IBMA comments on the EFSA Guidance on the characterisation and risk assessment of microorganisms used in the food chain

Part of the GD	**Subpart** (e.g. 3.1 taxonomic identification)	**IBMA Comment**
Abstract	No abstract available in the document. This is a general IBMA comment on the document	IBMA welcomes the communication within EFSA across the different panels and the major effort done to draft this guidance intended to applicants and assessor identifying potential hazards during the assessment process. We would suggest changing the title of the guidance to better express its purpose and content, to the following: "*Guidance on the identification of potential hazards of microorganisms used in the food chain*" The risk assessment is not covered by this Guidance Document, and it should be crossed referenced to the specific Guidance documents and Data Requirements currently in place for the different areas of use of microorganisms. It seems the Guidance Document has been driven by the QPS status and IBMA would like to point out that in the case of PPP uses, a risk assessment should be conducted irrespectively of the QPS status (not a requirement for PPPs). (Please note that an abstract is not available in the draft document)
1. Background and Terms of Reference as provided by EFSA	*It is not possible to leave comments on this section.*	*It is not possible to leave comments on this section*
2. Scope	Line 120-159	General comment on the Scope and applicability to PPPs: According to the document's scope and title, the Guidance should define a scheme to perform risk assessments. However, there is no mention in the document on how to decide what is an acceptable/unacceptable risk or decision impact. The Guidance Document seems to focus on a "no-risk" approach, identifying risk whenever a potential hazard is possible, and exposure is expected.

		PPPs Risk Assessment for the environment is out of the scope of the draft guidance but the consumer risk assessment, also covered by PPP legislation seems to be excluded and could lead to conflicting interpretations during the assessment of PPPs. A "no-risk" approach is followed across the guidance document, for the other sections, where the indication "…is considered (to be) a risk" appears numerous times. A zero-risk scenario is an idealized situation, that does not materialize in real-life situations. Regulation 1107/2009 and the PPP uniform principles do not follow a "no-risk" approach but referring to an "acceptable" or "unacceptable" risk. IBMA notes that the criteria for acceptability of risk are not defined in the draft guidance. For PPP, a reference to Reg.1107/2009 and all applicable legislation and guidance documents for PPP should be made to support applicants and assessors during the risk assessment process.
2. Scope	Line 125	The sentence "It provides the basis for the risk assessment of microorganisms." Can be misleading and interpreted as the Guidance Document is covering the risk assessment for all MO in the food chain, whereas the guidance is a starting point to support risk assessor identifying hazards that will require further considerations by the different risk assessors (the assessment process for risk is specific to the sector of application and applicable legislation regarding hazard, cut-off and risk assessment models connected to the use). IBMA suggests rewording the sentence as "It provides the basis to support hazard identification and support the risk assessment of microorganisms across the different food domains."
2. Scope	Line 128	Suggestion to clarify the resistance to antibiotics to avoid interpretation issues by the parties. IBMA suggests rewording the sentence: "investigate the presence of genes of concern involved in resistance to antimicrobials, production of antimicrobials of therapeutical interest, and the virulence potential of the microorganism;" as "investigate the presence of genes of concern involved in resistance to **medical important** antimicrobials (*), production of antimicrobials **used in therapeutical context in human**

		and animal heath, and the virulence potential of the microorganism. (*) reference to the WHO list. Please note that every microorganism will have genes against several antimicrobials – that's from where all antibiotics we have today in clinical use came from in the first place – thus the reference should be limited to the ones that could potentially interfere with the therapeutical use of antibiotics and for which resistances (according to the WHO list) are a concern. Note that these genes in the MOs are part of their survival mechanisms against other microorganisms, and most of these genes are also present in the human and animal microbiome gut.
2. Scope	Line 133	This sentence seems misaligned with the content provided by this Guidance document since the data required and the how to perform a risk assessment on the gut and food/feed microbiome is not provided. This requirement for a RA on gut and food/feed microbiome seems a very broad requirement: impact on the gut and food/feed microbiome (which is not characterise) seems impossible to address and this document does not provide guidance on how to address it, whereas it is stating it as a requirement for approvals. Please consider its removal or rewording accordingly to what guidance this Guidance Document provides in this matter and the characterisation of the protection goal, i.e. the gut (which animal?) and the food/feed microbiome (which crop? Under which conditions of growth? At each stage of cultivation/harvest/supermarket shelves?). At least for the PPPs, IBMA is of the opinion that without characterizing well the microbiome of the host/target intended to be protect, this question cannot be answered.
2. Scope	Line 143	IBMA suggest adding the non-QPS microorganisms, which are non-GM, and can be used as PPP, after the sentence: "A microorganism might be suitable for the QPS approach if it belongs to a taxonomic unit included in the most recent QPS list and fulfils all the qualifications set."

		By adding: "EFSA recognizes that the QPS status is not a requirement for the use of microorganisms across the food chain (e.g. as PPPs) but this guidance aims to support the identification of potential hazards for those microorganisms, which assessment falls under the rules established under Reg. 1107/2009, its procedures and applicable Regulations and Guidance Documents."
2. Scope	Line 157	Propose that clarification for the applicability of existing GD in the PPP framework is added beyond the environmental. Note that several GDs were developed taking into account the specific exposure of consumers and the environment following a PPP use. It would be useful to add a clarification here that such Guidance remain in place for the PPPs to avoid confusion between the parties when dossiers for PPP are submitted. A suggestion could be to state that this guidance will be helpful to identify hazards and risks, however the risk assessment should be conducted using the PPP applicable Guidance and the new data requirements for Microorganisms, approval criteria and the uniform principles (Reg. EU 2022/1438, Reg. EU 2022/1439, Reg. EU 2022/1441, and applicable SANTE Guidance Documents and Explanatory Notes).
3. Characterization of the microorganism	Line 198	A note should be made in the text to alert that interpretation of the genomic data should be made with care, since: 1) Taxonomy doesn't define pathogenicity; 2) Virulence is multifactorial and is dependent on the context of the biological host; 3) production of active toxin often requires the presence of accessory proteins for posttranslational modification and/or export, thus the detection of a toxin encoding gene is not necessarily predictive of virulence. Suggested reading on the subject: National Research Council. 2010. Sequence-Based Classification of Select Agents: A Brighter Line. Washington, DC: The National Academies Press. https://doi.org/10.17226/12970.
3. Characterization of the microorganism	Line 252: 3.2.1. Antibacterial resistance	Please make a note that final products that do not contain viable DNA are excluded from the scope of this assessment.

	(Figure 1 shows the decision tree on the AMR)	It would be useful to have a description of what analysis of AMR is required, particularly within context of how to interpret QPS (from EFSA BIOHAZ Panel 2023) – different descriptions are stated, intrinsic vs acquired AMR genes. IBMA would like to point out that for strains used in PPP, not adapted to mammals' ecological niche (e.g. entomopathogenic microorganisms) it is often difficulties to obtain reference genomes, i.e. of closely related spp. for comparison analyses.
	Line 253 to 257	IBMA suggests including a cross reference to the PPP legislation regarding bacteria and the approval criteria for all Microorganisms excluding virus (Reg. 2022/1438), which in Point 5.2.1 states that a bacteria can be approved if demonstrated sensitivity to 2 classes of antimicrobial agents can be demonstrate: "An active substance that is a micro-organism other than a virus may be considered a low-risk active substance unless its susceptibility to at least two classes of antimicrobial agents has not been demonstrated.")
	Line 257	IBMA proposes to add (end of Line 257) the following clarification: "For Microorganisms used as PPPs, the EU guidance document SANTE/2020/12260 (Guidance on the approval and low-risk criteria linked to "antimicrobial resistance" applicable to microorganisms used for plant protection in accordance with the regulation (EC) No 1107/2009) outlines the relevant parts of this guideline and antimicrobial agents that applies for microorganisms used as plant protection and provides a step-wise approach for the assessment of AMR in PPP uses."
	Line 267 Figure 1	The decision tree needs further consideration. Some suggestions: AMR gene would be relevant if they are of medical importance (WHO list). Just AMR gene is very broad, and the current decision tree is not very helpful since the "No" will never be an option because MO must have AMR genes to be able to survive in the ecosystem. Please add Further consideration is need regarding the transferability of gene, non-intrinsic, which might lead to a risk of horizontal transfer whereas such risk will be negligible for

		nontransferable genes. This is relevant for PPP which are not aimed at ingestion by/use on mammals but to be used on plants. Please also cite that for PPPs, an assessment should follow the current legislation and applicable Guidance for PPP on the risk assessment for an MO approval for use in a PPP product (COMMISSION REGULATION (EU) 2022/1439, of 31 August 2022). Transcription from the document: "Where the micro-organism is a bacterium, information on any resistance to relevant antimicrobial agents shall be reported at strain level, and information on whether the antimicrobial resistance genes are acquired, transferable and functional shall be reported. The information provided shall be sufficient to perform an evaluation as to the risks for human and animal health due to a possible transfer of relevant antimicrobial resistance genes."
	Line 270	Suggestion to harmonize the wording across the document when clinically relevant antimicrobial agents are mentioned. IBMA suggest using the WHO 2024 terminology: "medically important antimicrobials" (WHO 2024) instead of different terms that might lead to confusion.

	Line 277	The 2 years suggested do not seem to consider the time for PPP dossiers preparation since the WGS is done 4 years ahead of the submission date. This information will drive all studies and dossier preparation for PPP and the timeline is not suitable for MO dossiers for PPP uses. IBMA suggests at least 3 years to prevent applicants to redo the work once studies are already ongoing (tox, ecotox, analytics on metabolites, etc.). Please consider the time to prepare and contract studies based on the WGS information. The proposed 2 years is not adequate for the reality of the current PPP regulatory process and the preparations for submission.
	3.2.1.2. Discrimination between intrinsic and acquired AMR genes Line 288	IBMA suggests incorporating cross-references to the PPP legislation and guidance documents in place regarding the use of MO in Agriculture. After the sentence: "If uncertainty remains about the intrinsic nature of the AMR gene, the AMR gene will be treated as acquired and assessed accordingly (see Figure 1)." Please add: "For MO used as PPP under Reg. 1107/2009, a risk assessment should be provided in accordance with the Data Requirements (Reg EU 2022/1439) and the Approval criteria for Microorganisms used in PPP (Reg EU 2022/1438), and the respective Guidance Documents adopted by the EU Commission (e.g. Explanatory Notes PAFF-PPL-October 2023-Doc.A.07.01 of 12 October 2023; SANTE/2020/12260 of 23 October 2020, GUIDANCE ON THE APPROVAL AND LOW RISK CRITERIA LINKED TO "ANTIMICROBIAL RESISTANCE" APPLICABLE TO MICROORGANISMS USED FOR PLANT PROTECTION IN ACCORDANCE WITH REGULATION (EC) No 1107/2009"

	3.2.1.3 Phenotypic Testing Line 293	Please note that MIC reference values are only provided for MO with known or suspected adaptation to mammalian biological systems, which is not the case for the ones used in PPPs (adapted to soils, plants, insects, etc). The reference MIC values are not established for the common species used in PPPs and therefore a sentence should be added to the paragraph to state that for PPP, the RMS should assess the susceptibility based on expert judgment and considering the available information in the literature at the species level for the MO strain in PPPs. The values in Appendix D and E could be used as guiding value but obviously not as cut-off values since the presented MICs were not derived for non-clinical relevant species, which are the ones used in PPPs.
	Lines 299-327	IBMA suggests adding the following text: "for bacteria species not listed in Appendix D the activity or resistance to an antibiotic based on the MIC will be assessed case by case based on the available literature data at species level, if available." Please note that microorganisms used in agriculture are not adapted to mammals and therefore reference values are not available (and they are not listed in the appendix D nor E). This lack of values is understandable, due to the ecological niche of the MO used in PP, but it advisable to acknowledge it here by stating that for such MO (not human pathogens) MIC reference values might not be available, and sensitivity will need to be judge by expert judgment since there is no reference cut-off available for most of the non-human pathogen/potential pathogens.
	3.2.1.4. Interpretation of the results Line 303	For PPPs, consideration is needed regarding cut-off value applicable to mammals' host. Cut-off values (a.k.a. clinical breakpoints) are not fixed values, and vary with the test organism, the antibiotic, the animal species and infection site. Cut-off values are not available for all bacterial organism-animal species-site combinations. When Cut-off values are applied to different bacterial species, or different animal species, or different infection sites than the reference Cut-off values, the reliability of the interpretation result is reduced. This is particularly critical for MO used in PPP as they don't have a mammalian host and, according to this Guidance, should be linked to cut-off value provided in mammalian hosts. From an ecological point of view, the applicability of these criteria to the MO used

		in PPP is scientifically inappropriate. A note is needed for scientific accuracy and to express EFSA understanding of the relevance of a MIC value, how reference values are derived and their link to microbial ecology. Same applies to the fungi. Please note that this is even more relevant for fungi used in PPP because they are not closely related (taxonomically) to the list presented in the Appendix E.
	3.2.2. Antifungal resistance Line 336	Please note that fungi used in PPP are not listed in your Appendix E. This is understandable since they are poorly adapted to mammals and therefore reference values are not available. IBMA suggests referencing the assessment of fungi PPPs to the current Guidance (SANTE/2020/12260) and make a reference in the table in Appendix E, mentioning that for fungi used in PPP the MIC reference values might not be available and therefore sensitivity will be judged according to the PPP applicable provisions and RMS expert judgment. Note well that if a fungus is not a known pathogen to mammals, it will be difficult to find reliable MIC reference values to compare it to.
	3.3 Production of antimicrobial substances Line 340 (from)	**IBMA suggest that the PPP legislation and Guidance on Secondary Metabolites should be considered here for alignment or exclusion of PPP from this point.** **The entire section is not aligned with the PPP requirements, and it will generate enormous confusion between applicants and assessors. IBMA suggests an exclusion of PPP, since the PPP data requirements have been recently revised and the respective guidance on secondary metabolites**. IBMA proposes either a clear exclusion or adding a reference for PPPs to the applicable legislation and guidance documents (Reg. 2022/1439, Explanatory Notes PAFF-PPL-October 2023-Doc.A.07.01, SANCO/2020/12258 Rev 1 of 21 March 2024 GUIDANCE ON THE RISK ASSESSMENT OF METABOLITES PRODUCED BY MICROORGANISMS USED AS

		PLANT PROTECTION ACTIVE SUBSTANCES IN ACCORDANCE WITH ARTICLE 77 OF REGULATION (EC) No 1107/2009). Request for clarification: is this point only applicable to bacteria? Or for all MO except virus (not able to produce metabolites)?
	Line 341	IBMA suggested to add the sentence: "For PPP assessment the guidance on the risk assessment of metabolites produced by microorganisms used as plant protection active substances in accordance with article 77 of regulation (EC) No 1107/2009 (SANCO/2020/12258) applies." Justification: The guidance developed for microbial PPPs includes a detailed approach to assess the risk of microbial metabolites including antimicrobial substances that specifically considers the use as PPP and enable applicants to address the approval criteria set by the Reg. 1107/2009 and its amendments.
	Line 351	The sentence is not scientifically accurate. IBMA suggests rewording the sentence as "The assessment for the potential production of antimicrobial substances should identified based on the WGS analysis and in case needed, by phenotypic tests." As it is written now, it seems that a gene presence is enough for the production of a certain compound, which is not accurate since functionality and expression need to be considered.
	3.4 Toxigenicity and pathogenicity 3.4.1. Bacteria Line 404-420	IBMA understanding is that this section only applied to QPS MO. If this is the case, IBMA suggest that for non-QPS strains used in PPP, the applicable existing Guidance Documents developed under the Reg.1107/2009 framework will apply for PPP strains (non-QPS). This exclusion at the beginning will be helpful for applicant and assessors of PPPs.
	3.4.1. Bacteria Line 408 to 411	Better Guidance is needed regarding the expected methods to be use, such as antiSMASH, PRISM, and BAGEL, especially for less well-known species, where there isn't a

		specific group of toxins to target. What are EFSA recommendation regarding the approach in such cases?
	3.4.1.2. Bacillus spp Line 448	Comment: Although in-vitro cell-based cytotoxicity tests may be practical and useful for early-stage toxicity screening, these rather simple tests do not fully replicate the complexity of living organisms. Limitations of in vitro cell-based cytotoxicity tests include variability in assay methodologies, influence of drug solvents, concentration, and exposure duration, which can affect results. These important factors need careful selection and standardization to ensure reliable and applicable conclusions. In vitro human cell-based models, while useful, have limitations such as lack of systemic interactions, oversimplification of biological processes, and potential differences in cell behaviour compared to in vivo environments, which can affect the reliability of cytotoxicity conclusions. The guidance document should contain further information on the practical implications of a positive cytotoxicity test and how to advance from there, considering the biological systems of the host(s) and the microorganism ecology.
	Line 415	Please reconsider the example used here with the available evidence. Bacillus cereus can be responsible for two types of food poisoning, the emetic form due to food intoxication (gene for emetic toxin not found in Bacillus thuringiensis) and the diarrheal form emerging from food infections with enteropathogenic strains, also known as toxico-infections. The diarrheal type of food poisoning emerges after production of enterotoxins by viable bacteria in the human intestine. Basically, the manifestation of the disease is, however, the result of a multifactorial process, including B. cereus prevalence and survival in different foods (typically staple foods, not the ones Bt is applied on), survival of the stomach passage, spore germination, motility, adhesion, and finally enterotoxin production in the intestine. Moreover, all of these processes are influenced by the consumed foodstuffs as well as the intestinal microbiota which have, therefore, to be considered for a reliable prediction of the hazardous potential of contaminated foods.

		Considering all aspects mentioned, it becomes clear that the course of an infection with enteropathogenic B. cereus is hard to predict. On the one hand, there is a high variability of enterotoxin production between different strains, which is determined by complex and dynamic biological processes concerning gene transcription, post-transcriptional and post-translational modifications, as well as toxin secretion and stability, which we are, at the moment, only beginning to be understood. The same applies for the presence of further secreted virulence factors and their possible interaction with the enterotoxins (please see for e.g. Jessberger et al., 2020, The Bacillus cereus Food Infection as Multifactorial Process, Toxins 2020, 12(11), 701; https://doi.org/10.3390/toxins12110701). Non-haemolytic enterotoxin (Nhe), haemolysin BL (Hbl) and cytotoxin K (CytK)) are not validated effect biomarkers for an enterotoxigenic event. It is not proven either that these proteins alone are sufficient to cause a diarrhoeal event. Therefore, the request to demonstrate the “non-functionality of the genes” is not adequate to infer about a strain´s toxigenic properties.
	Line 418	The following sentence needs further elaboration to account for gene expression knowledge and burden of proof: “If there is evidence for similarity, the non-functionality of the genes should be demonstrated.” This is not always possible, mostly not, otherwise we would not be dying from diseases. Please refer to the entire research on cancer and the attempt to identify genes as biomarkers, which expression is still poorly understood. A Scientific document should recognize the limitation on the current science understanding of gene expression and consideration on phenotype, MO behaviour, its ecological niche and evolution to adapt to the host, as well as the environmental factors must be accounted for during an assessment of risk. The genes alone are of limited value for risk assessment and the proof of the negative – that gene expressing is not going to occur under any circumstances – is difficult to demonstrate by testing. A more science-based wording is advisable in the text to account for exclusion/presence of potential hazard and the identification and characterisation of the risk. Other factors,

		than genetics alone, play an important role in the common situations where the negative cannot be proved by testing. Genome similarity, based on whatever "similarity" criteria we wish to apply to establish similarity, seem to be used as the only criteria to identify a hazard. However, when we move to the risk characterization, expression and condition of expression need to be considered and at this point, the genome differences play a role on the risk characterization: what is different between a human pathogen and an insect pathogen? They might have evolved from the same line millions of years ago (like humans and chimpanzees), therefore the genomes will be very similar, but their behaviour and ecological niche are today very different and surely the minor differences in their genomes will explain why. Focus on similarity percentages alone is not useful to characterize a risk, particularly for MO with very different ecological niches and behaviour. A scientific guidance should cover and acknowledge this important aspect of MO ecology. It is in the differences between the human and chimpanzee genome that we have a species with destructive instincts and other that still lives in cohabitation with nature and the ecosystem, even though their genome qualifies as similar.
	3.4.2. Yeasts and filamentous fungi Line 453 to 454	Please note that for PPP (use on plants): the efficacy data should constitute evidence of the non-toxicity to plants under the conditions of exposure. It would be useful to add a cross reference here to PPP and its efficacy assessment (on plants) since the metabolites will be assessed under field/greenhouse testing conditions in the efficacy studies submitted in the BAD (Biological Assessment Dossier) for PPPs.
	3.4.3 Viruses Line 472	Suggestion for rewording the sentence to include virus used in PPPs: "the host range/infectivity of viruses should be indicated. In addition, the infectivity and the absence of adverse effects of viruses for non-intended species should be **justified and if needed** assessed on a representative set of species." Please consider the current scientific knowledge, already reflected in the EU Legislation on PPP, for the specificity of virus used in PPPs (baculovirus) for which the specificity of host is well understood, and assessment of data on non-targets might be redundant. It

		would be useful to reflect here EFSA understanding of baculovirus used in PPP, in alignment with the existing knowledge.
	3.4.4. Microalgae and other protists Line 500 to 503	The sentence has a dead end and further guidance is needed. Please revise to provide Guidance to applicants in case no information is available regarding the secondary metabolites; just stating that further studies might be needed to exclude safety concerns is not useful. Which studies? How to proceed if there is no starting point? If there is no information, how would you exclude the safety concern? Which conditions will then trigger studies? Which ones? How to exclude non-concern if no information is available? Or which threshold does EFSA recommends using to decide that no information can be concluded as a (no) concern?
4. Presence of viable cells and DNA in the final product	4.1. Presence of viable cells of the strain Line 615	IBMA does not follow the rational for the imposed restriction on "cultivation-independent' methods. Please note that for PPPs the current requirements allow for several microorganisms to be registered as single active substance (i.e. a MO consortium of organism under the Reg. 1107/2009), thus independent methods might be needed for the purpose of this point (analytics). Note well that the consortium does not have to be manufactured together and can manufactured using different medium condition. And also, if there are different organisms, would it be more appropriate to have independent media which are adapted to the growing conditions of each of the MO? If the intention is to state that only RNA/DNA measurements are accepted, IBMA suggests rewording the sentence for clarity that only non-plating methods are intended.
5. Environmental risk assessment	Line 673	This paragraph is confusing because it excludes PPP but provides an example of a PPP for the assessment ('It does not apply to non-GM microorganisms used as PPPs for which specific data requirements exist", but gives an example of a PPP) IBMA suggest a different example, one that is not excluded from this Guidance Document, to prevent confusion between applicants and assessors. Ideally a non-PPP use to support applicants in other domain of the food chain, not familiar with ERA.

5. Environmental risk assessment	Line 684	Please check if this QPS exemption can be applied to PPPs and the applicable legislation. As it stands, we read that for any QPS microorganism, an environmental risk assessment is not needed and IBMA is unsure how this links to any waiving possibilities of the PPP regulations in place and its requirements for a Risk Assessment based on the PPP use (i.e. there is no exclusion based on QPS status).
5. Environmental risk assessment	5.1. Non-GM active agents Line 688	"Common members of the microbiome(s)" is a broad term. IBMA suggest using: "species commonly present in the microbiome of the receiving environment(s)". This would add clarify by removing the need to classify species as common or uncommon (and no reference list exists for such judgement) and acknowledging that the relevance is their presence in the microbiome of the respective compartment(s).
5. Environmental risk assessment	5.3. GM active agents Line 715	The whole ERA process for a GM active agent seems less demanding than for a non-GM PPP. Is there a reason for more concerns with a non-GM organism than a native one used in PPPs? IBMA noted a discrepancy between the ERA requirements for a native MO used as PPP, under Reg. 1107/2009, and the ERA expectations from EFSA for a GM organism described here. Certain requirements applicable to non-GM PPP do not seem relevant for GM organisms, according to this proposal. IBMA would agree with a similar approach for non-GM microorganisms.
	5.3.1.1. Persistence and invasiveness, including selective advantage (1 and 2) Line 762	Please refer to previous comment on ERA for GM vs non-GM used in PPPs. It seems that a GM organisms will have less strict requirements than a non-GM one and not requiring chemical models (FOCUS) to be performed an ERA. IBMA support the approach but would suggest a similar assessment for non-GM organisms, which in our view should not have more restrictive criteria than GM ones in terms of ERA.
5. Environmental risk assessment	L774 to L776	A general reference is made to higher tier testing methods for the assessment of potential increased survival or selective advantage of the GM active agent in the receiving environment(s), naming competition experiments in microcosms under different biotic

		and abiotic conditions, mimicking the receiving environments. Currently there are no standardized methods and protocols to test in such systems and the interpretation of the results may differ between regulatory bodies. Suggested changes in text: "Currently there are no Examples of standardised methods suitable for the assessment of potential increased survival or selective advantage of the GM active agent in the receiving environment(s) including competition experiments in microcosms under different biotic and abiotic conditions, mimicking the receiving environments, that are mutually accepted by regulatory bodies. Therefore, results should be interpreted with caution and considered on a case-by-case basis."
5. Environmental risk assessment	L777 to L778	A general reference to alternatively, or additionally, considering modelling approaches in predicting the behaviour of the strain under a range of biotic and abiotic conditions, compared with the parental strain. Currently such approaches may not be considered robust or validated by many national regulatory bodies. Further guidance from EFSA is considered appropriate with specific examples or even case studies, in which these modelling approaches have or could be used. Suggested changes in text: Alternatively, or additionally, although modelling approaches can be helpful in predicting the behaviour of the strain under a range of biotic and abiotic conditions, compared with the parental strain such approaches may not still be considered robust or validated by many regulatory bodies. Therefore, it is recommended that novel modelling approaches need to be further investigated for the specific systems and considered on a case-by-case basis.
5. Environmental risk assessment	L779 to L780	A reference is made to the Test Guidelines (TGs) for Microbial Plant Protection Agents from the US EPA on how to test the ability of microorganisms to survive, persist and replicate in terrestrial and aquatic environments. The use of these TGs can be considered appropriate as the first step in hazard identification process in the problem formulation. Therefore, it is suggested that: L779 to L780 is moved to L774 instead.

6. Impact on the gut and food/feed microbiomes	L860	It is unclear how the analyses should be performed for secondary routes of exposure. No clear guidance is available for the impact on microbiome and each single fruit/vegetable will have its own microbiome. Clear guidance should be provided to enlighten what 'adverse effects' mean. What is required to show an adverse effect versus variation between specimens and natural variation between individuals? The use of "potential adverse effects" for the gut microbiome is difficult to handle with a regulatory context. Gut microbiome is not defined as an organ with standardized functional characteristics, it's highly variable between individual and influenced by diet and environmental conditions, thus difficult to interpret the meaning of any "potential adverse effect". IBMA does not see how this can be implemented in practice without a decision tree/tool to assess potential adverse effect. We comprehend that the effects of products made from or produced with microorganisms on food and feed should be understood in relation to their subsequent effects on the final consumers of the food or feed product. However, we do not consider the food/ feed microbiome to be a relevant target for risk assessment. IBMA suggest adding clarify for PPPs and cross-reference to the applicable risk assessment process by rewording Line 863: "The impact on the gut microbiome focuses on primary routes of exposure (e.g. when ingested through food or feed). For secondary routes of exposure by use as a plant biostimulant or PPP specific regulations with data requirements and specific GDs for the risk assessment apply. " (reason: As mentioned in the scope, the risk assessment of PPPs is conducted in line with the relevant regulatory framework. For biostimulants Regulation (EU) 2019/1009 applies.)
6. Impact on the gut and food/feed microbiomes	L873	It would be useful to clarify the criteria for a compound to "outcompete" commensal resident microorganisms of the gut microbiome. Does outcompete mean significant decrease on population or the disappearance of a species?

7. Outcomes	7. Outcomes Line 953-954; 967-968; 1037-1038; 1048-1049	Suggestion to replacing “clinically relevant antimicrobials” by “medically important antimicrobials”, as per the terminology used in the reference (WHO, 2024).
7. Outcomes	7. Outcomes Line 945	The sentence in line 945 is misleading to readers since the outcome of this Guidance is not a risk assessment. “The following sections are limited to the outcome of the risk assessment covered by this guidance”. Please reword to express the Guidance content. According to the document’s scope, the GD should define a scheme to perform risk assessments. However, there is no mention in the document on how to decide what is an acceptable/unacceptable risk or impact and the acceptable uncertainty associated. The guidance focus on identifying risk, whenever a potential hazard cannot be excluded, and exposure is likely to occur.
References	References Line 1055 (from)	Throughout the draft Guidance, the references to EFSA documents from same year are not clearly traceable when we look at the reference section (i.e. EFSA 2023a or EFSA 2023b are difficult to find on the reference list)
Glossary	Glossary Line 1190	Risk: under the General Food Law, ‘risk’ means a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard. An entry for “risk assessment” should also be added to the Glossary since this concept is contained in the draft Guidance Document. Risk assessment (as per the General Food Law: ‘risk assessment’ means a scientifically based process consisting of four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. It would be useful to include and define “toxicity” and “pathogenicity”; particularly to make the distinction between antimicrobial activity.

		Gut Dysbiosis: it is not clear from the document, definitions and the document references how the assessment of gut dysbiosis is to be done.
Appendix A – List of EFSA guidance documents impacted by this draft guidance	Lines 1270-1274	PPP Guidance documents are missing. Do they remain in place? It is not clear to IBMA which documents are superseded, and which ones have been considered for drafting this guidance. For PPPs particularly, there are considerable inconsistences (AMR, Metabolites, Assessment models) with the currently available Guidance and Legislative acts in force in the EU. Is this draft document replacing existing EU Commission guidance documents applicable to specific applications such as PPP? If they stay in place, which ones should be followed? (Clarification in the Guidance will be appreciated regarding what to use since they are not aligned for PPPs).
Appendix B – Recommended procedure for the phenotypic susceptibility testing to antibiotics and antimycotics	Appendix B Line 1275	The MIC cut-off values are not listed for most MO used in PPPs, they are not mammals hosts and therefore reference values are not established (particularly for fungi, the Appendix E is not very useful for PPPs assessments). Please consider exclusion of PPP and cross-reference to the respective GD under Reg. 1107/2009 or add a note for the MO used in PPP (poorly adapted to the mammalian systems and the used clinical growing media to derive the MIC values).
Appendix C – Recommended procedure for the detection of cytotoxicity in Bacillus and related species other than those of the Bacillus cereus group	Appendix C Line 1307	Cytotoxicity observed in Vero cells can provide valuable insights, but it doesn't always directly translate to specific effects in humans or animals. Vero cells, derived from the kidney of an African green monkey, are commonly used in research to assess the toxicity of various compounds.

Appendix C – Recommended procedure for the detection of cytotoxicity in Bacillus and related species other than those of the Bacillus cereus group	Appendix C Line 1307	Please note that the described procedure is not applicable to all environmental Bacilli; most likely not all Bacilli grow well on Brain Heart infusion media. There seems to be some concerns from labs in using the BHI medium (concern regarding mad cow disease and Creutzfeldt-Jakob disease) outside the clinical field, i.e. for PPP testing for example. Is there any suitable medium that could be recommended for PPP testing (non-clinical labs)?
Appendix D – Cut-off values (mg/L) for bacteria	Appendix D & E	Appendix D and E Please note that MO used in agriculture context are mostly missing in the tables since they are not adapted to mammals' system and MIC values are (mostly) not available. A note shall be made in both table, or cross reference to the PPP legislation and GD dealing with this topic. If some Bacillus can be handled using the proposed values, for most fungi (e.g. *Trichoderma)* and several other bacteria (e.g. pseudomonas) no MICs are presented in the table. IBMA would welcome a resolution for PPP by acknowledging the nature of MO used in PPPs for plant protection and insect control, i.e. neither adapted to the human nor other mammalian systems.
Appendix E – Cut-off values (mg/L) for fungi	Appendix D & E	Appendix D and E Please note that MO used in agriculture context are mostly missing in the tables since they are not adapted to mammals' system and MIC values are (mostly) not available. A note shall be made in both table, or cross reference to the PPP legislation and GD dealing with this topic. If some Bacillus can be handled using the proposed values, for most fungi (e.g. *Trichoderma)* and several other bacteria (e.g. pseudomonas) no MICs are presented in the table. IBMA would welcome a resolution for PPP by acknowledging the nature of MO used in PPPs for plant protection and insect control, i.e. neither adapted to the human nor other mammalian systems.

Appendix F - Protocol for Extensive Literature Search (ELS), relevance screening and article evaluation to establish microbiological cut-off values for antimicrobials.	*It is not possible to leave comments on this section*	*It is not possible to leave comments on this section*
Appendix G – Search strategies to establish microbiological cut-off values for antimicrobial resistance.	*It is not possible to leave comments on this section*	*It is not possible to leave comments on this section*