiotic production by a given microorganism. It should be noted that microorganisms can produce antibiotic compounds at levels that may not be biocidal, and which do not support or imply biocidal activity for a given MBCP.

Known Virulence Factors. The term "virulence factor" refers to compounds, structures, or regulatory elements which contribute to a microorganism's pathogenicity. While many of the items discussed above can be classified as virulence factors (e.g., toxins), virulence factors can also refer to various other aspects of microbial physiology that are less intuitively related to pathogenicity (e.g., ability to form biofilms, presence of quorum sensing systems, etc.). Virulence factors important to pathogenicity have been identified and characterized for a number of different microbial genera and species. As such, an evaluation of the presence or absence of such known genes encoding potential virulence factors is a prudent step to take when identifying the hazards associated with a given microbial ingredient. As mentioned above, such evaluations can be conducted through targeted testing and/or through assessing a given microorganism's genome and conducting a bioinformatics review against databases, such as the Virulence Factors Database (VFDB; as one example).

Mobile Genetic Elements. Mobile genetic elements (MGEs) represent a means by which genetic information can be transferred between microorganisms. MGEs are genetic material which can move within a genome or between bacteria. MGEs include, but are not limited to, plasmids, prophages, and transposons. For example, plasmids are small, circular Deoxyribonucleic acid (DNA) molecules that are physically separate from chromosomal DNA. They replicate independently from the bacterial chromosome and can be passed from one cell to another through HGT. In contrast, prophages are sequences of bacteriophage genetic material that have integrated into a host cell's genome and may carry genetic cassettes that have the potential to code for toxins, antibiotics, virulence factors, etc. Under certain conditions, lysogenic bacteriophages that present as prophages in a host genome can enter a lytic phase, which results in the expression of bacteriophage genes, bacteriophage replication within the host bacterium, and cell lysis resulting in release of bacteriophage progeny outside the cell. This process can generate viral particles which contain genes conducting virulence factors and can subsequently be transferred to other bacteria via the bacteriophage's lysogenic lifecycle.

An evaluation of the presence or absence of MGEs is important because a variety of genes conducting virulence factor, including those for production of some toxins, resistance to antibiotics, etc., can be spread through these elements. Accounting for mobile genetic elements helps ensure that relevant genes

encoding virulence factors are assessed during hazard identification. This accounting also provides information regarding the likelihood that virulence features potentially present in one microbial ingredient could be spread to other microorganisms which are more likely to exhibit pathogenic potential. The evaluation of transferrable genetic elements can include genetic analyses and a review of whether the genes of concern are present on MGEs.

Lifecycle. In addition to the above, because microbial ingredients of MBCPs are living organisms, the anticipated lifecycle of these organisms in a given use case should be assessed in order to contextualize how hazards may be actualized or changed (a) when formulated into a microbial cleaning product, (b) within the product's package during storage, and (c) following application of the product to treated surfaces.

Example Extrinsic Factors

Impacts on Microbial Communities: In addition to the intrinsic factors of MBCP ingredients discussed above, hazard identification should include an evaluation of how the assessed microbe may interact with or otherwise impact other microbial communities such as those associated with normal skin flora or the gut microbiome.

Contamination. Although not an intrinsic quality of a given microorganism used in a MBCP, the potential for that ingredient to be contaminated by potentially hazardous microorganisms should be considered as part of hazard identification and, thus, controlled. This should include an assessment of the possibility of contaminant introduction during the manufacturing process of the microbial ingredients and the finished product and result in identification of manufacturing and quality controls intended to mitigate contamination or rapidly identify contamination should it occur. Since the specific hazards associated with contaminating microorganisms may not be known in advance, it is advisable for manufacturers to develop standing policies and procedures to rapidly identify and respond to contamination events, should they occur.

Summary for Hazard Identification for Microorganisms

In summary, elements to consider for the purposes of hazard identification for microbial ingredients of MBCPs include:

- Microbial identification using a suitable approach.
- Toxin production determined using bioinformatic approach and validated using mammalian cell culture assays.
- Virulence factors identified and evaluated in terms of overall microbe/host interaction and infection.
- Acquired antibiotic resistance – Genes relevant to World Health Organization (WHO), Health Impact Assessment (HIA), and Critically Important Antimicrobials (CIA) lists identified and tested for relevance using MIC methods. Genes that confer resistance above breakpoints are evaluated at the bioinformatic level for intrinsic/acquired nature and potential mobility.
- Mobile genetic elements – Analysis of MBEs to understand their presence and genes they carry and the risk to transfer genes of concern to other microorganisms.

Example Tools for Evaluation of Intrinsic Factors

Many of the hazards discussed above can be identified using genetic approaches, like WGS coupled with bioinformatics, to enable accurate microbial taxonomic identification and provide a genetic basis for pathogenic and toxigenic potential in ed by comparing sequences to various databanks to screen for sequences of concern associated with antibiotic resistance, virulence factors and toxins. Examples of databases that are commonly used are:

- Antimicrobial resistance (AMR): The Comprehensive Antibiotic Resistance Database (CARD)
- AMR: ResFinder
- AMR: Antibiotic Resistance Gene Annotation (ARG-ANNOT)
- AMR: Center for Genomic Epidemiology
- AMR: Pathogenicity Island Database (PAIDB)
- Gene Transfer: PLSDB (uni-saarland.de)
- Gene Transfer: Insertion Sequence Finder (ISFinder)
- Gene Transfer: Phage Search Tool Enhanced Release (PHASTER)
- Gene Transfer: oriTfinder
- Gene Transfer: Integrative and Conjugative Elements (ICEberg)
- Virulence: Virulence Factors Database (VFDB)
- Virulence: Database of VF in Fungal Pathogens (DFVF)
- Virulence: The Toxin and Toxin Target Database (T3DB)
- Allergenicity: Allergen Online

In addition to the above, it is also important to be aware of scientific frameworks that can be used to evaluate the output of genetic analyses for hazard identification purposes (for example, see Negi et al., 2017 which describes a framework for toxin evaluations).

Perspective on Hazard Identification

Hazard identification is an integral part of a risk assessment because it helps determine the endpoints to focus on during risk characterization. Although the list above provides examples for hazards that should be considered during hazard identification for microbial ingredients, it is important to note that this list is non-exhaustive. Adequate review of the available scientific literature, as well as other data sources (internal testing, supplier information, etc.), can help to identify additional microorganism-specific hazards that need to be considered.

A hazard only becomes a concern if the exposure (or dose) resulting from product use is significant enough to cause an acute or chronic effect under normal conditions of use, and foreseeable misuse. As discussed in the next chapter, the potential for user exposure to any hazardous ingredient is based on the level of inclusion, product format, mode of use and frequency, and duration of use. These potential exposures can then be compared against established safe benchmarks, if available, to characterize the risks resulting from exposure.

Chapter 4 – Exposure Assessment

Exposure assessment evaluates the number and type of microorganisms the user may be exposed to during intended use and foreseeable misuse. Topics to consider when assessing exposure include product composition, concentration of microorganisms or spores, changes during storage over product shelf life, and usage and application including inadvertent direct and indirect contact by skin (e.g., pourable, spray, wipes), eyes and airways (aerosols both intended and inadvertent), and introduction to the gastrointestinal tract through contact (e.g., hand to mouth) during application. If the product is intended to remain for a period time on a surface or other object, the survival, regrowth, and exposure to the user by various routes following product application needs to be considered, as well as the potential for reapplication. In addition to direct user exposures, indirect exposures and potential exposures to individuals other than product users should be considered, as well. High levels of uncertainty in an exposure assessment may require measuring exposure under simulated use conditions or during actual consumer use to learn about potential microbial exposure when using a given product.

Determining the user exposure values is essential for thorough risk assessment. In the absence of good quality exposure data, conservative worst-case assumptions and uncertainty factors are employed, which may lead to an overestimation of exposure levels and thereby unnecessarily limit the microorganisms (e.g., type, quantity) that can be used in a product. Therefore, additional studies to reduce the uncertainties may be initiated by the risk managers to help reduce overestimation.

This chapter describes methods and approaches used to estimate exposure to microorganism from the use of microorganism-containing products.

Factors Influencing Exposure

Many factors related to product use or applications are important determinants of overall level, frequency and duration of exposure. Comprehensive answers to the following questions, as well as others, are needed to conduct optimal exposure assessment and risk assessment.

- What is the formulation and delivery mechanism of the product being assessed?
- How is the product going to be used under normal conditions, including frequency of use and duration?
- What may be the conditions of foreseeable misuse?
- Where will the product be used?
- What is the potential for user exposure to the product (direct or indirect)?
- Is the product concentrated such that dilution is required prior to use?
- What is the anticipated fate, persistence, and life cycle of the microbial ingredient post-application?

Product Formulation and Delivery Mechanism

Exposures to a given microbial ingredient can be impacted by the nature of the MBCP itself and how that product is delivered under recommended conditions of use.

Product Formulation: The physical and chemical properties of a formulation influence the potential for exposure (e.g., respiratory tract, skin, eyes). The potential for aerosolization of liquids (sprays), powders and foams leading to inhalation and contact with mucosal membranes should be evaluated during product development. This can be affected by the delivery mechanism and viscosity of the product. Aerosols

should be characterized in terms of their droplet or particle size and/or distribution; Droplet size along with their density, can determine their rate of settling and thus, the concentration of microorganisms in the air during and after use. Large droplets or particles have the advantage of settling out of the air quickly; however, droplet and particle size can change during application and may be dependent on the application method (e.g., trigger type). For example, liquid droplet size can decrease after impact on a surface during spray application, potentially leading to a higher percentage of particles that are of respirable size. An assessment of the particle size and distribution should be included in the product formulation evaluation to determine if inhalation risks exist. An assessment to the potential for exposure to skin and eyes should also be evaluated especially for those with active skin conditions or where the potential exists for surface-to-eye transfer.

Delivery System: When formulating a product, consideration should be given to how the design of a delivery system can affect user exposure. Packaging can have a significant impact on the extent and route of exposure to the product. Unit dose delivery systems provide an inherent reduction of exposure by design. A spray delivery system has the highest potential for inhalation exposure and should be designed carefully to minimize the production of inhalable mists.

Use Conditions

Normal Product Use: For product use under normal conditions, the amount of product used per application, duration of usage, frequency of use, and refilling of containers are factors that affect the exposure to the product. The refilling of product containers will need to be given careful consideration given the increased potential for exposure and contamination. Knowledge of the habits and practices of product users is important for a thorough understanding of potential exposure during a product's use. These data can be obtained by conducting market surveys and consumer tests (discussed below) to determine how the product will be used.

Potential Misuse: In addition to the above, the potential for intentional or unintentional product misuse should be considered during an assessment of potential exposures. Misuses may result in higher exposures than can be anticipated during recommended product use. These differences should be considered before extrapolating the results of any exposure assessment from one user group or geography to another. These differences should be investigated carefully to ensure proper characterization of exposures in all parts of the world where the product will be marketed. If appropriate, advisory statements could be applied to a particular product label to guard against misuse.

Use Location and Use Sites

There are many product types on the market today with varying use sites ranging from household and professional cleaners, animal housing products, drain and sewer cleaners, etc. The physical environment in which the product is used also influences the extent of exposure. Therefore, several factors should be considered with respect to the physical environment. For example, factors such as room size and ventilation will affect exposure. Use of a product outdoors can lead to a different exposure in the breathing zone of the user as compared to the use of a product in a small room with poor ventilation. The orientation of the consumer relative to the product during use (i.e., breathing zone relative to the source of microbial aerosols) will influence exposure as will use sites.

Potential for Direct/Indirect Exposure

The possible exposure routes for the product should be considered during the evaluation of exposure. Potential exposure routes may be impacted by the MBCP's formulation and delivery mechanism, use conditions (including potential misuse), and use locations/sites as described above. The most common routes of exposure are listed below.

Inhalation. A major route of exposure to be considered is inhalation. Inhalation exposure potential will be determined by the product format, use directions, and use sites. Exposure may arise from intentional pouring of powdered or liquid products, stirring or agitating product solutions (e.g., hand laundering), spray applications, vacuuming powder products or liquid products that have dried (e.g., carpet cleaning), or via other means.

Dermal. Skin exposure may occur during product use (e.g., hand laundering, surface cleaning) or from incidental exposure. Dermal exposures will also occur post-application for products that are applied to and remain on surfaces.

Oral Exposure/Ingestion. Direct oral exposure and potential ingestion of the product during its application may occur. Indirect oral exposure/ingestion may occur through various mechanisms including the treatment of food-contact surfaces, children mouthing previously treated surfaces (e.g., textiles), and hand-to-mouth activity following dermal exposures. Indirect oral exposures may also result from touching or other interactions with previously treated surfaces.

Ocular. Direct ocular exposures of the product during its application may occur due to splashing, spray dispersal, etc. Indirect ocular exposure may occur through various mechanisms, including dermal exposures on hands or the treatment of certain surfaces which may be touched prior to interactions with the eye (e.g., putting in contact lenses).

Product Dilution

Some MBCPs may be sold as concentrates that require dilution prior to use. Exposures to concentrated products may increase total exposure due to the presence of higher microbial concentrations in the concentrated form. Furthermore, the potential for intentional or unintentional misuse of a concentrated product exists. With concentrated products, the process of product dilution for the purposes of refilling a smaller container (e.g., trigger spray) may furthermore offer an opportunity for accidental exposures due to spilling, splashing, etc. As stated previously, refilling product containers will need to be given careful consideration given the increased potential for exposure and product contamination.

Post-Application Fate

Because microbial ingredients of MBCPs are living microorganisms, the anticipated fate, persistence, and lifecycle of these organisms both (a) within the product's package during storage, and (b) following application of the product to treated surfaces can influence exposure. The potential for the microorganisms to be in a steady state (e.g., spore form) or in a growth phase (e.g., vegetative cells) needs to be considered during the formulation process and assessed as part of the risk analysis.

Assessment of Consumer Exposure and Professional/Institutional Exposures

There are several approaches that can be taken to collect the information on product use and the influence of product use on exposure. Some approaches are described below:

Consumer Tests. The habits and practices of the product user can be evaluated by conducting tests in a setting where the product will be operated under circumstances of normal use. It may be beneficial to obtain product-specific exposure measurements during user tests and laboratory studies simulating in-use exposures. In addition, indirect exposures (e.g., deposition on fabric, glassware, utensils, solid surfaces) should be assessed, when appropriate.

Market Surveys and Questionnaires. Market surveys and questionnaires are used to evaluate parameters of product use. Generally, they are used to assess product efficacy and whether the product is being used safely as consumers can be asked questions on the use of the product (e.g., How much product was used for the task? How long did the task take? Were use instructions and precautionary labeling followed?). Feedback from consumers can be used to guide further product development, as well as validate and refine existing safety risk assessments. In addition, market surveys help identify potential non-recommended uses of the product. These non-recommended uses may increase exposure to levels that were not considered in the initial exposure assessment for foreseeable misuse, thereby warranting a reassessment of exposure.

Poison Control Centers. Poison control centers can collect data and also provide valuable information regarding trends, misuse or accidental exposures to products. In the United States, summarized poison control center data are published in the "Toxic Exposure Surveillance System." These data can be obtained by contacting the American Association of Poison Control Centers via email at aapcc@poison.org, or through their website: www.aapcc.org.

Manufacturer's Telephone Helpline. Consumer comments received via telephone helplines operated by product manufacturers provide additional useful information concerning use and misuse of products by consumers. This information is typically not found in the public domain.

Estimating Exposure

A risk analysis for microbial ingredients of MBCPs will require numerous individual scenarios of possible exposure and hazard assessment. A global assessment applicable to all MBCPs is probably not feasible to conduct and would likely be meaningless. For example, the scenarios of a child mouthing an article of treated clothing verses an elderly person treating a kitchen counter will have different outcomes and also different interpretations of risk and potential mitigations. The public and regulatory acceptance of these products will likely depend on the relatively few scenarios that have unacceptable risks.

Each scenario needs to be separated into a series of individual steps from manufacture to human exposure, and the changes in microbial populations need to be estimated at each step. For example, data are needed with respect to changes in microbial numbers as the product is in storage (and following MBCP application), how much of the liquid product remains on a user's hands after wiping a surface with the product, and how much liquid might then be transferred to a user's lips if they touch their lips with the wet hands. Some of this data will need to be determined on a product- and/or ingredient-specific basis while other data may be inferred from studies previously done for other purposes, such as the amount of water that remains on a user's hand.

For each of these steps, estimates of the most likely and worst-case values for all of the relevant parameters needs to be in the exposure assessment. Different trials within a scenario in which various values are tested can be used to calculate quantitative levels of exposure or provide relative exposures between trials that can be compared.

Exposure can be estimated initially from available data. The assumptions used in the estimation should be based on consumer habits and practices and the other factors referred to in the preceding paragraphs.

The first step in estimating exposure usually involves a conservative theoretical calculation using reasonable worst-case assumptions (e.g., using the entire packaged product at one time) while employing uncertainty factors. If there are insufficient data to allow a reliable estimate of exposure to be developed, then actual exposure measurements should be obtained before making a final risk estimation.

Measurements of Exposure

The exposure measurement process can be divided into simulation of the exposure, sample collection, measurement of microorganisms in the samples, background assessment, and carryover prevention. Relative to conservative theoretical calculations incorporating various uncertainty factors, actual measurements provide a more accurate assessment of the exposure and, thus, produce a more reliable basis for estimating the risk of using that product. Measurements can also be used to reduce uncertainty factors otherwise applied to the exposure estimation.

Simulation of the Exposure

Simulated use studies can be used to help understand the nature and extent of exposures resulting from product use. The exposure simulation procedure used should be developed based on habits and practices data, including visual observation of product use habits. This is important for the development of a procedure that provides an accurate representation of the consumer habits and is representative of consumer exposure.

Sample Collection

The procedures used for sample collection can vary depending on the type of exposure being assessed. It should be noted that the measurement procedure developed may need to be specific for the product, microorganism type (spore, vegetative, media requirements), and microbial level used. Validation of the procedure and equipment should be conducted prior to making the exposure assessment. Such validation is necessary to ensure that new data can be compared to values obtained previously.

Background Assessment

Before conducting any exposure measurements, assessment of the test area for the presence of contaminating microorganisms should be performed.

The results of the exposure assessment, along with the benchmark data, are utilized in risk characterization, discussed in Chapter 6.

Chapter 5 - Hazard Characterization

In this step of the risk assessment process, the relationship between the exposure and the specific biological effect is characterized. The dose-response assessment consists of determining the amount of exposure (i.e., the delivered dose) and the corresponding adverse effect for different subpopulations, such as neonates or immunocompromised individuals. The delivered dose will be a function of the level, duration, pattern, and route of exposure. This process is not trivial, since the dose-response relationship for many microorganisms is not clearly defined.

Dose-Response Estimations and Benchmarks

Dose-response estimation for populations is inherently a statistical process. Ideally, a quantitative mathematical model is developed that relates exposure to the likelihood of adverse effects. However, for microorganisms, there are often very little data on the dose-response, so a benchmark approach must be used to assess risk. This may be applicable for a closely related microorganism. In the absence of a level of predictability, exposures are estimated or measured empirically to establish uses that avoid adverse effects. As defined in Chapter 3, a benchmark dose is a value derived from a study or studies that is generally considered to be safe by scientific and medical experts and can be used as a pass/fail criterion. In the risk assessment process, the exposure level estimated for a use application is compared to benchmark values to assess risk.

Caution in the Use of Benchmarks

Caution should be used in the application of benchmarks. Exposure data needs to be relevant to a particular use or misuse for comparison to a newly derived exposure value. Furthermore, the limitations in measurement at the point of exposure may or may not relate to the actual internal body dose. As discussed in previous chapters, the actual dose is nearly impossible to obtain with current methodologies. Care should also be taken when extrapolating from one product type to another since the exposure conditions may be too different to be comparable.

Chapter 6 – Risk Characterization

Risk characterization is the examination of the relationship between human exposure (calculated or measured) and the inherent harm of a substance which is used to assess the likely incidence and severity of any effect. This step is important because it integrates information regarding the hazard identification and exposure assessment associated with use and foreseeable misuse of a product.

Overall risk is characterized in microbial risk assessments either qualitatively or quantitatively. The risk estimate is based upon exposure levels and frequency for the general population (e.g., non-immunocompromised) and sensitive subpopulations (e.g., immunocompromised, elderly, infants/children) for specific adverse health outcomes, such as a specific illness, allergic reaction, hospitalization, or death. For qualitative risk characterization, a risk estimate is typically expressed with a scale that ranges from 0 – 4: (0) negligible likelihood of occurrence, (1) very low, (2) low, (3) medium, or (4) high. This could be developed into a semiquantitative estimate, such as "low" equals 1 illness per 10,000 exposures. Quantitative risk estimates are more probabilistic and expressed for a given exposure as either the individual risk (probability of illness/number of exposures) or at a population level, such as the number of adverse outcomes per population size (e.g., 1.3 illnesses/100,000 population).

Current knowledge generally does not allow for quantifying hazards associated with microorganisms, for example, the production of a toxin or an allergen, in MBCP scenarios. Instead, for microbial ingredients of MBCPs the risk characterization process typically relies on comparing potential exposure to benchmark values or resorting to determining the likely presence/absence of a hazard. Here, a benchmark can be defined as the maximum hazard exposure generally considered to be safe by scientific and medical experts and used as a pass/fail criterion. In the latter case, the hazard identification - where the presence of virulence factors, toxin genes, allergens, etc. is determined - is the critical component in the risk characterization.

Although the information presented in this section is generally representative of current risk assessment practices, it should be recognized that this is an ever-evolving discipline. In the future, the methods and procedures used by practitioners should be modified, as necessary, to reflect the most current (and best) scientific practices. This may require a risk assessment to be re-evaluated for a particular product over the time it is in the marketplace.

Risk Characterization Process

As described above, the components necessary for risk characterization of microorganisms include hazard identification, a dose-response relationship, and an estimate of potential exposure. Other considerations include comparison of exposure to benchmarks and an application of general knowledge regarding hazard endpoints of microbial products.

The results of the risk characterization are an estimate of the likelihood and severity of the potential adverse events for the general population (e.g., non-immunocompromised) or a sensitive subpopulation (e.g., immunocompromised, elderly, infants/children). If the risk characterization can determine whether the risk generated for the new exposure is at or below an applicable no-effect benchmark, then the risk may be judged acceptable.

However, the setting of the significance of the risk for a product/scenario is the risk manager's decision. Safety is ultimately a judgement to be made by risk managers with communication and input from stakeholders.

Risk characterization provides a science-based support that risk managers can use to help control hazards. Prior to commercializing a product, it is often helpful to present the data gathered from the risk assessment to peers internally, if sufficient expertise exists in-house, or to experts external to the company. Conducting a peer review of the results of the risk assessment can provide different perspectives regarding the assumptions, methodology, and subjective interpretation inherent in the process. Once the risks are estimated and questions answered from the risk assessment, as summarized in the risk characterization, the risk managers may conclude whether the product is suitable for consumer use as is or if further studies or modifications are necessary.

Generation of Additional Data

Since risk characterization involves evaluation of substantial amounts of information from a variety of sources, there may be some uncertainty in the final assessment. Data quality and quantity are relative to the needs of the risk assessment. The overall confidence in the underlying data is judged to be too low to have confidence in the results, then consideration has to be given to obtaining additional data to better understand the exposure or dose-response. Judgment is required to decide whether or not refinement of the risk characterization is warranted and, if so, whether or not development of additional data is practical.

It is recommended that care be taken when considering new applications for an existing microorganism that may already have proved acceptable for another use. Data may need to be generated to support the safety of use in these new applications. For some new applications, a benchmark may not exist.

Chapter 7 – Risk Management

The objectives of the risk management process are fundamentally two-fold. First, before the risk assessment is started, there is the need to consider the necessity to conduct the risk assessment. Second, there is a need to define the questions to be answered, the goals of the risk assessment, and to interact periodically with risk assessors to ensure those goals are being met. At the end of the risk assessment, risk managers need to determine the significance of potential risks to human health, to ensure that the product use is and remains within the accepted risk levels, and to effectively work with risk communicators about potential risks and appropriate communication to target audiences about the proper use of the product.

Typically, risk managers are in the organizational position to provide staff, funds, and time for the risk assessors. Although risk management is sometimes thought of as a process that occurs after the risk assessment is completed, risk management decisions must be made at the beginning and reevaluated throughout the course of acquiring the risk assessment data. The initial risk assessment may reveal a lack of appropriate data necessary to reach the desired conclusion and the risk managers may initiate research to obtain that data.

Determining the Relevance of a Risk Assessment

Risk assessment provides useful information so that risk managers can weigh alternatives and analyze tradeoffs in addition to providing a means of organizing relevant information in order to estimate the potential impact on human health. In doing so, assessments may convey a level of precision that fails to reflect the shortfalls of the underlying assumptions and the uncertainties that may characterize the risk assessment. The quality and reliability of the risk assessment are dependent on, and are only as good as, the data used to conduct the assessment. Uncertainties may exist in dose-response relationships, defined benchmarks, exposure data, and estimates from exposure models. Assumptions and estimations need to be stated clearly as they can affect the reliability and quality of the risk assessment. It is important to consider these points when evaluating information from the risk assessment in determining whether or not the risk is considered acceptable.

Accepted Level of Risk

The setting of a benchmark or evaluating the significance of the risk for a product/scenario is the risk manager's decision. The risk assessment process does not define an acceptable level of risk; therefore, risk managers need to articulate what exposure to the hazard, if any, is acceptable in context of public perception and benefits of the product. No numerical level of risk will receive universal acceptance. Further, it is impossible to eliminate all risks associated with a particular activity, and this is also true for the use of microorganisms in finished products. Safety is ultimately a non-scientific judgement to be made by risk managers with communication and input from stakeholders.

Risk management approaches should be based on critical evaluation of the risks associated with the use of the product and the data generated from the risk assessment process. If screening level assessments based on estimates of exposure and available hazard information are not sufficient to support the safe use of the product, then the collection or generation of additional data as discussed in the risk characterization section could be considered. If the completion of the risk assessment results in the determination that the risk exceeds the accepted benchmark, then appropriate risk control measures should be carried out to reduce the exposure or hazard to within acceptable risk levels.

Risk assessments for a given product and usage may not be applicable for another product or application. It is important to understand these differences, as well as the effect of other exposure factors, such as frequency and duration of exposure, as these are important factors to consider in the risk management process and can further complicate the establishment of an acceptable level of risk.

If the risks associated with product use are acceptable and product is accepted, then surveillance of the marketplace experience should be used to assure that the risk assessment was correct and exposure to the microorganism(s) was indeed safe. If the risks associated with the product uses are not acceptable, product modification and re-evaluation of the risk characterization using information based on the modified product is recommended.

Risk Control

In general terms, the risk management process should strive to reduce the risk by assessing control options that limit exposure to microorganisms or other constituents from the product. Risk reduction options may include product modification, product use restrictions, labeling modifications, or a decision not to commercialize the microbial-based product.

As stated previously, the goal of risk control is to decide, based on an acceptable risk level, whether product modification or restrictions on its use is necessary. If product modification or restriction on use are not alternatives, and there is a likelihood of an adverse experience or event, not commercializing the product is also an option to be considered.

Risk Communication

An integral part of the risk analysis process is to effectively communicate the potential risks to appropriate audiences. There are two important audiences to target in designing a risk communication program: (1) users of the company's products and (2) other stakeholders, such as the general public and public interest groups.

Risk communicators may oversee preparing an interpretive summary and other publications, and in holding appropriate forums and public presentations, present the risk assessment and disseminate results.

Risk Communication to Product Users

Product labels and digital communications are the primary means of informing consumers. For MBCPs, as with all consumer and professional products, many countries require that the label include appropriate warning statements. In the U.S., the regulations of the Consumer Product Safety Commission apply; in Canada, those of the Consumer Chemicals and Containers Regulations apply; and in the European Union, those of CLP Regulation, Biocidal Products Regulation, and Detergent Regulation apply. In addition, there may be requirements to place handling instructions and information or first aid information on the label. Product manufacturers can also supplement this safety-related information, as needed.

To address other questions, manufacturers should have properly educated customer support personnel to provide answers to customers and effectively communicate issues to the public regarding the safety of microbial-containing products. Examples of essential information that customer support personnel should be able to communicate are as follows:

- Composition of the product;
- First aid information; and

- Use and handling guidelines, with detailed examples of correct use and concrete recommendations to steer consumers from misuses.

Further work is needed in risk communication to explain the benefits of the use of microorganisms in MBCPs to the general public since the understanding of the use and function of microorganisms in products varies. Labeling the product should be done carefully to address all of the information that is required under the relevant regulation (e.g., product use directions, use sites, precautionary language and first aid, etc.).

Risk Communication to Other Stakeholders

Other stakeholders may be governmental authorities, non-governmental organizations, or industry partners. An important route toward gaining acceptance of stakeholders is through interaction among experts in the field or industry, government authorities, and other interested parties, such as consumer associations, scientific journalists, and academia. The goal is to build confidence in the company and/or industry using the technology.

An attitude of openness and willingness to share information and data is essential, while recognizing the legitimate needs of companies to protect competitively sensitive information. The amount and detail of information that may be needed in dialogues with some stakeholders may be more extensive than what is provided to the general consumer. Position papers and dossiers giving details of the product with particular reference to the microorganisms used may be considered. In addition to information relevant to consumers and workers, product manufacturers should anticipate stakeholder requests related to the production process and containment procedures employed in the production facility, since levels of exposure in the workplace are generally higher than in the product use setting if exposure management steps are not taken. Finally, any document or dossier provided might be more readily accepted if it has been subjected to a peer-review process.

In short, an effective risk communication program requires the ability to provide useful information in response to worker, consumer, and other stakeholders' inquiries. By providing this information, it is possible to promote the safe use of the product and, in turn, reduce the risks associated with exposure to the microorganisms contained in the product.

Chapter 8 – Conclusion

Prior to introducing a microbial preparation into a product, a risk assessment should be conducted under the direction of the risk managers to ensure that any risks associated with the microbial component of the MBCP are identified, assessed, and minimized to the extent practicable and to ensure the outcome does not exceed accepted risk.

Three appendices are attached to facilitate utilization of the practices and principals discussed in this document. Appendix 1 includes a list of example questions that risk assessors and managers may use to facilitate evaluation of their MBCPs and microorganisms used in those products. Appendix 2 includes a checklist that may also be useful for this purpose, or otherwise adapted for use in a company's risk assessment program. Appendix 3 provides three high-level case studies which demonstrate in general terms how the different components of this document may be used to proactively assess MBCP risk prior to commercialization.

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APPENDIX 1
Example Questions to Support Risk Assessment

Appendix 1: Example Questions to Support Risk Assessment

Hazard Identification: Microbial Identity

- Is the microorganism(s) Risk Group 1?
  - Organism Type 1
  - Organism Type 2
- Is the micro-organism(s) in a Risk Group other than Risk Group 1 (e.g., 2, 3, 4)?
  - *In which risk group are they listed?*
- Is there a history of safe use of the species or strain?
  - *Which product or products?*
- Has the genome been sequenced?
  - *What method was used to sequence the genome?*
- Has the genome been annotated or otherwise evaluated for hazards?
  - *What method was used?*
- Is there a method to identify the strain with a high degree of certainty?
  - *What method was used to identify the strain?*
    Consideration should be given to the method(s) of identification i.e., one may not be sufficient (e.g., 16s), and their appropriateness for the microorganism.
- Are there genes present that could be involved in toxin production?
  - *Which toxins?*
- Are genes involved in potential toxin production expressed?
  - *Are the associated gene products excreted?*
- Are antibiotic resistance genes present?
  - *Which antibiotics resistance genes are present?*
  - *If present, are they intrinsic or acquired?*
- Is the antibiotic resistance phenotype expressed?
  - *If expressed, is it intrinsic or acquired?*
- Are antibiotics being produced?
  - *Which antibiotics are being produced?*
- Are there prophages present that carry genetic cassettes potentially coding for toxins, antibiotics, virulence factors, etc.?
- Are genes encoding virulence factors present?
- Are there plasmids or other mobile genetic elements?
- Were the plasmids or other mobile genetic elements genes characterized?
  - *How were the plasmids or other genetic element genes characterized?*
- Are the plasmids or other mobile genetic elements transmissible?
  - *How was the transmission (or lack of transmission) of the plasmids or other mobile genetic elements determined?*
- What is known about the life cycle of the strain: germination, growth rate of vegetative cells, sporulation?
- What is known about the intrinsic and extrinsic factors that influence growth and survival (e.g., generation time, growth temperature [optimum and range], pH, oxygen requirements, preferred energy and carbon sources, etc.).
- What is known about the strain's interaction with other microorganisms?

- What factors affect growth, survival, or reproduction (e.g., sporulation, encystment, other non-vegetative growth stages, ability to exist in the viable but non-culturable (VNBC) state, autotrophy, etc.)?

Hazard Identification: Host Factors

- Is there potential for infection, toxicosis, or allergic reaction in the general population (e.g., non-immunocompromised)?
    - *Which one(s) (infection, toxicosis, or allergic reaction)?*
- Is there potential for infection, toxicosis, or allergic reaction by at-risk sub-populations (e.g., immunocompromised, elderly, infants/children)?
    - *Which one(s) (infection, toxicosis, or allergic reaction)?*
- Will there be secondary exposure to non-users of the product but who may be exposed to the product?
- Will the product be used in commercial (e.g., retail, offices) applications?
- Will the product be used in residential (e.g., homes) applications?
- Will the product be used in healthcare (e.g., hospitals, clinics, nursing homes) applications?
- What potential adverse effects have been reported (e.g., literature)?
- Are potential adverse effects localized or systemic?

Hazard Identification: Formulation

- What ingredients are in the formulation?
    - What are the particular formulation components
    - What are the details for each of the components (e.g. CAS number and strain identification)?
    - What is the percentage of each of the components in the formulation?
    - What is the purpose of each of the formulation components?
- What are the product properties?
    - Viscosity, pH, Density, CFU/mL, Total Solids, Color Odor, etc.
- Are there sufficient preventative controls in place to prevent contamination of the finished product during manufacture?
- Does the shelf-life or storage conditions impact the number of micro-organisms present and their viability?
    - *If yes, describe.*
- Does the storage of the product provide a risk to contamination during use?
    - *If yes, describe.*
- Is the stability of the product compromised during storage of the product?
    - *If yes, describe.*
- What is the process used to produce the product (e.g., batch or continuous)?
    - *Describe the process*.
- What are the product directions for use, including frequency and duration of use including any recommendations for personal protective equipment (PPE) usage?
- What is the product mode of action?
- What is the intended shelf-life of the product?
- What are the recommended storage conditions of the product?

Exposure Assessment

- What is the product format (e.g. liquid, powder/solid, foam)?
- What is the mode of action (packaging) of the product including the application process and mode of use (e.g. trigger spray, wipe, pour, direct application (sponge, cloth, mop), or no direct contact (pod))?
- Is there potential for direct routes of exposure by applicators (dermal (chronic), dermal (acute), ocular, inhalation, oral/ingestion)?
- How often will the product be used (daily, weekly, monthly, one-time)?
- Is there potential for indirect routes of exposure by applicators (dermal (chronic), dermal (acute), ocular, inhalation, oral/ingestion)?
- Is there potential for direct routes of exposure by others (e.g., non-applicators) (dermal (chronic), dermal (acute), ocular, inhalation, oral/ingestion)?
- Is there potential for indirect routes of exposure by others (e.g., non-applicators) (dermal (chronic), dermal (acute), ocular, inhalation, oral/ingestion)?
- Is there potential for exposure concerns other than people (environment, pets, other animals, plants, other)?
- Does the physical environment affect exposure (indoor use, outdoor use, ventilation, air currents, temperature, humidity)?
- In what locations will the product be used (food contact surfaces (e.g. kitchen), non-food contact (e.g. bathroom), healthcare (e.g. patient rooms, common areas), other (e.g. outdoor)?
- Can the product be aerosolized (e.g., trigger, aerosol)?
    - *What is the droplet size and rate of settling?*
- If the product is aerosolized are the particles of inspirable size during direct application?
- If the product is aerosolized are the particles of inspirable size during indirect application?
- Does the product require dilution prior to use?
- Is the product a concentrate meant to be diluted and filled into other containers (e.g., refill for trigger bottles)?
- Is there a required delivery unit dose (e.g., grams per application)?
    - *Describe the unit dose requirements.*
- Is there a recommended reapplication rate?
    - *Describe the reapplication requirements.*
- What are the conditions of foreseeable misuse?
- How do the conditions of foreseeable misuse impact the potential for exposure?
- How many micro-organisms are estimated to be in contact with an individual for each exposure mechanism?
- What is the anticipated fate, persistence, and life cycle of the microbial ingredient post application?

Hazard Characterization: General

- What are the potential adverse health outcomes?
- Who are the at-risk subpopulations?
- Is there a quantitative pathway available to estimate dose-response?
    - *What is the pathway referenced?*

- Is the dose-response relationship well-characterized?
- Is there a known threshold of exposure to develop an adverse effect?
    - *What is the threshold?*
- Is it known how many exposures are required to cause an adverse effect?
    - *How many exposures? (1), (2), etc.*
- What is known about the dose-response relationship between exposure and each potential adverse effect for the general population and for each at-risk subpopulation?
- Is there a benchmark that can be used?
    - *What is the benchmark?*
- What is the expected outcome of the dose-response assessment?
    - *Risk of infection, risk of developing a specific symptom, allergic reaction, hospitalization, other (list).*

Hazard Characterization: Dose-Response Relationship

- Are the identity and amount of exposure to the hazards necessary to cause an adverse reaction been adequately described?
- Has a quantitative measure of the exposure, either level or total dose as appropriate been adequately described?
- Have the different human populations and their varying susceptibilities been adequately described?
- Have all possible routes of exposure (e.g., skin, inhalation, eyes) been adequately described?
- Have all possible durations of use for consideration of exposure (one-time, daily) been adequately described?

Risk Characterization

- Does the potential exist for both hazard and exposure?
    - Note to reviewer:
        - If yes, then the levels of exposure and the corresponding human response for the various scenarios are combined to estimate the likelihood that an adverse response occurs.
        - The human response may be quantitative (1 illness per 10,000 exposures) or semiquantitative (low to high likelihood).
        - A maximum exposure amount for the various different exposure doses, human populations and routes of exposure that can be judged as “safe” need to be determined.
- Is there adequate confidence, given the available data quality and quantity, in the risk characterizations for the various scenarios to allow the risk managers to make a decision on the safety of the product?
    - *What is the best approach to characterize the risk?*
- Can the risk event of interest be described simply by the nature, likelihood, severity, and consequences of the potential exposure to the hazard?
- Is there a quantitative approach available to characterize the risk?
    - *Is it better characterized qualitatively or descriptively?*

- Can the likelihood and severity of adverse events be estimated by the data available in this risk assessment?
- For a qualitative risk characterization, is the risk estimate (0) negligible likelihood of occurrence, (1) very low, (2) low, (3) medium, to (4) high?
- For a quantitative risk characterization can it be expressed as the estimated number of specific adverse events (illness, allergic reaction, deaths, etc.) per number of exposures for the general or at-risk subpopulations?
- Does the risk characterization show if the risk may be judged as acceptable?
- Has the risk assessment been presented to peers internally, if sufficient expertise exists in-house, or to experts external to the company?
- If different from the peer reviewers, have risk managers concluded whether the product is suitable for consumer use as is or if further studies or modifications are necessary?

Risk Communication

- Proper use of product is adequately communicated to users on product labeling (e.g., market label, supplemental material, websites, social media, etc.)?
    - Product composition
    - Storage and handling conditions
    - Where to use and where not to use product
    - How to use products
    - Personal protection required for use
- Label has appropriate cautions and actions to be taken if accidental events occur during use (e.g., skin contact, ingestion or eye contact with product)?
- Employees are informed of safety procedures to manufacture this product.
- Appropriately communicated with raw material suppliers about vendor qualifications established by the company?
- Develop and provide appropriate information to consumers/public about this product.
    - Product benefits
    - Mechanism of cleaning
    - Safety
    - Environmental implications

Regulatory and Registration Considerations

- Are there regulations or guidance documents from government sources that need to be considered?
- Are there regulations or guidance documents from non-governmental sources that need to be considered?
- Are there any pending regulations or guidance documents that need to be considered?
- Does the formulation contain any micro-organisms that are Genetically Modified (GMO)?
- Is the micro-organism(s) on the QPS list?

Risk Management: Vendor Qualification/Evaluation

- Have acceptable and documented quality control measures at the time of production for the micro-organisms?
- Control for strain purity?
- Control for contamination?
- Prevent cross-contamination if they manufacture multiple strains?
- Control for strain concentration?
- Provide a sufficient number of viable microorganisms per lot consistently?
- Have a history of strain stability?
- Documentation provided indicating each batch meets predetermined specifications and quality criteria?

Risk Evaluation

During risk evaluation, the following should be confirmed:

- Significance of potential risks to human health were appropriately evaluated.
- There was appropriate data to determine risk.
- The product use is within appropriate and acceptable risk levels.
- Are there benchmark doses that are generally considered to be safe by scientific and medical experts which can be used as pass/fail criterion?
- Risk communicators were briefed as to appropriate communication to target audiences.

Final Decisions

- The product is appropriate for its use. Hazards and risks have been sufficiently evaluated. Product may be commercialized.
- The product needs additional research to determine if it is appropriate for its use. Hazards and risks have not been fully evaluated. Continued research is recommended.
- The product is not appropriate for its use. Hazards and risks have demonstrated the product poses hazards and risks to human health and/or the environment. The commercialization of this product should be rejected.

APPENDIX 2
Checklist

APPENDIX 2: Checklist

Hazard Identification – Microbial Identity				
Considerations		**Yes**	**Investigate**	**No**
Is the microorganism(s) Risk Group 1?	Organism Type 1			
	Organism Type 2			
Is the microorganism(s) in a Risk Group other than Risk Group 1 (e.g., 2, 3, 4)? *In which risk group are they listed?*				
Is there a history of safe use of the species or strain? *Which product or products?*				
Has the genome been sequenced? *What method was used to sequence the genome?*				
Has the genome been annotated or otherwise evaluated for hazards? *What method was used?*				
Is there a method to identify the strain with a high degree of certainty? *What method was used to identify the strain? Consideration should be given to the method(s) of identification i.e., one may not be sufficient (e.g., 16s), and their appropriateness for the microorganism.*				
Are there genes present that could be involved in toxin production? *Which toxins?*				
Are genes involved in potential toxin production expressed? *Are the associated gene products excreted?*				
Are antibiotic resistance genes present? *Which antibiotics resistance genes are present?* *If present, are they intrinsic or acquired?*				
Is the antibiotic resistance phenotype expressed? *If expressed, is it intrinsic or acquired?*				
Are antibiotics being produced? *Which antibiotics are being produced?*				

Hazard Identification – Microbial Identity			
Considerations	**Yes**	**Investigate**	**No**
Are there prophages present that carry genetic cassettes potentially coding for toxins, antibiotics, virulence factors, etc.?			
Are genes encoding virulence factors present?			
Are there plasmids or other mobile genetic elements?			
Were the plasmids or other mobile genetic elements genes characterized? *How were the plasmids or other genetic element genes characterized?*			
Are the plasmids or other mobile genetic elements transmissible? *How was the transmission (or lack of transmission) of the plasmids or other mobile genetic elements determined?*			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Hazard Identification – Microbial Identity	
Considerations	
What is known about the life cycle of the strain: e.g., germination, growth rate of vegetative cells, sporulation? **Response:**	
What is known about the intrinsic and extrinsic factors that influence growth and survival (e.g., generation time, growth temperature [optimum and range], pH, oxygen requirements, preferred energy and carbon sources, etc.)? **Response:**	
What is known about the strain's interaction with other microorganisms? **Response:**	
What factors affect growth, survival, or reproduction (e.g., sporulation, encystment, other non-vegetative growth stages, ability to exist in the viable but non-culturable (VNBC) state, autotrophy, etc.). **Response:**	
Decision Points	
Continue evaluation	
Investigate further	
Stop evaluation	
Notes:	

Hazard Identification – Host Factors			
Considerations	**Yes**	**Investigate**	**No**
Is there potential for infection, toxicosis, or allergic reaction in the general population (e.g., non-immunocompromised)? *Which one(s) (infection, toxicosis, or allergic reaction)?*			
Is there potential for infection, toxicosis, or allergic reaction by at-risk sub-populations (e.g., immunocompromised, elderly, infants/children)? *Which one(s) (infection, toxicosis, or allergic reaction)?*			
Will there be secondary exposure to non-users of the product but who may be exposed to the product?			
Will the product be used in commercial (e.g., retail, offices) applications?			
Will the product be used in residential (e.g., homes) applications?			
Will the product be used in healthcare (e.g., hospitals, clinics, nursing homes) applications?			
What potential adverse effects have been reported (e.g., literature)? **Response:**			
Are potential adverse effects localized or systemic? **Response:**			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Hazard Identification – Formulation					
Considerations					
	What ingredients are in the formulation? For example:				
Formulation Component	**Formulation Component (e.g., CAS Number, Strain Identification)**	**Formulation Percentage (%)**	**Formulation Component Purpose**		
Ingredient #1					
Ingredient #2					
Ingredient #3 …					
Product Properties	Viscosity				
	pH				
	Density				
	CFU/mL				
	Total Solids				
	Color				
	Odor				
Considerations			**Yes**	**Investigate**	**No**
Are there sufficient preventive controls in place to prevent contamination of the finished product during manufacture?					
Does the shelf-life or storage conditions impact the number of microorganisms present and their viability? *If yes, describe:*					
Does the storage of the product provide a risk to contamination during use? *If yes, describe:*					
Is the stability of the product compromised during storage of the product? *If yes, describe:*					

Hazard Identification – Formulation	
What is the process used to produce the product (e.g., batch or continuous)? *Describe the process.*	
What are the product directions for use, including frequency and duration of use including any recommendations for personal protective equipment (PPE) usage? **Response:**	
What is the product mode of action? **Response:**	
What is the intended shelf-life of the product? **Response:**	
What are the recommended storage conditions of the product? **Response:**	
Decision Points	
Continue evaluation	
Investigate further	
Stop evaluation	
Notes:	

Hazard Identification – Exposure Assessment				
Considerations		**Yes**	**Investigate**	**No**
What is the product format?	Liquid			
	Powder/Solid			
	Foam			
What is the mode of action (packaging) of the product including the application process and mode of use?	Trigger spray			
	Wipe			
	Pour			
	Direct application: sponge/cloth/mop			
	No direct contact (pod)			
Is there potential for direct routes of exposure by applicators?	Dermal (chronic)			
	Dermal (acute)			
	Ocular			
	Inhalation			

Hazard Identification – Exposure Assessment				
Considerations		**Yes**	**Investigate**	**No**
	Oral / Ingestion			
How often will the product be used?	Daily			
	Weekly			
	Monthly			
	One-time			
Is there potential for indirect routes of exposure by applicators?	Dermal (chronic)			
	Dermal (acute)			
	Ocular			
	Inhalation			
	Oral / Ingestion			
Is there potential for direct routes of exposure by others (e.g., non-applicators)?	Dermal (chronic)			
	Dermal (acute)			
	Ocular			

Hazard Identification – Exposure Assessment				
Considerations		**Yes**	**Investigate**	**No**
	Inhalation			
	Oral / Ingestion			
Is there potential for indirect routes of exposure by others (e.g., non-applicators)?	Dermal (chronic)			
	Dermal (acute)			
	Ocular			
	Inhalation			
	Oral / Ingestion			
Is there potential for exposure concerns other than people?	Environment			
	Pets			
	Other animals			
	Plants			
	Other:			

Hazard Identification – Exposure Assessment				
Considerations		**Yes**	**Investigate**	**No**
Does the physical environment affect exposure?	Indoor Use			
	Outdoor Use			
	Ventilation			
	Air Currents			
	Temperature			
	Humidity			
In what locations will the product be used?	Food Contact surfaces (e.g., Kitchen)			
	Non-food Contact (e.g., Bathroom)			
	Healthcare (e.g., patient rooms, common areas)			
	Other (outdoor):			
Can the product be aerosolized (e.g., trigger, aerosol)? *What is the droplet size and rate of settling?*				
If the product is aerosolized are the particles of inspirable size during direct application?				
If the product is aerosolized are the particles of inspirable size during indirect application?				

Hazard Identification – Exposure Assessment			
Considerations	**Yes**	**Investigate**	**No**
Does the product require dilution prior to use?			
Is the product a concentrate meant to be diluted and filled into other containers (e.g., refill for trigger bottles)?			
Is there a required delivery unit dose (e.g., grams per application)? *Describe the unit dose requirements.*			
Is there a recommended reapplication rate? *Describe the reapplication requirements.*			
What are the conditions of foreseeable misuse? **Response:**			
How do the conditions of foreseeable mis-use impact the potential for exposure? **Response:**			
How many microorganisms are estimated to be in contact with an individual for each exposure mechanism? **Response:**			
What is the anticipated fate, persistence, and life cycle of the microbial ingredient post application? **Response:**			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Hazard Characterization				
Considerations		**Yes**	**Investigate**	**No**
Is there a quantitative pathway available to estimate dose-response? *What is the pathway referenced?*				
Is the dose-response relationship well characterized? **Response:**				
Is there a known threshold of exposure to develop an adverse effect? *What is the threshold?*				
Is it known how many exposures are required to cause an adverse effect? *How many exposures? (1), (2), etc.*				
Is there a benchmark that can be used? *What is the benchmark?*				
What is known about the dose-response relationship between exposure and each potential adverse effect for the general population and for each at-risk subpopulation? **Response:**				
What are the potential adverse health outcomes? **Response:**				
Who are the at-risk subpopulations? **Response:**				
What is the expected outcome of the dose-response assessment?	Risk of infection?			
	Risk of developing a specific symptom?			
	Allergic reaction?			
	Hospitalization?			

Hazard Characterization				
Considerations		**Yes**	**Investigate**	**No**
	Other: (list)			
Decision Points				
Continue evaluation				
Investigate further				
Stop evaluation				
Notes:				

Hazard Characterization - Dose-Response Relationship			
Considerations	**Yes**	**Investigate**	**No**
Are the identity and amount of exposure to the hazards necessary to cause an adverse reaction adequately described?			
Has a quantitative measure of the exposure, either level or total dose as appropriate been adequately described?			
Have the different human populations and their varying susceptibilities been adequately described?			
Have all possible routes of exposure (e.g., skin, inhalation, eyes) been adequately described?			
Have all possible durations of use for consideration of exposure (one-time, daily) been adequately described?			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Risk Characterization			
Considerations	**Yes**	**Investigate**	**No**
Does the potential exist for both hazard and exposure? **Note to reviewer:** • If yes, then the levels of exposure and the corresponding human response for the various scenarios are combined to estimate the likelihood that an adverse response occurs. • The human response may be quantitative (1 illness per 10,000 exposures) or semiquantitative (low to high likelihood). • A maximum exposure amount for the various different exposure doses, human populations and routes of exposure that can be judged as "safe" need to be determined.			
Is there adequate confidence, given the available data quality and quantity, in the risk characterizations for the various scenarios to allow the risk managers to make a decision on the safety of the product? What is the best approach to characterize the risk?			
Can the risk event of interest be described simply by the nature, likelihood, severity, and consequences of the potential exposure to the hazard?			
Is there a quantitative approach available to characterize the risk? Is it better characterized qualitatively or descriptively?			
Can the likelihood and severity of adverse events be estimated by the data available in this risk assessment?			
For a qualitative risk characterization, is the risk estimate (0) negligible likelihood of occurrence, (1) very low, (2) low, (3) medium, to (4) high?			
For a quantitative risk characterization can it be expressed as the estimated number of specific adverse events (illness, allergic reaction, deaths, etc.) per number of exposures for the general or at-risk subpopulations?			
Does the risk characterization show if the risk may be judged as acceptable?			
Has the risk assessment been presented to peers internally, if sufficient expertise exists in-house, or to experts external to the company?			

Risk Characterization			
Considerations	**Yes**	**Investigate**	**No**
If different from the peer reviewers, have risk managers concluded whether the product is suitable for consumer use as is or if further studies or modifications are necessary?			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Risk Communication				
Considerations		**Yes**	**Investigate**	**No**
Proper use of product is adequately communicated to users on product labeling (e.g., market label, supplemental material, websites, social media, etc.)?	Product composition			
	Storage and handling conditions			
	Where to use and where not to use product			
	How to use product			
	Personal protection required for use			
Label has appropriate cautions and actions to be taken if accidental events occur during use (e.g., skin contact, ingestion or eye contact with product)?				
Employees are informed of safety procedures to manufacture this product.				
Appropriately communicated with raw material suppliers about vendor qualifications established by the company?				
Develop and provide appropriate information to consumers/public about this product	Benefits of product			
	Mechanism of cleaning			
	Safety			
	Environmental implications			

Risk Communication	
Decision Points	
Continue evaluation	
Investigate further	
Stop evaluation	
Notes:	

Regulatory and Registration Considerations			
Considerations	**Yes**	**Investigate**	**No**
Are there regulations or guidance documents from government sources that need to be considered?			
Are there regulations or guidance documents from non-governmental sources that need to be considered?			
Are there any pending regulations or guidance documents that need to be considered?			
Does the formulation contain any microorganisms that are Genetically Modified (GMO)?			
Is the microorganism(s) on the QPS list?			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Risk Management			
Vendor Qualification / Evaluation			
Considerations	**Yes**	**Investigate**	**No**
Have acceptable and documented quality control measures at the time of production for the microorganisms?			
Control for strain purity?			
Control for contamination?			
Prevent cross-contamination if they manufacture multiple strains?			
Control for strain concentration?			
Provide a sufficient number of viable microorganisms per lot consistently?			
Have a history of strain stability?			
Documentation provided indicating each batch meets predetermined specifications and quality criteria?			

Risk Management			
Risk Management / Evaluation			
Considerations	**Yes**	**Investigate**	**No**
Significance of potential risks to human health were appropriately evaluated?			
There was appropriate data to determine risk.			
The product use is and remains within appropriate and acceptable risk levels?			
Are there benchmark doses that are generally considered to be safe by scientific and medical experts which can be used as pass/fail criterion?			
Risk communicators were briefed as to appropriate communication to target audiences?			
Decision Points			
Continue evaluation			
Investigate further			
Stop evaluation			
Notes:			

Final Decision	
The product is appropriate for its use. Hazards and risks have been sufficiently evaluated. Product may be commercialized.	
The product needs additional research to determine if it is appropriate for its use. Hazards and risks have not been fully evaluated. Continued research is recommended.	
The product is not appropriate for its use. Hazards and risks have demonstrated the product poses hazards and risks to human health and/or the environment. The commercialization of this product should be rejected.	
Notes:	

APPENDIX 3
Case Studies

Appendix 3: Case Studies

The following three case studies are provided as examples to help demonstrate the risk assessment and broader risk analysis principles described in this document. It should be emphasized that these case study examples represent hypothetical cases and products. With respect to the development of actual commercial products, individual risk assessments should be conducted for each potential product and/or microorganism during product development and prior to product commercialization. For products containing multiple microbial species and/or strains, an assessment of each individual strain, as well as for the product as a whole, should be conducted. Likewise, any pre- or post-commercialization changes being proposed for a product's formulation, packaging or delivery system, or labeling, including but not limited to labeling changes in directions-for-use, use rates and frequencies, use sites, and personal protective equipment (PPE), etc., trigger a need to re-assess risks. Each of these case studies assumes that the product in question has not yet been commercialized. Additionally, each of these case studies assume the intentional inclusion of a single microbial strain in the subject products. As described in detail below, each case study assumes use of a different strain of the same microbial species, *Bacillus subtilis*, in the subject product.

Case Study 1: Drain Cleaner Product

Case Study 1 encompasses a hypothetical consumer product intended for use in the mitigation or prevention of clogged water drains. The product is formulated as solid granules, which are intended to be poured directly into the drain of kitchen and bathroom sinks, bathtubs, or showers to clear minor blockages and generally maintain suitable operational characteristics for the treated drain. The product is labeled to specify use of rubber gloves and eye protection during application. Product labeling also specifies the use of water to flush product down the drain following product applications. In this example, the product is labeled to limit use to no more than twice per month. It is assumed that the manufacturer has conducted testing to determine that transport and/or storage of the product does not result in product granules breaking down into particles of respirable size.

The product includes viable spores from a proprietary strain of *Bacillus subtilis* as an intentionally added ingredient. It is assumed that this specific strain has no history of safe use in commercial products, that the genome of this strain has been sequenced and annotated using scientifically suitable methodologies, and that the quality control procedures of the supplier and/or manufacturer (e.g., purity tests, challenge tests, long-term stability tests, etc.) are sufficient to ensure the identity of the strain and prevent unacceptable levels of adventitious microbial contamination in the final product. It is likewise assumed that the species identification for the strain in question is robust and based on acceptable genomic analyses that extends beyond 16s ribosomal gene sequence alone. In this Case Study, it is assumed that genomic evaluation of the strain reveals genes with potential to produce subtilisins, a class of serine proteases common to many members of the *Bacillus* genera (Azrin et al., 2022), but that no other potentially hazardous features such as those detailed in Chapter 3 of this document are identified. Antibiotic testing for the *Bacillus subtilis* strain in question is assumed to have been conducted, with the resulting susceptibility profile demonstrating that the strain is susceptible to commonly used and clinically relevant antibiotics. With respect to potential hazards, *Bacillus subtilis* does not appear on the list of pathogens in Annex III of Directive 2000/54/EC and is not typically considered to be a frank human pathogen. *Bacillus subtilis* also has qualified presumption of safety (QPS) status in the absence of toxigenic

activity (EFSA, 2020; EFSA, 2022). QPS status indicates that the microorganism has been assessed for safety concerns for humans, animals, and the environment (EFSA, 2023). Subtilisins are commercially available for a wide variety of uses (CIPD, 2023). In general, these enzymes have low toxigenic potential but have been reported to elicit allergenic or hypersensitive responses from individuals exposed at high concentrations, particularly in industrial settings where the protease enzyme is produced for commercial purposes (EPA. 1997). Nevertheless, these enzymes are very widely, and safely, used in a variety of consumer goods including but not limited to laundry and cleaning products (CPID, 2023; HERA, 2007).

As previously described, the Case Study 1 product is labeled to limit use to no more than twice per month. While it is conceivable that consumer exposures could occur twice per month during or after application of the subject product, the product's use pattern (down-the-drain cleaner that is wash/rinsed down the drain following application) and its solid granular physical state minimizes exposure potential. The potential for dermal and ocular exposure is further mitigated by the consumer label language specifying the use of PPE during product application (rubber gloves and eye protection). Incidental direct or indirect oral exposures to the microorganism in question are unlikely to represent a significant hazard given the QPS status of non-toxigenic *Bacillus subtilis.*

The low hazard potential for this microorganism, in conjunction with low potential for exposure, suggests that use of the *Bacillus subtilis* strain in Case Study 1 is acceptable for this hypothetical product. An evaluation of hazards and risks associated with this hypothetical product therefore support product commercialization.

Case Study 2: General Purpose Spray Cleaner

Case Study 2 encompasses a hypothetical consumer product intended for use as general-purpose cleaner for use in residential and commercial settings. The product is formulated as a liquid, applied via trigger spray, and is designed to impart cleaning activity to treated surfaces. The product label specifies that direct contact with skin and eyes should be avoided; however, no provisions for PPE are specified.

This product is assumed to contain viable spores from a second proprietary strain of *Bacillus subtilis* that has no history of safe use in other commercial products. The strain of *Bacillus subtilis* used in the Case Study 2 product is not identical to the strain used in the Case Study 1 product, and as such, should be evaluated individually. It is likewise assumed that that its genome has been sequenced and annotated using suitable methodologies, that this annotation was used for species identification (beyond 16s sequence only), and that the supplier and/or manufacturer quality control procedures (e.g., purity tests, challenge tests, long-term stability tests, etc.) are sufficient to ensure the identity of the strain and prevent unacceptable levels of adventitious microbial contamination in the final product. It is again assumed that the annotated genome reveals no indication of potential toxin production, and that genomic evaluation of the Case Study 2 strain revealed genes with potential to produce subtilisins. However, in Case Study 2 the potential for production of the specific subtilisin, nattokinase, is identified. No other potentially hazards such as those detailed in Chapter 3 of this document, are identified following genome annotation and evaluations. Antibiotic testing for the *Bacillus subtilis* strain in question is assumed to have been conducted, with the resulting susceptibility profile demonstrating that the strain is susceptible to commonly used and clinically relevant antibiotics.

As with Case Study 1, the non-toxigenic *Bacillus subtilis* species in Case Study 2 has QPS status and is not generally recognized as a frank human pathogen, including in Directive 2000/54/EC. Nevertheless, the

Case Study 2 product does differ with respect to the type of consumer product and its use pattern, as well as the ability of the microbial ingredient to produce nattokinase, specifically. Subtilisins are safely used in many commercial products such as pet odor and stain removers (CPID, 2023; HERA, 2007), however, the identification of nattokinase in particular may warrant additional investigation for this microbial strain in this trigger spray cleaning product.

Nattokinase is believed to have similar allergenic potential to various soy-derived products, and the European Food Safety Authority (EFSA) concluded that some fermented soy products containing nattokinase are acceptable for use as food supplements (EFSA, 2016). Likewise, nattokinase evaluated as therapeutic agents in various clinical trials often showed few or no adverse events (Weng et al., 2017). Furthermore, at least some *Bacillus subtilis* strains isolated from natto (fermented soy) show no evidence of pathogenicity in rat models (Weng et al., 2017). That said, there is some evidence that sensitization reactions can develop in response to repeated nattokinase exposure, particularly for individuals with skin barrier disfunctions such as atopic dermatitis (Suzuki et al., 2023). There are reports of nattokinase exposure by oral ingestion of natto that have led to serious adverse events, including anaphylaxis (Awatani-Yoshidome et al., 2022).

Given its anticipated use pattern, the Case Study 2 product is likely to be applied frequently (e.g., weekly) as part of a routine cleaning. No PPE is specified on the product label, and application of the liquid product via trigger spray allows for potential oral, inhalation, ocular, and dermal exposures during product application. Inhalation exposures during application are of particular interest given method of application and allergenic potential of nattokinase. Post-application exposures may also occur following cleaning with the subject product.

The potential for repeated oral, inhalation, ocular, and dermal exposures, combined with the hazard potential of nattokinase (e.g., allergenicity), indicate that use of the subject *Bacillus subtilis* strain in the hypothetical Case Study 2 product warrants additional investigation. This investigation could include a more intensive literature review and/or testing to better understand hazards associated with oral, dermal, eye, and inhalation exposures. The addition of risk management measures such as label warnings to wear gloves and a face mask during application and/or user instructions disallowing applications to direct or indirect could likewise be considered as ways to reduce risk associated with the Case Study 2 product. Additional consideration could be given to labeling the product as “avoid contact if you have an allergy to soy”. Changing application methods for this product, for example, moving away from a trigger spray and towards a liquid product that can be applied by sponge, cloth, or mop, could help to reduce inhalation exposures and thereby mitigate risk. This product is not ready for commercialization until additional investigation is performed and acceptable risk mitigation measures are in place.

Case Study 3: Hand Dishwashing Detergent

The hypothetical Case Study 3 product is a manual dishwashing detergent that is formulated as a pourable liquid. The product is intended for use in daily manual dishwashing, and the product label does not include PPE provisions for product users.

The Case Study 3 product is assumed to contain spores from a third proprietary *Bacillus subtilis* strain that has no history of safe use in other commercial products. The *Bacillus subtilis* strain in Case Study 3 is not identical to that in Case Study 1 or 2 and should be assessed individually. As with Case Studies 1 and 2, it is likewise assumed that the genome of this strain has been sequenced and annotated using suitable

methodologies, that this annotation was used for species identification (beyond 16s sequence only), that the annotated genome reveals no indication of toxin production, and that the supplier and/or manufacturer quality control procedures (e.g., purity tests, challenge tests, long-term stability tests, etc.) are sufficient to ensure the identity of the strain and prevent unacceptable levels of microbial contamination in the final product. In this Case Study, the genome of the *Bacillus subtilis* strain in question shows the presence of subtilisins, as in Case Study 1. However, this genome evaluation also reveals the presence of genes for the expression of amylosin. No other potentially hazardous features such as those detailed in Chapter 3 of this document are identified during the genome evaluation. Nevertheless, the identification of amylosin production genes warrants additional investigation for the microbial strain in this particular product. Antibiotic testing for the *Bacillus subtilis* strain in question is assumed to have been conducted, with the resulting susceptibility profile demonstrating that the strain is susceptible to commonly used and clinically relevant antibiotics.

Amylosin is a heat stable toxin that has been associated with food poisoning cases involving *Bacillus subtilis* and other *Bacillus* species (Apetroaie-Constantin et al., 2009). It has likewise been hypothesized that inhalation of amylosin produced by other *Bacillus* species could contribute to inflammatory reactions of individuals residing in moisture-damaged buildings (Rasimus-Sahari et al., 2015). Some *in vitro* toxicological effects for amylosin have likewise been reported in the literature (for example, see (Mikkola et al., 2007).

The use pattern for Case Study 3 implies the potential for oral ingestion of the microbial strain in question, and such ingestion may occur on a daily or even more frequent basis. Likewise, daily (or more frequent) skin exposures are likely, and dishwashing activities may also result in splashing that could reasonably result in ocular exposures.

Based upon the high potential for exposure and the hazards associated with amylosin, there is a risk of adverse effects to consumers resulting from use of the Case Study 3 product. Based on the potential for exposure and the potential risks associated with amylosin, the *Bacillus subtilis* strain in question is likely inappropriate for use in the hypothetical subject product. Further literature review and/or testing may help define toxicologically insignificant doses which could be compared to anticipated exposures to better understand potential risks. However, in the absence of additional information and/or mitigation measures that adequately reduce potential hazards and/or risks, commercialization of this product should be rejected by a company's appointed risk manager.

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