



Convention on Biological Diversity

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**Subsidiary Body for Scientific,
Technical and Technological Advice**
Twenty-sixth meeting
Nairobi, 13–18 May 2024
Item 7 of the provisional agenda*
Detection and identification of living modified organisms

Synthesis of submissions of information on the detection and identification of living modified organisms**

Note by the Secretariat

I. Introduction

1. In decision [CP-10/11](#), the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety invited Parties and relevant organizations to submit information on their experience with new detection techniques, detecting newly developed and unauthorized living modified organisms (LMOs) and developing reference materials, as well as ongoing collaborations involving national and regional laboratories. The Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety also requested the Subsidiary Body on Scientific, Technical and Technological Advice at its twenty-sixth meeting to consider this information and to prepare a recommendation to the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety at its eleventh meeting regarding the need to update the training manual on the detection and identification of living modified organisms.¹ In addition, Parties were invited to submit information on their laboratories, including their specific activities, to the Biosafety Clearing-House using the laboratories for the detection and identification of living modified organisms common format.

2. In response to the above, the Secretariat issued notification No. 2023-100,² inviting Parties, other Governments and relevant organizations to submit information on the detection and identification of living modified organisms. To facilitate the collection of information, the Secretariat developed templates for the submissions of information, which can be found in annex I.

* CBD/SBSTTA/26/1.

** The present document is being issued without formal editing.

¹ Biosafety Technical Series 05: Training Manual on the Detection and Identification of Living Modified Organisms in the Context of the Cartagena Protocol on Biosafety is available at: <https://bch.cbd.int/en/database/VLR/BCH-VLR-SCBD-260177>

² Available at: www.cbd.int/doc/notifications/2023/ntf-2023-100-bs-en.pdf

II. Information received related to the detection and identification of living modified organisms

3. A total of five submissions related to the detection and identification of LMOs were received from:

- (a) Four Parties (Belgium, Brazil, Germany and Thailand);
- (b) One Organization (Outreach Network for Gene Drive Research).

4. The original submissions have been made available on the Biosafety Clearing-House.³ In addition, information on laboratories submitted to the Biosafety Clearing-House using the laboratories for the detection and identification of living modified organisms common format can be found through the search interface on the Biosafety Clearing-House.⁴

5. When inviting Parties, other Governments and relevant organizations, the Secretariat requested information on the following areas:

- (a) New techniques or tools for the detection and identification of living modified organisms
- (b) Experience with:
 - (i) New detection techniques
 - (ii) Detecting newly developed and/or unauthorized living modified organisms
 - (iii) Developing reference materials
- (c) Collaborations or agreements between national and/or regional laboratories

6. Information relevant to the areas above is detailed in the sub-sections below. Bibliographic references provided through the submission of information process is contained in annex II below.

A. New techniques or tools for the detection and identification of living modified organisms

7. Under this first category, Parties and organizations were asked to provide information on new techniques or tools for the detection and identification of LMOs. In terms of the new techniques, strategies and tools for the detection and identification of LMOs, no new tools were shared. Rather, the submissions suggested that further refinement and continued development of digital PCR and next-generation sequencing strategies and methodologies have occurred.

8. In German laboratories, highly reproducible next-generation sequencing techniques in combination with continually improving bioinformatic analysis pipelines were being evaluated for their potential to act in complement to and as a guide for PCR-based tools. Approaches, such as targeted sequencing, could be applied for the simultaneous detection and multiple known LMOs. However, for unknown LMOs, next-generation sequencing using primer walking or whole genome sequencing strategies could be applicable for unknown or unauthorized LMOs. In addition, it was further suggested that if there was a continual collection of next-generation sequencing data, there could be the potential to develop artificial intelligence tools and models to detect unknown LMOs. Furthermore, for organisms produced through genome editing and have a single nucleotide change, detection might rely on next-generation sequencing to distinguish single nucleotides. However, these tools were noted as being at a research and development stage and may not be easily implemented at the moment due to the requirement of specialized technical equipment.

9. Regarding the detection of organisms produced through genome editing, two recently published techniques were shared in the submissions. The first was a detection strategy based on targeted high-throughput sequencing, which is based on a prior PCR-based enrichment step to amplify regions of interest, (ii) a sequencing step, and (iii) a data analysis methodology to identify

³ Available at: <https://bch.cbd.int/en/submissions-to-notifications?schema=submission¤tPage=1¬ification=2023-100>

⁴ Available at: <https://bch.cbd.int/en/search?currentPage=1&schema=laboratoryDetection>

single nucleotide variants of interest. It was suggested that this strategy could be used for detecting organisms produced through genome editing. The second was a duplex digital droplet PCR approach based on the use of two different oligonucleotide labeled probes,⁵ which were designed using prior knowledge of the genome-edited line and differed only by a single variation point. Due to the differences in the probes, a study was able to distinguish between the wild-type and the genome-edited rice.

B. Related experience

10. Next, Parties and organizations were requested to share information on their experiences with new detection techniques, detecting newly developed and unauthorized LMOs and developing reference materials to support the detection and identification of LMOs.

1. New detection techniques

11. Despite recent developments with respect to digital PCR, isothermal techniques and next-generation sequencing, the some of the submitting countries noted that LMO analysis still relied on real-time PCR methodologies, such as those published by the International Organization for Standardization and the European Union Reference Laboratory for Genetically Modified Food and Feed. In this regard, it was mentioned that there was experience with developing new detection methodologies based on real-time PCR. For example, it was highlighted that Thai Department of Agriculture developed an in-house method for multiplex real-time PCR for screening of living modified crops, based on the Cauliflower mosaic virus 35S promoter, *Agrobacterium tumefaciens* nopaline synthase terminator and the appropriate endogenous reference gene for the matrix (e.g., maize high mobility group gene) according to the ISO17025 standards. Since its development in 2018, it has been utilized for over 6000 samples per year in Thailand.

12. In terms of digital PCR and next-generation sequencing, the submitting Parties have varied experience. For example, German control laboratories have begun implementing both of these tools and are currently evaluating highly reproducible next-generation sequencing techniques in combination with continually improving bioinformatic analysis pipelines. In another example, it was mentioned that despite having experience with next-generation sequencing and digital PCR for pest identification and food fraud, Brazilian laboratories have not yet implemented these two techniques for the analysis of LMOs or products obtained through the use of new genomic techniques. However, the laboratories have over 10 years of experience with Sanger sequencing. In addition, two recent research publications were also shared regarding Belgian experience on the detection of genome-edited organisms containing a single nucleotide variant using sequencing and digital PCR strategies (also see paragraph 9 above).

2. Detecting newly developed and/or unauthorized living modified organisms

13. To overcome the challenges with the increasing number of newly developed and/or unauthorized LMOs, German laboratories have adopted the following strategies:

- (a) New genetic element and/or construct-specific screening methods are regularly developed and validated before being added to the German table of screening methods;⁶
- (b) Pre-spotted plates with oligonucleotides for events authorized or in the process of being authorized in the European Union have been developed by the European Union Reference Laboratory for Genetically Modified Food and Feed;
- (c) Official methods for unauthorized LMOs in the European Union have been developed;

⁵ One probe was labelled using hexachlorofluorescein and the other was labelled using 6-carboxyfluorescein.

⁶ Available at: https://euginius.eu/euginius/pages/methodSearch_searchview.jsf (select method set "ABC").

(d) Event-specific detection methods are being developed for genome-edited organisms and for the detection of scaffold RNA of CRISPR/Cas9⁷ nucleotide sequences;⁸

(e) Next-generation sequencing approaches are being implemented for the simultaneous detection of multiple known LMOs, molecular characterization and identification of unauthorized LMOs and detection of organisms produced through genome editing (if possible).

14. For Brazilian laboratories, it was mentioned that screening was based on seven different LMO sequences in order to identify possible events and then followed up using event-specific methods in the case of positive results. For the detection and identification of unauthorized LMOs, the analysis strategy is performed on a case-by-case basis depending on species and the origin of the material. For newly-developed LMOs, which have been recently authorized, the applicants were noted as providing methodologies for the detection and identification of the LMO event, as well as reference materials for the official laboratory such that the methodologies can be validated.

15. Regarding the detection of organisms produced through genome editing, some recent publications were additionally shared related to the development of a targeted high-throughput sequencing and a digital droplet PCR protocol for distinguishing single nucleotide variants (kindly refer to para. 9 above).

3. Developing reference materials

16. Regarding experience with developing reference materials, experience varied. For example, it was noted that LMO analysis in Brazil is conducted with purchased certified reference materials and reference materials that have been provided by the developer. In another example, the Laboratory of Genetically Modified Detection in the Thai Department of Agriculture has developed an in-house reference material for living modified wheat event MON71800. The reference material is based on a plasmid containing Cauliflower mosaic virus 35S promoter, *A. tumefaciens* nopaline synthase terminator, a MON718000 event specific element and plastid acetyl-CoA carboxylase-1 (functions as a positive control).

17. In the European Union, it was noted that certified reference materials are available and provided by a few accredited producers. However, the access to suitable reference materials and sufficient information for unauthorized LMOs remains a challenge. In Germany, reference materials for unauthorized LMOs are produced by the German National Reference Laboratory for Genetically Modified Organisms on an ad hoc basis. Procurement for biomass and/or genomic DNA from the LMO producer is also done, but remains challenging. In addition, it was noted that if biomass of the LMO is available, parameters, such as extractability of the DNA, homogeneity, zygosity and the number of insertions must be determined, but having biomass most similar to the analytical sample is preferred. For pure genomic DNA, long shelf-life is an advantage, but it may be unsuitable for next-generation sequencing as DNA extraction and purification must follow specific protocols. If neither biomass or pure genomic DNA can be obtained, artificial plasmids are synthesized, containing sequences of the known genetic modification. However, it was noted that these may behave differently than the sample material during analysis.

C. Collaborations or agreements between national and/or regional laboratories

18. Regarding collaborations between national and regional laboratories, the submissions noted several national, regional and international collaborations or agreements.

19. For Brazilian national laboratories, LMO analysis is conducted by a network of laboratories, which have been accredited to the National Institute of Metrology, Standardization and Industrial Quality based on ISO 17025:2017 standards. Further, Brazilian laboratories also actively participate

⁷ Clustered regularly interspaced short palindromic repeats (CRISPR); CRISPR-associated protein 9 (Cas9)

⁸ Not for routine official control at the time of submission.

the Network of official laboratories for detection of GMOs in Latin America and the Caribbean region.

20. In Thailand, it was noted that the Laboratory of Genetically Modified Detection in the Department of Agriculture participates in the Association of Southeast Asian Nations Genetic Modified Food Testing Network.⁹ Further, it was noted that the Central laboratory (Thailand) Co. Ltd. Bangkok Branch participates in proficiency testing with Fapas, an accredited proficiency testing provider located in the United Kingdom of Great Britain and Northern Ireland.¹⁰

21. At a national level, German laboratories work in working groups, such as the § 28b GenTG and § 64 LFGB working group, which were founded on a legal mandate and consist of national laboratories, as well as representatives of industry and academia. It was highlighted that their work involves developing and/or validating analytical methods for control activities and published in the Official Collection of Methods. At a European regional level, German laboratories are members of the European Network of GMO Laboratories, which develops, harmonizes and standardizes the means and methods for sampling, detection, identification and quantification of LMOs in Europe. Further, it was noted that German laboratories maintain national, European and international level accreditation. Further, the Federal Office for Consumer Protection and Food Safety and the Wageningen Food Safety Research Group collaborate on the EUGenius database,¹¹ which provides information on authorization status, reference materials and detection methods for LMOs

22. Within Belgium, it was noted that some professionals participate in the following projects:

(a) Belgian project GENEDIT: Development and evaluation of approaches for detection of plants modified by new genome editing techniques (2021-2024);

(b) European Union project DARWIN: Transition to safe & sustainable food systems through new & innovative detection methods & digital solutions for plant-based products derived from new genomic techniques, under a co-creation approach (2024-2027);

(c) European Network of GMO Laboratories working group on method performance requirements (including criteria for methods detecting genome edited plants);

(d) European Network of GMO Laboratories working group on genome edited plants, animals and microorganisms.

D. Additional relevant information

23. In addition to the information requested by Parties, other Governments and relevant organizations, considerations were also provided related to LMOs containing engineered gene drives and the need to update Biosafety Technical Series 05: Training Manual on the Detection and Identification of Living Modified Organisms in the Context of the Cartagena Protocol on Biosafety.

24. Regarding LMOs containing engineered gene drives, it was noted in one submission that although LMOs containing engineered gene drives would not present significant challenges for detection and identification compared to other types of LMOs, different strategies may need to be adopted as it could be important for monitoring the LMOs containing engineered gene drives in the environment, ecological impacts and potential transboundary movements. Some specific considerations could be:

(a) Establishment of validated methods, including inter-laboratory proficiency testing and standardized reagents (e.g., PCR primers), among others, to ensure harmonized testing and reliability;

⁹ Available at: https://org.doa.go.th/aseangmf/?page_id=5

¹⁰ Available at: <https://fapas.com/>

¹¹ Available at: <https://www.euginius.eu/euginius/pages/home.jsf>

(b) Applicable detection and identification approaches that work across species where the potential for hybridization exists, such as a living modified *Anopheles gambiae* containing an engineered gene drive and the *Anopheles* species complex;

(c) Consideration for different types of LMOs containing engineered gene drives (e.g., the recipient species);

(d) Establishment of standards regarding storage and sample stability and building suitable storage facilities (e.g., -80°C freezers);

(e) Availability of reference materials, such as the strains of LMOs containing engineered gene drives and the equivalent reference wild-type organism;

(f) Establishment of appropriate identifiers or genetic sequences specific to the construct, which might be more appropriate for certain types of engineered gene drives systems (e.g., split drives);

(g) Robust and statistically defensible sampling strategies to support monitoring, including the establishment of limits of detection thresholds, sampling LMOs containing engineered gene drives in nature over large areas (compared to agricultural LMOs, which would be sampled at port-of-entry or defined sample spaces).

25. In one submission, it was suggested that due to digital PCR and next-generation sequencing being in a stage of research and development for their applicability to LMO analysis, there was not an urgent need to update Biosafety Technical Series 05: Training Manual on the Detection and Identification of Living Modified Organisms in the Context of the Cartagena Protocol on Biosafety. Further, it was suggested that the manual continues to provide a sufficient overview of the current methods for LMO analysis. In the submission, it was concluded that an update should be re-evaluated at a later date.

Annex I

Template for submitting information on detection and identification of living modified organisms pursuant to paragraph 3 of decision CP-10/11

Part I. Endorsement of submission

Name of Country/Organization:

Name of Cartagena Protocol Focal point/Head of Organization endorsing:

Signature of the Cartagena Protocol Focal Point/ Head of Organization:

Date:

Part II. Submission of information

In decision CP-10/11, the Conference of Parties serving as a meeting of the Parties to the Cartagena Protocol on Biosafety (COP-MOP) invited Parties and relevant organizations to submit information on their experience with new detection techniques, detecting newly developed and unauthorized living modified organisms and developing reference materials, as well as ongoing collaborations involving national and regional laboratories. At its twenty-sixth meeting, the Subsidiary Body on Scientific, Technical and Technological Advice to consider the information submitted and prepare a recommendation on the need to update Biosafety Technical Series 05: *Training Manual on the Detection and Identification of Living Modified Organisms in the Context of the Cartagena Protocol on Biosafety*.

Based on this, please submit information on the following areas:

1. New techniques or tools for the detection and identification of living modified organisms
2. Experience with:
 - a. New detection techniques
 - b. Detecting newly developed and/or unauthorized living modified organisms
 - c. Developing reference materials
3. Collaborations or agreements between national and/or regional laboratories

Submission of supporting documentation

For any publication that you may want to share as part of your submission, kindly include:

1. Name of publication(s), author, date and DOI or URL link.
2. Attach in pdf format any publication you have listed above.

Annex II

Bibliographic references

Fraiture, Marie-Alice and others. ddPCR strategy to detect a gene-edited plant carrying a single variation point: Technical feasibility and interpretation issues. *Food Control*, vol. 137, No. 108904 (July 2022).

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