

Transitional Guidance on the Biocidal Products Regulation

Transitional Guidance on Efficacy Assessment for Preservatives

May 2014

LEGAL NOTE

This document contains Transitional Guidance on Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (Biocidal Products Regulation, the BPR).

Users are reminded that the text of the BPR is the only authentic legal reference and that the information in this document does not constitute legal advice. Only the Court of Justice of the European Union can give authoritative interpretations on the contents of Union law.

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European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland

Visiting address: Annankatu 18, Helsinki, Finland

PREFACE

This Transitional Guidance is to be applied to applications for active substance approval and product authorisation submitted under the Biocidal Product Regulation (the BPR). This document describes the BPR obligations and how to fulfil them

This Guidance replaces the Technical Notes for Guidance (TNSG) on Data Requirements (EU, 2008a) in support of Directive 98/8/EC (Biocidal Product Directive - BPD).

A "Transitional Guidance" is a document that has been initiated under the "old" Biocidal Products Directive and because it has been finalised before the relevant new Biocidal Products Regulation guidance document has been fully developed, it is being made available as a Transitional Guidance document until such time as the relevant new document is ready for publication.

This Transitional Guidance document has had a Public Consultation by the Commission and this document is now finalised and waiting for inclusion into Volume II Part B of the new BPR guidance structure: there will be no further consultation on this document and it will be added by a corrigendum when Volume II Part B is available.



NOTE to the reader:

This Transitional Guidance will be reformatted when it is incorporated into the New Guidance Structure. When this is completed, the finalised version will be uploaded onto the website of ECHA. No consultation will be made to do this

General principles and practical considerations for testing the efficacy of preservatives

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1. Scope

The aim of this guidance is to help with the practical aspects of designing and performing laboratory trials for testing the efficacy of preservatives. Other applications of biocides than preservation are only mentioned to point out the differences between the major groups. The product types are not discussed separately. It is the intention of this guidance to make the general testing principles understood so that they can then be applied in all types of preservative testing.

2. Introduction

Biocides are needed to solve problems in a large number of different environments. Some products are used to protect humans or animals, in other cases a closed industrial process needs to be protected. It is essential to define the problem for which the biocide in question is supposed to provide a solution. Aim and intention of using a preservative need to be stated. The claim of the product shall reflect this.

Biocides against micro-organisms can act in different ways and the purpose of using a biocide should be clear:

2.1 Preservation

The aim of preservation is to prevent microbial spoilage, decay or conglomeration of biomass which is detrimental to the functionality of an item or material. Detrimental effects can be caused by proliferation of cells or by metabolic activity of cells not involving cell division. It is not the intention of preservatives to transfer their effects to other materials or the environment, but to protect the material itself. A long term effect is required. A preservative can have a reversible action on microorganisms (e.g. by stress or cell damage without total loss of viability). Other than in disinfection there is no specification of the level of reduction/remaining population that can be defined to prevent spoilage.

A biocide against fungal decay of wood is one example for a preservative. While the wood and its function (e.g. carrying load) are preserved against wood decaying fungi other microorganisms (e.g. algae, bacteria) can settle on its surface.

2.2 Liquid Disinfection

The aim of disinfection is the reduction of the number of micro-organisms in or on an inanimate matrix- achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose. The "irreversible action" and the reduction to an "appropriate level" are specific to disinfection. The action of a disinfectant, to meet the reduction criterion, is usually fast, between a few seconds and a few hours for the most extreme cases. The purpose of their use is to kill a certain fraction of living cells in a short time no matter whether they spoil or decay the material they are applied on. Also here, the intention is not to transfer their effects to other materials or the environment. E.g. a surface once disinfected can become contaminated again immediately afterwards with a bacterial population. A liquid disinfectant is applied to a surface or to a liquid, but not incorporated into a material during its production. The goal of a disinfectant is not to protect the material, but to protect people, animals and food in contact with the material from transfer of microorganisms.

2.3 Reduction of microorganisms via a treated article

Such applications are applied for under both main groups, as disinfectants and as preservatives. An example for preservatives is application on textiles (PT 7 or 9) to prevent deterioration of the material. But also applications as disinfectants, e.g. PT 1 or 2 are common. The commonality is that biocides are incorporated in or applied on a solid material. This shall either prevent the deterioration of the material itself by micro-organisms or prevent the transfer of micro-organisms to animals, food or humans. In the latter case, the target organisms have not necessarily an effect on the treated item. Unlike in liquid disinfection the kill rate can be much lower, but the treated article shall have a long lasting effect. An example would be a biocide treated kitchen work-top claiming hygienic effects. In case of treated articles it is particularly important to define the claim carefully and to set up an appropriate test scheme to prove that claim.

2.4 Borderline cases

2.4.1 Algicides To Prevent Surface Growth

If algae are expected not to destroy the material as such, but to cause unwanted visual and hygienic effects, algicides are not considered to be acting as preservatives in the sense of the current BPD, but as general purpose biocides within product-type 2.

In the Biocidal Product Regulation, products used against algae for remedial treatment of construction materials belong to product type 2 "Disinfectants and algaecides not intended for direct application to humans or animals" whereas products with protective function are considered as products belonging to the product-types, like 7, 9 or 10.

If biocides are used against algae damaging materials and affecting their function they belong to the preservatives. For example, surface coatings for external use are often formulated with both a fungicide and an algaecide. The purpose of a surface coating is to both protect and enhance the visual appearance of a surface. The growth of both fungi and algae can deteriorate the surface (algae can etch surfaces and penetrate films – e.g. *Trentopohlia* spp.) and will affect the aesthetic appearance of the film (indeed this is the major objective of film biocides) and result in premature re-decoration. The algaecide, like the fungicide, is performing a preservative function in the coating and is thus covered by PT7. Similarly, algaecides are incorporated into plastics (e.g. electricity pylon insulation sleeves - to prevent growth that would otherwise cause arcing and system failure) and material used in aquatic and marine environments (including some cementitious materials). Algae are a problem in many water-based cooling systems and water-based process systems (e.g. paper making).

2.4.2 Treated articles with external effect: changing from materials preservation to hygienic effects

Biocidal products contained in treated articles with external effect are not preservatives in the sense of the BPD. They belong to the main section 1 "Disinfectants and general biocidal products". Their effect can be both aimed at preservation and hygienic effects. Label claims and intended use determine which Product Type of the BPD is relevant.

3. Tiered approach to testing preservatives

To structure the complex issues of product claims regarding efficacy of a biocide, a tiered approach (see Ref. 1) is, in general, accepted by the involved authorities. In the Biocidal Product Directive this is reflected by distinguishing between the evaluation of a biocidal

active substance and a biocidal product. A biocidal active substance is often required to show efficacy in a model matrix of the product type it claims to protect (e.g. synthetic coatings) against the type of organisms it claims to be active against. Its performance regarding leachability, UV-stability etc in this model matrix is not being questioned at this stage. These questions will arise when a biocidal product is to be addressed where weathering and/or other environmental conditions that occur during the use of a product can be of importance.

This tiered approach can be summarised very briefly:

- A) Tier 1 Proof of principle: Does the biocidal active substance work in principle in its appropriate model matrix?
- B) Tier 2 Product Authorisation: Does the biocide show efficacy under the real life conditions and for how long?

When moving up from tier 1 to tier 2 a test design has to be more tailored to the field of application envisaged. While in tier 1 some existing standards are basically suitable when the biocide is tested in a relevant matrix with defined organisms and under reproducible conditions (which are normally only to be found in a laboratory) testing for tier 2 is more complex and specific standards often do not exist. There may be a need for weathering cycles, wind tunnel tests, cleaning regimes etc. to evaluate whether the biocide maintains its efficacy when exposed to the environmental stresses it is likely to face in service. Similarly, soiling and the influence of other microorganisms can be of more significance. When aging is performed in the field or under in use conditions the reproducibility can become a difficult issue, as the aging factors such as e.g. evaporation and soiling are difficult to reproduce and can influence the results. This is particularly true for preservatives, and for disinfectants in treated articles, as liquid disinfectants are intended for short-term effects.

4. Preservation- efficacy testing

4.1 How to test the efficacy of active ingredients in the laboratory (Tier 1)

The aim of any preservation is to preserve the present stage of a material or matrix and with it its functionality. To show the efficacy of a biocide used as a preservative it is essential to show that the material or its function can be changed by microbial growth. This can be done in several ways, if for instance organisms can grow in a matrix it has to be assumed that they metabolised certain components of the matrix. To determine microbial activity in a biocide-free material the method of measuring colony forming units is the most common approach to prove that a preservative is needed. Other parameters indicating metabolism can also be documented like e.g. changes in pH, in viscosity, in colour. Data needs to be recorded from the beginning of the test (incubation time 0) and before and after each new inoculation. Not inoculated samples with and without biocide need to be prepared as aging-control samples to monitor the changes of the chemical components over the duration of the test.

Often a fungicidal or bactericidal claim needs to be supported. For this purpose a species can be tested singly or, as it is good practice in many test protocols, in mixed solutions of either bacterial species or fungal species. Bacteria and fungi are not to be mixed in these solutions. However, filamentous fungi ("moulds") and non-filamentous fungi ("yeasts") can be mixed.

Many microorganisms are able to form dormant cells or spores to survive unfavourable environmental conditions. These resting cells do not proliferate and show no significant metabolic activity until they find a suitable environment. It is therefore possible that vital

and active cells, being exposed to an unfavourable environment e.g. a synthetic paint containing solvent or a preservative, are transformed into dormancy. Only when a sample of the material is taken out of this environment and is spread onto a nutrient medium, the cells start to grow and to build new colonies. This underlines that the appearance of colony forming units (CFU) on a nutrient media is not necessarily sufficient evidence that growth had been occurring in the matrix used in the test. Growth can only be determined by counting CFUs when the number of colonies increased during incubation, compared to the recovery rate immediately after inoculation. Any smaller numbers of CFU than the initially counted recovery rate document survival, but not necessarily growth.

A relevant study that **proves the need of a biocide** and its efficacy **as a preservative against bacteria** must have the following features:

- a. The test must be performed in all relevant model matrixes that the claim of efficacy is made for (e.g. dishwasher liquid, paints, glues etc).
- b. Control samples without the addition of a biocide must be included during the whole test. These control samples must be handled identically to the other samples, except that they have no biocide included.
- c. When the control samples and the biocide-containing samples have been contaminated with organisms the recovery rate should be recorded measuring CFU.
- d. When bacteria are the contaminating agent the control samples have to show growth (e.g. indicated by an increased number of CFU), during incubation which has to be documented. If no growth in the control samples can be seen, this could indicate that only the dormant stages of bacterial cells, without active metabolism, are in the matrix.
- e. Only, if growth cannot be proved by increase in CFU other factors like e.g. CO₂-emission, O₂ depletion, the change of pH, colour change or disintegration of matrix can be recorded to demonstrate the need of preservation of a matrix by the active ingredient or preservative.

A relevant study that **proves the need of a biocide** and its efficacy **as a preservative against filamentous fungi** is in many ways the same as for bacteria, but an attempt to count colony forming units of thread-like mycelia after incubation is bound to fail for several reasons:

- It is impossible to take an aliquot from the incubated test vessel since the mycelia tend to conglomerate into pellets of different sizes (often blocking the tip of a pipette).
- Different seized fragments of mycelia and so far dormant spores form colonies on a petri dish and there is no way of knowing what is what

However, CFU are sensible for looking at the recovery rate of spores from just inoculated liquids before spore germination in the matrix and for unicellular yeasts. At this stage no mycelia have formed in the liquid and no fragments can be counted as CFU and wrongly interpreted as growth. Therefore, after the control samples and the biocide-containing samples have been contaminated with spores the recovery rate can be recorded measuring colony forming units.

Ascomycetes and fungi imperfecti form thread-like hyphe and spores. Spores serve as dormant stages when environmental conditions are detrimental to growth. When growth conditions are favourable the spores germinate and form a mycelium and maybe other spores. In liquids the fungal growth tends to form pellets. These can be very small or up to several millimetres in diameter. Furthermore, it is possible that a visible biofilm accumulates at the sides of the test vessel, e.g. an Erlenmeyer flask. Both phenomena

are visible by the naked eye and clearly demonstrate that the fungus has grown. To quantify this growth the whole contents of the test vessel has to be filtered to determine the amount of growth as dry weight.

4.2 Practical aspects for testing of preservatives with bacteria and fungi

Showing growth is essential for proving metabolism of the microorganisms. It is then assumed, if not proven in every case, that changes have taken place induced by microbial growth that can be prevented by the use of a biocide acting as a preservative. Often, when growth could not be proved this is caused by an unnecessarily high inoculation rate. If, at the beginning of the test, a recovery rate of e.g. 10^4 CFU for bacteria is adjusted, a growth from 10^5 to 10^6 can often easily be shown during the test period. When the recovery rate is adjusted to e.g. 10^6 after the inoculation, growth is much harder to detect.

Threadlike mycelia of fungi and single cellular cells of yeasts are often used in the same solution for inoculation. As said before, after inoculation it is not possible to use CFU to describe the growth of thread-like mycelia – neither qualitatively nor quantitatively. For yeast cells on the other hand, a CFU counting-method can be used. When aliquots are taken from a matrix incubated with mycelia forming fungi and with unicellular yeasts and spread out onto a nutrient media, the yeasts can visually be separated from the randomly appearing mycelia, which often also appear a few days later than the yeast colonies. Growth of filamentous fungi can be stated by weighing the mycelia and by determining an increase in weight. A series of concentrations of the active substance or the biocidal product should be measured in order to investigate which concentration achieves which level of efficacy. It is likely that the application rate in practice varies depending on the in-use conditions of a biocidal product even though the matrix is identical, e.g. in a metal working fluid.

4.3 How to test the efficacy of biocidal products for preservation in the laboratory (Tier 2)

Testing at the level of tier 1 and tier 2 follows the same principles. The fundamental difference is that at the level of Tier 2, for product authorisation, additional questions have to be addressed. Depending on the label claim accelerated aging tests with e.g. UV, temperature changes, leaching and wind-channel-test, before microbiological testing have to be performed. It has to be considered which environmental conditions are relevant for simulating aging factors in realistic in-use conditions. These conditions cannot be defined in this guideline, because label claims for products are too variable. Several examples shall be given to demonstrate how varied claims and approaches for generating data to prove such claims can be. The applicant should justify why they used specific performance tests and how they mimic in-use conditions.

Example 1: In-Can-preservation for containers holding materials e.g. spray foams, paints, glues, silicone.

Many water-based products are susceptible to bacterial or fungal growth. The growth can be sustained by the matrix itself or by soiling of the matrix during the production process.

In a tier 2 test (biocidal product, efficacy under real-life conditions) more parameters have to be considered than in a tier 1 test (active ingredient, proof of principle) to test efficacy of the preservative. Most commonly aging procedures are being applied to help to establish e.g. a shelf life by considering parameters like frost, high temperatures and condensation.

Useful field tests are relatively rare in such systems, but can be of value in applications that are difficult to simulate in the laboratory.

Example 2: Fungicide to protect plastic benches e.g. in the changing room of a public swimming pool from mould growth

Plastic benches attract a multitude of soiling material during use that can act as nutrients for microorganisms: from body lotion, skin cells, to sticky food. This nutrient film can easily support a growing biofilm when water is present. Mould fungi, e.g. *Aspergillus* sp., might damage the material by emitting organic acids or spores might permanently discolour the surface by resting in small pores and fine cracks of the plastic. In order to prevent these effects a fungicide can be incorporated into the plastic.

A solid matrix is in some ways more of a challenge to protect by incorporating a biocide than a liquid matrix. The keyword here is “bioavailability”. The biocide has to get into contact with the microorganism to act. If the biocide molecule is “stuck” in the matrix it cannot act (unless the molecule is on the very surface of the plastic). To make the biocide available as a constant film on the surface it can either be applied as such or diffusion must occur from the inside of the material to the outside.

A tier 2 test might include aging of the material by bringing it into contact with acidic or alkaline substances or by leaching in water before the biological test is performed. If the plastic bench were outside, UV-treatments might be appropriate to test the stability of the biocide under this condition.

5. Conclusion

What do biocides need to do in synthetic materials? They have to solve a problem. The first question therefore always is: what is the problem?

- Can it be demonstrated that there is a problem?
- Can it be demonstrated that there is less or no problem when a biocide is used?

The use of a preservative has to be justified by sound data that also proves its efficacy.

6. References

1. Analysis and Assessment of Current Protocols to Develop Harmonised Test Methods And Relevant Performance Standards For The Efficacy Testing of Treated Articles / Treated Materials, OECD Environment Directorate Joint Meeting of The Chemicals Committee and the Working Party on Chemicals, Pesticides And Biotechnology Monograph; Document ENV/JM/MONO(2007)4 - JT03222464

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
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