Annex

QUESTIONNAIRE FOR THE TESTING OF THE GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

GENERAL INFORMATION ABOUT THE TESTING	
Q1. These results are being submitted on behalf of a:	☐ Party. Please specify: <Country's name> ☐ Other Government. Please specify: <Country's name> ☒ Organization: Please specify: Global Industry Organization
Q2. When was the testing of the Guidance conducted?	Please enter date: November 2011
Q3. Type of event where the testing of the Guidance was conducted?	☐ Group event (e.g., workshop, training course, meeting). Please provide the title of the event and name of organizer: <Type here> Type of meeting: ☐ Face-to-face ☐ Online ☐ Individual exercise. Please provide your name, occupation and affiliation: <Type here> ☒ Other: Please specify: During opportunity for review
Q4. Which sections of the Guidance were tested?	☒ Part I: The Roadmap for Risk assessment of LMOs Part II: Specific types of LMOs or Traits: ☒ Risk assessment of LMOs with stacked genes or traits ☒ Risk assessment of LM crops with tolerance to abiotic stress ☐ Risk assessment of LM mosquitoes

OVERALL EVALUATION					
	Very poor	**Poor**	**Neutral**	**Good**	**Very good**
Please indicate the level of agreement you attribute to each of the questions in the left column.					
Q5. How do you evaluate the level of consistency of the Guidance with the Cartagena Protocol on Biosafety, particularly with its Article 15 and Annex III?	☐	☒	☐	☐	☐
Q6. How do you evaluate the usefulness of the Guidance as a tool to assist countries in conducting and reviewing risk assessments of LMOs in a scientifically sound and case-by-case manner?	☐	☒	☐	☐	☐
Q7. How do you evaluate the usefulness of the Guidance as a tool to assist countries in conducting and reviewing risk assessments of LMOs introduced into various receiving environments?	☐	☒	☐	☐	☐

PART I: ROADMAP FOR RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

Please answer each of the questions in the left column with "yes" or "no" and add comments if needed.

Q8. Does the Roadmap provide useful guidance for conducting risk assessments of LMOs in accordance with the Protocol?

☐ Yes
☒ No

Comments:

On different aspects the Roadmap either provides a particular interpretation of the Protocol or goes beyond what is indicated in the Protocol. Furthermore, as guidance, the roadmap appears too theoretical. Providing examples, rather than special cases in Part II, would be useful for understanding some of the abstract concepts and could help less experienced risk assessors to understand the logic of the process. The following table provides some examples:

Line	Referenced text	Comment
0074	The choice of protection goals may, in addition to environmental considerations, also be based on societal and economic considerations (see Related Issues section) and may be informed by Annex I of the Convention on Biological Diversity.	The reference to the related issues could be interpreted as indicating that these are now eligible as protection goals in the risk assessment whereas they are on purpose excluded and only mentioned as "related issues". Art 26 Protocol limits the use of socio-economic considerations: - in application: in reaching a decision - in scope: only socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities. The Roadmap reference broadens the scope beyond the Protocol. Suggest to replace by: *The choice of protection goals may be informed by Annex 1 of the Convention on Biological Diversity as relevant to the Party.*
0091	Similarly, the issues mentioned in the 'Setting the context and scope' section below may be taken into consideration again at the end of the risk assessment process to determine whether the objectives and criteria that were set out at the beginning of the risk assessment have been met.	This is not supported by practice and remains very theoretical as no examples are provided. Can an example be provided of a case where, after setting the context and scope, and subsequently proceeding with the risk assessment, it would be concluded that the objectives and criteria were not met? How will this be done? This step is confusing and, if maintained would benefit from real world examples of regulatory submissions.
0095	In the decision-making process, other Articles of the Protocol or other relevant issues may also be taken into account and are listed in the last paragraph of this Roadmap: 'Related Issues'.	This confuses Articles of the Protocol and other issues for which there is no basis in the Protocol. As such this goes beyond the scope of the Protocol. It is now also mentioned that socio-economic considerations as defined in Art 26 may influence the decision-making but should not get mixed in the risk assessment process.
0114	For example, data may be considered relevant if they are linked to protection goals or assessment endpoints, contribute to the identification and evaluation of the potential adverse effects of the LMO, or if they can affect the outcome of the risk assessment or the decision.	In this case it may be more useful to indicate when data would not be considered relevant. Extend this example by: *For example, data may be considered relevant if they are linked to protection goals or assessment endpoints, contribute to the identification and evaluation of the potential adverse effects of the LMO, or if they can*

		affect the outcome of the risk assessment or the decision. Data would be considered irrelevant if they merely answer questions of scientific curiosity or if potential adverse effects cannot be logically linked to the LMO.
0128	This would include ensuring the accessibility of data by the risk assessors (e.g. the availability of relevant, required data or information or, if requested and as appropriate, of sample material),	This explanation moves beyond the information requirements included in the Protocol. It is incorrect to present the provision of sample material as a principle of scientific quality.
0139	Availability of independent experts with the relevant background in the different scientific disciplines needed to conduct risk assessments or to provide input into the risk assessment process**.**	This point is listed among issues that should be considered to ensure the quality and relevance of the information used as well as the outcome of the risk assessment. Whereas the previous "issues" handle information, this point refers to availability of experts without justifying their role in the risk assessment process. Would the risk assessment be less relevant if independent experts with relevant backgrounds are not available? What is meant by "independent"? What are the "the different scientific disciplines needed to conduct risk assessments"?
0178	…and starts by setting its context and scope in a way that is consistent with the country's protection goals, assessment endpoints, risk thresholds, management strategies and policies.	We understand the context and scope to be determined in function of the protection goals and policies of the Party. It is not clear how this should be consistent with "assessment endpoints". The reference to risk thresholds and management strategies seems inappropriate (also refer to definitions provided at the end of the document) or should be further explained with an example.
0191	iii) Protection goals, assessment endpoints, risk thresholds and management strategies as laid down, for instance, in the relevant legislation of the Party;	This citation refers to some of the aspects of existing environmental and health policies and strategies that can be taken into account when setting the context and scope. It would assist readers to understand how risk thresholds and management strategies should be interpreted in this sentence. There seems to be confusion with the definitions included at the end of the document.
0208	Means of describing the level of the potential adverse effects of LMOs and its transfer, handling and use, as well as the terms that are used to describe the likelihood (step 2), the magnitude of consequences (step 3) and risks (step 4) and the acceptability or manageability of risks (step 5; see risk assessment steps below) as well as the criteria used to distinguish between the terms	The term "means of describing" is not clear in this sentence. We assume that it refers to the terminology like in the subsequent part of the sentence. As in each of the steps a possibility is already indicated, it would be better to either leave it out here or to include a reference to the specific sections.
0232	In risk assessments where the (near-) isogenic non-modified recipient organism is used as the comparator, additional comparators may prove useful depending on the biology of the organism and types of modified traits under assessment.	Not clear how this provides guidance. How may it prove useful? How is this related to biology of the LMO or to the type of modified traits?
0381	This can be done by building conceptual models describing relationships between the LMO, and pathways of exposure and potential effects in the environment. For example, concerning an LMO producing a potentially toxic gene product, oral, respiratory or dermal exposure could be relevant.	The second sentence is indicated to provide an example of how a conceptual model can be built. Assuming that this is step 2, it should be known to what degree the gene product is toxic or not. The potential to show the toxic effect depends on the exposure of the sensitive organisms to a certain dose. It should also be known in what form the toxin will act and therefore the choice of exposure routes may be limited by that determination. Finally, the example lacks any mention of the potential effect.
0384	Models, including conceptual ones, …	It would be interesting to include examples of non-conceptual models that are used in risk assessment.

0394	The levels of likelihood may be expressed quantitatively or qualitatively, for example, by the terms 'highly likely', 'likely', 'unlikely', 'highly unlikely'. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.	See comment on line 0208.
0442	The use of well-formulated risk hypothesis (step 1) may be helpful in assessing the consequences of potential adverse effects.	Can an example be provided on how a risk hypothesis, which is supposed to help testing if a risk may occur, may help in assessing the consequences of a potential adverse effect?
0455	The evaluation of the consequence of adverse effects may be expressed qualitatively or quantitatively. For instance, terms such as 'major', 'intermediate', 'minor' or 'marginal' may be used. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.	See comment on line 0208.
0495	A description of the risk characterization may be expressed qualitatively or quantitatively. Terms such as 'high', 'medium', 'low', 'negligible' or 'indeterminate' (e.g. due to uncertainty or lack of knowledge) have been used to characterize the overall risk of an LMO. Parties could consider describing these terms and their uses in risk assessment guidelines published or adopted by them.	See comment on line 0208.
0535	The recommendation on the acceptability of risk(s) should take into account a scientific benefit analysis as well as risks associated with other existing user practices and habits and also acknowledge the identified uncertainties.	The Protocol doesn't foresee benefit analysis and it is rather simplistic to give a single statement by the end of step 5 that a scientific benefit analysis should be taken into account, where no indication has been provided before. Without further indication, this could refer to benefits for the environment and human health (*e.g.*, change in the use of crop protection products, reduction of infections in the case of mosquitoes), but it might also refer to socio-economic considerations that can be taken into account in decision-making. This statement also suggests that there may be risk associated with other user practices and habits. It is confusing to suggest that there would be other risks that have not been addressed before.
0543	Monitoring can be applied as a tool to detect unexpected and long-term adverse effects. Monitoring can also be a means to reduce uncertainty, address assumptions made during the risk assessment and to validate its conclusions on a wider (e.g. commercial) level of application and to establish a causal link or pathway between LMOs and adverse effects. Monitoring may also be used as an instrument for effective risk management, including the detection of adverse effects before the consequences are realized.	Note that in the context of Annex III of the Protocol, monitoring is only included as one of the possible actions to take in case of scientific uncertainty. We suggest that any other possible use of monitoring is omitted from the Roadmap as it confuses the issue. A reference could be made to the specific document that is being developed on monitoring.
0548	The issues mentioned in the 'Setting the context and scope' section may be taken into consideration again at the end of the risk assessment process to evaluate whether the objectives and criteria that were set out at the beginning of the risk assessment have been met.	See comment on line 0091
0560	(c) Scientific benefit analyses carried out using similar principles of sound-science as those used throughout the risk assessment	See comment on line 0091
0584 - 0590	Section on Related issues	Risk Management is already captured in step 5 of the risk assessment.

		The Protocol doesn't foresee that Capacity-building; Public Awareness and Participation; and Liability and Redress are considered during decision-making. Socio-economic Considerations are covered within the limits provided by the Protocol. As they may be considered during decision-making they should not be included as protection goals. Co-existence and Ethical issues are not within the scope of the Protocol and should not be included in this section at all.

We remain concerned that uncertainty receives too much attention in this version of the Roadmap
It seems that given that no risks have been identified, the risk assessors are guided to consider uncertainty as risk. Although several statements are made that "uncertainty should not automatically be considered as a risk per se", there is an overemphasis on dealing with uncertainty that is inherent to science.

The detailed comments further illustrate that either we consider uncertainty as part of the quality of information (so called "overarching issues") or as a point to consider at each step. The former seems more justified and practical in our experience.

Line	Referenced text	Comment
0142	Identification and consideration of uncertainty	Why is it considered an overarching issue? Annex III places uncertainty as an element for consideration in point 8f in the risk assessment.
0147	According to the Protocol, "where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies or monitoring the living modified organism in the receiving environment". The issue of uncertainty is dealt with – sometimes differently – in each international instrument incorporating precautionary measures.	Must introduce the fact that not all uncertainties are relevant for risk assessment Suggest to add the following sentence to this paragraph: *Irrespective, it should be recognized that it is not necessary to eliminate all uncertainty in order to arrive at a valid risk assessment.*
0366	(p) Consideration of uncertainties arising in step 1 (see "Identification and consideration of uncertainty" under the section "Overarching Issues in the risk assessment process").	Must include the fact that not all uncertainties are relevant for risk assessment In addition it is incorrect to add this to the list of points to consider regarding the potential adverse effects resulting from the interaction between the LMO and the receiving environment.
0433	(i) A consideration of uncertainty arising in step 2 (see "Identification and consideration of uncertainty" under the "Overarching issues in the risk assessment process").	Must include the fact that not all uncertainties are relevant for risk assessment In addition it is incorrect to add this to the list of points to consider as equal to the other points.
0479	(f) A consideration of uncertainty arising in step 3 that may significantly impact the evaluation of consequences should the adverse effects be realized (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).	Must include the fact that not all uncertainties are relevant for risk assessment In addition it is incorrect to add this to the list of points to consider as equal to the other points.
0490	… and also taking into consideration any relevant uncertainty that emerged in the preceding steps.	It is not very helpful to indicate that uncertainty must be taken into consideration without further clarification. In each of the previous steps uncertainty has already been addressed or it has been addressed as an overarching issue. At this step the consideration should be limited to verify if an uncertainty would be relevant for the outcome of the risk assessment. It would be better to indicate "identified" than

		"emerged".
0509	(g) A consideration of uncertainty arising in this and the previous steps (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).	Must include the fact that not all uncertainties are relevant for risk assessment In addition it is incorrect to add this to the list of points to consider as equal to the other points.

The section on "Choice of comparators" covers different aspects of the comparative approach that is at the basis of most risk assessments conducted to date. It is of interest to point out that a choice of comparators exists and that a careful, justified selection should be made on a case-by-case basis. The text as presented now, focusing strongly on the (near-) isogenic line provides a certain logic, which may not be justified in all cases. We recommend to adapt this important section and suggest listing different options for comparators, each with their strengths and potential drawbacks. This would help risk assessors to get insight in the relative importance of any difference that is observed in the comparison.

Line	Referenced text	Comment
0220	The comparative approach aims at identifying changes occurring between an LMO and its comparator that may lead to adverse effects.	Replace by: *The comparative approach aims at identifying changes between an LMO and its comparator that may lead to adverse effects.*
0227	Some risk assessment frameworks use the (near-)isogenic non-modified organism as the primary choice of comparator	Why point out that this is primary choice? We suggest listing different options.
0228	To account for natural variation, as the same organism grown under different environmental conditions can lead to significant differences, the comparators that are going to provide the basis for comparison should be grown or should live at the same time, location and physiological conditions as the LMO under consideration.	Although some authorities may desire comparative testing, there is no reason why comparison of data from the LMO grown in different locations compared with generally accepted baseline databases would not be sufficient. Therefore presenting the simultaneous growing as an obligation is limiting the options without scientific justification.
0232	In risk assessments where the (near-) isogenic non-modified recipient organism is used as the comparator, additional comparators may prove useful depending on the biology of the organism and types of modified traits under assessment.	The (near-) isogenic organism is used to identify if there are differences that could cause adverse effects between the LMO and the comparator. Similarly, using other comparators may serve to identify such differences. This sentence incorrectly puts other comparators as "additional". We suggest that these are treated as equally valid and that the choice must be justified.
0234	In practice, the (near-)isogenic non-modified organism is used in step 1 and throughout the risk assessment.	Not clear what "in practice" means. Also not clear why a reference is made to step 1 and throughout the risk assessment. Again, this statement incorrectly suggests that there is a hierarchy between comparators and that the near-isogenic is the most prominent one and that it must be used.
0235	When the likelihood and potential consequences of adverse effects are evaluated, broader knowledge and experience with additional comparators such as reference lines may also be taken into consideration, as appropriate, along with the non-modified recipient organism.	The main point remains the identification of differences between the LMO and a justifiable relevant comparator that could have an adverse effect. This is not relevant at step 2 or 3. We propose to clarify this by following modification: *If a difference is identified between the LMO and its comparator, then broader knowledge and experience with additional comparators such as reference lines may be used to determine the relevance of the difference.*
0240	In certain cases, the (near-)isogenic non-modified comparator may not be sufficient to establish a good basis for a comparative risk assessment, such as for the risk assessment of LM plants tolerant to abiotic stress, stacked LMOs and certain LM mosquitoes (please refer to Part II of this Guidance for some examples).	This statement is incorrect by its generality and seems to be conflicting with the subsequent sentence (0244). There will be cases of plants to abiotic stress, stacked LMOs and LMO mosquitoes for which a (near-) isogenic comparator will be perfectly suited. The sentence is misguiding, is based on false presumptions and digresses from the case-by-case approach.

0244	In other risk assessment frameworks, the choice of an appropriate comparator will depend on the specific LMO being considered, the step in the risk assessment and on the questions that are being asked.	This sentence captures well the actual situation: depending on the case a justified choice must be made on which comparators to use. We suggest to start this section on choice of comparators with this sentence, and then discuss several options, including the (near-)isogenic material, other reference materials, other LMOs.
0284	In this step, a comparison of the LMO may be carried out with the non-modified recipient or parental organisms in the likely potential receiving environment, taking into consideration the new trait(s) of the LMO	The focus must be on the identification of differences that may have an adverse effect. This may be by comparison based on simultaneous testing of LMO and comparator(s) in containment or in releases in the environment as long as the obtained information is relevant for the purpose of the risk assessment. Consequently, the reference to the non-modified recipient or parental organism (previously not mentioned as such in the section on comparators), the indication of "in the likely potential receiving environment" and " taking into consideration the new traits of the LMO" are confusing and provide no concrete guidance.
0557	(b) Any relevant experience with the non-modified recipient organism(s) and practices associated with its use in the likely potential receiving environment which were used to establish the baseline for the risk assessment;	Step 5 is the first and only time in the main text of the roadmap that a "baseline" is mentioned. Why is it introduced so late and why in this context?

Q9. Is the Roadmap useful to risk assessors who have limited experience with LMO risk assessment? ☐ Yes ☒ No Comments: <Type here>

The wording of the Roadmap should be improved in order not to confuse risk assessors with limited experience with LMO risk assessment on basic elements of the risk assessment. In some cases, more information will be required to fully understand why statements are made or what they mean in practice. The following table provides some examples of this point:

Line	Referenced text	Comment
0062	… a structured process conducted in a scientifically sound and transparent manner	A much (mis)used and confusing term. Suggest: *.. a structured, science based process conducted in a transparent manner"*
0073	What is considered an adverse effect as well as an "acceptable risk"…	Many aspects relate to protection goals, but linking consideration of an effect as adverse and the acceptability of a risk would at least require further explanation. They are very different in nature and are considered at quite different moments in the risk assessment. The relationship between adverse effects and protection goals is very different than between acceptable risk and protection goals. This sentence can be clarified in the following way: *In order to determine if a potential effect should be considered adverse, the potential impact on protection goals needs to be evaluated. This is assessed taking into account appropriate assessment endpoints*.
0101	**OVERARCHING ISSUES**	There is nowhere in the guidance or in the documents, which the guidance is expected to complement, a definition of an "overarching issue". In common language it could refer to an issue that is dominating or embracing all else. We believe that this is incorrect for the issues that are indicated. It would be better to talk about quality aspects, as both scientific quality and uncertainty can be related to the quality of the information.
0124	Risk assessments frequently require that data	Confusing sentence would require at least an example.

	generated from multiple scientific fields, which are sometimes diverging or even contradictory, be used and analyzed.	What does "frequently" mean? Can an example be provided of "diverging" and "contradictory" data? As well as an example of how they can be analyzed??
0280	It may be important to define a causal link or pathway between a characteristic of the LMO and a possible adverse effect, which may be direct or indirect, immediate or delayed,[19] otherwise the risk assessment may generate information that will not contribute to reaching a recommendation that will be useful for the decision-making process.	Footnote 19 refers to article 2, paragraph 2(b) of the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress. It is not clear why this is relevant at this point in the text.
0289	The novel characteristics of the LMO that may cause adverse effects may be intended or unintended, direct or indirect, immediate or delayed, combinatorial or cumulative, as well as predicted or unpredicted.	This statement is very confusing. A characteristic may be intended or unintended. However when indicating direct or indirect, immediate or delayed, combinatorial or cumulative, reference is made to possible (adverse) effects.
0335	… intrinsic level of confinement (such as biological confinement)..	What is meant by "intrinsic level of confinement"? The fact that biological confinement is indicated with "such as" suggests that there are other forms of intrinsic confinement. Including biological confinement as a point for consideration for the receiving environment is misleading.
0344 - 0365	Points h) to o)	This list provides points to consider regarding the potential adverse effects resulting from the interaction between the LMO and the receiving environment. It is not a useful list as it is an amalgam of overall objectives (e.g. protection goals); biology of the LMO (e.g. survival, outcrossing, horizontal gene transfer) and potential adverse effects (e.g. on non-target organisms, on human health, ..) The presentation is very confusing and lacks clear guidance on how to consider these aspects.
0389	Examples of issues to be considered in this step include (i) the potential of the LMO (or its derivatives resulting from outcrossing) to spread and establish in and beyond the receiving environment (in particular into protected areas and centres of origin and genetic diversity), and whether that could result in adverse effects; and (ii) the possibility of occurrence of adverse (e.g. toxic) effects on organisms (or on organisms other than the 'target organism' for some types of LMOs (e.g. those producing insecticidal proteins).	Not clear why this is included in this way, in particular as a few paragraphs later the list with points to consider is provided. Confusing.
0422	(g) When assessing the likelihood of outcrossing and outbreeding from the LMO to sexually compatible species, …	The term "Outbreeding" is not commonly used in this context. It is artificial to introduce this difference and can only confuse people.
0444	In this step, results of tests done under different conditions, such as laboratory experiments or experimental releases, may be considered.	Why only in this step? This suggests that in (some) other steps this would not be acceptable? In our experience, the tiered approach is relevant at any step of the risk assessment.
0445	The scale of the intended use (e.g. small or large) should be taken into account.	This seems more related to likelihood or to exposure assessment.
0472	(d) Results from laboratory experiments examining, inter alia, dose-response relationships (e.g., EC50, LD50), sub-chronic effects and immunogenic effects as information elements in the context of determining effects on non-target organisms, and from field trials evaluating, for instance, potential invasiveness;	"Inter alia" would suggest that all of these are necessary as well as other result. This would not be in line with the case-by-case approach. We suggest replacing "inter alia" by "as appropriate".

0476	(e) For the case of outcrossing to sexually compatible species, the possible adverse effects that may occur, after introgression, due to the expression of the transgenes in the sexually compatible species; and	This point seems to be in the wrong list. It belongs to step 1 to identify if such adverse effects may arise.
0505	(d) Risk management options, if identified in step 5;	Unclear how step 5 will occur before step 4.
0507	(f) Broader ecosystem and landscape considerations, including cumulative effects due to the presence of various LMOs in the receiving environment; and	This must have been considered in step 1. Why would it be included in step 4?

Also the structure of some sentences is very complex and convoluted. Some sentences may be wrongly constructed as a result of several rounds of editing. Taking into account that for many people English may not be their first language, an effort should be made to simplify and to correct the text. Some examples:

Line	**Referenced text**	**Comment**
0070	The novel combination of genetic material in an LMO and its use may lead to environmental effects which may vary depending on the LMO itself, the environment exposed to the LMO and how the LMO is used.	Complex sentence. Replace by: *The potential environmental effects of the use of an LMO may vary depending on the characteristics of the LMO, on how the LMO is used and on the environment exposed to the LMO.*
0072	The effects may be intended or unintended, beneficial, neutral or adverse.	Complex sentence. Replace by: *A potential effect may be intended of unintended. Depending on the impact on a protection goal the potential effect may be considered beneficial, neutral or adverse.*
0122	Adequate statistical tests should be used to generate statistically meaningful results. Where appropriate, in the risk assessment and be described in the risk assessment report. d	This is badly formulated. We also see no benefit of describing the analysis in the risk assessment provided that it will be described in the information that is submitted in support of the risk assessment. We assume that the following is intended: *Where appropriate, adequate statistical tests should be used to strengthen the scientific conclusions that will be used in the risk assessment. Such methods should be described in the supporting documentation*.
0228	To account for natural variation, as the same organism grown under different environmental conditions can lead to significant differences, the comparators that are going to provide the basis for comparison should be grown or should live at the same time, location and physiological conditions as the LMO under consideration.	Complex sentence. Replace by: *To account for variation due to interaction with the environment, studies may include the LMO as well as the comparator(s) tested at the same time, location and physiological conditions.*
0266	The purpose of this step is to identify potential adverse effects that may result from changes due to the genetic modification(s) compared to the non-modified recipient organism, and identify what, if any, of those changes could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.	Circular logic and redundancy. We suggest to replace by: *The purpose of this step is to identify changes due to the genetic modification(s) compared to the non-modified organism that could cause adverse effects within the context of the Protocol when the LMO is used.*
280	It may be important to define a causal link or pathway between a characteristic of the LMO and a possible adverse effect, which may be direct or indirect, immediate or delayed, otherwise the risk assessment may generate information that will not contribute to reaching a recommendation that will be useful for the decision-making process.	Complex sentence and use of verbs leaves uncertainty on what is intended or when this would be relevant. We suggest rephrasing: *Possible adverse effect may be direct or indirect, immediate or delayed. It is important to define a causal link or pathway between a characteristic of the LMO and a possible adverse effect, otherwise the risk assessment may generate information that will not contribute to reaching a recommendation useful for the decision-making process*.
0376	One aspect to be considered is whether the receiving	This sentence should be rephrased to make it clearer.

	environment will be exposed to an LMO for which adverse effects have been identified taking into consideration the intended transfer, handling and use of the LMO, and the expression level, dose and environmental fate of transgene products as well as plausible pathways of a hazard leading to adverse effects.	
0379	In determining the route of exposure to the LMO being assessed or its products, when possible, the causal link between the LMO and the potential adverse effect should be established.	Delete the "when possible". If it is not possible to establish a causal link, then it will also not be possible to determine the route of exposure. The contrary would suggest logic opposite of the risk assessment process.
0384	Models, including conceptual ones, tested through experimental studies complemented by expert input, may be used for an assessment of the potential level and kind of exposure, combined with the use of statistical tools relevant for each case.	Complex sentence, unclear what the point is.
0398	(a) Information relating to the type and intended transfer, handling and use of the LMO, …	Not clear to what "type" refers in this sentence.
0407	(c) Levels of expression in the LMO and persistence and accumulation in the environment (e.g. in the food chain) of substances with potentially adverse effects newly produced by the LMO, such as insecticidal proteins, toxins and allergens.	There seems to be a bias against insecticidal proteins. By putting these at the same level as toxins and allergens they are regarded as substance with known adverse effects. For toxins and allergens negative effects are documented by definition and the safety of their use will be determined by the dose to which an organism will be exposed. Suggesting that all insecticidal proteins are de facto in the same category is misleading.

Q10. Is the Roadmap organized in a logic and structured manner? ☐ Yes ☒ No Comments: <Type here>

Whereas the logic and structure of the risk assessment process on a case-by case basis is coherent with what is presented in Annex III, the other elements would require better positioning.
As pointed out, "Overarching issues" should be reconsidered, as they are more of a general nature ensuring the quality of the information, rather than overarching. In any case, they should not be repeated in each step of the risk assessment.

It would be more logical to include the section on the choice of comparators as a general element on quality of information.

The difference between setting the scope & context is an important overall element, which should happen irrespective of specific cases. It is not clear how revisiting of the objectives at the end of the risk assessment should be organized.

Related issues and its links are confusing and should be removed.

Q11. Is the Roadmap user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity? ☐ Yes ☒ No Comments: **See comments provided on Q9**

Q12. Is the Roadmap applicable to all types of LMOs (e.g. plants, animals, microorganisms)? ☐ Yes ☒ No Comments: <Type here>

The Roadmap was written based on experience with certain crop plants. Even for plants, it can be argued that not all aspects are covered (e.g. a lot of attention on outcrossing which is not relevant for plants that are vegetatively propagated). When considering LM animals, there is a bias on mosquitoes whereas other animals may be at least as advanced and present different potential environmental effects. There is little information that would be applicable for micro-organisms.

Q13. Is the Roadmap applicable to all types of introductions into the environment (e.g. small- and large-scale releases, placing on the market/commercialisation)? ☐ Yes ☒ No Comments: <Type here>

The document provides different indications that it may be applicable to any type of introduction of LMOs in the environment. At several points there are indications that the amount and type of information needed may vary depending on the type of the release. These are important indications ensuring that the assessment is tailored to the specifics of the transfer, handling and use.

However, overall the document lacks practical indications on how limited information can be used in a risk assessment. The document also introduces terminology that differs from the Protocol and that is not further specified, e.g. import only, environmental release of limited duration and scale, field testing, field trials, release at early experimental stage, large scale releases and commercial use. We suggest to define a few of these terms and to use them throughout the document.

Finally, it is noted that the particular LMOs and traits addressed in Part II only cover aspects of commercial or large scale release.

Line	Referenced text
0057	The Roadmap may be applied to all types of environmental releases of LMOs, including those of limited duration and scale as well as large scale releases
0197	… the scale and duration of the environmental exposure, e.g. whether it is for import only, field testing or for commercial use.
0199	For small scale releases, especially at early experimental stages, the nature and detail of the information that is required or available may differ as compared to the information for large scale or commercial environmental release.
0301	… such as small-scale trials, especially at early experimental stages. Likewise, in cases where the exposure of the environments to the LMO is limited, such as for some early-stage experimental releases,…
0400	For example, in the case of field trials, the level of exposure in the receiving environment may be low due to the scale of the release, its temporary nature and the implementation of management measures;
0409	In the case of field trials, the level of persistence and accumulation in the receiving environment may be low due to the scale of the release, its temporary nature and the implementation of management measures;
0445	The scale of the intended use (e.g. small or large) should be taken into account.

Q14. Is there any other issue or concept that you would like to see included in the Roadmap? ☒ Yes ☐ No Comments: <Type here>

The following concepts have not been defined:

- (near-) isogenic: although used throughout the document there is no description of what is meant by isogenic or near-isogenic organisms.
- "Familiarity": this pivotal concept was introduced in early discussions by the OECD as a basis for the comparative assessment in relation to environmental impact of an LMO. A risk assessor should not remain unfamiliar with this concept.
- "Substantial equivalence": This is another essential concept in relation to the comparative assessment. It is the basis of LMO legislation in several countries and it would be strange to have a guidance document assisting risk assessors without at least positioning how "substantial equivalence" is relevant for the risk assessment of LMOs.
- "Tiered approach to safety studies": the tiered approach is now well established in risk assessment data gathering and is included in guidance documents. Since the Roadmap indicates at several instances points to consider, an explanation of how the tiered approach can be implemented to provide relevant information would be useful to less experienced risk assessors.

As stated in the first sentence of the document, the objective of the Protocol is "to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms. At some points in the text it is omitted that the risk assessment always takes into account 3 aspects: the LMO, the intended (and related) activities and the likely potential receiving environment. It is confusing to give the impression that the overall safety of the LMO would need to be evaluated while in other points the use seems to be an important factor.

Line	Referenced text	Comment
0005	…an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms	
0009	…of making informed decisions regarding living modified organisms	*…of making informed decisions regarding **the safe use of** living modified organisms*
0044	… environmental risks of living modified organisms	*… environmental risks of **the use of** living modified organisms*
0070	The novel combination of genetic material in an LMO and its use may lead to environmental effects which may vary depending on the LMO itself, the	

	environment exposed to the LMO and how the LMO is used.	
0193	Intended handling and use of the LMO, including practices related to the use of the LMO, taking into account user practices and habits	
0196	…depend on the biology/ecology of the recipient organism, the intended use of the LMO and its likely potential receiving environment	
0208	Means of describing the level of the potential adverse effects of LMOs and its transfer, handling and use, ..	Replace by *Terminology for describing the level of the potential adverse effects of the transfer, handling and use of the LMOs, ..*
0220	Risk assessments can be done in a comparative manner where risks associated with an LMO are considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.	*Risk assessments can be done in a comparative manner where risks associated with **the use of** an LMO are considered in the context of the risks posed by **the use of** the non-modified recipients or parental organisms in the likely potential receiving environment.*
0222	The comparative approach aims at identifying changes occurring between an LMO and its comparator that may lead to adverse effects.	*The comparative approach aims at identifying changes between an LMO and its comparator that may lead to adverse effects **when transferred, handled or used**.*
0278	…whereby novel characteristics of the LMO, as well as its transfer, handling and use,…	*…whereby the transfer, handling and use of an LMO with novel characteristics…*
0398	(a) Information relating to the type and intended transfer, handling and use of the LMO, …	

Cumulative vs combinatorial effects
The terminology of cumulative and combinatorial effects needs further clarification and consequent application throughout the Roadmap.

The first concept is that of effects that may not be observed in limited releases, but that may become visible after repetitive, large-scale introduction. These accumulated effects are in some legal frameworks referred to as "cumulative" effects (e.g. EFSA opinion on environmental risk assessment).

A second consideration would involve the simultaneous presence of different LMOs, each having potential effects on the environment. The combined potential effect may be different from the potential effects of the individual LMOs. We understand from the definition provided in line 1408 of the Roadmap that this situation is indicated as "cumulative" effects, i.e. effects that occur due to the presence of multiple LMOs or their products in the receiving environment. One could argue that as it is a combined effect, the term "combinatorial" would be more applicable.

Finally, the case is described where the presence of two (or more) genes in one organism results in a new effect different from the effect expected on the basis of the individual genes. The effects may occur at the level of gene expression, or through interactions between RNA, or among gene products. This case is indicated as "combinatorial" effect as defined in line 1418. As the genes need to be present in the same LMO, this situation is identical to what is described in Part II for the specific case of stacked genes. Given that this is under natural conditions, only the case of stacking via crossing would have to be considered.

Q15. Does the flowchart provide a useful graphic representation of the risk assessment process as described in the Roadmap?

☐ Yes
☒ No

Comments: <Type here>

There are several flaws in the flow chart
1) Overarching issues : The title is misleading as well as the positioning in the chart. It seems that a risk assessor should first indicate the criteria of acceptance of information, whereas this can happen independent of any specific case.

2) Planning phase: The difference between setting the scope & context, which is of a generic nature, and the choice of comparators, which will be case-by-case must be emphasized.

3) Related issues and its links should be removed

4) Step 5 covers a recommendation as to whether or not the risks are acceptable or manageable, including, where necessary,

identification of strategies to manage these risks.
The 3 questions included are irrelevant:
Objectives and criteria set at the beginning of the risk assessment: There is no example of such case
New information: always triggers an analysis if it concerns information that brings new insights in elements relevant for the risk assessment. This is not just relevant when arriving in step 5
New management options: This would not be relevant provided that the agreed management options –if any- are adequate. Improvements can always be analyzed like any new information.

5) The scheme doesn't position the recommendation on additional information or monitoring.

6) The final step is decision-making including decision on monitoring and management measures.

PART II: SPECIFIC TYPES OF LIVING MODIFIED ORGANISMS OR TRAITS

Risk assessment of living modified organisms with stacked genes or traits

Please answer each of the questions in the left column with "yes" or "no" and add comments if needed.

Q16. Does this section provide useful guidance when conducting risk assessments of LMOs with stacked genes or traits in accordance with the Protocol?

☐ Yes
☒ No

Comments: <Type here>

We repeat the recommendation that the Part II on specific types of LMOs and traits should be handled separately and after the Roadmap has been fully established. It is premature to fully comment on Part II.

Furthermore Part II risks digressing from the case-by-case approach by creating types of LMOs and traits that all will require a specific treatment. While it may be of interest to highlight that certain aspects may require special attention, it is against the case-by-case approach to make generalizations

Line	Referenced text	Comment
0619	As such, risk assessments of this type of LM plants also follow the general principles outlined in the Roadmap, but take into account the specific issues outlined in this section of the present document	We find many of the aspects indicated in this Part not de facto applicable and/or not specific for compared with other LM plants. We indicate this comment throughout the document.
0632	Likewise, the scope of this section is restricted to those LM plants generated through the methods of modern biotechnology as defined in Art. 3(i)(a) of the Protocol. LM plants derived from fusion of cells are not covered in this guidance.	This statement should be clarified. Does the fusion in the second sentence refer to one of the techniques that will not lead to an LMO, therefore the combination between two plants being fusion products will not be considered in this section? Or does it refer to a way to combine two LM plants and does it suggest that a fusion product of two LM plants is not considered a stack in the context of this Part II?
0635	This guidance also includes some considerations on unintentional stacked events as the result of natural crossings between stacked events and other LMOs or compatible relatives in the receiving environment.	As pointed out in comment on line 0761, unintentional stacks should be excluded from this Annex.
0761	A set of new stacked LMOs may arise in the environment through crossings between the stacked event LMOs and other LM plants or sexually-compatible non-modified relatives in the receiving environment. These crossings can be controlled (i.e. mediated by man) or uncontrolled (i.e. natural outcrossings through pollination) and, depending on the segregation patterns, the new stacked LMOs could contain new and/or different combinations of transgenes and DNA fragments that could result in cumulative effects.	Stacks mediated by man are the topic of this Annex. In consequence they are covered and do not trigger new issues. Crosses with sexually-compatible non-modified relatives are not stacks in the meaning of this Annex. They are covered by the general Roadmap. Uncontrolled stacking between transformation events can happen via cross pollination of approved LM plants. In our experience, during the evaluation of new transformation events the potential impact of such crosses is included in the risk assessment. If a reference is to be made then it should be to the Roadmap. Suggest to delete lines 0758 - 0790

Q17. Is this section of the Guidance useful to risk assessors who have limited experience with risk assessments of LMOs with stacked genes of traits?

☐ Yes
☒ No

Comments: <Type here>

The wording should be improved in order not to confuse risk assessors with limited experience with LMO risk assessment on basic elements of the risk assessment. In some cases, more information will be required to fully understand why statements are made or what they mean in practice. The following table provides some examples of this point:

Line	Referenced text	Comment
0612	Stacked LMOs can be produced through different approaches. In addition to the cross-breeding of two LMOs, multiple traits can be achieved by transformation with a multi-gene transformation cassette, retransformation of an LMO or simultaneous transformation with different transgene cassettes (i.e., co-transformation).	The goal is to stack traits, stacking of LMOs can only be done via breeding or fusion! Replace by: *The presence of multiple traits in an LMO can be achieved by transformation with a multi-gene transformation cassette, simultaneous transformation with different transgene cassettes (i.e., co-transformation), retransformation of an LMO or by cross-breeding of two LMOs.*
0670 - 0694	Rationale for "Sequence characteristics at the insertion sites, genotypic stability and genomic organization"	To improve the logic of this section, we suggest to move lines 0691 – 0694 ("Transgenes with similar..") to line 0674. The molecular characterization of the stacked events is not a specific issue for stacked events.
0710	Previous risk assessments of the parental LMOs provide useful information on the mode of action and molecular characteristics of the individual genes as a starting point to assess the potential for interactions. In addition to information about the characteristics of the parental LMOs, specific information on potential for interactions between the altered or inserted genes and DNA elements (e.g. promoters and other regulatory elements), proteins, metabolites or modified traits and endogenous genes and their products in the stacked LM plant should be considered and assessed.	Information of mode of action of the individual genes is indeed the starting point, but this would not require a previous risk assessment. A different trait can only arise when there is an interaction between the newly combined modes of action. The indication of "should be considered and assessed" is not warranted for most stacks and we like to add "in case an interaction between the traits at molecular or biochemical level is expected".
0725 - 0733	Points to consider	Points a) and b) are generally not relevant for the case. Point d) Please clarify the idea of comparing the level of expression of the transgene with the non-modified recipient organism. We would rather keep reference to comparators.
0782 - 0811	Methods for distinguishing the combined transgenes in a stacked event from the parental LMOs	This section is highly descriptive and holds no practical guidance for risk assessors. It also confuses the detection of transgenes and specific LM plants. It fails to note why and when a detection of the stack would be needed over the detection of individual transformation events. This section is hardly helpful.

Q18. Is this section of the Guidance organized in a logic and structured manner?

☐ Yes
☒ No

Comments: <Type here>

One problem is that this section should address specific issues, whereas most of the issues are not specific for stacked events and therefore their inclusion and description may create additional confusion for less experienced risk assessors.

Line	Referenced text	Comment
0647	In cases of parental LMOs that have highly heterozygous genomes or significantly differ from each other, the resulting stacked LMOs will display high variability and a vast range of phenotypes. This variability should be taken into account during the establishment of a baseline for a comparative risk assessment.	This is not unique for stacked events, but may occur in any other LM plant that is a result of line selection or is produced as hybrids. It is incorrect to present it as a specific issue.
0650	(Near-)isogenic lines to be used as comparators may be lacking which may present challenges to the interpretation of data when establishing the baseline for the risk assessment of a stacked LM plant.	This is not unique for stacked events. This paragraph should not be presented as specific for stacked events.

	Therefore, in risk assessment frameworks that rely on the (near-)isogenic non-modified recipient organism as the primary comparator, it may be useful to use the closest available non-modified genotype as comparator.	
0655	Moreover, stacked LM plants produced may be the result of multiple rounds of cross-breeding among many different genotypes and possibly involve several stacked events. In such cases, choosing the appropriate comparators among the single transformation LMOs and the intermediate stacked events that gave rise to the stacked LM plant under assessment may not be a straight forward action and the choice of comparator should be justified.	This is not unique for stacked events. This paragraph should not be presented as specific for stacked events. We suggested that on a case-by-case basis proper comprators should be chosen in function of the objective, i.e. to determine if the LM plant (or the stack of LM plants) has different characteristics that could lead to potential adverse effects.
0660 - 0665	Points to consider	As argued before, non of these are truly specific issues for stacked events an should be omitted.

Q19. Is this section of the Guidance user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity?

☐ Yes
☒ No

Comments: <Type here>

See previous comments.

Also providing examples to illustrate the concepts would be welcomed and particularly useful for les experienced risk assessors.

Line	Referenced text	Comment
0644	the LMOs that were involved in the cross-breeding process leading to the stacked LM plant under consideration may also be used as comparators, as appropriate and according to national regulations.	It would be illustrative to indicate examples where this could be useful.

Q20. Is there any other issue or concept that you would like to see included in this section of the Guidance?

☒ Yes
☐ No

Comments: <Type here>

In spite of a very descriptive text, we miss a clear indication of the potential risk that is associated with a stack. In fact, there is no indication that most stacks will have no new effect at all. *E.g.* combining two herbicide tolerance traits, providing tolerance to two different active ingredients via different pathways, will have no different phenotype or effect than the individual transformants.

The section on combinatorial and cumulative effects does not help to clarify these concepts.

Line	Referenced text	Comment
0734 - 0766	**Combinatorial and cumulative effects**	This section is to a large extent redundant with the previous section "Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment".
0728	Assessment of combinatorial and cumulative effects is based on the environmental risk assessment data for the stacked event LM plant in comparison to the closely related non-modified recipient organism(s) and the parental LMOs in the likely receiving environment, taking into consideration the results of the genotypic and phenotypic assessments outlined above.	It is not clear what this sentence means in practical terms. The genotypic and phenotypic assessment will confirm if any combinatorial effects are expected. Only if such an effect may occur, then additional information may be required to support the risk assessment.
0732	Proteins and metabolites produced due to the insertion of multiple transgenes in the same stacked LM plant can interact between themselves as well as with endogenous genes and metabolic pathways.	Redundant with section "Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment". Delete.
0733	These interactions could lead to unpredicted combinatorial effects. For example, the impact on non-target organisms could be broader than the sum of the individual parental LMOs, or the evolution of resistance in target organisms (e.g. insect pests) could	Unpredicted effects are handled in general in the Roadmap. This section fails to show specific unpredicted effects for stacked traits. Interactions are handled in section "Potential

	happen faster than in the case of single event LMOs.	interactions between combined genes and their resulting phenotypic changes and effects on the environment". The statement on impact on non-target organisms is not supported by evidence. On the contrary, combinatorial effects of multiple pest protection strategies are introduced as an accepted way to reduce the chance for resistance development.
0737	Possible interactions on DNA- or RNA-level and/or between proteins and metabolites could be investigated and the potential adverse effects arising from them may be thoroughly assessed.	Redundant with section "Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment". Delete.
0738	An assessment of potential combinatorial and cumulative effects may be performed, for instance, by conducting phenotypic and compositional analyses, toxicity tests on non-target organisms and any other study that integrate these multiple and interacting factors to predict the adverse effects.	This statement seems to plead for testing irrespective if there is an assumption of a combinatorial or cumulative effect. If the safety of the individual LM plants has been accepted and there is no reason to assume an interaction between the stacked traits, then such test should not be performed systematically.
0741	Also, indirect effects due to changed agricultural management procedures, combined with the use of the transgenic stacked event LMOs, may occur.	It would be helpful to provide clear examples of cases where this is expected. Again this issue is not specific for stacked events.
0747 - 0757	*Points to consider*	Redundant with section "Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment". Delete.

Risk assessment of living modified crops with tolerance to abiotic stress

Please answer each of the questions in the left column with "yes" or "no" and add comments if needed.

Q21. Does this section provide useful guidance when conducting risk assessments of LM crops with tolerance to abiotic stress(es) in accordance with the Protocol?	☐ Yes ☒ No	Comments: <Type here>

We repeat the recommendation that the Part II on specific types of LMOs and traits should be handled separately and after the Roadmap has been fully established. It is premature to fully comment on Part II.

Furthermore Part II risks digressing from the case-by-case approach by creating types of LMOs and traits that all will require a specific treatment. While it may be of interest to highlight that certain aspects may require special attention, it is against the case-by-case approach to make generalizations

Line	Referenced text	Comment
0841	In plants, any gene or gene combinations providing increased tolerance to some abiotic stress may have pleiotropic effects on the stress physiology of the plant, e.g. drought, temperature and salt stress are interconnected and plant responses to these stresses share multiple components and genes. Such pleiotropic effects may be classified as "unintended predicted effects" (see the Roadmap, step 1) and may be inferred during the risk assessment by considering the crosstalk between different stress responses of the plant and assessing if the identified changes may cause adverse effects.	Potential for pleiotropic effects is not limited to abiotic stress tolerance. The concept of “cross talk” should be explained.
0850	These variations pose difficulties in (i) controlling/measuring these conditions in field experiments to analyse the phenotype of the LM plant and generate data for the risk assessment, and (ii) defining the phenotype of the LM plant itself, which in many cases may be subject to the interaction between external and physiological parameters.	Comment on field trials is similar to what is indicated for line 0838. Point on defining the phenotype is relevant, but may not be clear enough for users of the Roadmap. A scientifically correct example would be useful.
0854	Some of the potential adverse effects to be evaluated in the risk assessment, from the introduction of LM plants tolerant to abiotic stress into the environment may include, for example: a) increased selective advantage(s) other than the intended tolerance trait that may lead to potential adverse effects; b) increased persistence in agricultural areas and increased invasiveness in natural habitats; c) adverse effects on organisms exposed to the LM plant; and d) adverse consequences of potential gene flow to wild or conventional relatives. While these potential adverse effects may exist regardless of whether the tolerant plant is a product of modern biotechnology or conventional breeding, some specific issues may be more relevant in the case of abiotic stress tolerant LM plants.	Why is this list included? All these points are covered in the Roadmap and there is no indication why they would be particular for abiotic stress tolerance. The last sentence should also be further explained. Why are some specific issues (which?) more relevant in the case of abiotic stress tolerance? As the document is intended to help less experienced risk assessors, such statements should be based on easily understandable examples.
0937	If the stress tolerance trait leads to an increased physiological fitness, introgression of the transgenes for stress tolerance may occur at higher frequencies than observed among non-modified plants.	There is no indication why this would be specific for abiotic stress tolerance and is adequately covered in the Roadmap. The question must also be asked if an introgression then leads further to an adverse effect. This is not developed in the text. Delete here
0952	(c) A change in the substances (e.g., toxin, allergen, or nutrient profile) of the LM plant that could cause adverse effects.	There is no indication why this would be specific for abiotic stress tolerance and is adequately covered in the Roadmap. Delete here.
0974 - 0988	Points to consider a) to D)	We fail to see why these are specific or different for abiotic stress tolerance traits. This is adequately covered in the Roadmap.
0991 - 1024	Increased persistence in agricultural areas and invasiveness of natural habitats	There is no indication why this would be specific for abiotic stress tolerance and is adequately covered in the Roadmap. Delete here

Q22. Is this section of the Guidance useful to risk assessors who have limited experience with risk assessments of LM crops with tolerance to abiotic stress(es)?

☐ Yes

☒ No

Comments: <Type here>

The wording should be improved in order not to confuse risk assessors with limited experience with LMO risk assessment on basic elements of the risk assessment. In some cases, more information will be required to fully understand why statements are made or what they mean in practice. The following table provides some examples of this point:

Line	Referenced text	Comment
0841	In plants, any gene or gene combinations providing increased tolerance to some abiotic stress may have pleiotropic effects on the stress physiology of the plant, e.g. drought, temperature and salt stress are interconnected and plant responses to these stresses share multiple components and genes. Such pleiotropic effects may be classified as "unintended predicted effects" (see the Roadmap, step 1) and may be inferred during the risk assessment by considering the crosstalk between different stress responses of the plant and assessing if the identified changes may cause adverse effects.	Potential for pleiotropic effects is not limited to abiotic stress tolerance. The concept of "cross talk" should be explained.
0862 - 0874	Questions that may be relevant..	Again this part does not address anything specific that would not be covered in the Roadmap already. It also fails to indicate why the question would be specific or different for abiotic stress tolerance traits.
0889	Comparisons with the observed range of changes in the non-modified plant in different environments, also provides baseline information.	We commented before that there are other valid ways to set baselines e.g. by reference to published information.
0913	On a case by case basis in the future, information available from "omics" technologies, for example, "transcriptomics" and "metabolomics", as it becomes available, may help to detect phenotypic and compositional changes (e.g., the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison between field grown plants at a suboptimal condition.	Reference to future, yet unvalidated and unavailable tools is not relevant at this time. Since the roadmap is a living document, the use of omics can be added in the future if it becomes a widely accepted tool for risk assessment. Furthermore, the essential element of the comparison is overlooked by a focus on the tool. If a suitable comparator is not available, then how will phenotypic and compositional changes be detected and assessed? The LM plant and comparators grown under non-stress conditions would be a relevant comparator to identify if any relevant change has occurred due to stress conditions.
0923 - 0944	Unintended characteristics including crosstalk between stress responses	This description points out the possible complexity of stress tolerance traits, whereby a single function can elicit a broader response to different stressors. While valuable to point out the complexity, it should be also pointed out that this is not always the case and that the effectiveness of multiple responses doesn't result in tolerance to all stress factors.
0927	..(i.e. crosstalk),..	This is not correct. There are different mechanisms that can lead to different stress responses, cross-talk is one, with a clear definition. It is confusing to provide this here in this way.
0979	…, differences in occurrence or in the number of generations of target organisms,	Given that the trait is abiotic stress tolerance, we don't see how target organisms can be defined. Please clarify or delete

Q23. Is this section of the Guidance organized in a logic and structured manner?

☐ Yes
☒ No

Comments: <Type here>

One problem is that this section should address specific issues, whereas most if the issues are not specific for stacked events and therefore their inclusion and description may create additional confusion for less experienced risk assessors.

Line	Referenced text	Comment
0823	The considerations in this guidance complement the Roadmap for Risk Assessment of LMOs and aim at	There is a difference between providing a general overview of issue that may be relevant or giving

	providing a general overview of issues that may be relevant when assessing the risks of LM plants with tolerance to abiotic stress(es).	emphasis to issues that are of particular relevance (as in the case of stacked events). Please align the objectives. In our comments we have assumed that issues of particular relevance are intended.

Q24. Is this section of the Guidance user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity?	☐ Yes ☒ No	Comments: <Type here>

See previous comments.

Also providing examples to illustrate the concepts would be welcomed and particularly useful for les experienced risk assessors.

Q25. Is there any other issue or concept that you would like to see included in this section of the Guidance?	☒ Yes ☐ No	Comments: <Type here>

It is pointed out that there are very diverse abiotic stresses, yet the trait as such is called abiotic stress tolerance possibly creating the illusion that the LM plant would be tolerant to all if not to a diversity of stresses. This is highly unlikely. It fails to correctly characterize the reality that organisms have inherent and natural tolerance to stress.

Secondly it is important to remind readers that an abiotic stress tolerance means that a plant has acquired the capacity to cope to some extend with the abiotic stress. It would be incorrect to believe that the plants are completely insensitive to the stressor or survive any level of stress.

Some concepts, e.g. “cross-talk”, should be correctly defined.

In line 0829 it is indicated that increased tolerance to abiotic stress has long been a target of plant breeders working towards improved crops. The trait is of primary importance given the increasing environmental, geographical and climatic limitations that agriculture faces. We suggest highlighting the importance of abiotic stresses.

Risk assessment of living modified mosquitoes

Please answer each of the questions in the left column with “yes” or “no” and add comments if needed.

Q26. Does this section provide useful guidance when conducting risk assessments of LM mosquitoes in accordance with the Protocol?	☐ Yes ☐ No	Comments: <Type here>
Q27. Is this section of the Guidance useful to risk assessors who have limited experience with risk assessments of LM mosquitoes?	☐ Yes ☐ No	Comments: <Type here>
Q28. Is this section of the Guidance organized in a logic and structured manner?	☐ Yes ☐ No	Comments: <Type here>
Q29. Is this section of the Guidance user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity?	☐ Yes ☐ No	Comments: <Type here>
Q30. Is there any other issue or concept that you would like to see included in this section of the Guidance?	☐ Yes ☐ No	Comments: <Type here>

ADDITIONAL COMMENTS

Please add any additional comment you may have regarding the "Guidance on Risk Assessment of Living Modified Organisms" below.

Q31. <Please type your comments here>
