GeneWatch UK comments on the EFSA GMO Panel's Scientific Opinion on New developments in biotechnology applied to microorganisms

April 2024

Summary

This document contains GeneWatch UK's response to the European Food Safety Authority (EFSA)'s consultation on genetically modified (GM) micro-organisms (GMMs), including those created using new genomic techniques (NGTs), such as gene editing (referred to as NGT-Ms in the consultation).[1] The Opinion considers different types of GMMs (including NGT-Ms), such as viruses, bacteria, yeasts, filamentous fungi and algae.

A narrower range of GMMs are currently widespread in contained use facilities (under Directive 2009/41/EC) for production of, e.g., additives and enzymes for use in food/feed or industrial products such as detergents. However, new GMM products are now being developed for commercial open release into the environment (under Directive 2001/18/EC). The Opinion and this response focus on two types of GMMs:

- Category 3: Products derived from GMMs in which GMMs capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present (e.g., heat-inactivated starter cultures);
- Category 4: Products consisting of or containing GMMs capable of multiplication or of transferring genes (e.g., live starter cultures for fermented foods and feed).

To date, there have been no applications or approvals for commercial open release of GMMs in the EU, outside the field of medicinal and veterinary products (the live cholera vaccine, Vaxchora, contains GMOs[2]).[3] Medicinal products are outside the scope of this Opinion. In the draft Opinion, attention is focused largely on technical changes in genetic engineering tools, i.e., the shift from the use of established genomic techniques (referred to in the Opinion as EGTs) to the use of new genomic techniques (so called NGTs). Insufficient attention is paid in the Opinion to the potential shift from contained use to open release of GMMs, including living (category 4) GMMs, which can reproduce as well as spread in the environment.

The draft Opinion also wrongly interprets EFSA's mandate (which is not limited to agri-food applications) and hence fails to consider some major issues of importance, such as:

- the need to consider a wider variety of potential applications, beyond agriculture and food/feed (e.g., aquaculture, bioremediation, and virus-induced genome editing in a variety of applications), that may have a direct or indirect impact on food and feed safety, and to include a wider range of receiving environments, including freshwater and marine environments;
- the need to assess the risk of cross-border (transboundary) movements of living genetically modified organisms (GMOs), including those developed using NGTs.

In relation to Category 3 and 4 GMMs, EFSA has rightly drawn attention to a number of important gaps in existing guidance, including that some proposed GMM applications have no comparator with a history of safe use in food or feed production. The Opinion notes that

there are many gaps in knowledge regarding, e.g., exposure assessment: allergenicity assessment; impacts on the microbiome (gut bacteria in humans and animals); horizontal gene transfer (from the GMM to other organisms), and environmental impacts. The Opinion also notes that Category 3 GMMs, although not living organisms, can transfer genes containing harmful traits, such as antimicrobial resistance, to other micro-organisms. However, the discussion of potential risks is not extensive (e.g., just over a page each on the gut microbiome and on potential environmental impact) and omits important issues. In particular, category 4 GMMs (which are living GMOs) can reproduce and spread in the environment and will co-evolve with existing micro-organisms (for example, in the human gut, soils and watercourses) in ways that are poorly understood and unpredictable.

Beyond EFSA's remit, but nevertheless relevant to decision-makers, are issues of risk management, such as traceability, labelling and liability for future harms to the environment and/or human or animal health. Open releases of GMMs (including NGT-Ms) pose major challenges to risk managers because worst-case scenarios include the spread of harmful, self-replicating organisms, including potential new pathogens, on a global scale.

EFSA is wrong to suggest that the relevant gaps in knowledge associated with the open release of GMMs can be addressed simply by developing new guidance. Allowing open releases of GMMs into the environment risks permanently (and negatively) altering complex ecosystems. The need for a precautionary approach is enshrined in Directive 2001/18/EC and in the Cartagena Protocol on Biosafety (implemented by Regulation (EC) No 1946/2003). Correctly interpreted, these legal instruments should lead to the conclusion that GMMs (including NGT-Ms) should not be deliberately released into the environment, due to the inability to predict and/or manage future adverse effects on human and animal health and the environment.

Comments on the EFSA Opinion, by Section

1 Introduction

1.1 Background and Terms of Reference as provided by the requestor

[It is not possible to leave feedback on this section].

1.2 Definition of new developments in biotechnology for the Terms of Reference

EFSA has interpreted the Terms of Reference too narrowly, by emphasising only the shift to new genetic engineering methods (New Genomic Techniques), rather than the important shift towards proposing open releases of GMMs (rather than contained use). The proposal to release GMMs into the environment is novel, as current applications all occur within contained use facilities. Industry researchers claim that there is sufficient evidence to support open releases of GMMs into the environment.[4] However, this paper is based on limited and speculative evidence, much of which is out-of-date and ignores the complexities which can give rise to unexpected risks (particularly in the gut microbiome, see comments on Section 3.2.3.6, and the wider environment, see comments on Section 3.2.3.10). By framing the Scientific Opinion in this way, EFSA has given the misleading impression that

relevant gaps in knowledge (some of which it has identified) can be addressed merely by developing new guidance.

1.3 Interpretation of the Terms of Reference

The earlier EFSA GMM Guidance (EFSA GMO Panel, 2011) covers only products intended for food and feed. The current mandate extends to all products of category 4 (i.e., live GMMs) to be released into the environment, as well as category 3 or 4 food and feed products. Under Regulation (EC) No. 178/2002 (Article 22), EFSA's mission covers "*all fields which have a direct or indirect impact on food and feed safety*". Whilst EFSA's remit does not extend to medicinal products, it is also not limited to agri-food products alone, yet EFSA appears to have wrongly interpreted the mandate in this way (i.e., including biofertilisers and biopesticides as well as food and feed, but no non-agricultural applications). In the search terms in Appendix A, aquaculture is omitted and applications such as biofuels are excluded (although production, in some cases, might use open ponds). Most obviously, EFSA has failed to consider any examples of GMMs (including NGT-Ms) that might be released directly into aquatic environments. For example, gene editing research is taking place in marine microalgae, which might be used as a source of lipids, and research is also taking place using marine bacteria.[5,6] More broadly, open releases of live GMMs in fields such as bioremediation[7], biomining[8], biosequestration (of greenhouse gases)[9] and biofuels production (including in open pond systems[10]) may also have an indirect impact on food and feed safety, via environmental contamination. The Opinion also omits any consideration of applications that use open releases of viral vectors as transient delivery vehicles of CRISPR-Cas components to plants (or other organisms), known as virus-induced genome editing (VIGE).[11] The release of novel genetically modified (GM) virus applications into the environment for agricultural, veterinary, and nature-conservation purposes may have a high probability for transmission and spreading, including transboundary movements and a high potential to result in adverse environmental effects.[12] Thus, a broader range of potential applications should have been considered, consistent with the mandate (which covers any GMMs for open release under Directive 2001/18/EC).

1.4 General outline of risk assessment for genetically modified microorganisms

2 Data and Methodologies

[It is not possible to leave comments on this section.]

2.1 Ad hoc expert Working Group and its methodology

[It is not possible to leave feedback on this section.]

2.2 Consultations

2.3 Horizon scanning Contractor and call for data

As noted in the comments on Section 1.3, the search terms (in Appendix A) have been wrongly limited to agricultural applications, excluding aquaculture and a broad range of potential GMMs that might be developed for commercial release into the environment (including aquatic environments).

2.4 Selection and description of the case studies

As noted in the comments on Section 1.3, the selection of case studies is too narrow. It is also unclear why no GMM biopesticides have been included (targeting insect pests, for example[13]). This leads to a number of unfortunate effects, including:

(i) Some environments (particularly aquatic environments) are not adequately considered, particularly in relation to pervasive uncertainties likely to arise when conducting Environmental Risk Assessments (ERAs);

(ii) A false impression is given that the relevant regulatory requirements might be delivered via a product-based approach, when, in reality, a very broad range of products (extending way beyond agriculture) need to be considered in the context of the risks that GMMs may pose to human health and the environment.

3 Assessment

3.1 ToR1: Novel potential hazards and risks that new developments in biotechnology applied to microorganisms could pose for humans, animals and the environment

3.1.1 AQ1. What are the new techniques/approaches developed since 2001 (namely, new developments in biotechnology) which could be applied/are applied to microorganisms?

3.1.1.1 CRISPR-Cas technology

3.1.1.2 New technologies for mutagenesis

3.1.1.3 Other site-directed nucleases

3.1.1.4 Synthetic biology

3.1.1.5 Genome minimization and genome design

3.1.1.6 Enabling technologies-DNA sequencing

3.1.2 AQ2. Are there any novel hazards that these new developments in biotechnology applied to microorganisms could pose to humans, animals and the environment, as compared to established genomic techniques and conventional mutagenesis?

3.1.3 AQ3. Are there any novel risks that these new developments in biotechnology applied to microorganisms could pose to humans, animals and the environment, as compared to established genomic techniques and conventional mutagenesis?

As noted in comments on Section 1.3, the Opinion omits any consideration of applications that plan to use open releases of viral vectors as transient delivery vehicles of CRISPR-Cas components to plants or animals, known as virus-induced genome editing (VIGE). The release of novel genetically modified (GM) virus applications into the environment for agricultural, veterinary, and nature-conservation purposes may have a high probability for

transmission and spreading, including transboundary movements and a high potential to result in adverse environmental effects.[14] Similarly, example 7 in Table 1 of the draft Opinion involves using bacteria-delivered genome-editing to edit live *Salmonella* bacteria in chickens. Introducing bacterial- or viral-delivered genome editing into open environments raises potential novel risks, due to the lack of ability to assess the potential intended or unintended effects prior to release, e,g., on- or off-target changes induced by genome editing techniques. It also exposes non-target organisms to genetic engineering machinery, raising novel risks, such as the potential generation of novel unintended GMOs (including pathogens).[15] These changes involve a shift from genome editing in a controlled laboratory environment to editing in the open environment. At the same time, there is a shift from genome editing mainly crop plants to editing a wide range of wild organisms in natural environments, which is also novel.

3.2 TOR2: Applicability and sufficiency of the existing guidelines for risk assessment of GMM to risk assess new developments in biotechnology applied to microorganisms

3.2.1 AQ1 and AQ2. What kind of GM microorganisms and GM microbial products within the EFSA remit have been identified and can be expected in the next 10 years that were developed using developments in biotechnology

As noted in the comments on Section 1.3, the search terms (in Appendix A) have been wrongly limited to agricultural applications, excluding aquaculture and a broad range of potential GMMs that might be developed for commercial release into the environment (including aquatic environments). In addition, applications involving virus-induced genome editing in the environment have been neglected from consideration. Whilst new products will not necessarily be developed within the next 10 years, it is important to include a broad range of potential applications because this affects the receiving environments that might need to be considered (e.g., aquatic environments), exposure pathways, and the relevance of existing product-based regulations in a wide variety of fields.

3.2.2 AQ3. Which are the existing guidelines to be used for the risk assessment of these GMMs?

The list of current GMO legislation should include Regulation (EC) 1946/2003, which implements the Cartagena Protocol to the Convention on Biological Diversity in the EU. This is relevant because the requirement for transboundary notifications of cross-border movements of GMMs is challenging and likely impossible to fulfil in the case of commercial open releases of GMMs into the environment. In relation to the product-based regulations in Table 2, only regulations relating to food, feed and agricultural products appear to have been considered: a considerably wider range of potential products are under development that could lead to the open release of GMMs into the environment (as noted in comments on Section 1.3). Although outside the scope of this consultation, it is important that the European Medicine Agency (EMA)'s Guidance is also outdated and inadequate to cover the potential open release of GM viruses in the context of medicinal or veterinary products. [16,17]

3.2.3 AQ4. Are the existing guidelines for risk assessment applicable, fully or partially, and sufficient for the risk assessment of GMMs generated with the use of the new developments in biotechnology?

As noted in comments on Section 1.3, the selected case studies are insufficient to identify all the gaps in relation to the current guidance (which covers only food and feed).

3.2.3.1 Comparative approach: use of a comparator

EFSA has rightly noted that the current guidance is insufficient. However, it is incorrect to imply that updated definitions are sufficient to cover cases where a comparator with a history of safe use is not available. In such cases, there is no scientific basis to allow open releases, given the requirement for a precautionary approach. Even where a comparator is available, current scientific knowledge is insufficient to ensure the required high level of protection of human health and the environment. This is due to the considerable complexity and very high level of uncertainties, in relation to the risk assessments needed, particularly in relation to the gut microbiome (see comments on Section 3.2.3.6) and the wider environment (see comments on Sections 3.2.3.10 and 3.2.3.11). Further difficulties occur in the case of GM bacteriophages (viruses that infect bacteria), for example, due to difficulties regarding their taxonomic classification and their ability to carry potentially harmful traits.[18]

3.2.3.2 Microbial characterisation

EFSA has rightly identified that the existing guidance is not sufficient, particularly in relation to antimycotic resistance, and to the characterisation of microalgae and viruses.

3.2.3.3 Information relating to the manufacturing process and product specifications

EFSA is correct to note that the current guidance is insufficient because it fails to consider microalgae or viruses. However, a much broader range of products (including non-agricultural products) should have been considered in the case studies, to likely identify more gaps (see comments on Section 1.3).

3.2.3.4 Compositional analysis

The Opinion rightly notes the difficulties in making a comparative compositional analysis where there is no comparator with a history of safe use. However, the idea that the strategy laid down in the Novel Food Guidance could be used as a basis for this is totally inadequate because:

(i) Not all GMMs to be considered for potential open release are foods (see comments on Section 1.3);
(ii) The Novel Food Guidance has not been developed with GMMs in mind – in fact, it explicitly refers back to relevant guidance for GMMs.

It is likely to be impossible to assess of substantial importance, such as impacts on the gut microbiome (see comments on Section 3.2.3.6) and the wider environment (see comments on Sections 3.2.3.10 and 3.2.3.11) in the case of living (category 4) GMMs, and this would be

particularly difficult in cases with no comparator with a history of safe use. In many cases, the composition of the product may be unstable, and will also liable to change as it evolves in the environment.[19]

3.2.3.5 Toxicology

The Opinion rightly notes that the current guidance does not cover viruses, and that newly expressed proteins, mutated proteins, new metabolites and altered levels of constituents other than proteins all need to be assessed. However, it underestimates the difficulties of doing so, due to the complexities of altered metabolic pathways, which may depend on the environment (see also comments on Section 3.2.3.6).[20] For example, the detection of toxin genes does not always provide a reliable approach to predict the pathogenic potential of bacteria.[21] These difficulties will be exacerbated in cases where there is no comparator with a history of safe use.

3.2.3.6 Gut microbiome

The Opinion identifies important gaps in the evaluation of intended and unintended effects on the gut microbiome, correctly stating that existing guidance is insufficient for all case studies. However, the difficulties in assessing potential risks due to the evolution of the GMM, or of the microbiome in response to the GMM, have not been properly considered. Micro-organisms continuously divide inside the guts of humans and animals and hence microbiota can evolve over time, through a mixture of within-host evolution and the invasion of external strains.[22] Understanding the evolution of microbiota is at a very early stage of research and shows considerable complexity. The effects of introducing GMMs into this environment are poorly understood. Although the Opinion has rightly identified the risk of GMMs increasing antimicrobial resistance (AMR), the introduction of new genetic variants can also alter metabolism, the breakdown of drugs, or colonization resistance against pathogens.[23] The human gut microbiome is a complex community with a vast network of microbe–host interactions and horizontal gene transfer (HGT) in the microbiome has profound consequences for human health and disease.[24] As well as the transfer of specific traits from GMMs to other micro-organisms, the whole gut ecosystem may evolve, leading to significant changes in species composition. Although it is to be hoped that risk assessment processes might avoid the direct introduction of new GMM pathogens, existing pathogens also evolve within human and animal guts, and their evolution may utilise any newly introduced traits from GMMs in unpredictable ways.[25,26] The processes of domestication, horizontal gene transfer and microbial succession might be important mechanisms behind the many pathogen spill-over events driven and accelerated by climate change, biodiversity loss and globalization.[27] Given this complexity, potential changes in virulence or pathogenicity are highly unpredictable. It is therefore highly implausible, given the current state of scientific knowledge, that new guidance could be developed that adequately addresses all the gaps.

3.2.3.7 *Allergenicity*

The Opinion rightly notes the difficulties in assessing allergenicity and that existing guidance is not sufficient. In particular it notes that substances other than proteins may hold

adjuvanticity (the ability of a substance to augment the body's immune response to an antigen) and that the assessment of adjuvanticity is not sufficient because it would also be necessary to consider the functional features of GMMs, or GMM-derived metabolites, that are linked to adjuvanticity. However, it is unclear how the recommended update to the guidance can adequately assess these concerns.

3.2.3.8 *Nutritional assessment*

The Opinion underestimates the difficulties of assessing the nutritional impact in cases where there is no comparator with a history of safe use. Further, the complexities of interactions with the gut microbiome should also have been considered, given that GMMs could also impact on metabolism (see comments on Section 3.2.3.6).

3.2.3.9 *Exposure assessment*

The Opinion correctly notes that GMMs used as bio-fertiliser expose the environment to potential adverse effects, and also expose human and animal guts via consumption of food/feed treated with fertiliser. Existing guidance is insufficient for open release applications of GMMs and both primary and secondary exposure routes need to be assessed (also including viruses). However, the Opinion is again over-optimistic that this can be achieved simply be developing new guidance. Many proposed applications for commercial open release require GMMs to persist and/or spread in the environment and to compete with indigenous microorganisms in order to achieve the desired result.[28,29,30,31,32] Whilst, in practice, this may mean that GMMs do not deliver on the promises made for this technology, open releases of GMMs could also lead to 'living pollution', which may spread and reproduce in the environment and lead to unpredictable exposures (including transboundary movements from one country to another). This risks permanently (and negatively) altering complex ecosystems.

For GMMs used in food or feed (including fish feed), or in agriculture or aquaculture, or which reach human or animal guts via other pathways, human or animal faeces may spread GMMs onto agricultural fields or into watercourses, including the marine environment. This will include any micro-organisms that have co-evolved in the gut to include perhaps harmful traits (see comments on Sections 3.2.3.6 and 3.2.3.11) Antibiotic resistance genes and mobile genetic elements can spread through wastewater treatment sites and rivers.[33,34,35] Bacterial aerosols can spread from landfill sites.[36,37,38] Changes in the environmental fitness of GMMs can be dependent on the environmental conditions, further adding to complexity.[39] In some cases, GMMs may be designed to be self-spreading, or to be spread by insects or other environmental mechanisms, in ways that are highly unpredictable. [40]

3.2.3.*10 Potential environmental impact of GMMs and their products*

The Opinion correctly notes that the existing guidance on environmental risk assessment (ERA) is insufficient for all category 4 GMMs (living GMMs), and that it is recommended to elaborate on all the relevant areas of risk as per Directive 2001/18/EC. However, the Opinion itself does not elaborate on these areas of risk and this leads to the erroneous conclusion that updating the existing guidance is sufficient. In reality, few relevant studies

are available and existing knowledge is unlikely to be able to ensure the necessary level of protection of human and animal health and the environment, due to the complexities of microbial interactions and evolution (as discussed in comments on Section 3.2.3.6). Some of the gaps in research have already been identified in the EFSA Scientific Committee's SynBioM ERA Opinion of 2020, so it is surprising that they are not reiterated here. Open releases of GMMs risk permanently (and negatively) altering complex ecosystems in ways that may be unpredictable.

The Opinion also states that the existing guidance is sufficient for category 3 products, as only ERA of horizontal gene transfer (HGT) is necessary: however, the ERA of HGT discussed in the Opinion is inadequate because it is limited to antimicrobial resistance (ARMs) and neglects the potential transfer of other harmful genetic traits (see comments on Section 3.2.3.11).

3.2.3.11 *Horizontal gene transfer*

The Opinion rightly notes the importance of assessing the risk of transfer of antimicrobial resistance genes (ARMs) from category 3 or 4 GMMs to humans, animals or the environment. However, it should go further and require the industry to phase out all remaining production strains of GMMs (in any category, 1 to 4) containing antibiotic resistance marker genes. These are not necessary and pose unnecessary risks to human and animal health and the environment.

The Opinion wrongly proposes weakening existing guidance to focus solely on the horizontal gene transfer of ARMs. As the Opinion itself recognises, genes encoding harmful traits may spread in the microbiota and may provide a selective advantage to some of their members, thereby reducing or displacing other microorganisms with beneficial properties. Yet, no means to assess these risks, or even to consider risks other than the transfer of antimicrobial resistance, appears to be proposed. Virulence factors that determine a bacterial strain's pathogenicity can also be transferred horizontally, as can genes involved in metabolic functions, including the breakdown of certain sugars or the breakdown of drugs.[41,42] Similar concerns apply to viruses.[43] By omitting consideration of the implications of HGT for any of these traits, the Opinion does not adequately consider the consequences of HGT for human or animal health, or for the environment.

3.2.3.12 *Post-market environmental monitoring*

The Opinion proposes weakening the requirements for post-market environmental monitoring. This is unacceptable, given the many potential hazards and uncertainties identified above (see comments on Sections 3.2.3.6, 3.2.3.9, 3.2.3.10, 3.2.3.11). Open releases of GMMs risk permanently altering ecosystems in ways that may not be reversible. In addition, monitoring is very important for GM virus applications, due to their high potential for survival and spread, as well as their ability to quickly mutate and evolve. [44] It is difficult to see how adequate monitoring can be achieved in practice for open release GMM applications, as these organisms spread, replicate and evolve in the environment.

3.3 *ToR3: In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to identify on which aspects existing guidelines should be updated, adapted or complemented.*

3.3.1 AQ1. Which aspect (if any) of existing guidelines should be updated, adapted, or complemented?

The Opinion is correct to conclude that existing guidance is inadequate. However, the many gaps in scientific knowledge cannot be addressed simply by proposing updates to the guidance. GMMs (including NGT-Ms) should not be deliberately released into the environment, due to the inability to predict and/or manage future adverse effects on human and animal health and the environment. In addition, industry should be required to phase out all remaining production strains of GMMs containing antibiotic resistance marker genes. These are not necessary and pose unnecessary risks to human and animal health and the environment, even in contained use production systems.

3.3.2 AQ2. What recommendations can be formulated for future guidance updates?

Although many of the proposed updates are necessary (in the sense that existing guidance is inadequate), updating guidance will not deliver the required level of protection for human and animal health and the environment. In addition, proposals to weaken requirements for post-market environmental monitoring (PMEM), and to limit consideration of horizontal gene transfer (HGT) to antimicrobial resistance genes (ARMs) are not consistent with the evidence.

3.3.2.1 Comparative approach
3.3.2.2 Microbial characterisation
3.3.2.3 Toxicology
3.3.2.4 Gut microbiome
3.3.2.5 Allergenicity
3.3.2.6 Nutritional assessment
3.3.2.7 Exposure
3.3.2.8 Environmental Risk Assessment (ERA)
3.3.2.9 HGT
Proposals to limit consideration of horizontal gene transfer (HGT) to antimicrobial resistance genes (ARMs) are not consistent with the evidence.

3.3.2.10 PMEM
Proposals to weaken requirements for post-market environmental monitoring (PMEM) are not consistent with the evidence.

3.3.3 Future recommendations

The recommendations here are limited to requiring a common risk assessment approach for GMMs and NGT-Ms. The issues to be assessed are indeed the same (as outlined in Directive 2001/18/EC, regarding open releases of GMMs into the environment, and Regulation (EC) 1829/2003, regarding genetically modified food and feed). However, elsewhere in the

Opinion, EFSA has concluded that there are significant gaps in the knowledge needed to conduct risk assessments (Section 3.2), and has outlined how these will be addressed by adopting new Guidance (Section 3.3). In reality, there are major additional gaps (see comments above) and there is no realistic prospect of addressing these gaps by the means proposed, and hence no means to ensure that the environmental release of GMMs does not pose unacceptable risks to human and animal health and the environment. Allowing open releases of GMMs into the environment risks permanently (and negatively) altering complex ecosystems. In addition, some proposals in Section 3.3 are deregulatory (such as proposals to limit post-market monitoring). EFSA should instead acknowledge that these gaps in knowledge cannot be addressed merely by developing new guidance and, as a result, GMMs (including NGT-Ms) cannot lawfully be deliberately released into the environment. In addition, industry should be required to phase out all remaining production strains of GMMs containing antibiotic resistance marker genes. These are not necessary and pose unnecessary risks to human and animal health and the environment, even in contained use production systems.

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