CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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Agenda item 7 **CX/PR 24/55/6**

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GUIDELINES FOR MONITORING THE STABILITY AND PURITY OF REFERENCE MATERIALS AND RELATED STOCK SOLUTIONS OF PESTICIDES DURING PROLONGED STORAGE

(At Step 4)

(Prepared by the Electronic Working Group chaired by India and co-chaired by Argentina and Singapore)

Codex members and observers wishing to submit comments at Step 3 on the guidelines as presented in Appendix I should do so as instructed in CL 2024/45-PR available on the Codex webpage[1]

BACKGROUND

1. The Codex Committee on Pesticide Residues (CCPR51, 2019)[2] considered an additional request related to the shelf-life of certified reference materials (CRMs) raised by some delegations as follows:

   - CRMs were used for many purposes e.g., Good Agricultural Practices (GAP), supervised field trial data, monitoring of import/export samples, etc.
   - Most of the CRMs remained stable after their expiry period mentioned in their Certificate of Analysis (CoA).
   - The limitation of the use of CRMs after the expiry date led to recurring high costs for laboratories, consideration should therefore be given to including guidance on monitoring of purity of CRMs of multi-class pesticides during prolonged storage.

2. The CCPR51 agreed to request Argentina and India to prepare a discussion paper regarding monitoring of stability and purity of CRMs of multi-class pesticides during prolonged storage for consideration at CCPR52.

3. At CCPR52 (2021)[3], India on behalf of Argentina, introduced the item and reminded CCPR of the background for the work, the work process followed in the development of the discussion paper and key issues discussed in the paper. The Delegation informed CCPR that further work was needed on this topic and recommended that an Electronic Working Group (EWG) be established to further develop the discussion paper for consideration by CCPR53.

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1 Codex webpage/Circular Letters: http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/.
Codex webpage/CCPR/Circular Letters: https://www.fao.org/fao-who-codexalimentarius/committees/committee/related-circular-letters/jp/?committee=CCPR

2 REP19/PR51, paras. 182-184 & 186

3 REP21/PR52, paras. 198-201

4. At CCPR53 (2022)[4], India, as Chair of the EWG and on behalf of the co-Chairs Iran and Argentina, introduced the item and recalled the request regarding limitation of the use of CRMs after the expiry date leading to high recurring costs for laboratories and trade disruption, and thus the need for harmonized guidance on monitoring of stability and purity of CRMs of multi-class pesticides during prolonged storage. Such guidance would enable the use of CRMs after the expiry date when verification was performed as per the international guidance provided by Codex. Use of expired CRMs with verified purity would have economic impact by saving the purchasing cost of fresh CRMs especially by developing countries.

5. Keeping in view the comments received from Mexico, Germany, USA, Uruguay, Thailand, Chile, China, United States of America (USA), Canada, Mauritius and Institute of Food Technologists (IFT) during EWG discussions, the discussion paper was revised to highlight the relevance and objective more clearly with the scope of guidelines focusing on reference materials (RMs) having known purity specified by the Reference Material Producer (RMP) in the certificate of analysis (CoA). Comments received on Circular Letter CL 2023/38-PR from Brazil, Canada, Chile, Cuba, Egypt, Ghana, Indonesia, Iraq, Japan, Kenya, Saudi Arabia, Singapore, Uruguay, USA, European Union (EU), China, Philippines, Peru, Republic of Tanzania, South Korea, Ecuador, Guatemala, AgroCare Latinoamerica and International Commission for Uniform Methods of Sugar Analysis (ICUMSA) were also taken into consideration.

6. At CCPR54 (2023), India as Chair of the EWG and the in-session WG established by CCPR, and on behalf of the co-chairs Argentina and Iran, introduced the item, recalled the background to the work and the mandate of the EWG, explained the work process and summarized key points of discussion, conclusions, and recommendations of the EWG. While highlighting the importance of these guidelines, the Chair emphasized that such guidance would allow the extended use of the RMs which are stable with acceptable purity beyond their expiry dates specified by RMPs for robust residue analysis.

7. Following the detailed deliberations on the proposed work held during CCPR54, it was agreed by the CCPR to re-establish the EWG chaired by India and co-chaired by Singapore and Argentina to[5]:

   i. Develop the guidance procedures for monitoring the stability and purity of multi-class pesticide reference materials and their stock solutions during prolonged storage based on the outline provided in CX/PR 23/54/14, Appendix III and taking into account comments submitted in reply to CL 2023/38-PR; and

   ii. Submit the report of the EWG and the proposed guidance procedures to the Codex Secretariat for circulation for comments at Step 3 and consideration by CCPR55 (2024).

WORK PROCESS

8. Based on the outline for new work provided in CX/PR 23/54/14, Appendix III and taking into account comments submitted in reply to CL 2023/38-PR and during CCPR54, the EWG Chair and co-Chairs prepared a draft guidance document for monitoring the stability and purity of multi-class pesticide reference materials and their stock solutions during prolonged storage and circulated it on the Codex forum inviting comments from the EWG members. During the first round, comments were received from Singapore, Canada, Chile, Thailand, United States of America (USA), Uruguay and Institute of Food Technologists (IFT). The EWG members, in general, supported the development of the guidelines.

9. Based on the comments the document was revised to improve the analytical protocol for better understanding and clarity and uploaded by the EWG Chair inviting second round of comments from the EWG members and observers. In the second round Canada, Germany, Uruguay, USA, and China provided their comments on the forum.

10. The document as revised by the EWG is presented in Appendix I: Guidelines for monitoring the stability and purity of reference materials and related stock solutions of pesticides during prolonged storage; Annex for definitions and Annex II which provides the list of participants.

4 REP22/PR53, paras. 235-242
5 REP23/PR54, paras. 254-259

KEY POINTS OF DISCUSSION

11. The following are the key points of discussion based on the comments received in CCPR54 and during two rounds of circulation of the document in EWG.

    1. Refine and extend the scope of guidelines to include stock solutions in the guidance document.
    2. Align the acceptability criteria for difference in purity between the expired and unexpired RM as per SANTE guidelines.
    3. Exclude the criteria of proficiency testing for monitoring the stability and purity of RMs from the analytical protocol.
    4. Stability and purity of RMs and related stock solutions may continue to be monitored up to 10 years (as per SANTE) provided the RMs and stock solutions continue to maintain the acceptability criteria.
    5. RMs and stock solutions should be stored at sub-zero temperatures in deep freezer. If a laboratory maintains storage conditions more protective than those recommended by the RMPs (i.e., temperature lower than recommended without exposure to light and moisture, etc.), the rate of degradation of the RMs is significantly minimized as long as these conditions do not contradict those indicated in the product information sheet by the RMP.
    6. The exposure of RM and stock solutions to high temperatures and light should be kept as short as absolutely necessary.
    7. The daily record of the storage conditions (temperature) and date of use of the RMs should be maintained.
    8. Stock solution of the RMs need to be first brought to room temperature and then opened for analytical work. The room temperature at which the RM bottle is opened should also be recorded.
    9. The acceptable criterion for replicate measurements has been included as %RSD ≤ 10%.
    10. The inclusion of the use of internal standard (unexpired) in Approach II to account for any change in the response of the equipment. The deviation in the peak area ratio of the target analyte to internal standard should be ≤ 10% as mentioned in Approach II.
    11. General detectors such as HPLC-DAD / GC with FID detector/LC-MS and GC-MS in full scan mode or the detectors mentioned in the product information sheet/CoA may be used for assessing the stability and purity of RMs. The selective detectors have been excluded from the list of detectors recommended for analysis of the RM.

CONCLUSIONS

12. The EWG observed that there is a general support for the guidance document. Based on the discussions held at CCPR54 and comments received from the EWG and forum members, the guidance document has been revised.

13. Two approaches (analytical protocols) have been proposed for monitoring the stability and purity of RMs and their use beyond the expiry date. If the stability and purity of RMs continues to meet the acceptability criteria, they may be considered suitable for use up to a maximum period of 10 years provided these are stored under conditions specified in the guidelines.

RECOMMENDATIONS

14. CCPR is invited to

    i. consider the proposed guidelines as set out in Appendix I and provide general and specific comments on the document including its readiness for advancement in the Step Procedure; and

    ii. identify key issues or sections in the document that may need further consideration or development in order to progress with the finalization of the guidance document.

APPENDIX I

GUIDELINES FOR MONITORING THE STABILITY AND PURITY OF REFERENCE MATERIALS AND RELATED STOCK SOLUTIONS OF PESTICIDES DURING PROLONGED STORAGE

(For comments at Step 3)

PREFACE

1. Pesticide residues in food commodities have become a worldwide agricultural trade-concern, which has led to enforcement of strict pesticide regulations. More than 1200 pesticides are available globally to control the pests on different food commodities. Analyses of pesticides at trace level in the food chain requires the use of specific Reference Materials (RMs) of known chemical purity manufactured by the Reference Material Producers (RMPs) to ensure the reliability of the test results. Accurate determination of pesticide residues in food commodities is important for food safety control and fixation of pesticide MRLs thereby overcoming the related trade barriers. RMs with specified purity are also required for accurate qualitative and quantitative analysis of pesticide active ingredient(s) in technical products, formulations, and stock solutions.

2. Limited shelf life, diminishing purity, and high recurring cost of RMs act as major impediments for performing regular pesticide residue analysis. These problems are magnified for multi-pesticide residue analysis by testing laboratories situated in developing countries as they are required to allocate a large part of their funds to the frequent procurement of expensive RMs. Furthermore, use of RMs is restricted by the expiry dates specified by the RMPs in the information sheet (either certificate of analysis (CoA) or product information sheet) which provides the value for purity, expiry date and measurement uncertainty of the RMs. Many times, laboratories cannot afford frequent purchase of high-cost RMs for their pesticide residue control work.

3. Furthermore, due to supply chain constraints, some laboratories may receive RMs close to their expiry date as mentioned in the product information sheet. In such situations the laboratories are forced to buy new standards and prepare new stock solutions more frequently than necessary. This leads to insurmountable extra work and increased laboratory costs, especially for compounds for which stability is well-understood. Additionally, shipping of RMs by the suppliers to laboratories increase the acquisition time for procurement (few weeks to months), creating hurdles in sustainable pesticide residue control program.

4. Many RMs stay stable even after the expiry dates stated in the product information sheet with no significant change in the purity. Some studies[1,2,3] have also reported that if RMs are stored at better storage conditions than recommended by the manufacturer, provided that these conditions do not contradict those indicated by the RMP in the product information sheet, the RMs are stable for much longer than the expiry dates indicated by the RMPs. Such RMs may technically be allowed to be used beyond their expiry dates if laboratory checks are in place to demonstrate that they are stable and continue meeting the purity requirements. However, the lack of data on the stability and purity of RMs during prolonged storage and absence of guidance procedures for monitoring prevents their use beyond the expiry dates.

5. This document represents a first step towards developing comprehensive harmonized guidance which would enable the laboratories to monitor the stability and purity of the pesticide RMs and their stock solutions during prolonged storage. The document would provide guidance to the accredited laboratories to monitor the stability and purity of RMs for their possible use beyond their expiry dates as well as for continued use of stock solutions which retain their stability and purity.

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1 de Kok, A., de Kroon, M. and Kiedrowska, B. (PO 005 pdf, 2019). Stability of pesticides reference standards and stock solutions Part 1. GC-pesticides NVWA - Netherlands Food and Consumer Product Safety Authority, Laboratory of Food and Feed Safety-Chemistry Laboratory, National Reference Laboratory (NRL) for Pesticide Residues in Food and Feed, Wageningen, The Netherlands.

2 de Kok, A., de Kroon, M. and Scholten, J. (PO 006 pdf, 2019). Stability of pesticides reference standards and stock solutions Part 2. LC-pesticides NVWA - Netherlands Food and Consumer Product Safety Authority, Laboratory of Food and Feed Safety-Chemistry Laboratory, National Reference Laboratory (NRL) for Pesticide Residues in Food and Feed, Wageningen, The Netherlands.

3 Sharma, K. K., Tripathy, V., Gautam, R., Gupta, R., Tayade, A., Sharma, K., Yadav, R., Shukla, P., Devi, S., Pandey, P., Singh, G., Kalra, S., Walia, S. (2020). Monitoring of purity of CRMs of multi-class pesticides during prolonged storage before and after expiration. Accreditation Qual. Assur., 25 (10), 89-97. 10.1007/s00769-019-01411-w.

SCOPE AND OBJECTIVE

6. The purpose of this document is to furnish a framework which would assist the laboratories in monitoring the stability and purity of individual reference material (RM) of pesticides during prolonged storage and identify expired RMs with continued stability and purity. The general criterion of the proposed new work is to monitor and verify the stability and purity of individual RMs before and after expiration through robust analytical protocols so that such materials that retain their purity as per the product information sheet/CoA even after expiry can continue to be used as valid RMs. The guidance document does not cover mixture of standards. Another aspect of the proposed work is to monitor the stability of the stock solutions used for pesticide residue analysis so that those solutions which continue to be valid can be used for the accurate and reliable determination of pesticide residue levels.

7. This document is applicable to reference materials (RMs) of pesticides and their individual stock solutions of known purity specified by a reference material producer (RMP).

8. These guidelines will enable the pesticide residue laboratories to overcome the shortcomings associated with RMs and use them beyond their expiry dates mentioned in the product information sheet. After the expiration date, the RMs retaining the purity specified in the product information sheet can be used as RMs or as quality control materials (QCM) for the analysis of pesticides provided that these are stored under conditions specified in the guidelines.

9. The guidelines cover the storage conditions that should be maintained, and quantitative measurements that should be performed to monitor the purity of RMs before and beyond their expiration period.

GENERAL CRITERIA

1. The analysis should be conducted in an ISO/IEC 17025 accredited laboratory with the scope relevant to the measurement concerned.

2. The RMs should be procured from a RMP who is accredited according to ISO/IEC 17034 to ensure analytical traceability.

3. For ensuring traceability, the analytical balances used should be calibrated with weights traceable to the national/international standards.

4. Calibrated class A glassware or appropriate electronic pipettes should be used for volumetric measurements.

5. The instrumentation used in purity tests must have comparable sensitivity/specificity to those used in the product information sheet/CoA.

CRITERIA FOR STORAGE CONDITIONS FOR PESTICIDE REFERENCE MATERIALS AND THEIR STOCK SOLUTIONS

6. The storage conditions of RMs are specified by RMPs in the product information sheet/CoA as these are susceptible to degradation at high temperature and other environmental factors.

7. If a laboratory maintains the RMs at storage conditions which are better i.e., more protective than those recommended by the RMPs (i.e., temperature lower than recommended without exposure to light and moisture, etc.), the rate of degradation of the RMs is significantly minimized as long as these conditions do not contradict those indicated in the product information sheet/CoA by the RMP. Under such conditions, the expiry date as recommended by the RMPs may be extended as appropriate for a RM by a date allowing for storage up to 10 years or as long as the purity mentioned in the product information sheet/CoA holds good (≤ ±10%) (SANTE[4], 2022). Another study revealed the stability of pesticide reference standard up to 15 years or in stock solution up to 10 years[6,7].

8. To avoid any cross contamination or degradation of RMs, the vials must be placed in airtight capped tube/sealed pouch and immediately stored in the freezer at conditions better than those recommended by RMPs; preferably at subzero temperature. The stock solutions must also be stored in airtight capped glassware (preferably

4 SANTE/11312/2021, Implemented by 01/01/ 2022, European Commission Directorate General for Health and Food Safety.

volumetric flask). Storage conditions should be monitored with appropriately calibrated equipment and should be controlled and recorded.

ANALYTICAL PROTOCOL FOR MONITORING THE STABILITY AND PURITY OF PESTICIDE REFERENCE MATERIALS AND INDIVIDUAL STOCK SOLUTIONS

9. Two analytical approaches may be considered for monitoring the stability and purity of RMs and their use beyond the expiry date provided the purity of RM is acceptable.

   In Approach 1, the stability of new and expired RMs/ new and old stock solutions is determined simultaneously, and it is applicable for neat standards and their related stock solutions. The comparisons of peak area or concentration should be run as close to repeatability conditions as possible, and mitigate other sources of variation in instrument response, such as use of internal standard, if applicable. If the deviation (in peak area/purity) after expiration is found within 10%, the analyte in the RM is acceptable and therefore can be considered for continued use as a RM. For neat standards and stock solutions, monitoring of stability & purity may be continued regularly up to a maximum of 10 years (SANTE) provided the purity remains acceptable[5,6,5.] This approach is based on SANTE guidance document (SANTE/11312/2021). Here new RM would be required for the comparison purpose.

10. As per Approach 2, whenever a fresh RM is procured by any laboratory, its purity is monitored as per the analytical conditions of the product information sheet/CoA and the purity is monitored periodically before and after expiry using the same analytical conditions. Here, new RM need not be procured. An unexpired internal standard can be used to account for any change in the response of the equipment. This approach is applicable only for the neat RMs accompanied by Product Information Sheet/CoA of RM.

Approach 1: Comparing the stability of old and freshly acquired pesticide reference standards; applicable to neat standards of reference materials and related stock solutions

11. Prepare fresh stock solution of the old (or expired) and newly acquired RM standard of appropriate concentration. Appropriate concentration will depend on the response of the RM in the detector. Generally, for HPLC-DAD/GC-FID, good response is obtained between 10 ppm to 100 ppm. Higher concentration of the RM may lead to saturation of the detector.

12. Inject the standard solution of the new RM prepared from the stock solution at an appropriate concentration into the instrument (HPLC[6]-DAD[7] /HPLC-UV[8] /GC[9]-FID[10] /LC-MS[11] and GC-MS in full scan mode) and record the peak area. Perform a minimum of five replicate measurements to obtain an acceptable level of variation (% RSD ≤ 10%). The mean value of the peak area for the new stock solution is taken to be 100% and is also used as a basis for the calculation of the percentage-difference.

13. Inject the old standard solution prepared from the stock solution at the same concentration as the new RM into the instrument and record the peak area. Perform a minimum of five replicate measurements to obtain a mean value of the peak area for the old standard with %RSD ≤ 10%.

14. If the mean peak area of the old stock solution shows a deviation ≤ 10% in comparison to the new stock solution, the old (expired) standard may be considered suitable for use.

15. The old standard should be compared with the unexpired standard at regular intervals of one year provided the recommended storage conditions are maintained.

16. The gravimetric records should be maintained for RMs (opened or unopened), both solid and liquid and their respective stock solutions during storage. Before recording the weight, the volumetric flask should be allowed to

5 EURL DataPool, https://www.eurl-pesticides-datapool.eu/
6 High-performance liquid chromatography
7 Diode-Array Detection
8 Ultra-violet spectroscopy
9 Gas chromatography
10 Flame ionization Detector
11 Mass Spectrometry

attain room temperature/ambient temperature and wiped to remove any adhering moisture. The exposure of RM and stock solutions to high temperatures and light should be kept as short as absolutely necessary.

17. The daily record of the storage conditions (temperature) as well as the date of use of the RM and their stock solution should be maintained. Also, the temperature at which the RMs and their stock solutions are opened for use should be recorded.

Approach 2: Verification of purity of neat standards of pesticide reference materials during prolonged storage (not suitable for verification of stock solutions)

18. To verify the purity of the RM, chromatographic assay should be performed as per the analytical conditions mentioned in the product information sheet/CoA by the RMP. The verification of RM purity is performed by considering the purity (in terms of percent peak area) mentioned in the product information sheet/CoA as the reference value.

19. Prepare fresh stock solution of the newly acquired neat standards of RMs and internal standard (a different unexpired RM) of appropriate concentration in a suitable organic solvent. Appropriate concentration will depend on the response of the RM in the detector. Generally, for HPLC-DAD/GC-FID, good response is obtained between 10 ppm to 100 ppm. Higher concentration of the RM may lead to saturation of the detector.

20. The standard solution of the RM prepared at an appropriate concentration from the stock solution is injected into the instrument (HPLC-DAD /HPLC-UV /GC-FID/ LC-MS and GC-MS in full scan mode) as per the analytical conditions mentioned in the product information sheet/CoA and the percent peak area so obtained is recorded as percent purity. A minimum of five replicate measurements should be performed to obtain a mean value of percent purity and the %RSD should be ≤ 10%. The instrument should be calibrated as per the conditions recommended by the manufacturer.

21. Compare the mean value of verified purity obtained from the laboratory analysis with the reference value of purity provided in the product information sheet/CoA. Significant deviation should not be observed in the purity figure provided by the manufacturer and the purity obtained in the laboratory.

22. Spike a different RM (unexpired) into the standard solution of the analyte RM. This different RM serves as an internal standard. Inject the solution and record the peak area of the RM and the internal standard and calculate the average ratio of internal standard area to RM area. The internal standard peak should have a similar abundance to the RM being verified and it should not interfere with the analysis of the target analyte.

23. Repeat the same procedure at regular intervals of one year using a new stock solution of the RM, particularly before and after expiry of the RM to monitor its stability during prolonged storage.

24. After expiry of the RM, if the mean value of percent purity in terms of percent peak area obtained for the RM during analysis shows a deviation ≤ 10% from the reference value and the ratio of peak area for the RM compared to the internal standard is ≤ 10%, the RM may be considered suitable for use in the laboratory.

25. The gravimetric records should be maintained for RMs (opened or unopened), both solid and liquid during storage. Before recording the weight, the volumetric flask should be allowed to attain room temperature/ambient temperature and wiped to remove any adhering moisture. The exposure of RM and stock solutions to high temperatures and light should be kept as short as absolutely necessary.

26. The daily record of the storage conditions (temperature) as well as the date of use of the RMs should be maintained. Also, the temperature at which the RMs are opened for use should be recorded.

ANNEX

DEFINITIONS

Certified Reference Material (CRM): Reference material (RM) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

Multi-class Pesticides: Multi-class pesticides include insecticides, fungicides, bactericides, nematicides, herbicides, etc. belonging to different chemical groups.

Product Information Sheet or Certificate of Analysis (CoA): A document that provides the relevant information about certified purity, concentration, date of expiry, and measurement uncertainty of an RM which in compliance with the requirement in the ISO 17034 and ISO Guide 31.

Purity: Characteristic of a reference material which indicates the proportion of the stated component of interest in relation to the total substance. The purity is typically expressed in percentage and should be considered when preparing standard solutions.

Quality Control Material (QCM): Reference material used for quality control of a measurement.

Reference Material (RM): Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Reference Material Producer (RMP): Body (organization or company, public or private) that is fully responsible for project planning and management; assignment of, and decision on property values and relevant uncertainties; authorization of property values; and issuance of a reference material certificate or other statements for the reference materials it produces.

Stability: Characteristic of a reference material, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period of time

Stock Solution: A solution of a reagent, of high concentration, from which appropriate dilutions can be made at the time of use

APPENDIX II

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