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**GUIDANCE DOCUMENT ON STORAGE STABILITY OF MICROBIAL PEST CONTROL
PRODUCTS**

**Series on Pesticides
No. 85**

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**OECD Environment, Health and Safety Publications
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No. 85**

**GUIDANCE DOCUMENT ON STORAGE STABILITY OF MICROBIAL PEST
CONTROL PRODUCTS**

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INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

**Environment Directorate
ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT
Paris 2016**

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FOREWORD

This document provides guidance to both industry and regulatory authorities on storage stability for different types of microbial pesticide formulations. It includes guidance on the physical and chemical parameters to be assessed for different formulation types before and/or after the storage period, and also describes specific and adequate criteria for the determination of storage stability. The document was prepared with the aim of providing guidance on the information/data requirements for microbial pesticide formulations and to facilitate the submission of a more complete data package/dossier which will in turn facilitate the review and decision-making process.

The document has been developed within the framework of the OECD BioPesticides Steering Group (BPSG), a sub-group of the OECD Working Group on Pesticides (WGP), which helps member countries to harmonise the methods and approaches used to assess biological pesticides and to improve the efficiency of control procedures. The International Biocontrol Manufacturers Association (IBMA) served as the initial author of the guidance document, which has been reviewed and further developed by the BPSG.

The present guidance document received final approval of the OECD BPSG in November 2015 and of the OECD WGP by written procedure in July 2016.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be declassified and made available to the public.

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INTRODUCTION

1. Test guidelines for storage stability have been established only for plant protection products based on *chemical active substances* (hereafter referred to as "chemical formulations"). However, these guidelines have also been applied to products based on *micro-organisms as active substances* ("microbial formulations") without providing clear guidance and taking into consideration the fact that different methodologies are needed for assessing the (biological) properties of living microbial pesticides. For example, unlike chemical substances, micro-organisms can die, survive or proliferate (OECD, 2014). Also, the physical stability of microbial formulations may be significantly different from that of chemical formulations.

2. Furthermore, when interpreting analytical data derived from (micro-) biological test methods, it is worth considering that these quantification methodologies are generally less precise than analytical methods for chemical substances, e.g. analysis by HPLC-UV or GC-MS. Cell and spore counts often have considerable relative standard deviations (RSD) with respect to the nominal value, whereas for bioassays the precision is difficult to standardize due to the complexity of interactions in host-pathogen relationships. Another important difference is that microbial formulations are often not milled and, therefore, the particle size may be larger compared to formulations containing chemical active substances. Consequently, microbial formulations may not always comply with general limits for physical parameters (e.g. min. 60 % suspensibility) implemented for chemical formulations.

3. Requirements concerning the development of storage stability data on microbial formulations vary across OECD countries. In Canada, the Health Canada Pest Management Regulatory Agency (PMRA) provides guidance for designing a study on storage stability for microbial pest control products (MPCPs). No reference to chemical methods (accelerated or otherwise) is made in the Canadian microbial guidelines (PMRA Regulatory Directive DIR2001-02). In the European Union, Commission Regulation (EU) N° 284/2013, part B, includes specific data requirements for plant protection products which are preparations of micro-organisms including viruses. In the introduction to Part B, it is stated that, pending the acceptance of specific guidelines at international level, the information required shall be generated using test guidelines accepted by the competent authority (e.g. US EPA guideline). As regards storage stability, Part B of the Regulation indicates that useful information is given in GIFAP Monograph No 17 (GIFAP, 1993). However, the US Environmental Protection Agency (EPA) and the GIFAP Monograph No 17 do not have strict storage stability requirements for microbial pesticides. Thus, in the absence of an alternative, some regulators have requested that storage stability studies be conducted according to the adapted chemical substance guidance provided by the international association, CropLife (CropLife International, 2009).

4. The present guidance document describes specific criteria for the determination of storage stability for different microbial formulation types. It also includes guidance on the physical, chemical and biological parameters to be assessed for different formulation types before and/or after the storage period, which can be used to support the registration of plant protection products. It is acknowledged that some specific cases or aspects may not be covered in this document and that authorities and applicants need to find pragmatic ways to approach such cases.

GENERAL INFORMATION ON STORAGE STABILITY REQUIREMENTS

Considerations for testing of shelf-life

5. Formulation details should be provided for all storage stability studies so that a study can always be traced back to the formulation. These details should include:

1. Trademark and/or formulation code if available
2. Active substance and content (OECD, 2011)
3. Type of formulation [Wettable powder (WP); Water dispersible granules (WG); Suspension concentrate (SC); etc.]
4. Batch identifier, e.g. batch code number
5. Manufacture date of tested batch(es)

Specification of technical characteristics of the formulation

6. The composition of the formulation and the packaging used in shelf-life studies should be provided. [Due to the integrated nature of some products it may not be possible to accurately define the exact composition of the MPCP (MPCA + dead cells, fermentation residues of nutrients, substrate, etc.). In such cases for these integrated products all biological components should be considered as a single line item in the composition. The composition of the MPCP should mention the content of the MPCA and all actively added ingredients. Relevant aspects of the MPCP (e.g. potential microbial contamination) should be addressed separately].

Methods of analysis

7. The method of analysis for the content of an active substance in the formulation should be determined and validated prior to the start of the shelf-life study. Where validation is not feasible or relevant for microbial formulations, this must be identified and justified.

Physical methods

8. Testing of physical methods should follow the requirements and test methods described for chemical formulations, e.g. as described in the *Manual on development and use of FAO and WHO specifications for pesticides* (WHO, 2010). Where physical tests are not applicable to microbial formulations, this must be identified and justified.

SHELF-LIFE TESTING OF MICROBIAL FORMULATIONS

Shelf-life testing

9. Shelf-life testing may not be applicable to microbial formulations in the same way as for chemical formulations due to the fact that these formulations contain living organisms and are able to proliferate in logarithmic scales, and taking into account the variability of the analytical methods and inter-laboratory variations for determining the content of active substance in the microbial formulations.

Analytical methods for determining the content of active substance

10. In most microbial formulations, the biological activity is based on the micro-organism itself. Hence, the micro-organism represents the active substance and its content should be determined.

11. Depending on the micro-organism, such (micro-) biological test methods may include:

- determination of the total number of viable spores [total spore count (typically by use of a counting chamber) multiplied by the ratio between viable and non-viable spores (different than CFU)],
- determination of the viable spore count (typically by plating on a Petri dish with a growth medium). The total number of viable spores is usually reported as colony forming units (CFU) per unit of weight or volume,
- an assay for concentration(s), where appropriate in combination with microbial cell count,
- a specific biopotency assay for assessing the potency of the active substance, e.g. in the case of viruses where viable and non-viable occlusion bodies or polyhedral bodies cannot be distinguished.

12. Weight based methods are not appropriate for determining the content of the MPCA during the product shelf-life. The weight might not change even if the biological activity of the MPCA decreases simultaneously. Furthermore, the weight of an active substance in a MPCP can, in most cases, only be derived indirectly. For example, the potency (through bioassay), cell/spore, toxin or CFU number per weight or volume unit is the relevant value related to product efficacy. Therefore, weight-based methods are not recommended for assessing the content of the MPCA. It should be taken into account that:

1. it might not be possible to reconcile the weight based content with a CFU or cell/spore count because the weight based content could potentially include the non-viable part of the active substance as well, and
2. in many typical production processes the weight based content of the active substance cannot be measured but only calculated.

13. If the proposed active content (e.g. CFU or spore count or biopotency) has not changed over time, potential corrections of MPCA weight based values should not be considered as “formulation changes”.

14. When interpreting analytical data based on such (micro-) biological test methods, it merits considering that cell and spore counts often have considerable relative standard deviations (RSDs) with respect to the nominal value, whereas for bioassays it is difficult to standardise the precision due to the complexity of interactions in host-pathogen relationship [e.g. different host insects, different breeding

conditions, different pathogen (host) species]. Due to the nature of some specific micro-organisms and as generally accepted in microbiology, limit indications on a logarithmic scale should be acceptable, if the applicant can demonstrate that the label claims of MPCP are within the indicated range. If necessary, other parameters such as RSD should be considered.

15. The release of the stored microbial formulation should not be of concern because loss of biological activity of microbial MPCA is unlikely to have adverse health effects. Although a reduction in the biopotency of the active substance of microbial formulations may be determined in the specific bioassay used for quantification of the content, this may not necessarily reflect a proportional loss of field efficacy.

16. The ability of some micro-organisms to reproduce, for example in the soil or on foliage, after application can possibly compensate for any decrease in active substance content during storage below limits specified on the product label. However, the focus of storage stability testing on MPCPs is on maintaining the labelled shelf-life claims and the minimum or nominal guarantee. At the end of the storage period, the content of the biological active substance(s) should not be below the minimum content specified on the label. However, if the MPCA shows poor stability during storage, which means that the content (e.g. cfu/g or viable spores/g etc.) decreases below the specified minimum limit, it should be possible to adapt the specified minimum content if it is still sufficient to achieve the claimed effects (i.e., efficacy) in the field.

17. In cases of large deviations during the stability study, acceptable justification should be provided.

Non-functional spore or cell products

18. In the European Union, according to Regulation (EC) No 1107/2009, micro-organisms shall be, per definition, living organisms capable of replication. Therefore, non-viable spores or cells incapable of replication or of transferring genetic material would not be registered following the data requirements for Microbials in Regulation (EU) No. 284/2013, Part B. Inactive spore or cell products work through the “biological compounds” products (enzymes and secondary metabolites), which were produced by the microbial when it was still active. The content of such “biological compounds” is assessed in the same way as for microbial formulations containing similar “biological compounds”. The U.S. and Canada do not differentiate these products in the same way as the EU. The US EPA regulates killed or inactivated micro-organisms as microbial pesticides and Canada’s PMRA regulates them as microbial pesticides, but with additional short-term toxicology data requirements reserved for biochemical (non-conventional) pesticides to account for potential effects of the metabolic by-product(s) responsible for the MPCP’s pesticidal mode of action.

GLP-Requirement

19. In general, tests and analyses should be conducted in accordance with the principles of Good Laboratory Practice (GLP) only where test results are necessary to evaluate the safety with respect to human or animal health and the environment. Other tests, e.g. related to application, can be performed under non-GLP conditions and following existing guidelines.

20. Under exceptional circumstances the latter tests could be performed in a non GLP test facility but only when a full justification is made explaining why they could not be performed in existing GLP certified laboratories. For the determination of the content of active substances during shelf-life, GLP is generally only required where hazardous compounds are formed during storage. However, in the case of micro-organisms, some specific methodologies can only be performed by specialist laboratories where the respective analytical method for the micro-organism is available or can be established and where

experienced staff are available to perform these particular tests. Such specific expertise may sometimes only be available from the manufacturer and may only be possible in laboratories, which are not GLP certified.

21. Where GLP is not established or cannot be established with acceptable effort, non-GLP data could be considered acceptable but a quality standard similar to GLP should be applied: a study report, including study plan, comprehensive method description, validation, and raw data. Furthermore, regulatory authorities should consider that many contract laboratories will also not be able to perform specific tests under GLP.

Ambient and cold temperature stability testing

22. Due to the diversity of micro-organisms, it is not possible to recommend one fixed temperature scheme for all cases. Storage temperatures for stability testing of microbial formulations will need to be adapted on a case-by-case basis and supported with a rationale. Storage stability testing should be conducted according to the recommendations of the manufacturer and should represent the required storage conditions stated on the product label. Where specific storage conditions are required, these should be detailed and supported. Storage stability testing should be conducted according to the type of microbial formulation and in accordance with the storage schemes/conditions recommended by the manufacturer.

23. Cold stability testing should be considered on a case by case basis only where cold storage may negatively affect the physical stability of the formulation. No negative impact is expected for the biological activity of most micro-organisms in microbial formulations when stored at 0 °C. When storage stability for shelf-life is shown at low temperature, e.g. refrigeration/freezing, no additional cold stability testing should be performed at 0 °C.

Storage period

24. Shelf-life may differ depending on the microbial formulation and this must be reflected in the storage scheme. As it is the case for some less stable chemical formulations, a suggested shelf-life below 2 years can also be acceptable for microbial formulations as long as this is specified on the label.

25. It must be considered that each micro-organism is different and that any decrease in the content of an active substance may not necessarily occur in a linear way. During storage, a micro-organism may retain good biological efficacy against the target organism even after an initial drop in the content of active substance. Furthermore, it can be seen in bioassays that the biological efficacy may drop with respect to the standard test species, but may retain potency to target species under field conditions.

Accelerated storage stability testing

26. MPCPs are typically temperature-sensitive. Therefore, standard accelerated storage stability methodologies defined in CIPAC MT 46.3 (CIPAC, 2000) and referred to in many guidelines (WHO, 2010; US EPA, 2012; EU, 2013; CropLife International, 2009) are not appropriate to assess MPCP stability over the shelf-life. The accelerated storage stability testing that might be conducted voluntarily for assessing the stability of a formulation should not necessarily be required in assessing the microbial activity. Test regimes at temperatures lower than the standard 54 °C (e.g. 18 weeks at 30 °C) may be feasible for some micro-organisms and could be used in individual cases. Suitability of accelerated storage test parameters must be related to the specific micro-organism and/or formulation type. There is no general rule whether liquid or solid formulations of the same organism will be more compatible with accelerated storage conditions.

27. Higher temperatures often do not allow storage for a duration relevant for practical conditions, but may be used to calculate time dependent variable mortality rates or strain specific mortality rate using e.g. the Arrhenius equation (Pauling, 1988) which are reported to work well for some microbial formulations. The results allow calculations on storage stability at realistic temperatures. However, this calculation method does not allow cross-reading conclusions from one microbial strain to another.

28. Alternative temperature/time regimes may be proposed but reasons and conclusions should be supported by a reasoned scientific case.

29. For the purpose of minor changes in formulation, physical testing only for assessing stability of the microbial formulation should be performed.

30. The biological activity should be assessed unless a robust rationale can be provided by the applicant in terms of why the formulation change is unlikely to impact the biological activity/viability of the organism over time. The potential influence of the change should be provided (e.g. change of co-formulants, protectants, adjuvants, packaging material, etc.).

Packaging requirements

31. Testing for interaction with the packaging and its stability during storage can be monitored for microbial formulation in the same way as for chemical formulations following UN standards for transport safety.

32. Packaging must be designed to adequately contain the microbial formulation.

Microbial contamination

33. Preservative properties of MPCPs, including the presence of contaminating micro-organisms (where appropriate), should be considered in the design of storage stability tests. If a formulation is not intended to be stored under conditions that will prevent or inhibit microbial growth (e.g., refrigerated temperatures) or does not contain microbial growth inhibiting inerts/formulants, then contaminants should be checked before and after storage and/or at appropriate time intervals during storage stability testing. OECD *Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products* (OECD, (2011) provides detailed guidance on microbial contaminant testing of microbial products, and includes acceptance limits for hazardous micro-organisms.

34. Internationally accepted methods such as AOAC (Association of Analytical Communities), US FDA BAM (Bacteriological Analytical Manual) ISO / DIN or those quoted in European and US Pharmacopoeia for example are acceptable and do not require validation.

Special requirements:

35. Other formulation specific properties, e.g. for water soluble bags, are considered as for chemical formulations.

36. Physical and chemical properties of the microbial formulation should be tested before and, if applicable, after storage for microbial formulations following chemical products guidelines for the particular formulation type (WHO, 2010). Integrity of the commercial packaging material should also be assessed throughout the testing period.

37. When assessing the physical stability of microbial formulations, it must be considered that some properties may be significantly different from chemical formulations. For example, microbial formulations

are not milled and therefore the particle size may be larger compared to formulations containing chemical active substances. As a consequence, microbial formulations may not always comply with general limits for physical parameters (e.g. min. 60 % suspensibility) that were developed for chemical formulations. In case of such findings, fitness for use shall be shown or appropriate scientific reasoning be given.

SUMMARY

38. Testing of shelf-life stability for microbial formulations requires taking into consideration the fact that specific methodologies are needed for assessing the (biological) properties of living microbial pesticides. While many physical property testing methods for individual formulation types can be based on existing guidance for chemical formulations, individual formulations do not fit in the framework and require, adaption of the methods and thresholds which then has to be justified appropriately.

39. It is important to consider essential differences with respect to the determination of the active substance content and the storage conditions for micro-organisms.

40. For the determination of active substance content in microbial formulations, a specific testing methodology is necessary, e.g. use of bioassay, cell counting or other valid methods. In general, tests and analyses should be conducted in accordance with the principles of GLP. Under exceptional circumstances these tests could be performed in a non GLP test facility following similar standards but only when it is fully justified.

41. Generally, accelerated storage stability testing at higher temperatures is not appropriate for microbial formulations, but could be used in individual cases.

42. For microbial formulations the applicant should justify a shelf-life programme based on the stability profile of the individual microbial formulation in its commercial packaging. It is understood that this programme may well include temperatures other than ambient temperature, e.g. refrigeration or freezing and storage periods other than those currently required by existing guidelines for chemical formulations.

GLOSSARY AND ABBREVIATIONS

TERM	DEFINITION
Active substance	The component of a formulation which is responsible for the biological activity of a formulation.
Bioassay	Methodology to assess the potency or efficacy of a microbial product
CFU	Colony forming unit
Chemical formulations	Formulations containing active substance(s) which are natural or synthetically derived chemicals
GLP	Good Laboratory Practice
MPCA	Microbial pest control agent A micro-organism (e.g. bacterium, fungus, protozoan, virus, viroid, mycoplasma, algae) and any associated biological compounds/toxins, fermentation residues and contaminants as manufactured, to which the effect of the pest control is attributed.
MPCP	Formulated product containing a MPCA
Microbial formulations	Formulations containing active substance(s) which are micro-organisms, e.g. bacterial or fungal spores, viruses, etc.
Biological compounds	Biological compounds (secondary metabolites) which can be produced by micro-organisms and which are not directly involved in the normal growth, development or reproduction of the micro-organisms in which they occur, but may play an important role in stress tolerance and their ecological interaction with other (soil-) organisms including plants (Gunatilaka and Wijeratne, 2011)
Strain	A strain is a population of an organism that descends from a single cell or a pure culture isolate. Typically, it is the result of a succession of cultures ultimately deriving from an initial single colony. For the purpose of this document 'strain' refers to a culture that is specifically linked to a collection number.

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