*Annex*

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

## GENERAL INFORMATION ABOUT THE TESTING

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| Q1. These results are being submitted on behalf of a: | ☐ Party. Please specify: <Country's name><br>☐ Other Government. Please specify: <Country's name><br>☒ Organization: Please specify: Public Research and Regulatin Initiative |
| Q2. When was the testing of the Guidance conducted? | Please enter date: <30 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><br>Type of meeting: ☐ Face-to-face ☐ Online<br>☒ Individual exercise. Please provide your name, occupation and affiliation: Hector Quemada, Biosafety Resource Network Director, Donald Danforth Plant Science Center, on behalf of Public Research and Regulation Initiative<br>☐ Other: Please specify: <Type here> |
| Q4. Which sections of the Guidance were tested? | ☒ Part I: The Roadmap for Risk assessment of LMOs<br>Part II: Specific types of LMOs or Traits:<br>☒ Risk assessment of LMOs with stacked genes or traits<br>☐ Risk assessment of LM crops with tolerance to abiotic stress<br>☐ Risk assessment of LM mosquitoes |

## OVERALL EVALUATION

| | Very poor | Poor | Neutral | Good | Very good |
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| *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.*

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| Q8. Does the Roadmap provide useful guidance for conducting risk assessments of LMOs in accordance with the Protocol? | ☐ Yes<br>☒ No | Comments: The roadmap left open numerous questions that required clarification before it could be used to guide a risk assessment:<br>Lines 469-470: Must any and all interactions between the transgene and other genes in the plant genome be investigated? What combinatorial and cumulative effects are relevant for risk assessment?<br>Line 494: Please provide references to examples of how these risk estimation tools have been used by regulators to assess LM risks.<br>Line 506: Where are these defined to enable their use in the context of this document?<br>Lines 509-510: How would a consideration of uncertainty be used in the assessment of overall risk? Please provide an example.<br>Line 543: How would a consideration of uncertainty be used in the assessment of overall risk? Please provide an example.<br>Lines 546-547: Where is the basis for this use of monitoring in the text of the Protocol?<br>Line 557: How is one to determine what is relevant experience?<br>Line 560: What are the elements that should be taken into consideration in this benefits analysis? What scientific benefits should be considered?<br>Lines 562-563: Does this not describe the process of risk assessment and determination of risk management? Please explain this in the context of determining acceptabilty of risk.<br>Lines 571-575: Where is environmental monitoring as a risk management measure described in Annex III of the Protocol?<br>Lines 579-580: Why would one take the trouble to describe risk management measures that are not feasible? Would one not only consider feasible risk management measures? What is the difference between measuring efficacy and measuring effectiveness? |
| Q9. Is the Roadmap useful to risk assessors who have limited experience with LMO risk assessment? | ☐ Yes<br>☒ No | Comments: See answer to Q8 |
| Q10. Is the Roadmap organized in a logic and structured manner? | ☒ Yes<br>☐ No | Comments: <Type here> |
| Q11. Is the Roadmap user-friendly taking into account that risk assessment is a complex scientific | ☐ Yes<br>☒ No | Comments: See answer to Q8 |

and multidisciplinary activity?

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| Q12. Is the Roadmap applicable to all types of LMOs (e.g. plants, animals, microorganisms)? | ☐ Yes<br>☒ No | Comments: <Type here> |
| 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<br>☒ No | Comments: <Type here> |
| Q14. Is there any other issue or concept that you would like to see included in the Roadmap? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q15. Does the flowchart provide a useful graphic representation of the risk assessment process as described in the Roadmap? | ☐ Yes<br>☒ No | Comments: Lines 579-602: Why are there double headed arrows between steps 1 and 2/3, 2/3, 4 and 5? |

## 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.*

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| Q16. Does this section provide useful guidance when conducting risk assessments of LMOs with stacked genes or traits in accordance with the Protocol? | ☐ Yes<br>☒ No | Comments: This section left open numerous questions that required clarification before it could be used to guide a risk assessment:<br><br>Lines 629-631: What are the differences between LMOs stacked by retransformation and those through conventional breeding that allows one to use the Roadmap in the former case but not in the latter case?<br><br>Lines 633-634: Since the Protocol also covers LM plants derived through fusion between cells of plants beyond taxonomic families, why are they specifically excluded in this part of the guidance? Which raises the question : does the Roadmap itself cover this type of LMO?<br><br>Line 635: Why only some? What are the differences in risks presented by intentional versus unintentionally stacked events?<br><br>Lines 655-659: Is this not essentially saying the same thing as the previous paragraph?<br><br>Lines 661-664: Are these not just two different manifestations of the same phenomenon? Please explain the difference.<br><br>Lines 676-678: When is molecular characterization needed? When not? How does one judge the needed extent?<br><br>Lines 687-688: What methods are referred to here? Would not the same methods that were used to characterize the parental LMOs be used to characterize the stacked LMO, if needed?<br><br>Line 689: Why always consider reliability of detection methods and not only when needed in the context of risk management measures?<br><br>Lines 693: Would be a point to consider, to check if it's a case where biochemical pathways or specific analyzable characteristics may be known to be in the same biochemical pathway….that might interact and these characteristics analyzed? i.e. identification of characteristics that might have adverse effect?<br><br>Lines 692-693: Under what circumstances would one require a sequencing of the genes introduced into the stacked LMO? This point to consider is not one mentioned in the Roadmap itself and so would not necessarily be available for comparative purposes if it were not done for the parental LMO. When would it be necessary to provide sequence information from the stacked or even the parental LMO?<br><br>Lines 697-698: The definition of trans-regulation |

involves a specific means of interaction that does not seem to be the intent here. Please clarify.

Lines 701-702: Why would this be a problem specifically in stacked events?

Lines 706-707: Aren't these most of the cases? How would one know that there are reasonable chances of relevant interactions not detectable through typical phenotypic characterization ?

Lines 718-720: When is important to consider this point?

Lines 723-724: Wouldn't the non-modified recipient organisms always have no expression of the transtenes? Why would it be necessary to do this comparison? Does this mean altered levels of expressions that leads to a toxic level of a produced e.g. protein?

Lines 732-733: In the beginning of this specific guidance, genes stacked by transformation are clearly distinguished from genes stacked by traditional breeding methods. However, the guidance at this point mentions insertion of multiple transgenes in the same stacked LM plant. Does this not relate to transgenes stacked through transformation? And once again, given the apparent confusion introduced here, what is the distinction between the two types of stacking that prompts separating the two?

Lines 743-743: Please provide an example of this type of effect.

Lines 756-757: What is the difference between « interactions » in the previous paragraph and « combinatorial and cumulative effects »? What types of interactions would not be covered under « combinatorial and cumulative effects »?

Line 772: Why are these points to consider entirely different from those pertaining to intentionally stacked LMO's? Why would intent result in such a drastic change?

Line 773: How would the ecological function of a sexually compatible non-modified relative be taken into consideration for a stacked event? Please provide an illustration.

Lines 786-787: Can you give specific examples of LMOs approved that stacking offers an unacceptable risk and there is no acceptable detection method for carrying on management strategies?"

Lines 789-796: Is this not a situation relevant to labeling requirements rather than LMO risk assessment?

Lines 803-804: Can you give examples of risk management strategies to reduce unwanted stacking to occur?

Line 809: Isn't this important only in cases when risk management strategies might be useful to have

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| | | detection of the LMO?<br><br>Lines 810-811: Isn't it more important to consider risk management strategies than monitoring? |
| 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<br>☒ No | Comments: <Type here> |
| Q18. Is this section of the Guidance organized in a logic and structured manner? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q19. Is this section of the Guidance user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q20. Is there any other issue or concept that you would like to see included in this section of the Guidance? | ☐ Yes<br>☒ No | Comments: <Type here> |
| **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<br>☒ No | Comments: This section leaves numerous questions that need clarification:<br><br>Lines 838-840: Isn't this always the case for all sorts of LMOs? Why specific for abiotic stress tolerance?<br><br>Lines 841-842: Are not pleiotropic effects not a consideration with all LMOs?<br><br>Lines 847-850: Is this not also the case for other traits introduced into LM plants?<br><br>Lines 855-856: What are examples of selective advantage(s) other than the tolerance trait that would be the result of an introduction of an abiotic stress tolerant trait.<br><br>Lines 860-861: Could you please explain why and when there are specific issues for LMOs? Please give examples<br><br>Lines 864-874: How is this more relevant for an LM plant as opposed to one derived through traditional breeding?<br><br>Lines 906-907: How would you use such comparators?<br><br>Line 910: Why are statistically meaningful differences of interest here? Are not biologically meaningful differences more relevant to risk assessment?<br><br>Line 915: How are these traits relevant to the type of risk assessment within the scope of the Cartagena Protocol?<br><br>Line 920: Whether comparators are available or |

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| | | which are the most suitable comparators?<br><br>Lines 927: How is this "crosstalk" different from a pleitotropic effect?<br><br>Lines 934-935: Aren't these characteristics always considered for modified crops (modified for abiotec or other trait)?<br><br>Lines 940: How are interaction, cross-talk, and other terms used in this guidance such as combinatorial, cumulative, and pleitropic, distinguished from one another?<br><br>Line 952: Aren't these considerations taken to all other types of LMOs?<br><br>Lines 960-962: Why is this particularly important with respect to LM plants with tolerance to abiotic stress, as opposed to other LM plants?<br><br>Lines 963-965: Is this not true of other LM plants as well?<br><br>Lines 968-972: Is this not true of other LM plants as well?<br><br>Lines 979 - 980: Since the trait is abiotic stress tolerance, why would one be concerned with the occurrence of number of target organisms? Organisms are biotic components, not abiotic. What would be the target organisms of an abiotic stress tolerant trait?<br><br>Lines 984-986: Would this not be applicable to risk assessment of other LM plants?<br><br>Lines 987-988: Would this not be applcable to risk assessment of other LM plants?<br><br>Lines 991-992: Isn't it necessary to consider not only the coparative potential adverse effect that might arise from LMOs, but also consider the level of probability (likelyhood-step 2) that the adverse effect will occur? Why are steps jumped here?<br><br>Lines 1022-1024: Would these not be relevant to other LM plants as well?<br><br>Lines 1027-1028: aren't the cultivation of any organism capable of causing changes in the receiving environment? Please explain how to know when they are important<br><br>Line 1038: isn't a wished characteristic of a LMO made to tolerate abiotic stress to be able to use land that was not possible to use before? Isn[t a benefit to extend arable land to those places where biodiversity is lower?<br><br>Lines 1041-1042: What if they do not exist? Does it mean that one should not have any abiotic resistnat crop obtainded through conventional breeding or recombinant biotechnology released? |
| 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 | ☐ Yes<br>☒ No | Comments: <Type here> |

stress(es)?

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| Q23. Is this section of the Guidance organized in a logic and structured manner? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q24. Is this section of the Guidance user-friendly taking into account that risk assessment is a complex scientific and multidisciplinary activity? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q25. Is there any other issue or concept that you would like to see included in this section of the Guidance? | ☐ Yes<br>☒ No | Comments: <Type here> |
| **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<br>☒ No | Comments: There are several points that need clarification, such as:<br>Lines 1064-1067: Not necessarily true. RIDL is a platform technology that can be adapted for different circumstances; some of the introduced traits target females ( such as flighlessness), but others introduce conditional lethality (or sterility) to both males and females. However in both cases « sterile « males are the GM mosquitoes that are released into the environment. Please clarify.<br>Lines 1079-1080: Isn't it that HEG's can be used for population suppression, and is not necessarily self sustaining? why not use a better example of self-sustaining type strategies as MEDEA?<br>Lines 1099-1101: There appears to be some redunancy here, surely » case by case » risk assessment could include these other approaches and doesn't RA already include gathering data obtained from the laboratory and confined trials ?<br>Lines 107-1109: Shouldn't this be made clear in the paragraph above where paratransgenisis is included as it gives the impression the guidance will cover it?<br>Lines 1119-1122: These two sentences appear to be conflicting, surely itif it is well known in regions of the world, these are likely to be the same regions where mosquitoes are a problem and these would be the same areas where LM mosquitoes would be released. Why would more information be required for these regions ? This is confusing to territories that might be new to risk assessment of these organisms.<br>Lines 1150-1152: How would selection affect insertion sites or transpositon?<br>Lines 1156-1157: Isn't this the specific aim of LM mosquito interventions ? You want to either reduce the population or replace it with a less harmful form. Therefore should't the RA focus on non-target |

species and the role of the mosquito in the ecosystem? The use of the word natural here needs removal or definition as urban environments, tyre dumps etc where mosquitoes breed cannot be regarded as natural.

Lines 1166-1168: This is extremely unlikely to happen in the duration of a « field trial » therefore shouldn't this be considered when appropriate as point for post market monitoring rather than pre-trial approval?

Lines 1169: Aren't mosquitoes pests already ?

Lines 1185-1186: Why would this be a concern if not specifically altered in the LM mosquito?

Lines 1196-1198: Are mosquitoes significant pollinators in any ecosystem?

Lines 1199-1201: If this is true, why is a discussion of ecosystems services of mosquitoes contained in this guidance?

Lines 1218-1219: Given lines 1200 – 1201, why is this included as a point to consider?

Line 1220: Does this mean any mutation that might occur in the mosquito? How would interactions with other organisms be mutagenic?

Lines 1224-1226: Isn't this better if addressed in PMM, when relevant, as the timescale of this occuring is likely to be beyond that of a release?/ Epidemological data cannot be collected from a trial dealing with entomological objectives.

Lines 1127-1128: And if it does ?? shouldn't this point should relate to potential risks rather than « can it move by these means »?

Lines 1261: What microorganism is meant here? If this sentence refers to the use of Wolbachia, this technology does not qualify as an LMO and is therefore out of scope of the Protocol.And paratransgensis is excluded from the scope of this document

Lines 1267: What is undesirable ? and who decides what is desirable or undesirable ?

Line 1274: Please provide a reference of horizontal gene transfer between mosquitoes and Wolbachia .

Lines 1274-1276: Why would the risk to Wolbachia from transgenes be any differnt from the risk presented by any of the other genes in the mosquito genome?

Lines 1310-1315: These are both likely to be long-term effects and not apparent during a trial. This guidnace should consider the type of data that would be required to determine these effects and that it should be gathered during PMM rather than in any pre-trial RA.

Lines 1325-1326: Dispersal per se is not necessarily a risk. Why not only if a negative consequence has

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| | | been determined in the RA?<br><br>Lines 1339-1341: How is monitoring alone a risk management strategy? The protocol wisely does not equate monitoring with risk management. Is this not a precursor for implementing a risk management strategy?<br><br>Lines 1349-1350: Sexual dimorphism is completely species dependent, and therefore this statement needs to be better formulated. Please clarify.<br><br>Line 1351: The control programme is not necessarily compromised if some females are released. It is certainly desirable that no females are released but it is rarely a « requirement ».<br><br>Lines 1371: What is the definition of paratransgensis? It is stated earlier that paratransgensis is not in the scope, why does it come here?<br><br>Lines |
| Q27. Is this section of the Guidance useful to risk assessors who have limited experience with risk assessments of LM mosquitoes? | ☐ Yes<br>☒ No | Comments: <Type here> |
| Q28. Is this section of the Guidance organized in a logic and structured manner? | ☐ Yes<br>☒ 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<br>☒ 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<br>☒ 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. *Several terms in the glosssary need to be better defined, among them:*

*Lines 1399-1403: Is this not a contradition to the use of the term baseline in the guidance documents, where the comparator is designated as the baseline?Moreover, measuring "the existing conditions" of an environment or its components without a link to relevant protection goals and the results of the risk assessment would not serve a meaningful purpose*

*Lines 1404-1406: 'each LMO' should be replaced by: "an LMO or trait, or category of LMOs or traits".*

*Lines 1408-1412: Whether or not and in which cases such effects should be further analysed is something to address in the roadmap, not in a glossary.*

*Lines 1413-1414: the CPB speaks of 'the extent'; adding "outcome" and "severity" confuses*

*Line 1416: the CPB speaks of "traditional breeding and selection" it is best to stick to the terminology of the CPB. The term "conventional " in the roadmap is used in various ways, some of which are not very clear*

*Lines*

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