Evaluation Manual for the Authorisation of plant protection products and biocides

EU part

Biocides

Chapter 7 Efficacy

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Board for the Authorisation of plant protection products and biocides

Chapter 7 Efficacy
Category: biocides

Main group 2 Preservatives
PT6 In-can preservatives

Contents

GENERAL INTRODUCTION

This chapter describes the data requirements for the assessment of the efficacy of a biocide and the active substance within PT 6, and which evaluation methodologies are applied for the EU framework. This chapter is derived from the TNsG on product evaluation (appendix to chapter 7 on Product Type 6 In-can preservatives).

In December 2012 a new chapter of the TNsG was endorsed describing requirements for the main group of preservatives (PT6 to PT13). See this chapter for more recent requirements. Where the chapter below is in contradiction with the general chapter the general chapter will prevail.

1.1.1. PRODUCT TYPE 6 – IN-CAN-PRESERVATIVES

1 INTRODUCTION

This technical annex provides background information regarding in-can (and in-tank) preservative use, and amplifies the nature and extent of data that should be available to support efficacy claims relating to these substances.

1.1 USE OF IN-CAN PRESERVATIVES

In-can preservatives are included in many manufactured products, including paints, adhesives and binders. They are used to control micro-organisms that may be present in the product and which may cause deterioration prior to use. They therefore help to ensure product integrity during normal shelf life. For example, food preservatives and cosmetics preservatives, which are used exclusively in these two product types are not included in Product Type 6.

1.2 THE NEED FOR IN-CAN PRESERVATIVES

In order to grow in a manufactured product, a micro-organism must have access to both moisture (water) and a nutrient source.

Water may be an integral part of a particular manufactured product. However it may be present in a product as a result of the moisture content of various components, and not added separately.

An extremely wide range of substances can act as a source of nutrition. These substances may be utilised by micro-organisms as they are, or following some form of conversion or degradation.

Utilisation of nutrition sources by micro-organisms results in the loss from the product of one or more components, leading to reduced integrity and spoilage. By-products of microbial growth also contribute to spoilage. Thus vulnerable products require an in-can preservative content for protection during the wet state, prior to use.

1.3 NATURE OF THE MICROBIAL PROBLEM

Bacteria are the major group of spoilage organisms, but other causes of problems are yeasts and moulds. The consequences of uncontrolled microbial growth in the wet state are varied, but include:

Discolouration (many bacterial cells are pigmented)
Gassing
Malodour

Loss in viscosity*
Ropiness (certain bacteria produce slime)
Phase separation

*Liquefaction of cellulosic thicken agents can be caused by enzymes which are produced by bacteria and fungi. Such enzymes (cellulases) are capable of exhibiting their bio-catalytical activity at concentrations as low as 10-5 units ml-1. Since enzymes cannot be inactivated by subsequently adding preservatives in the usual doses, it is recommended that preventative measures are taken at an early stage in production.

Such microbial damage is irreversible and therefore steps to control microbial growth/spoilage must be taken at the earliest opportunity.

Examples of some spoilage micro-organisms are presented in Table 1:

Table 1. Common spoilage micro-organisms associated with in-can products

Bacteria	Fungi	Yeast
Alcagenes species		Candida albicans
Micrococcus luteus	Aspergillus spp.	Rhodotorula rubra
Escherichia coli	Geotrichium candidium	Saccharomyces cerevisiae
Proteus vulgaris	Penicillium spp.	-

1.4 EFFICACY TESTING OF IN-CAN PRESERVATIVES

1.4.1 LABORATORY TEST METHODS

1.4.1.1 MIC determinations

MIC (Minimal Inhibitory Concentration) determinations are conducted in the laboratory on active substances. A dilution series of the active substance identifies the minimum amount of biocide that is required to inhibit microbial growth, under defined laboratory conditions.

In such tests, efficacy is assessed against a range of bacterial, fungal and yeast spoilage organisms. MIC values are usually presented as ppm required to inhibit the growth of a particular test organism; however only the bioavailable amount is effective. The values are useful for determining the spectrum of activity of an active substance.

1.4.1.2 Challenge testing

The usual method for evaluating in-can preservatives in paints or other aqueous products is the challenge test. Typically microbial cells are deliberately added to the test sample. The survival or death rate of these cells is monitored with respect to time. Under certain test protocols, the sample may be re-challenged several times.

1.4.1.3 Heat stability testing

An important property of an in-can preservative is heat stability. In this test the level of active substance is usually measured accurately by a suitable chromatographic method at time zero; the test is not always required. The product is then incubated at an elevated temperature for a defined period of time. The level of active substance is measured again after the incubation period in order to determine whether the biocide has degraded. The length of this test as well as the temperature may vary. The results are useful as an indication of the stability of the active substance in a particular product formulation.

1.5 AVAILABLE STANDARD TEST METHODS

There are a limited number of National standard test methods currently available which claim to assess the effectiveness of biocides for the 'in-can' protection of liquid coatings such as paints, adhesives and thickeners. However, they are either test methods limited to the determination of MIC in artificial matrices, or tests that do not give sufficient detail or guidance to carry out a properly controlled challenge test.

One example of a National standard is ASTM D 2574. This ASTM method utilises only one test bacterium; others use a mixture of fungi, or both bacterial and fungal cells.

An exhaustive list of currently available test standards for use in efficacy testing of In-can preservatives is presented in Appendix 1 of this document.

1.6 CURRENT EPA ASSESSMENT CRITERIA FOR IN-CAN PRESERVATIVES

The EPA requires that active substances proposed for use in preserving water-based products should show effectiveness in controlling spoilage or deterioration caused by bacteria in at least two representative formulations in which the biocide is intended for use.

Tests should be carried out in at least three replicates of each of the two product formulations using pertinent micro-organisms and adequate controls. Actual bacterial isolates (identified at least to Genus) from spoiled product and/or ATCC (American Type Culture Collection) spoilage bacteria should be employed as test inocula. Mixed bacterial and fungal inocula are not acceptable in demonstrating bacterial deterioration.

Efficacy data should be derived from simulated-use type tests with quantitative bacteriological sampling and concurrent observations of product quality. Both test and control samples should be tested for a period of 6 months to 1 year. The test protocol, including such elements as frequency of repeated bacterial challenge, must be appropriate to the intended active substance use pattern.

1.6.1 SUGGESTED EPA PERFORMANCE STANDARD

The data should show control of bacterial growth and control of bacterial-caused deteriorative (physical and chemical) changes in the treated products during the test period. The data from control products should show not only survival of test bacteria but also significant growth and resultant deteriorative (physical and chemical) changes.

APPENDIX 1

CURRENTLY AVAILABLE STANDARD TESTS FOR IN-CAN PRESERVATIVES

STANDARD	DATE	TITLE
AFNOR NF X 41-520	03/68	Test method for the resistance of paints to microorganisms and their protective capabilities.
DIN 58 940 Part 5	08/79	Method for the determination of susceptibility of pathogenic bacteria to chemotherapeutic agents; determination of MIC by broth dilution method.
SABS 1102	1987	Bacterial efficacy of biocides used in water-based emulsion paints.
ASTM D 2571-86 or ASTM D 2574-93	1986	Test method for the resistance of emulsion paints in the container to attack by microorganisms.
ASTM D 4783-89	1989	Test methods for resistance of adhesives preparations in container by bacteria, yeast and fungi.b

6. References

1 Biocidal directive (BPD) (98/8/EC)

2 Technical Notes for Guidance: TNsG on Product Evaluation; Common principles and practical procedures for the authorisation and registration of products. Available at: http://ecb.jrc.it/biocides/