GUIDANCE

Guidance on the Biocidal Products Regulation

Volume II Efficacy Assessment and Evaluation (Parts B & C)

DRAFT Version 1.0
August 2016

LEGAL NOTICE

This document aims to assist users in complying with their obligations under the Biocidal Products Regulation (BPR). However, 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. Usage of the information remains under the sole responsibility of the user. The European Chemicals Agency does not accept any liability with regard to the use that may be made of the information contained in this document.

Guidance on the BPR: Volume II Efficacy - Parts B & C Assessment and Evaluation

Reference:	xxxxxxxx
Cat. Number:	xxxxxxxx
ISBN:	xxxxxxxx
DoI:	xxxxxxxx
Publ.date:	xxxx 201x
Language:	EN

© European Chemicals Agency, 201x

If you have questions or comments in relation to this document please send them (indicating the document reference, issue date, chapter and/or page of the document which your comment refers) using the Guidance feedback form. The feedback form can be accessed via the ECHA website or directly via the following link: https://comments.echa.europa.eu/comments_cms/FeedbackGuidance.aspx

European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland
Visiting address: Annankatu 18, Helsinki, Finland

DOCUMENT HISTORY

Version	Comment	Date
Version 1.0	First edition	xxxx 2017

PREFACE

This document describes the BPR obligations and how to fulfil them.

NOTE FOR PEG CONSULTATION

The text for the Preface is under development and will be added during the PEG consultation and be available for the PEG meeting.

This will include an explanation of adding Parts B and C together and will also include explantion of sections that are under development as well as those sections that still refer to the "old" TNsG.

Table of Contents

LEGAL NOTICE 2
DOCUMENT HISTORY 3
PREFACE 4
LIST OF ABBREVIATIONS 8
1. GENERAL INTRODUCTION 9
2. LABEL CLAIMS 9
2.1 INTRODUCTION 9
2.2 LABEL CLAIMS AND DIRECTION FOR USE 10
3. GENERAL CONSIDERATIONS FOR THE DEVELOPMENT AND REPORTING OF EFFICACY DATA 10
3.1 EFFICACY 10
3.1.1 Efficacy tests 10
3.1.2 Test report 11
4. ACTIVE SUBSTANCE APPROVAL 12
4.1 INTRODUCTION 12
4.2 GENERAL PRINCIPLES 13
4.2.1 Intended use 13
4.2.2 Evaluation of efficacy 13
4.2.2.1 Active substance efficacy (part A): 13
4.2.2.2 Product efficacy (part B): 14
4.2.3 Overall evaluation for active substance approval 15
4.2.4 Link to risk assessment 15
4.3 ACTIVE SUBSTANCES WHICH ARE NOT INTENDED TO BE USED IN ISOLATION 15
4.4 "DUMMY PRODUCTS" 16
4.5 TREATED ARTICLES 17
4.5.1 Definition 17
4.5.2 Product authorisation or active substance approval? 17
4.5.3 The PTs significance for assessing efficacy on active substance level 18
5. PRODUCT AUTHORISATION 18
5.1 GENERAL INTRODUCTION 18
5.1.1 Evaluation of efficacy at product authorisation stage 18
5.2 PRODUCT FAMILIES 19
5.2.1 Background 19
5.2.2 Worst case testing 20
5.2.3 Take formulation types and chemical composition into account 22
5.2.4 Allowing for the addition of new products 23
5.2.5 Deviation in *meta* SPC's 24
5.2.6 Minimum concentration needed 25
5.3 TREATED ARTICLES 26
5.3.1 The basic distinction between material protection and protection of humans or animals 26
5.4 DISINFECTANTS 28
5.4.1 General 28
5.4.2 Materials and Articles Treated to Protect Humans or Animals 28
5.4.2.1 Determining the purpose of the Treatment 28
5.4.2.2 Effects Intended to Inhibit Microbial Growth 29

5.4.2.3 Effects intended to Kill Microorganisms through Contact 30
5.4.2.4 Norms and Criteria 30
5.4.3 PT1 35
5.4.4 PT2 35
5.4.5 PT3 35
5.4.6 PT4 35
5.4.7 PT5 35
5.5 PRESERVATIVES (MAIN GROUP 2) 36
5.5.1 Distinction between preservation/curative treatment and disinfection 36
5.5.1.1 Curative uses 37
5.5.1.2 Borderline case: Algaecides 37
5.5.1.3 Borderline cases: Treated articles 38
5.5.2 Principles for testing preservatives 38
5.5.3 Tiered approach to testing preservatives 39
5.5.4 Standard Test Methods 39
5.5.4.1 Practical aspects for testing bacteria 40
5.5.4.2 Practical aspects for testing fungi 40
5.5.5 Wet-state preservation (PT 6, 11, 12, 13) 41
5.5.5.1 PT 6 41
5.5.5.2 PT 13 43
5.5.6 Curative Treatments (PT 6, 7, 11, 12, 13) 43
5.5.7 Protection of solid material: PT 7, 8, 9, 10 43
5.5.7.1 Model matrices 44
5.5.7.2 Representative species 44
5.5.7.3 PT 7 and PT 9 Material protection 45
5.5.7.3.1 Simulation Tests 45
5.5.7.3.2 Tests based on artificial growth media 47
5.5.7.3.3 Tier 2 Testing 48
5.5.7.3.4 Tier 3 Testing 49
5.5.7.4 PT 9: Prevention of Odour by odour-producing microorganisms 49
5.5.7.4.1 Agar plate-based tests 49
5.5.7.4.2 Suspension tests 50
5.5.7.4.3 Tier 2 testing 51
5.5.7.4.4 Tier 3 testing 52
5.5.8 PT6 54
5.5.9 PT7 54
5.5.10 PT8 54
5.5.11 PT9 54
5.5.12 PT10 54
5.5.13 PT11 54
5.5.14 PT12 54
5.5.15 PT13 54
5.6 PEST CONTROL 54
5.6.1 General 54
5.6.2 PT14 54
5.6.3 PTs 15, 16 & 17 54
5.6.4 PT18 + PT 19 on arthropods 55
5.6.5 PT19 other non-arthropods 55
5.6.6 PT20 55
5.7 OTHER BIOCIDAL PRODUCTS 55
5.7.1 PT21 55
5.7.2 PT22 55
APPENDIX 1. CLAIMS MATRICES 56

APPENDIX 2. STANDARDS AND TESTING METHODS FOR EFFICACY-TESTING OF DISINFECTANT BIOCIDAL PRODUCTS (PT 1-5) 56
APPENDIX 3. TABLE OF REFERENCE TEST ORGANISMS 56
APPENDIX 4. OVERVIEW OF STANDARDS, TEST CONDITIONS AND PASS CRITERIA ...56
APPENDIX 5. EXAMPLES OF VIRUSES SORTED ACCORDING TO THEIR PRESENCE IN THE HUMAN BODY IN CASE OF VIRUS INFECTION 56
APPENDIX 6. METHODS TO MEASURE THE EFFECTS OF PRESERVATIVE/CURATIVE ACTION IN LIQUID MATRICES 57
APPENDIX 7. METHODS TO MEASURE THE EFFECTS OF PROTECTING MATERIAL....59
APPENDIX 8. METHODS TO MEASURE ANTIMICROBIAL ACTIVITY....74

Figures

Figure 1: Decision scheme to distinguish between claims for material protection and claims for protection of humans and animals 27
Figure 2: A Test for Antibacterial Activity in Wet Conditions 29
Figure 3: Simulated Splash Model Non-Porous Materials....31
Figure 4: Simulated Splash Model Porous Materials 32
Figure 5: Printing Model 33
Figure 6: Decision scheme for the distinction between preservation and disinfection...37
Figure 7: Example of a Simulated Growth Test....46
Figure 8: An Example of an Agar Plate Based Test....48
Figure 9: OECD/IBRG Tier 1 Textile Test 51

Tables

Table 1: Example ready-to-use disinfectants with pre-cleaning*. 20
Table 2: Example concentrated disinfectants 21
Table 3: Example surface disinfectants ready-to-use: more PT's 22
Table 4: Example insecticide: take target organisms and application method into account....23
Table 5: Example disinfectant: take formulation into account....24
Table 6: Example anti-fouling product: Different ratio's of two (or more) active substances. 25
Table 7: Protection of Humans or Animals – Example Claims, Problems and Testing Approaches 34
Table 8: Basic Requirements for a Valid Test Protection of Humans or Animals 35
Table 9: Examples....44
Table 10: Basic Requirements for a Valid Test Protection....49
Table 11: Odour: Example Claims, Problems and Testing Approaches 52

NOTES to the reader:

Sections xxxx are under development and will be added at a future update.

In this document text cited from the Biocidal Products Regulation (EU) No 528/2012 is indicated in green boxes.

This symbol highlights text to be noted.

NOTE FOR PEG CONSULTATION

The List of Abbreviations and the Glossary of Terms will be elaborated during the PEG consultation and will be available for the PEG meeting.

List of Abbreviations

Standard term / Abbreviation	Explanation

Glossary of Terms

Standard term / Abbreviation	Explanation

1. General Introduction

Efficacy data are a fundamental component in the regulatory management and decision making process for biocidal products. Efficacy data are required to establish the benefit arising from the use of biocidal products and must be balanced against the risks their use poses to man and the environment.

Authorisation of a biocidal product will only be granted according to Art. 19 (1) b of the BPR if that product is shown to be sufficiently effective.

Even for the requirement to limit the use to the minimum necessary and the general requirement of sustainable use of biocidal products (Art. 17 and 18 BPR), it is crucial that the biocide in questions delivers the expected effect.

The information and data required relevant to the effectiveness of the active substance(s) to be employed in biocidal products are outlined in Annex II, BPR, title 1 No. 6 and 7 and title 2 No 5 and 6. For biocidal products the data required are set out in Annex III, Title 1 No 6 and 7, and title 2, No 6 and 7.

2. Label claims

2.1 Introduction

The evaluation of the efficacy of a biocidal product is carried out in order to determine whether the claims made for the activity of the active substance or the product are supported by suitable efficacy data

Claims should comprise of the description of the problem and the way it is suggested to be solved by the biocidal treatment. Claims include information given in an active substance dossier, information on the label of a product, information provided on a web-site or in product-associated leaflets. All claims should be consistent.

Claims can range from simple to complex, depending on the activity and benefits the applicant wishes to claim as resulting from the use of the active substance/biocidal product.

This includes as a minimum:

- The purpose of the claim (e.g. prevent destruction of material by insect infestations, decrease risk of infection by bacterial contamination)
- The function of the product (e.g. insecticide, wood preservative, disinfectant, etc.)
- The (group of) target organisms which will be controlled
- In-use concentration
- Use conditions and area of use
- The effect which will result from using the product on the target organisms (e.g. kill, control, repel, prevent, etc.)
- Any products, organisms or objects to be protected

Some examples are available in the different claim matrices and PT specific guidances.

However this basic information can be supplemented by additional claims which further describe the effects of the active substance/product where appropriate, such as:

- How fast the effect is produced
- The duration of the effect (residuality) or lifespan

- The types of surface on which the product can be used (e.g. hard porous and non-porous surfaces, soft furnishings)
- etc.

For products used to treat articles, additional information should be provided:

- Durability of the effect in relation to the expected life-span of the treated article
- Resilience towards ageing, weathering or other use conditions as for instance washing
- Where relevant, leaching/migration data for different materials or different use conditions

All claims made should be supported by data or a suitably robust scientifically based reasoned case.

2.2 Label claims and direction for use

A label claim is information which is provided to the user which describes the biocidal effects that will result from using a product under its normal conditions of use (e.g. when it is used at the recommended dose/application rate, by the recommended application method(s) and in the appropriate areas, etc.).

Label claims should be as specific as possible, or if more general claims (such as "fast acting") are made, then they should be further clarified on the label where possible (e.g. "fast acting – acts within 5 minutes"). If no clarification is provided, the evaluating Competent Authority should ask the applicant to specify the claim. A judgement as to what a normal user would reasonably expect from the claim should be made. Evaluation should be made according to this claim and the directions for use should be taken into account.

An application for product authorisation must include a copy of the draft product label containing the claims made for the product.

Applications for product families should include the entire range of the claims proposed for the products within the family.

3. General considerations for the development and reporting of efficacy data

3.1 Efficacy

Efficacy is defined as the ability of a product to fulfil the label claims for it on the proposed product label: Is the product actually sufficiently effective against the claimed organisms under the conditions specified? The applicant must provide sufficient information to clearly specify the field of use of the product. In addition, studies must be provided to demonstrate that the product, when used in accordance with the use instructions (concentration, application method, etc.), is sufficiently effective.

3.1.1 Efficacy tests

The applicant must submit studies which clearly demonstrate the efficacy of the active substance/product.

We distinguish various types of studies:

- Screening tests
- Laboratory tests
- Simulation tests in laboratory

- Field tests

Screening tests are usually not related to practical/field conditions and are often not implemented with the complete product but only with the active substance. Such tests are therefore primarily useful for providing supplementary information, for example to demonstrate that the concentration used is optimal.

Laboratory studies are performed to validate the efficacy in lab according to criteria defined. These tests permit to validate for example a level of mortality during a given time, a KD effect and if need be the palatability of the product.

Simulation tests are more linked to practical/field conditions and can, in some cases, be sufficient for demonstrating the efficacy. Simulation tests can include factors like ageing, weathering, UV, washing, etc.

Example: For disinfecting products aimed at controlling bacteria on hard surfaces, it is sufficient to carry out a suspension test and a surface test in accordance with the relevant EN standards.

Screening tests, laboratory tests and simulation tests must always include an untreated control test without active substance (i.e. a negative control). This can be, for instance, a control test with only the solvent, e.g. water. There are few exceptions to this rule (e.g. EN disinfection test), this should be justified by the methodology.

In particular cases where the action of co-formulants needs to be determined (e.g. in PT18 or PT19 products where essential oils/terpenes of potential insecticidal or repellent activity are used as co-formulants for their fragrance), the product should be tested without the active substance (i.e. negative control).

Field tests provide a good indication of how the product works in practice/under field conditions, to evaluate how the efficacy can be affected by a variety of factors (the weather, population density, natural fluctuation of the population over time etc.). The experimental setup is important in these tests. The results of the tests should be compared to the results achieved with a control object which has not been treated or with the situation prior to treatment.

In all types of tests, a relevant reference product (authorized, commercially available) should preferably be used at label rates as positive control to assess the level of efficacy of the product.

Tests should preferably be carried out in accordance with standard protocols, e.g. ISO, OECD, CEN, , ASTM, etc. If standard protocols are not available or are not suitable for the field of use concerned, other methods may also be used on condition that the studies concerned have a sound scientific basis. Preferably, available standard methods should be modified to meet the actual application in such cases. The tests may be carried out in accordance with Good Laboratory Practice (GLP) or similar quality assurance systems (ISO), although this is not mandatory for efficacy tests.

3.1.2 Test report

Some standard tests (e.g. EN tests) contain examples of appropriate reports, which should be used as a template. In all other cases the test report must contain the following elements:

- introduction
- materials and methods
- results and raw data
- conclusion/discussion based on criteria defined in guidance

The introduction must indicate the goal of the test. When a standard test is used the name and/or number of the test should be stated. The section on materials and methods must provide a complete description of the test method. If an internationally recognised standard method is used, it is sufficient to provide a brief description of the test. The product used and the concentration of the active substance must be specified. If the name of the product tested is not the same as the product for which the application is being submitted (e.g. a name used outside the EU or an internal company code for the product), the complete composition of the product tested must be provided in a separate document. The test organisms used must correspond to the organisms against which the product is intended to be used, or they must be adequate representatives. For example, if a product is intended for use against bacteria in hospitals, it is not possible to test the product on all possible species of bacteria. Instead, four standard species of bacteria are usually tested. The conditions under which the negative control tests were carried out must also be described (e.g. treated with product not containing the active substance, not treated, or treated with water for example).

The materials and methods should be described well. In case of standards test protocols all the deviations should be indicated and justified.

The section on the results of the test must provide quantitative data. It is not sufficient to present only tables or figures in which the results have been processed. The raw data must also be included. In case of repetitions performed in the test, the results should also be subjected to a statistical analysis. At the end of the report, a conclusion must be presented. Sometimes, it is necessary to discuss and/or present further arguments for the conclusion. For field tests in particular, the results obtained in repeated tests may differ. If an explanation is provided for such differences in results, a test may possibly still be approved.

Example: In test 1, the product was "washed away by rainfall" and was therefore not effective, but tests 2 and 3 do demonstrate the efficacy. In such case the tests can be accepted and a remark will be made on the SPC that the product should not be used when rain is expected within x hours, because this will influence the efficacy negatively.

4. Active substance approval

4.1 Introduction

According to Article 4 of the BPR, an active substance shall be approved if at least one biocidal product containing that active substance may be expected to meet the criteria laid down in point (b) of article 19(1), and more particularly for the context of this guidance the paragraph (i), which says "the biocidal product is sufficiently effective".

During the review of an active substance at the active substance approval stage, both the efficacy of the active substance and a representative biocidal product are assessed. At this approval stage, it is the activity of the active substance which must be demonstrated, both in its own right and when formulated into a biocidal product.

Although a biocidal product containing the active substance is evaluated at the active substance approval stage, this part of the BPR process is concerned primarily with the efficacy of the active substance itself. The purpose of this section is to provide guidance for applicants and competent authorities on the principles for evaluation of efficacy at the active substance approval stage, and to help determine whether the information provided in an application for approval of an active substance is sufficient for inclusion of the substance in the Union list. For guidance on data requirement see Volume II Part A of ECHA's guidance under the BPR.

4.2 General principles

4.2.1 Intended use

When making an application for approval of an active substance, the applicant must clearly describe the uses for which the active substance is intended when it is incorporated into biocidal products and treated articles.

This information is required to allow a proper evaluation of the efficacy to be carried out, and must include, for every product type separately:

- The purpose of the claim (e.g. prevent destruction of material by insect infestations, decrease risk of infection by bacterial contamination)
- The function of the active substance (e.g. bactericide, fungicide, rodenticide, insecticide)
- The (group of) target organism(s) to be controlled
- The effects on representative target organism(s) (e.g. attracting, killing, inhibiting)
- Any products, organisms or objects to be protected
- The likely concentration at which the active substance will be used in products and, where appropriate, in treated articles. This likely concentration should be demonstrated according to the requirements described in section 4.2.2.1.

In the application, the applicant may choose to provide information on all of the intended target organisms at the active substance approval stage, or on only a few.

However, in order for approval of the active substance to be granted, efficacy must be demonstrated for at least one main target organism (or group of target organisms (e.g. bacteria)). Use against additional target organisms may be applied for at the product authorisation stage.

For use in treated articles, which can be very varied, efficacy for a category of articles with similar use conditions has to be demonstrated. For instance, for active substances which can be used in different polymer articles, efficacy needs to be demonstrated for a set of use conditions, like concentration in the polymer, presence of liquids, ageing or weathering, if relevant. The efficacy demonstrated cannot be extrapolated to other use conditions, but efficacy must be shown for every set of use conditions independently. For example, if a certain effect is demonstrated for a polymer which is in constant contact with water, this cannot be transferred to a polymer which is used under humid or dry conditions.

4.2.2 Evaluation of efficacy

Efficacy of an active substance has to be demonstrated both in part A of the CAR (related to the intrinsic efficacy of the active substance) and in part B (where the active substance is incorporated in a formulated product). Evaluation of each part is described below.

4.2.2.1 Active substance efficacy (part A):

As the testing of an active substance is normally carried out using the technical active substance, or a simple dilution of the active substance in water or an appropriate solvent (so that the testing is carried out in the absence of other substances which may affect the efficacy), an extensive data package and evaluation is not required at this stage.

However, efficacy studies should be submitted on the active substance, and these data should be capable of demonstrating the innate activity of the active substance against representatives of the proposed target organisms at the concentration relevant for the

risk assessment. For that purpose, innate activity of an active substance could be defined as the capacity of an active substance to provide a sufficient effect on one or several relevant target organisms, for each activity, for the use considered.

The following minimum requirements should be fulfilled to demonstrate innate activity:

- For main group 1 (disinfectants: PT1, 2, 3, 4 and 5), innate activity is at least a "cidal" activity demonstrated in a suspension test and has to be demonstrated against one or more representative target organism(s) for the activity claimed (e.g. bactericide, yeasticide), preferably according to the CEN norms (phase 1 tests and phase 2 step 1 tests). Test organism(s) should be that or those specified in the respective norm. Phase 1 tests are sufficient for the active substance if a phase 2 step 1 test is available for a product.
- For main group 2 (preservatives: PT6, 7, 8, 9, 10, 11 and 12), innate activity is generally a static activity demonstrated in challenge tests on several and relevant target organisms, in the relevant matrix. To demonstrate efficacy against one target organism only could also be acceptable in the case of a strictly defined claimed use relevant for the PT (for instance the control of Legionella in cooling water in PT11). For PT8, CEN norms are available to support efficacy testing and give indications on representative target organisms to be tested.
- For main group 3 (pest control: PT14, 15, 16, 17, 18, 19 and 20), innate activity can be demonstrated for one target organism only (for instance, control of mice or control of bedbugs).
- For main group 4 (other biocidal products: PT21 and PT22), innate activity is generally supported on a taxonomic group of organisms (algae, animals, bacteria) and examples of appropriate target organisms are available in the Efficacy guidance for PT21 and PT22.

When minimum requirements are not met this should be justified.

Generally, efficacy data are generated from laboratory tests, performed by the applicant. Nevertheless efficacy data from literature could also be acceptable if the application rate, target organisms and area of use are relevant. This should be agreed between the applicant and the eCA on a case by case basis.

The level of efficacy demonstrated at this stage of the process need not be high, as an active substance in a simple solution may not be as effective as when it is used in a fully formulated product. For that reason an active substance should still be considered suitable for approval if the levels of efficacy demonstrated fulfill the minimum requirements above. In the case where the levels of efficacy of the active substance alone are lower than expected, efficacy tests performed with the representative product has to show a sufficient/basic efficacy, according to the requirements above. If both are insufficient, approval for the Union list should not be proposed.

If no efficacy tests with the active substance itself (in water) are available, but only tests with a formulation, a justification has to be given by the applicant regarding the possible influence of co-formulants on the efficacy. If the co-formulants used potentially have biocidal activity, it is essential to demonstrate that the efficacy is due to the active substance and not to the co-formulants, e.g. a control should be performed with all co-formulants but without the active substance.

4.2.2.2 Product efficacy (part B):

Although approval for the Union list is primarily concerned with the active substance, efficacy data is also required for a product. Ideally efficacy data on an existing biocidal product should be submitted. If this is not possible data on a dummy product could be acceptable in order to demonstrate that the active substance is capable of producing an

effect on the target organism and in a relevant matrix according to the proposed use, when included in a formulated product.

However, a detailed evaluation of the effectiveness of the product (including an evaluation of the proposed label claims) is not in all cases required at the active substance approval stage. This may for example be the case where no marketed product is available.

Nevertheless, the level of efficacy (e.g. the kind of activity "biocidal" or "biostatic") have to be consistent with the uses claimed and fulfill the minimum requirements mentioned in the active substance part (part A).

Where the levels of efficacy demonstrated are low enough to raise concerns by the evaluating Member State, the applicant should be asked to justify why the result should still be considered acceptable. Two specific reasons are discussed below: the use of 'dummy products' and the case of active substances not used alone but always in combination with other active substances.

4.2.3 Overall evaluation for active substance approval

It is concluded that efficacy data are required on the active substance, to demonstrate on the one hand the innate activity of the substance (either the technical grade active substance or a dilution in water or a solvent) and on the other hand the efficacy of the representative product against one or more of the proposed target organisms. Efficacy should be demonstrated in accordance with the use(s) considered in the risk assessment. If for some justified reasons, the results of the biocidal product do not completely fulfil the requirements described above, this could still be acceptable as long as the results of the active substance are sufficient to demonstrate efficacy. The other way around, if the results of the active substance do not fulfil the requirements described above acceptable data of the biocidal product may be sufficient as long as it can be excluded that the co-formulants contribute to the efficacy of the product.

Where the levels of efficacy demonstrated are low enough to raise concerns by the evaluating Member State, the applicant should be asked to justify why the result should still be considered acceptable. Two specific reasons are discussed below: the use of 'dummy products' and the case

4.2.4 Link to risk assessment

There is an essential link between efficacy testing and the risk assessment for human health and the environment at the active substance approval stage:

- Efficacy has to be proven for active substance concentrations used in the risk assessment
- Efficacy has to be sufficient for the use assessed in the risk assessment, e.g. biostatic or biocidal activities related to the PTs.

The information on efficacy is relevant in assessing the dose recommended for the use(s) applied for. The dose (or the "likely concentration(s) at which the active substance will be used" as stated in Annex II 6.4 of the BPR) is the starting point in the exposure assessment for human health and the environment.

4.3 Active substances which are not intended to be used in isolation

This section is developed to deal with active substances which are not intended to be used as the sole active substance in a product.

At the active substance approval stage, the following should be demonstrated:

- **in part A** (dedicated to the active substance), the innate activity of the active

substance should be demonstrated against target organism(s) relevant for the field of use envisaged.

The evaluation should demonstrate that the active substance is capable of producing an effect on its own or when formulated into a very simple product. Due to the absence of the other active substance(s), the formulation may have only a limited, rather than broad based, spectrum of activity, or a lower level of efficacy.

Evaluation of the data will be done on a case by case basis.

Some examples where limited efficacy could be acceptable:

- for wood preservatives with fungicidal activity where different fungicides are active against different groups of target fungi and therefore two or more fungicides would be included in a product to produce the full spectrum of antifungal activity;
- for insecticides that are used in combination with other active substances to improve the insecticidal performance of the latter as they exert a synergistic effect;
- for insecticides used in combination with a synergist that is not itself an active substance.
- combined *in situ* production of the active substances

However, an appropriate argumentation is always required in order to justify situations with a more restricted level of efficacy. The minimum requirements in section 4.2 have always to be fulfilled.

- **in part B** (dedicated to the accompanying/representative product), the efficacy of a product where the active substance is formulated in combination with other (active) substances should be demonstrated against target organism(s) relevant for the field of use envisaged. Relevant efficacy tests should be used and structured to allow evaluation of the contribution of the active substance to the overall efficacy. This is particularly important if efficacy data have not been submitted in Part A.

Efficacy data packages for formulations containing two or more active substances are not fully suitable for determining the activity contribution from the active substance under evaluation. For that reason great attention should be paid to justify the contribution of the active substance under evaluation to the total efficacy of the product. Information about the mode of action/function of the other active substances present in the product is also requested.

The submitted data should allow the definition of an effective concentration (i.e. the concentration of active substance at the efficient application rate of the product) that can be used for the risk assessment. If in part B a formulation is introduced with additional co-active substances, this formulation will only be considered for efficacy testing and for setting a likely in-use concentration of the active substance, which will not be used in isolation.

A statement should be added in the BPC opinion in order to stress that the active substance is intended to be used in combination with other active substances or synergists.

4.4 "Dummy products"

A "dummy product" is a product that is not fully formulated. It is not intended to be placed on the market.

In order to satisfy the requirement of the BPR, a dossier of an active substance for inclusion in the Union list (or in Annex I of active substances referred to in Article 25a of the BPR) may be accompanied by such a product as the associated biocidal product. To the extent possible, data from real products are nevertheless recommended.

While some dummy products may be very similar to a fully formulated product, others may be a very simple formulation that bears little resemblance to the product which will finally be placed on the market. The latter may be used where the applicant has limited experience in formulating products, for example by applicants who only manufacture active substances.

At the active substance approval stage, the following should be demonstrated:

The evaluation should demonstrate that the active substance under evaluation is capable of producing an effect when formulated into a very simple product (active substance alone or diluted in a solvent) and to define an application rate, which is consistent with the intended use(s) claimed by the applicant, and that can be used for the exposure assessment.

If a dummy product is used, a more restricted levels of efficacy could be acceptable if an appropriate and detailed justification is given by the applicant. However, the minimum requirements mentioned in section 4.2 have always to be fulfilled.

4.5 Treated articles

Treated articles have been included into the biocides legislation on 1 September 2013 with the BPR (Biocidal Products Regulation). This requires different considerations and testing approaches as compared to the BPD.

4.5.1 Definition

'treated article' means any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products; (BPR, Art. 3 (1) l)

A treated article that has a primary biocidal function shall be considered a biocidal product. (Art. 3(1) a)

A biocidal product, in contrast, is any substance or mixture with a biocidal function. Consequently, efficacy testing and assessment is not principally different for biocidal products and treated articles. Both categories can take different forms (liquid, solid) and can concern different materials. In both cases efficacy has to be shown for normal conditions of use and against an untreated control. The untreated control should demonstrate the problem which is to be solved by the biocidal treatment.

4.5.2 Product authorisation or active substance approval?

For biocidal products placed on the market in the EU, the authorisation requirements of the BPR apply, including testing efficacy. For treated articles imported into the EU, there is only the active substance approval stage to test efficacy. In this respect, it is particularly important to evaluate and assess use in treated articles at the active substance approval stage.

Where claims to treat articles are made for active substance or biocidal products, efficacy data to support these claims have to be submitted (see *Annex II, Title 1, 6.6* and *Annex III, Title 1, 6.6 and 6.7*). If claims are made on active substance level, efficacy assessment of the use in treated articles has to be part of the active substance evaluation.

4.5.3 The PTs significance for assessing efficacy on active substance level

For active substances notified for certain PTs it is obvious that they are mainly or exclusively used to treat articles as for example for PTs 6, 7, 8, 9, 10. Thus, efficacy testing with respect to use to treat articles is a natural part of the active substance evaluation. In such cases use concentrations and standard use conditions for use in treated articles have to be taken into account in assessing efficacy. The biocidal function of the PTs within main group 2 is usually protection of specific materials from biodeterioration, in some cases odour prevention. The state of the articles treated can be solid or liquid. The use conditions can be dry, humid or wet, which can be quite crucial for the release of the active substance out of the matrix. Thus, the representative product should show the claimed effect(s) in the range of uses and use conditions which are described and in the type of matrixes applied for. Use conditions like ageing, weathering or washing should be simulated as appropriate, to demonstrate the duration of the effect in relation to the life-span of the article treated.

Active substances notified for PTs 1-5 are usually used in (liquid) biocidal products as for instance hand disinfection or surface disinfection products. These products are clearly considered biocidal products. But sometimes active substances belonging to PTs 2, 3 or 4 are incorporated into textiles and other solid materials, and claims for health protection are made. Not the protection of the material itself is intended, but a new property is introduced to an article, intended to protect its user. In these cases, it has to be carefully considered whether such products are biocidal products or treated articles; that is whether they have a primary or secondary biocidal function. For these health protection claims, testing is particularly challenging and the specific conditions of use have to be considered when designing the efficacy testing. Please read more about how to design such tests in section 5.3 However, even at active substance level, the representative product should show the claimed effect(s) in the range of uses and use conditions which are described and in the type of matrixes applied for. Particularly the wet state of the use conditions (dry, humid or wet) needs to be taken into account, as this is crucial for the release of the active substance out of the matrix and thus for the efficacy of the representative product. Furthermore, use conditions like ageing, weathering or washing should be simulated as appropriate, to demonstrate the duration of the effect in relation to the life-span of the article treated. Use conditions for which no efficacy of the representative product could be demonstrated shall be excluded from the approval as appropriate.

Active substances belonging to PTs 18 and 19 and used to treat (solid) articles can have different purposes. The treatment can be intended to protect the material (for instance a carpet treated with an insecticide to prevent moth damage) or it can be intended to protect humans or animals against insects (for instance clothes treated with a repellent). Again, in the latter case it has to be carefully considered whether such a product fulfils the definition of a biocidal product and has to undergo an authorisation procedure. At the active substance approval stage, any claims made should be demonstrated with appropriate efficacy tests on the representative product, taking into account the specific conditions of use (e.g. regular washing for clothes) and the availability of the active substance to the target organisms, which can differ in different matrices.

5. Product authorisation

5.1 General introduction

5.1.1 Evaluation of efficacy at product authorisation stage

The Product Authorisation stage is the point in the evaluation process where the efficacy of the biocidal product should be looked at in detail. At this stage, it is not the properties

of the active substance which are of interest, but instead the properties of the fully formulated product, which may contain more than one active substance.

Therefore, this is the stage at which a detailed evaluation of the efficacy of the formulated product should be carried out, and where the efficacy is evaluated in relation to the label claims made for the product. This evaluation should include all relevant target species (or representative species), the effects of using the product, the duration and speed of effect, any claims for residual action, together with any other specific claims.

For biocidal product authorisation

At biocidal product authorisation, the applicant must clearly describe the uses for which the product is intended when it is used under normal conditions, at the appropriate application rate and in accordance with the label instructions.

This information is required to allow a proper evaluation of the efficacy to be carried out, and must include, for every product type separately:

- The function of the product (e.g. bactericide, fungicide, rodenticide, insecticide)
- The organism(s) to be controlled
- The effects on representative target organism(s) (e.g. attracting, killing, inhibiting)
- Any products, organisms or objects to be protected
- The concentration at which the active substance will be used (the use concentrations for different targets should be stated for each use and method of application, if appropriate. Applicants should also indicate if the use concentrations should be different in different parts of EU).

At the product authorisation stage, efficacy must be demonstrated against all claimed target organisms. Use against additional target organisms (i.e. which were not supported at the active substance approval stage) may be applied for at this stage.

For biocidal products used to treat articles, the above mentioned principles apply.

5.2 Product families

5.2.1 Background

A product family is a group of products with the same active substance(s) and similar use, but small differences in the formulation, which do not significantly reduce the efficacy of the product.[1]. When authorisation is requested for a product family efficacy should be demonstrated for the whole group but not necessarily of each product. A product family can be divided in different *meta* SPC's[2], , and all products in the *meta* SPC have the same hazard and precautionary statements. It should thus be noted that the efficacy evaluation of the product family should be made in conjunction with the other parts of the evaluation (e.g. ENV, HH and phys-chem) and that an overall assessment of the division into *meta* SPC's should be made taking all areas into account. This guidance is specifically aimed at an evaluation of differences in efficacy claim, which could lead to certain structures of the BPF and *meta* SPC's. Therefore, some of the following examples could result in other structures of the *meta* SPC's when ENV, HH and phys-chem are taken into account.

[1] see article 3 of the BPR for the full definition of a BPF.

[2] See for the definition of a *meta SPC* CA-Nov15-Doc_4_3Update_note_for_guidance_on_BPF_concept.docx

5.2.2 Worst case testing

The BPF concept allows read-across of data between similar products within and across *meta*SPCs. Efficacy tests can be done on the product with the lowest concentration of the active substance, under the worst case circumstances. The influence of the co-formulants on the efficacy should be taken into account. A justification should be given for the product and circumstances taken.

Tests and criteria for testing efficacy of products in a family are the same as for single products. For the data requirements and test criteria, please see the specific sections per PT.

Applicants need to ensure that all products within a family have been supported, in terms of:

- target organisms
- concentrations / application rates
- influence of the co-formulants
- application methods
- field of use / use conditions
- other label claims
- formulations
- any other relevant information

Table 1: Example ready-to-use disinfectants with pre-cleaning*.

	Family A Concentration AS: 1-4%		
	***meta* SPC 1**		***meta* SPC 2**
	Product 1	**Product 2**	**Product 3**
concentration AS	1%	1.2%	4%
target organisms	bacteria yeasts	bacteria yeasts	bacteria yeasts viruses
use conditions	apply after pre-cleaning	apply after pre-cleaning	apply without cleaning

NOTES to Table 1

In this example one worst case for efficacy cannot be identified. Product 1 should be tested against bacteria and yeasts under clean conditions (also supporting product 2), and product 3 should be tested against bacteria, yeasts viruses, under dirty conditions.

Since these are all ready-to-use products, and presuming that 1% is not efficacious against viruses, product 1 and 2 should be in a different *meta* SPC than product 3 since they are not efficacious against viruses. The *meta* SPC of products 1 and 2 will state as target organisms bacteria and yeasts and the *meta* SPC of product 3 bacteria, yeasts and viruses.

***** In the examples, only the information given in the table is taken into account for the deviation in *meta* SPC's, presuming that all other factors are the same for the different products or of no influence. In practice other factors relating to the products will also need to be taken into account.

In some cases it is not possible to identify one worst case scenario for a combination of products and use conditions: where such a single "worst case" scenario at *meta* SPC level cannot be identified, an assessment of the minimum efficacy levels that might be relevant for the uses covered by a *meta* SPC has to be performed. For instance, the family contains products (1) and (2) with low active substance (AS) concentration which will be used as disinfectant under clean conditions and only for the control of bacteria and yeast, while another product (3) with a higher concentration of AS is used under dirty conditions for the control of bacteria, yeast, and viruses. Product (1) and (2) will not be sufficiently efficacious against viruses, so it cannot be used to demonstrate efficacy for all the uses. In this family, product (1) should be tested under clean conditions against bacteria and yeast (and cover product (2)) and product (3) should be tested under dirty conditions against bacteria and yeast and viruses (see Table 1). Tests done for a product in one *meta SPC* can, where relevant, be used to support a claim for a similar product in a different *meta SPC*, provided that variations in co-formulants have no influence on efficacy. Justification may need to be provided to allow read across.

In some product families several combinations of products and uses should be tested, to demonstrate efficacy for all combinations of products and use conditions (see Tables 2, 3, and 4).

Table 2: Example concentrated disinfectants

	Family B Concentration AS: 10-40%		
	meta SPC Product: 10-40% AS Dilute product to use concentration: bacteria: 1% AS fungi: 1% AS viruses: 4% AS		
	Product 1	**Product 2**	**Product 3**
concentration AS	10%	20%	40%
target organisms	bacteria fungi	bacteria fungi	bacteria fungi viruses

NOTES to Table 2

In this example all products are concentrates to be diluted before use. The applicant only claims efficacy against bacteria and fungi for product 1 and 2 and in addition viruses for product 3. Presuming all products only differ in the concentration active substance, testing can be done with either of the products at use concentration: product diluted to 1% active substance should be tested against bacteria and fungi, and product diluted to 4% active substance should be tested against viruses.

Since all concentrated products can be diluted to an efficacious concentration, when used according to the instructions on the *meta* SPC, all products can be in one *meta* SPC.

Table 3: Example surface disinfectants ready-to-use: more PT's

	Family C Concentration AS: 10%		
Option 1	***meta* SPC 1** Use #1: PT3, bacteria, fungi Use #2: PT4, bacteria, fungi, viruses		
Option 2	***meta* SPC 1** Use #1: PT3, bacteria, fungi		***meta* SPC 2** Use #2: PT4, bacteria, fungi, viruses
	Product 1	**Product 2**	**Product 3**
concentration AS	10%	10%	10%
target organisms	bacteria fungi	bacteria fungi	bacteria fungi viruses
PT	PT3	PT3	PT4

NOTES to Table 3

In this example all products are ready to use and have the same use concentration, they only have a different use claim. It is presumed that the products only slightly differ in their composition and that it is demonstrated that this does not influence the efficacy. In this case either of the products can be tested under worst case conditions (justification should be given that PT3 soiling and temperature is the worst case). A representative products should be tested against the specified bacteria and fungi required for PT3, and against the specified bacteria and viruses required for PT4. Since the fungi that have to be tested for PT3 and PT4 are identical, one test performed under the worst case conditions is sufficient. Since this *meta* SPC can be split into 2 uses, one for PT3 and one for PT4, and all products are efficacious against all uses, it is possible to put all three products in one *meta* SPC, (option 1). All possible products in this *meta* SPC will be efficacious against use #1 and use #2. Efficacy against viruses in PT3 is not demonstrated, however, since this is not in one of the uses in the *meta* SPC, this is acceptable. On the product label only the specified uses, combination of PT and target organisms, can be claimed. However, an applicant might consider it easier to split the family in 2 *meta* SPC's , one per PT (option 2)

When a family contains more than one active substance it might not be sufficient to test the products to be authorised in a *meta* SPC, in some cases it is necessary to test a 'dummy' product to cover all products in one *meta* SPC (see Table 6). Alternatively, they could be authorised in separate *meta* SPC.

5.2.3 Take formulation types and chemical composition into account

While the active substance is the most important constituent for efficacy of a biocidal product, the effect of the formulation of the product on the efficacy must also be taken into account. Therefore, the justification should be given for the product used in the test, taking into account the formulation. If the product contains more than one active substance, the synergistic effect between different active substances will be considered.

In the case of products having different formulation types (e.g. Wettable Powder and Water dispersible Granuals for PT18), bridging studies with these products can be used

to substantiate that the products are equivalent in terms of their efficacy. Bridging studies should involve worst case circumstances (after appropriate justification).

Depending on the influence of the ingredients (chemical composition) on the efficacy either the product with the lowest concentration of all the ingredients should be tested or several products, together including the whole spectrum of the formulations, should be tested (see Table 5).

5.2.4 Allowing for the addition of new products

In general the (*meta*) SPC(‘s) of a family will give a range for the concentration of the active substance(s) and co-formulants. After authorisation of the family it is possible to add new products to the family, as long as their composition falls into the range for the (*meta*) SPC. For these new products no evaluation will be done. Therefore, efficacy testing should be done in such a way that efficacy against all possible new products will be demonstrated.

For instance, in the example in Table 5, a new product with 70% active substance and the lowest concentration of both acids could be added. Efficacy of this product should be demonstrated, or the two products should be put into different *meta* SPC’s. Another example is explained in Table 6.

Table 4: Example insecticide: take target organisms and application method into account.

	Family D Concentration AS: 1-4%		
	***meta* SPC 1** Conc. AS: 1%	***meta* SPC 2** Conc. AS: 1%	***meta* SPC 3** Conc. AS: 1%
	Product 1	**Product 2**	**Product 3**
concentration AS	1%	1%	4%
target organisms	moth	moth and mosquitoes	ants
application method	paper in wardrobe	electric device in wardrobe or room	bait box with sugar

NOTES to Table 4

In this example one worst case for efficacy testing cannot be identified and all products should be tested for all target organisms and uses.

All three products should be in different *meta* SPC’s because of the different application methods and organisms.

Table 5: Example disinfectant: take formulation into account.

	Family E Concentration AS: 70-85% Concentration acid 1: 1-4% Concentration acid 2: 2-5%		
Option 1	***meta* SPC 1** Concentration AS: 70-75% Concentration acid 1: 1-4% Concentration acid 2: 2-5%		***meta* SPC 2**
Option 2	***meta* SPC 1**	***meta* SPC 2**	***meta* SPC 3**
	Product 1	**Product 2**	**Product 3**
target organisms	bacteria fungi	bacteria fungi	bacteria fungi virus
Active substance	70%	75%	85%
Acid 1	1%	4%	1%
Acid 2	5%	2%	5%

NOTES to Table 5

In this example both acids are pH regulators. It is presumed they are not considered active substances in this formulation (in some cases this should be demonstrated with tests), however, both acids might enhance the efficacy to some extent (i.e. formulation effect). Since it cannot be ruled out that there is a difference in effect between these two acids, this should be taken into account in the efficacy testing.

When product 1 and 2 are placed in one *meta* SPC (option 1) it should be considered that it is possible to add a new product in this *meta* SPC with 1% acid 1 and 2% acid 2. In that case it is not sufficient to test product 1 (with lowest concentration AS), but a 'dummy' product should be tested, with 70% AS, 1% acid 1 and 2% acid 2.

To prevent testing with 'dummy' products, it might be easier to place products 1 and 2 in separate *meta* SPC's, without a range for the acids(option 2). Also in that case, read across between product 1 and 2 is not possible. Both product 1 and 2 should be tested, to rule out the effect of the formulation with different acid concentrations.

In all cases product 3 should be tested against viruses, and put in a different *meta* SPC (assuming 85% is necessary for viruses). The test with product 1 or the 'dummy' product can be used to demonstrate efficacy against bacteria and fungi for *meta* SPC 2 (product 3).

5.2.5 Deviation in *meta* SPC's

When dividing a product family in *meta* SPC's, it must be taken into account that all (possible new) products will be efficacious for all uses, target organisms, etc.. Worst case testing must make sure that all possible new products will be efficacious. Where needed/possible new *meta* SPC's should be made for a different group of target organisms, a different use, different application method, etc.

This means for the example family in Table 4, that all products should be in a different *meta* SPC.

In Table 1 product 1 and 2 should be separated from product 3, because these are not efficacious against viruses and therefore not against all target organisms in this *meta* SPC.

However, in some cases it might be possible to not deviate in more *meta* SPC's but give a good description in the *meta* SPC, making sure that all products will be efficacious. For instance, in the examples in Tables 2 and 3, which are very similar to Table 1, the product with a virus claim can be in the same *meta* SPC. This is acceptable because all possible products are efficacious when used according to the use description in the *meta* SPC, either because all products can be diluted to an efficacious dose, or by making separate use numbers. In these cases some of the products in the *meta* SPC have a limited claim (i.e. fewer organisms, fewer PT's).

When the different uses results in a too complicated *meta* SPC, with several different use numbers, it is better to divide such a *meta* SPC in more simpler *meta* SPC's.

When dividing into meta SPC's the applicant must make sure that the text in the *meta* SPC's is unambiguous, and consider that no products can be added to the family that have not been supported in the efficacy testing (see Tables 3 and 4).

Table 6: Example anti-fouling product: Different ratio's of two (or more) active substances.

	Family Concentration.AS 1: 5-10% Concentration.AS 2: 2-7%	
Option 1	***meta* SPC 1** Concentration.AS 1: 5-10% Concentration.AS 2: 2-7%	
Option 2	***meta* SPC 1** Conc.AS 1: 10% Conc.AS 2: 2%	***meta* SPC 2** Conc.AS 1: 5% Conc.AS 2: 7%
	Product 1 RTU	**Product 2 RTU**
target organisms	Macro fouling	Macro fouling
Active substance 1	10%	5%
Active substance 2	2%	7%

NOTES to Table 6

In this example testing product 1 and 2 is not sufficient to cover the worst-case situation of this *meta* SPC. The worst-case would be a product 5% active substance 1 + 2% active substance 2. This 'dummy' product should be tested to demonstrate efficacy for this *meta* SPC (option 1). Alternatively, product 1 and 2 can be put into different *meta* SPC (option 2), and efficacy test using prod 1 and 2 can be provided.

5.2.6 Minimum concentration needed

While products authorised on their own are evaluated on their merits and not in comparison to other products, this is not the case in a product family. Since all products are presented at the same time a comparison can be made. The BPR Annex VI art. 77 of the common principles state: the recommended dose is the minimum necessary to achieve the desired effect.

For historical reasons it is possible that products on the market in one EU country contain a higher concentration of AS than another product with the same intended use in another country. When this is the case the applicant should request for authorisation for the products with the lowest concentration of AS or give a good justification why it is relevant to have different formulations.

CA's should consider that a product on their market might better be replaced by another product in the product family when this other product contains a lower concentration of AS and is efficacious for the same intended use.

5.3 Treated articles

A treated article according to Article 3(1)(a) of the BPR is any substance, mixture or article which has been treated with or intentionally incorporates one or more biocidal products. A treated article with a primary biocidal function is considered a biocidal product. Treated articles with claims to protect humans or animals can have a primary biocidal function. Please consider the CA Note for Guidance "Frequently asked questions on treated articles"[3] on this matter.

There are some exemptions the definition given in Art. 3(1)(a): Articles such as paper or carton, where the pulp has been treated with a biocide during manufacture, and where the biocide is not intended to have a function in the final good are not considered treated articles. Another example are articles with print on it or with glue holding it together which have been treated with an in-can preservative. However, the preservative doesn't have any function in the final article as soon as the ink or adhesive is applied and dried. In contrast, an article like a table made of a composite material with wooden legs painted with a film preservative containing coating, is considered a treated article, as the coating still has a biocidal function in the final article.

Generally, there is no difference in efficacy testing of treated articles or biocidal products. Specific requirements apply, however, when the efficacy of solid material or articles has to be tested. Treated articles with claims to protect humans or animals fall under this category. In these cases, use conditions, most importantly humidity, have to be specified. Materials can be used in articles with a wide range of use conditions, and these have an effect on efficacy. For example, for a polymer article permanently exposed to water the conditions for bacterial growth are much more favourable, and different requirements apply as compared to a polymer article which is generally dry and is only exposed to occasional splashes or to the humidity which comes from touching it. But more importantly, humidity has an effect on the availability of the active substance, because it has to be released out of the matrix somehow. Another example are clothes treated with repellents; also in this case use-conditions do influence efficacy. Wearing and tearing and washing have to be taken into account to assess the efficacy. Complete protection time needs to be defined in terms of the life-cycle of the treated clothes.

Consequently, if efficacy is demonstrated for a certain set of use conditions, this cannot generally be transferred to another set of use conditions. The possible limits of the use conditions have to be reflected in the approval/authorisation decision. In the following, guidance is given for the testing of (solid) materials with claims to protect humans or animals.

5.3.1 The basic distinction between material protection and protection of humans or animals

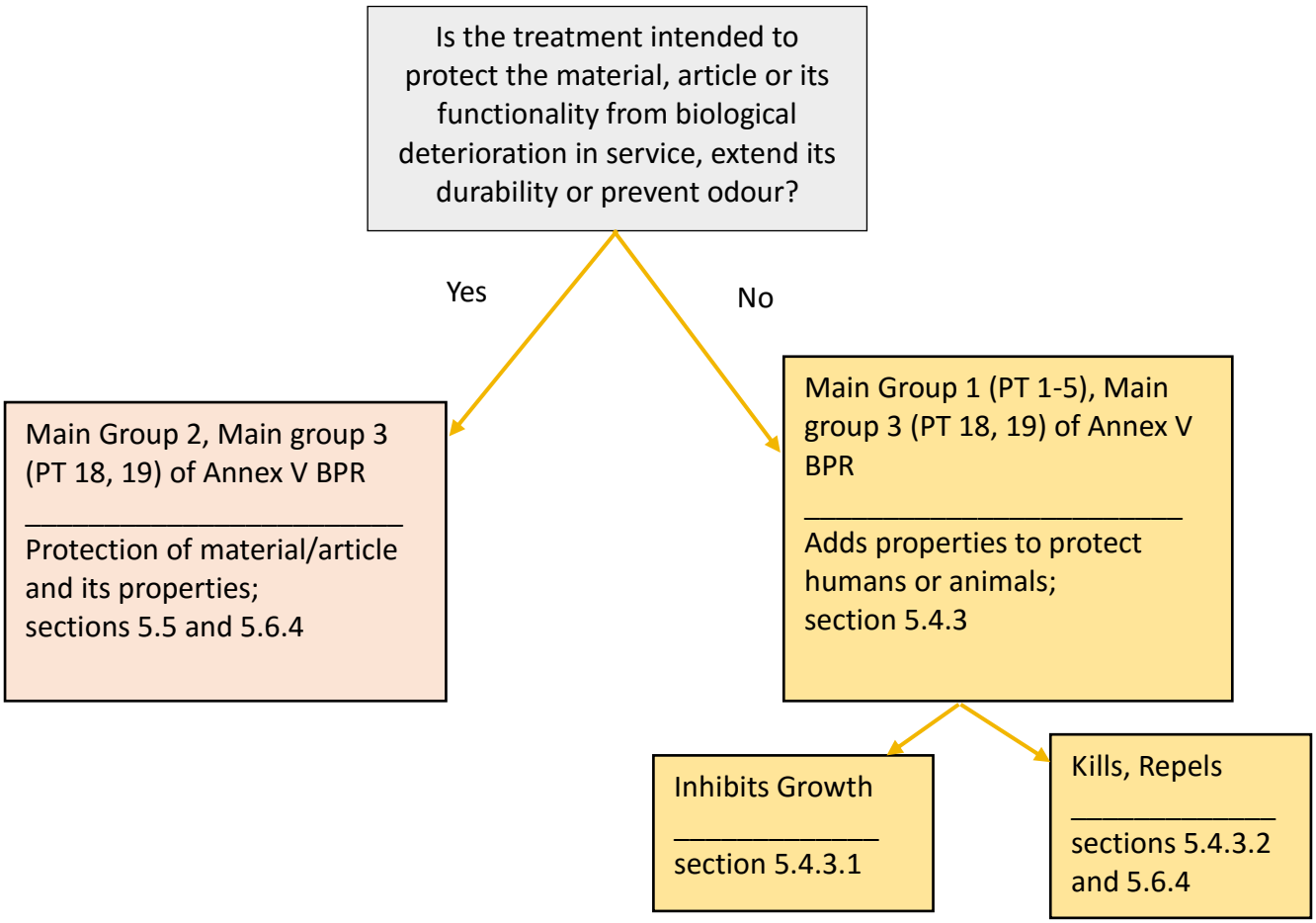
When biocides are incorporated into materials or used in the production of treated articles they are applied with two purposes:

[3] CA-Sept13-Doc

- To protect the materials used in the article or the properties of the article in service.
- To protect humans or animals from the unwanted effects of organisms. The treatment is directed towards targets organisms which have no adverse effect on the item/material treated.

The following scheme gives an overview and decision help:

Figure 1: Decision scheme to distinguish between claims for material protection and claims for protection of humans and animals



Guidance for the testing of biocidal products with a claim to protect humans or animals is given in sections 4.5.3 and 5.6.4. Guidance for material protection is given in section 5.5.

5.4 Disinfectants

5.4.1 General

NOTE FOR PEG CONSULTATION

The text for this section has been published in a Transitional Guidance (May 2016). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The TG document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_disinfectants_pt1-5_en.pdf]

5.4.2 Materials and Articles Treated to Protect Humans or Animals

For testing materials and articles with claims to protect humans or animals, a tailored approach is compulsory. The testing strategy entirely depends on the specific claim made. In the majority of cases, a claim can only be made for a specific type of final article, as use area and use conditions are decisive for describing the problem which the biocide shall solve, and to demonstrate efficacy in exactly those conditions is necessary. Consequently, this section describes testing principles and strategies rather than recommending specific tests.

A tiered approach has to be followed in demonstrating claims for protection of humans or animals:

- Tier 1 - Proof of principle: Tier one tests should document the efficacy of the incorporated biocide in the treated article against relevant target organism(s) under relevant conditions (e.g. humidity, temperature).
- Tier 2 - Simulated Use: Tier two tests should document the efficacy of the incorporated biocide in the treated article under real-life conditions (e.g. way of contamination, cleaning regimes, speed requirements) and for how long.

Depending on the claim made (e.g. "kills bacteria on door-handles to prevent cross contamination", "protects against mosquito-bites"), even Tier 3 testing can be necessary:

- Tier 3 - In-Use Evaluation/Field studies: To substantiate health benefit claims, treated and untreated articles would be tested via statistically designed use trials by a representative user group.

Generally, the principle applies that only claims can be made which have been demonstrated.

5.4.2.1 Determining the purpose of the Treatment

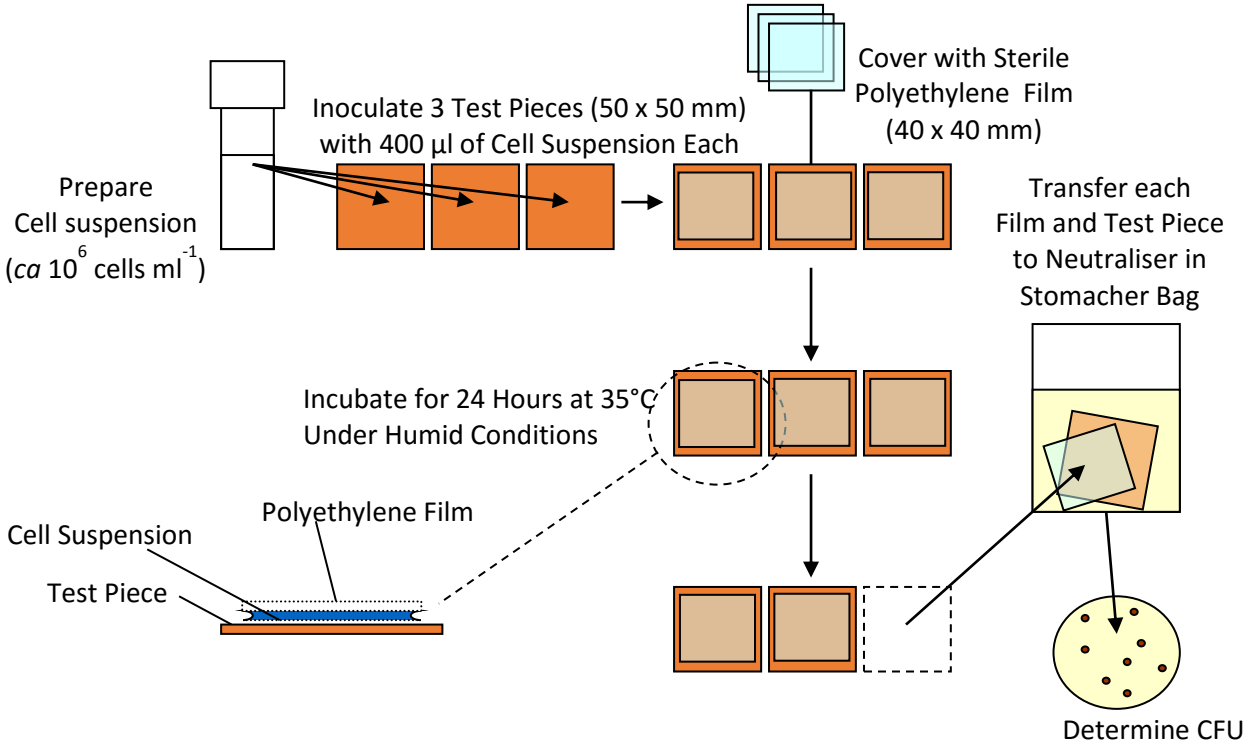
The effects of articles with a disinfection claim cannot be detected by changes in appearance, mechanical properties or odour. The precondition for demonstrating efficacy is a clear description of the purpose of the treatment. Often, claims are unclear about whether the treatment prevents growth or kills bacteria on contact. On most articles, no bacteria will grow under normal conditions of use. Nevertheless, antibacterial claims (such as 'anti-bacterial', 'hygienically clean', 'free of bacteria', 'prevents the spread of hazardous bacteria') are made, insinuating that bacteria will be killed on the material, though only growth inhibition tests have been carried out. In most environments, the sheer presence of bacteria does not present a problem. If this is a problem, it is in most cases much more effective to use traditional disinfection methods like cleaning with a liquid disinfectant. In most cases, the treatment of articles should not be used as the only measure of disinfection, but should be combined with a disinfection management regime.

5.4.2.2 Effects Intended to Inhibit Microbial Growth

Under the majority of indoor situations, most microorganisms will not grow on environmental surfaces due to lack of humidity. To make a claim for growth inhibition, wet or at least humid conditions are a precondition. To demonstrate such a claim, sub-samples of treated and untreated material of the article in question could be tested using a method adapted from ISO 22196 (see Figure 2). Soiling conditions, temperature, test species and contact time have to be adapted to mimic a realistic in-use situation (Tier 1). Possibly, the impact of in-use conditions like ageing or cleaning regimes on the effect would have to be included in the testing (Tier 2). The minimum requirements for disinfection are laid down in the Claims matrix for treated articles (see Appendix 1) with claims to protect humans or animals [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy].

Figure 2: A Test for Antibacterial Activity in Wet Conditions

ISO 22196



ISO 22196, Method Outline:

An aliquot (usually 400 µl) of a log phase bacterial cell suspension (*ca*10^5 cells ml^{-1}) in $^1/_{500}$ Nutrient Broth are held in intimate contact with each of 3 replicates of both treated and untreated variants of the test materials using a 40 mm x 40 mm polyethylene film (*e.g.* cut from a sterile Stomacher bag) for 24 hours at 35°C. Usually, *S. aureus*, *P. aeruginosa*, *Enterococcus hirae* and *E. coli* should be tested (see Appendix 3). The

populations are then recovered using a neutraliser solution and the size of the surviving populations are determined as colony forming units (CFUs) using a dilution plate count method. Additional replicate unfortified samples are also inoculated in the same manner but are analysed immediately to determine the size of microbial population present prior to incubation. The differences between the initial and final population as well as between the treated and untreated materials are used to assess the basic antibacterial properties of the test materials.

5.4.2.3 Effects intended to Kill Microorganisms through Contact

Claims made for materials and articles to kill on contact to prevent cross-contamination are not easy to demonstrate. Mostly, the effect will require the release of the active substance from the surface of the material; this release needs to be triggered somehow. In the majority of cases, water or other liquids are the crucial component to facilitate such release and transfer. If the event that caused the deposition of the target organism does not introduce moisture and the normal exposure conditions of the material or article are dry (or only subject to normal, ambient indoor humidity), the effect of the treatment will probably be limited.

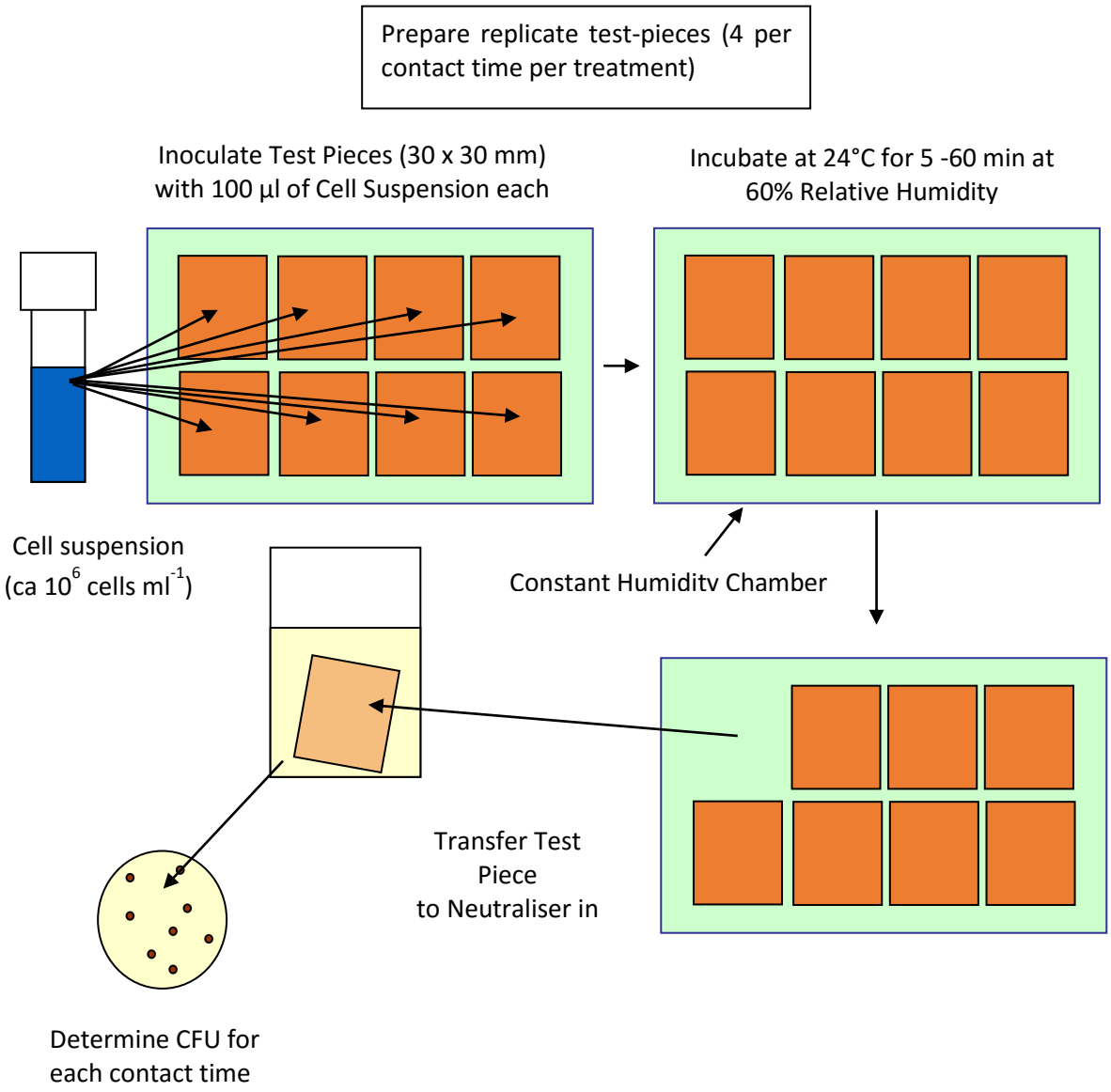
Another issue is the speed of activity needed to inhibit cross-contamination. If for instance door handles in a hospital would be treated with an active substance to kill deposited pathogenic organisms, the effect would have to be sufficiently fast to prevent the next person using the door handle from cross-contamination. In combination with the little moisture which is deposited in the event, it will be challenging to demonstrate a satisfying effect. The minimum requirements for disinfection are laid down in the Claims matrix for treated articles (see Appendix 1) with a claim to protect humans or animals. Additional requirements may apply depending on the claim made.

Testing could be carried out with the example protocols given below. Again, care must be taken to adapt test conditions to realistic in-use conditions. Figures 3 and 4 show the approach used for non-porous materials and for absorbent materials, respectively, both intended to simulate contamination through contact with splashes of contaminated liquids. Figure 5 illustrates a protocol intended to simulate contamination through, for example, hand/gloved hand contact.

5.4.2.4 Norms and Criteria

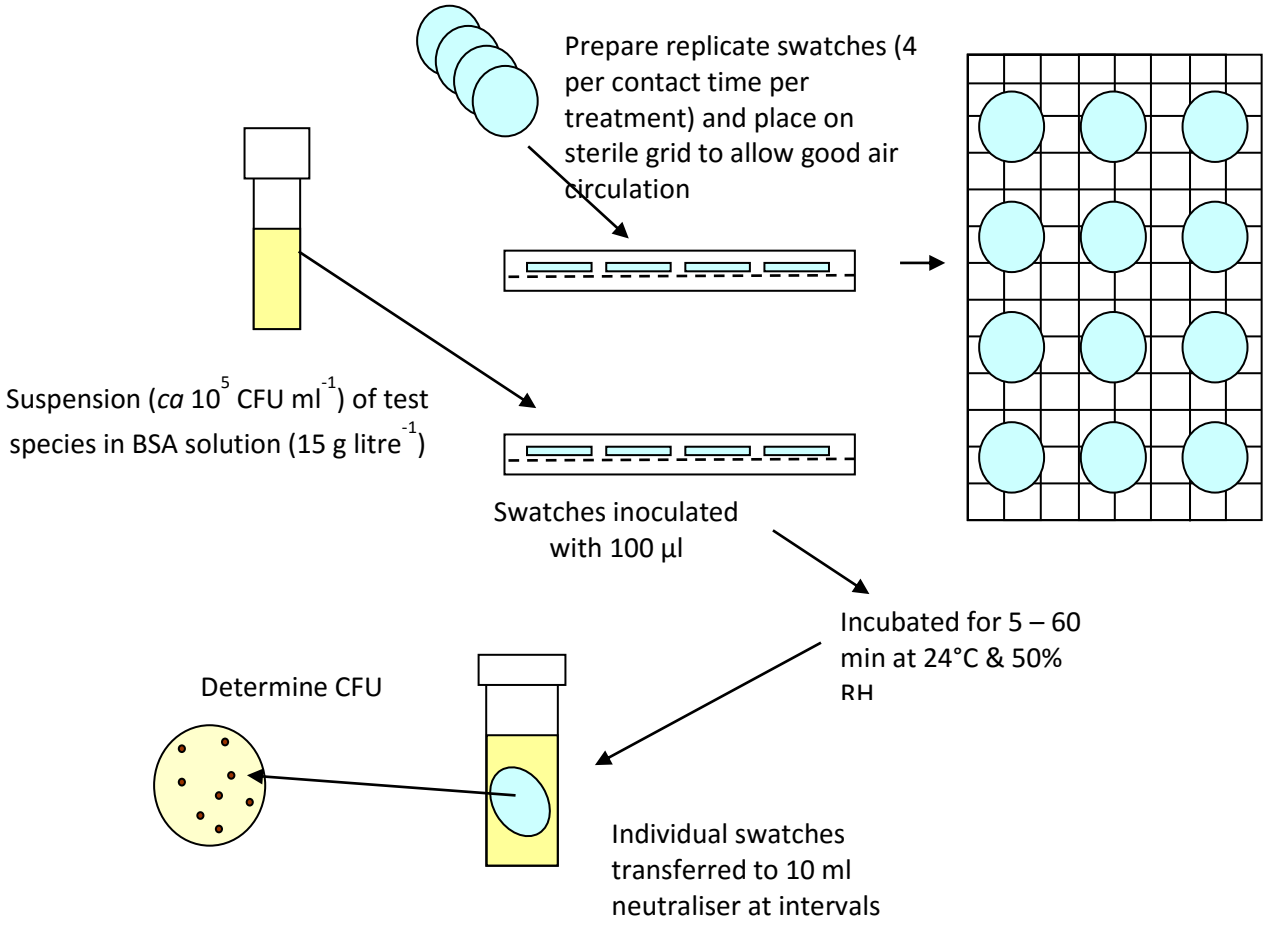
Generally, the norms and criteria used for liquid disinfectants can be used as supportive information to e.g. choose appropriate testing organisms (see Appendices 3 and 4). As the performance criteria for treated articles are generally lower than for liquid disinfectants, the treatment of articles should generally not be used as the only measure of disinfection, but should be combined with a disinfection management regime.

Figure 3: Simulated Splash Model Non-Porous Materials



CFU= colony forming units

Figure 4: Simulated Splash Model Porous Materials



CFU= colony forming units, RH= relative humidity,
BSA= Bovine Serum Albumine

Figure 5: Printing Model

TVC= total viable count
CFU= colony forming units

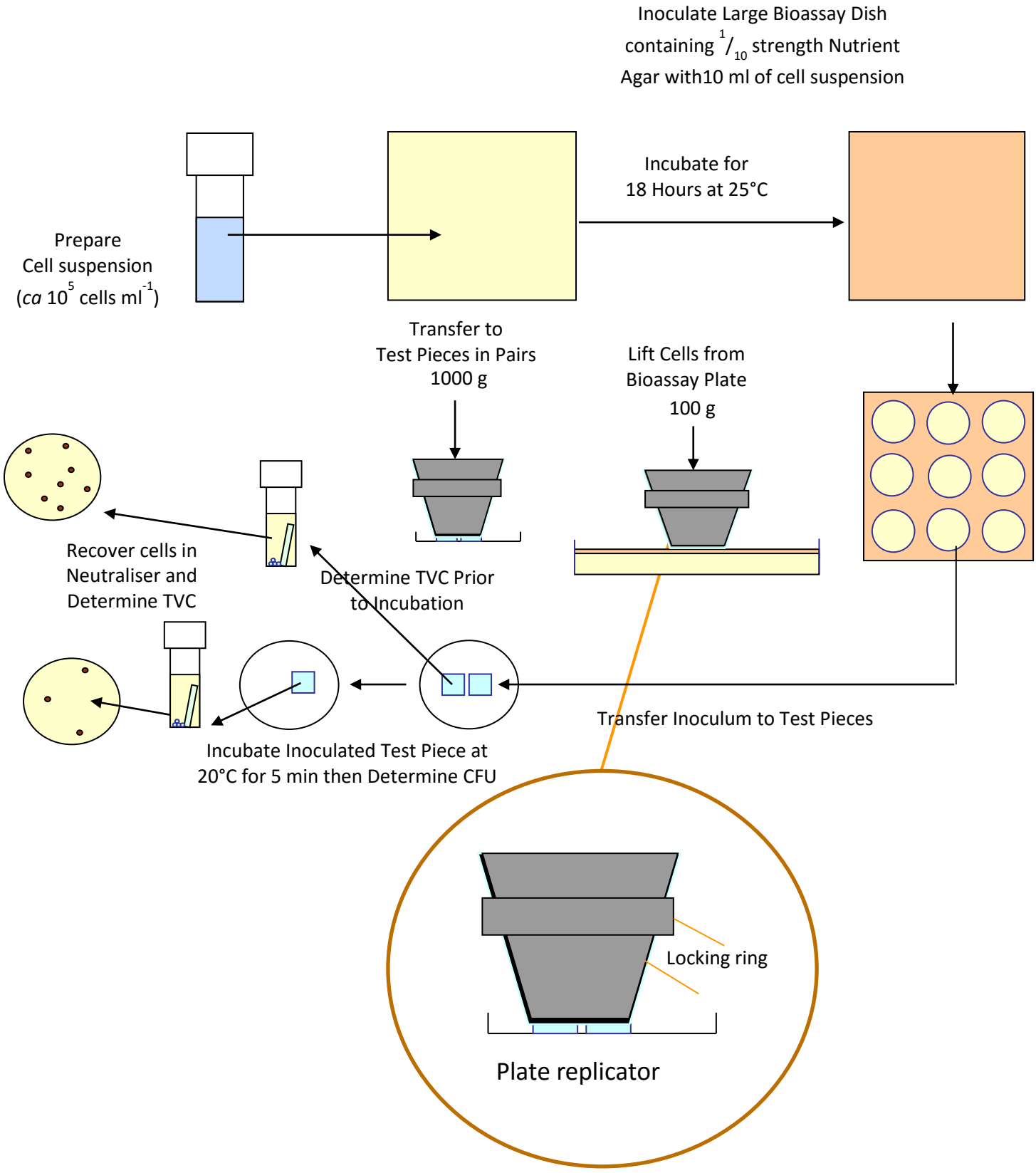


Table 7: Protection of Humans or Animals – Example Claims, Problems and Testing Approaches

Claim	PT	Proof required	Example method
Bedside cabinet for use in hospitals that has been treated to reduce infections by killing 'bacteria on contact'.	2	Data should show that microorganisms, when deposited through skin contact (even under simulated conditions) and through the deposition of fine aerosols are killed within a time-frame that would prevent the surfaces becoming a vector for cross-contamination.	Plaques made of the identical material used for the cabinet are employed in the test. Both treated and untreated variants are used.
		Skin contact	The method described in Figure 5 is employed to deposit bacteria onto test plaques. A range of contact times between 5 minutes and 1 hour are used. A log reduction of 3 should be achieved.
		Aerosol	The method described in Figure 3 is adapted for use by employing multiple droplets of 1 µl on each test plaque. A range of contact times between 5 minutes and 1 hour are used to explore activity. A log reduction of 3 should be achieved.
A plastic conveyer belt is treated to prevent the growth of bacteria between cleaning intervals in a food factory.	4	Data should show that relevant bacteria grow on an untreated conveyer belt under normal conditions of use during a 6 hour interval. Significantly reduced growth should be demonstrated on the treated belt.	Plaques made of the identical material used for the belt are employed in the test. Both treated and untreated variants are used. ISO 22196 is adapted to simulate a moist conveyor belt. A soiling agent relevant to the end use is included. A contact time and temperature equal to that encountered in practice are employed.

Table 8: Basic Requirements for a Valid Test Protection of Humans or Animals

The following summary provides a guide to the basic requirements for a valid test:

i. The test should be carried out on the type of final article.
ii. A test which mimics the way of deposition and the type of material needs to be chosen.
iii. An untreated variant of the test material must be included such that the impact of the treatment can be demonstrated.
iv. Test conditions should reflect normal conditions of use in terms of humidity, temperature, soiling, contact frequency, etc.
v. The test should employ organisms that are relevant to the end use of the article and the purpose being claimed.
vi. Tests that employ a single species of organisms should be favoured over those that use consortia.
vii. A minimum of three replicate test pieces of both treated and untreated materials should be employed.
viii. The final data should include either some indication of the impact of service conditions on the performance of the treated material/article or data from an ageing study. The intention is to demonstrate how long the claimed effect will be sustained.
ix. If claims are made which require a field test, relevant data including statistical evaluations have to be provided.

NOTE FOR PEG CONSULTATION

The text for the following sections 5.4.3 – 5.4.7 (Disinfectants PT1 – PT5) has been published in a Transitional Guidance (May 2016). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation. Please note that for PT5, the text will be updated (by a Guidance Update procedure) after the results and recommendations of the "ECHA disinfectants project" are available, foreseen in 2017.

The TG document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_disinfectants_pt1-5_en.pdf]

5.4.3 PT1

5.4.4 PT2

5.4.5 PT3

5.4.6 PT4

5.4.7 PT5

5.5 Preservatives (Main group 2)

NOTE FOR PEG CONSULTATION

The text in this section will supercede the published Transitional Guidance on Efficacy Assessment of Preservatives. The Transitional Guidance document will be made obsolete when this Guidance document is published.

The TG document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_preservatives_en.pdf]

Preservatives in main group 2 are intended to prevent the biodeterioration of a material or a matrix. Wood can lose stability by the action of microorganisms or insects, fabric can be destroyed by fungi, and even polymer-based plastics are prone to biological deterioration. Plasticised PVC would soon become fouled by surface growths of fungi, lose plasticity and crack without the inclusion of a fungicide. A water-based paint, free of volatile organic compounds (VOCs), could not be stored without the use of a biocide. Polyurethane, for example as used for the soles of shoes, can become colonised by fungi and actinomycetes. The heat exchangers in cooling towers have to be kept free from microbial growth to enhance performance by treatment of the cooling liquid.

5.5.1 Distinction between preservation/curative treatment and disinfection

Preservatives are directed towards the protection of a *material*. If the material itself is not affected by the target organisms, the claim does not belong in main group 2. The aim of preservation is to prevent microbial spoilage, decay or the accumulation of biomass that is detrimental to the functionality of an item, material or system. Detrimental effects can be caused by proliferation of cells or by the metabolic activity of cells and may not necessarily involve cell multiplication. The presence of microorganisms can result in either a degradation of the matrix in which they are present or damage to the system in which they are present either due to their metabolic activities (e.g. corrosion) or by fouling or blocking pipes, forming biofilms on heat exchangers etc. It is not the intention of preservatives to transfer their effects to other materials, humans or animals, but to protect the material itself. A long-term effect is generally required. A preservative can have a reversible effect on microorganisms (e.g. by causing stress or cell damage without total loss of viability). In contrast to disinfection no level of reduction is defined for a set of predefined claims.

Curative treatments are also directed towards material protection and therefore likewise fall into main group 2[4]. The aim of a curative action is to either cure microbial spoilage which has already occurred or to eliminate / reduce populations in materials and systems prior to them being treated with a preservative (in some instances a biocidal product can have both curative and preservative functionality).

The level required to prevent spoilage in different media/conditions will be defined by the individual claim made. This will also be the case when the treatment is intended to achieve a curative action.

The claim made will define in which of the PTs an application will fall. The following data is needed:

- A problem description: Scale, speed and type of effect required and what would happen if the biocide was not present

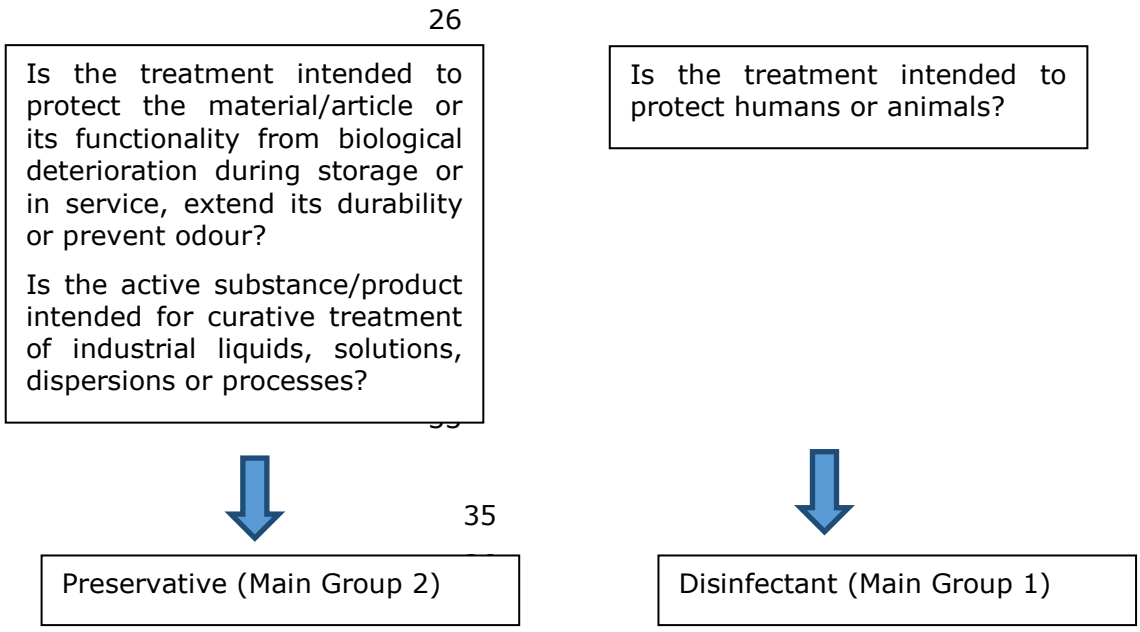
[4] See CA-Sept15-Doc.8.3 – Curative use of preservatives

- The target organisms
- Categorisation of the material/matrix to be treated including dose-rate/concentration of the biocide in the material/matrix.
- The intended use pattern of the treated material/matrix including service-life, weathering conditions, leaching (intended or unintended).

5.5.1.1 Curative uses

Curative uses often require rates and speeds of effects that are similar to those required for disinfectants but do not have prescribed performance standards. Such uses are nevertheless intended to cure (eliminate or reduce) contamination in materials, matrices or systems. They therefore fall under main group 2. Performance requirements will be defined by the requirements of either the matrix or the process involved. A curative effect and a preservative effect may sometimes be achieved using the same biocidal product, only the concentration may differ. In other cases active substances with curative properties will be combined with those that have preservative. Curative and preservative effects need to be demonstrated separately and different methods need to be employed. When claims are made for curative uses, it is important to carry out the health and environmental risk assessment with any higher doses that may be required. A typical example of curative action is the treatment of a contaminated product prior to packaging and sale (in some cases in addition to a preservative – in other cases the curative product may be capable of achieving both a preservative and a curative effect). Another example is the treatment of a contaminated system by reducing the microbial population it contains to limits that are acceptable to the process (e.g. on a paper mill). Please read more about testing of curative uses in section 5.5.6.

Figure 6: Decision scheme for the distinction between preservation and disinfection



5.5.1.2 Borderline case: Algaecides

If algae are expected not to destroy the material or damage its function, algaecides are not considered to be preservatives. Products used against algae for treatment of swimming pools, aquariums and other waters and for the *remedial* treatment of

construction materials belong to product type 2 in main group 1, whereas products with *protective* function are considered as products belonging to main group 2.

For example surface coatings for outdoor use are often formulated with both a fungicide and an algaecide. The algaecide, like the fungicide, is performing a preservative function in the coating and is thus covered by PT 7. 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), where either a preservative or a curative action may be required. Such applications likewise belong to main group 2.

5.5.1.3 Borderline cases: Treated articles

Treated articles can both belong to Main Group 2 or Main Group 1 (and even to Main Group 3 or 4). Please refer to section 5.3 on treated articles and section 5.4.2 on materials and articles treated to protect humans or animals.

5.5.2 Principles for testing preservatives

The aim of any preservation is to maintain the present state/properties of a material or matrix along 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: 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, i.e. the population needs to be shown to increase in size in the untreated material. The production of a biofilm or an increase in biomass may also be appropriate. 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. Uninoculated 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.

Showing growth / metabolism of the microorganisms in the untreated system is an essential requirement of any demonstration of effectiveness of an active substance or biocidal product. It is then assumed, if not proven in every case, that changes have taken place that were induced by microbial growth and that this can be prevented by the use of a biocide acting as a preservative. Often, when growth cannot be proven this is caused by an unnecessarily high inoculation rate. If, at the beginning of the test, an inoculum of e.g. 10^4 CFU for bacteria is employed, an increase to 10^5 - 10^6 can often easily be shown during the test period. When a higher inoculum density e.g. 10^6 is employed, growth is much harder to achieve due to limitations in the supply of nutrient etc. An important consideration is to use a model substrate that can support growth readily rather than attempt to achieve growth in a final product that is less susceptible to the non-acclimated species employed in laboratory tests (i.e. it is often nearly impossible to replicate the failure phenomena observed in practice in a laboratory).

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 suspensions of either bacterial species or fungal species. Bacteria and fungi are not to be mixed in these suspensions, but filamentous fungi ("moulds") and non-filamentous fungi ("yeasts") can be mixed in the inoculum. However, for determining growth different methods need to be applied for yeasts and filamentous fungi.

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

vegetative and active cells, being exposed to an unfavourable environment e.g. a synthetic paint containing solvent or a preservative, are forced into dormancy. Only when a sample of the material is taken out of this environment and is spread onto a nutrient medium do 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 CFU and demonstrating that the number of CFU increased in the untreated matrix during incubation, compared to the number measured immediately after inoculation. The same or a smaller number of CFU than measured initially demonstrates survival, but not necessarily growth. However, for testing solid material, showing growth by adding a nutrient medium to the material is not necessarily enough. It needs to be shown that the material itself is damaged or loses its functionality. Please read more in section 5.5.7.

5.5.3 Tiered approach to testing preservatives

A tiered approach should be followed for testing biocidal products:

Tier 1 - Proof of principle: Tier one tests should document the biocidal efficacy of the incorporated biocide in a relevant model matrix against the target organism(s) under relevant basic environmental conditions (e.g. temperature, humidity).

Tier 2 - Simulated Use: The biocide should demonstrate efficacy under real life conditions relevant to its anticipated service life. Factors such as weathering, UV-stability, extended ageing or leaching should be considered.

Tier 3 – In-use evaluation/field studies: to substantiate specific claims, treated and control articles/products can be tested via statistically designed in-use trials by a representative user group, or by other appropriate methods.

In a Tier 1 test, the damage should be shown in a model matrix and demonstrate how the inclusion of the biocide prevents it (often with the help of an inoculum representing the organisms that cause the damage). In a Tier 2 test, damage or impact of the target organisms under either simulated use conditions or in a manner that simulates an anticipated shelf life should be shown, and even sometimes without the use of an inoculum (soil burial). When moving up from tier 1 to tier 2, a test design has to be more tailored to the field of application envisaged. In tier 1, existing standards are often suitable when the biocide is tested in a relevant matrix with defined organisms and under relevant and reproducible conditions (which are normally only to be found in a laboratory). In tier 2, testing is more complex and often specific standards do not exist. However, sometimes the same standards can be used as for tier 1 tests, simulating use conditions by employing pre-treatment of the matrix. There may be a need for weathering cycles, wind tunnel tests, cleaning regimes etc. Similarly soiling and the influence of other microorganisms can be of more significance. Accelerated aging tests may have to be performed before microbiological testing to allow for factors such as UV, temperature changes, leaching etc. Consideration must be given to which environmental conditions are relevant for simulated aging in realistic in-use conditions. When aging is performed in the field or under in-use conditions, 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. Generally, the applicant should be able to justify how the specific conditions used in testing relate to the in-use conditions relevant to the product or active substance. Tier 3 testing entirely depends on the claim made. The results have to be relevant for that claim and to be scientifically sound.

5.5.4 Standard Test Methods

A list of the most common standard test methods can be found in Appendices 6, 7 and 8. In contrast to disinfection, there are no specific tests allocated to the different tiers. Often the same test can be employed for tier 1 and tier 2, and only the pre-treatment of

the matrix will differ. Different factors can trigger the choice of a test: In some cases the choice of one type of method over another is related to the speed with which it generates results. Often, a method is 'known' to be capable of guiding the choice and concentration of a biocide for a certain material through experience within an industry. However, this may not necessarily mean that the method is suitable for demonstrating the claim made.

Care has to be taken as to whether the test method is appropriate for the testing of preservatives, or if it is intended to prove a curative/sanitising activity of a biocide. Generally, for preservative action growth needs to be shown in the untreated controls. Furthermore, the demand to have at least 3 replicate samples is not always met in all methods.

Nevertheless, an existing test method can form a good basis regarding the parameters of choice of microorganisms, temperature, and choice of neutraliser. If necessary, these methods need to be amended by adding untreated control samples, determining the numbers of organisms that can be recovered immediately after inoculation (0 hours incubation), use of a neutraliser, and the use of a smaller sized inoculum etc. Particularly for tier 2 and 3 testing, it is important that the chosen adaptations reflect the relevant conditions for which the claims shall apply.

Specific tests which are recommended for certain uses are described under the sections for the different PTs.

5.5.4.1 Practical aspects for testing bacteria

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

a. The test must be performed in a range of relevant model matrixes that the claim of efficacy is made for (e.g. dishwasher liquid, paints, glues, textiles, etc).

b. The test has to be performed in relevant environmental conditions (temperature, type of matrix, humidity)

c. 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 shall have no biocide included. The study must include replicate sub-samples for each treatment (minimum of 3).

d. For preservative uses, the control samples should typically show growth (e.g. indicated by an increased number of CFUs) during incubation and this 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 present in the matrix. The treated samples should show statistically significant effects as compared to the controls.

e. Only if growth cannot be proven by increase in CFU, data concerning other factors like e.g. CO_2-emission, O_2 depletion, change of pH, colour change or disintegration of the matrix should be used to demonstrate the need of preservation of a matrix by the active ingredient or preservative.

f. Relevant bacteria for the intended use have to be tested.

5.5.4.2 Practical aspects for testing fungi

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 in liquid systems is bound to fail for several reasons:

- It is impossible to take a representative 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 mycelium and spores that are dormant in the matrix form colonies on a petri dish and their origin cannot be differentiated and so their numbers do not reflect the increase in biomass that has occurred.

However, counting CFU is a practical option to measure the recovery rate of spores inoculated into liquids before spore germination (time 0 analysis) and for unicellular yeasts. At this stage, no mycelia have formed in the liquid, so no fragments will be counted as CFU and wrongly interpreted as growth. Therefore, after the control samples and the biocide-containing samples have been inoculated with spores, the recovery rate can be recorded by 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 will accumulate at the sides of the test vessel, e.g. an Erlenmeyer flask or on the surface of the matrix. Both phenomena are visible by the naked eye and clearly demonstrate that the fungus has grown. In highly fluid materials this growth can be quantified by filtering the whole contents of the test vessel and then determining the amount of growth as dry weight. The use of replicates (minimum 3) is an important factor in such tests.

For testing solid materials, fungal growth is often assessed by optical appearance, using a rating scale from 0 (no growth) to 5 (>70% cover).

5.5.5 Wet-state preservation (PT 6, 11, 12, 13)

Challenge tests are generally employed for preservatives which shall preserve liquid matrices, dispersions or fluids used in systems. The inoculum used and the strength of the inoculum depends on which claim shall be supported. For preservation claims, growth needs to be shown in the untreated samples and prevention of growth in the treated samples. A larger population (generated by prior growth in an untreated matrix) may be more appropriate for demonstrating a curative effect. Some methods for wet-state preservation are compiled in Appendix 6.

A series of concentrations of the active substance or the biocidal product should be employed in order to investigate which concentration achieves which level of efficacy. It is likely that the application rate in practice will vary depending on the in-use conditions of a biocidal product even though the matrix is identical, e.g. in a metal working fluid, where the in-use concentration is achieved by diluting the product at the point of use.

5.5.5.1 PT 6

The broad group of wet-state preservatives for the purpose of storage prior to use has been divided into the sub-categories and sub-scenarios:

PT6.1 Washing and cleaning fluids and human hygienic products

6.1.1 Washing and cleaning fluids (human hygienic products)

6.1.2 Washing and cleaning fluids (general) and other detergents

PT6.2 Paints and Coatings (PN)

PT6.3 Fluids used in paper, textile and leather production (P)

6.3.1 Fluids used in paper production (Bulk raw materials in storage)

6.3.2 Fluids used in textile production (Bulk raw materials in storage)6.3.3 Fluids used in leather production (Bulk raw materials in storage)

PT6.4 Metal working fluid

6.4.1 Lubricants (P)

6.4.2 Machine oils (P)

PT6.5 Fuel

PT6.6. Glues and Adhesives

PT6.7 Mineral slurries and other matrices

Each of these sub-scenarios can be tested as described above. This can be summarised as follows.

- A relevant matrix must be chosen according to the intended use. This matrix should be selected in a way that it can easily support growth if no biocide is present. A reasonably high water content and organic matter (either from the matrix itself or added as a soiling agent) will allow for growth.
- If available, a standard that covers the matrix must be chosen (e.g. for glues you might choose ASTM standard D 4783). From this test protocol the test organisms, the method of cultivating the test organisms, duration of the incubation, incubation temperature, etc. can be extracted and integrated into a test protocol that follows the principles outlined above (e.g. by reducing the size of the inoculum).

Examples for test protocols that follow these principles are:

- A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Polymer Dispersions, IBRG[5] PD16/001/1.01 (February 2016);
- A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Paints, (IBRG[2] P16/001/1.01 (February 2016));
- Tier 1 Basic Efficacy Method for Biocidal Active Substances used to Preserve Aqueous-Based Products, (IBRG[2], IBRG PDG16/007/1.1)

These documents describe methods for determining the basic efficacy of biocidal active substances in an aqueous based matrix and are intended for the generation of tier 1 data. For tier 2, the impact of additional factors like temperature and chemical stability etc., depending on the claim, would need to be tested.

When a claim of an active is to reduce bacterial growth, all 3 methods work according to the same principles, but differ in the bacteria used as they are specific to the matrix and the strength of the inoculum (also refer to 5.5.4.1). When the active substance also claims to reduce fungal growth, it will be necessary to differentiate between unicellular yeasts and filamentous fungi as yeasts can be counted as colony forming units, whereas filamentous fungi cannot (also refer to 5.5.4.2).

The filamentous fungus *Geotrichum candidum* is an organism that forms filamentous chains of fragmented cells. These are special in so far as they disintegrate easily into single arthrospores. Enumeration of growth of this fungus can therefore be performed in the same way as for unicellular yeasts. Details for culturing this fungus are given in method ii (Paints).

Whereas methods i) deals with polymer dispersions and ii) deal with paints, the efficacy of preservatives in all other matrices in PT 6 are at this point tested according to a

[5] International biodeterioration research group (IBRG): www.ibrg.org

generic method shown under iii) above. It provides a unified approach and is for use with those materials that do not (yet) have a specific method available (e.g. surfactants, cleaning products, mineral slurries etc.). It is designed to satisfy the basic requirements described in this document. As with the above tests, it is based on a challenge test (multiple inoculations at weekly intervals) and has the same basic requirements.

5.5.5.2 PT 13

PT 13 deals with preservatives for metal working fluids during their use in industrial processes. IBRG[6] developed a method that allows to test the efficacy of active substances in a model matrix ("A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Metal Working Fluids for their Protection in Use, IBRG[3] FFG 16/001/1.02 (January 2016).

5.5.6 Curative Treatments (PT 6, 7, 11, 12, 13)

Suspension tests are generally employed for curative treatments of liquid matrices, dispersions or systems. A curative treatment might be applied to a system to reduce a population prior to employing a maintenance regime / treatment (e.g. PTs 11, 12 and 13) or it might be used prior to the addition of a preservative in either a final product, intermediate or a raw material (e.g. PT 6). A model matrix that has been inoculated with microorganisms appropriate to the claim to achieve either growth or a stable population shall be treated with the active substance / biocidal product and the effect measured after an appropriate contact time using a dilution plate count (methods described for wet state preservation can be employed to generate the model contaminated matrices / systems). The inoculum can comprise of aerobic or anaerobic bacteria, endospore forming bacteria, yeasts, fungal spores and / or mycelial growth as appropriate to the claim. A log-reduction relevant to the matrix and its use needs to be shown in the treated samples. Viability / growth should be shown to be maintained in the untreated samples. Replicate sub-samples shall be employed (minimum of 3) and any differences that result should be shown to be statistically significant. Data from samples treated under field conditions can be used as supporting evidence provided that any effects shown can be attributed to the treatment applied.

5.5.7 Protection of solid material: PT 7, 8, 9, 10

This section describes the nature and extent of data which should be made available to support the label claims for biocidal products within PT 7 through PT 10. The common denominator of these PTs is that they concern the treatment of solid material where use conditions can vary considerably, depending on the site and type of use of the material (e.g. treated wood to be used in constant contact with water compared to use in dry conditions; a film preservative to protect a bathroom sealant compared to protecting a house-façade). In contrast to liquid disinfectants or preservatives belonging to PTs 11, 12 and 13, where application often takes place on-site (that is where the target organisms occur), the treatment of materials can take place anywhere, for example where the material is manufactured or at a specific-treatment site. This may not necessarily be within the EU.

Use conditions are much more variable for these product types than they are for liquid disinfectants and liquid preservatives. Often, many different materials can be treated with the same biocide, and even more different articles can be manufactured from the treated materials, which are used in a wide variety of conditions. For instance, water absorption properties of different polymer materials vary and so does the release of the biocide. The concentration of the biocide has to be adapted accordingly. Biocides can be applied as a coating to fabrics or can be incorporated into the material by adding the

[6] International biodeterioration research group (IBRG): www.ibrg.org

biocide to the polymer before spinning or extrusion. This alters the fixation in or on the material and has an impact on performance. Materials and articles can be used indoors, outdoors, in wet, humid or dry conditions and at varying temperatures. All of this has an impact on performance. Simulating service life, as length, weathering conditions, temperature, leaching, laundering, etc. is crucial for testing of products within these PTs. Thus, efficacy testing for PT 7 through 10 requires a good description of the frame in which the biocide shall perform. In many cases it will be impossible to test every material/substance combination; it might be feasible, however, to categorize different parameters: material, concentrations ranges, use (outdoor, indoor, temperature, humidity, use for load-bearing components, etc.) and to try to test representative, preferably worst-case, examples for every category. It is important though, to describe and justify which range the tested sample represents.

5.5.7.1 Model matrices

The array of possible material and biocide combinations is vast and phenomena observed in practice cannot always be reproduced in the laboratory. A model matrix has to be chosen which represents a certain type of material and which is relevant to the intended use. For example, plasticised PVC and polyurethane would be useful models for rigid or semi-rigid polymers and a room temperature vulcanised silicone would provide a useful model of a sealant etc. Relevance is the key factor. Thus, if a treatment is intended to protect natural fibres in service then a natural fibre should be employed as the model. When more than one type of material (*e.g.* plastics, paints and synthetic fibres) can be protected by the biocide, then representative matrices that demonstrate the range of protection should be employed. Different materials can require different biocide concentrations due to varying release behaviour. It is also important to consider what the purpose of the end use is (*e.g.* in one application the biocide may provide essential protection of a matrix whereas in another it may increase durability). The objective is to in any case to support the claims made.

5.5.7.2 Representative species

The species employed in any test should be relevant to the intended use (*i.e.* fungi should be employed if the material is affected by fungal growth, bacteria to be found on the skin should be employed for odour testing, etc.). Consortia rather than individual species should be employed (although mixing bacteria with fungi, algae *etc.* should, in general, be avoided, see 5.5.2). The species employed in the tests should be relevant to the material under investigation especially where the prevention of the degradation of a material is intended. In many cases the organisms will be specified with the method. Very limited ranges of model organisms should be avoided where possible (*e.g.* the use of *A. brasiliensis* as the sole fungus). The test should include replicates (at least three) for both the treated and untreated variants.

Table 9: Examples

Claim	PT	Example Problem	Example Method
Fungicide is used to treat paint to prevent mould growth in service	7	Painted panels exposed to weather become stained by mould growth and have to be re-painted more often.	BS 3900 Part G6 Painted panels inoculated with a mixture of spores of fungi known to colonise paints exposed to humid conditions for up to 12 weeks should show visual appearance of fungal growth. The treated sample

Claim	PT	Example Problem	Example Method
			should be free of it.
	8		
Fungicide is used to treat paper goods to prevent mould growth in service.	9	Labels used on wine and beer bottles become degraded and stained by fungi and difficult to read when stored in cellars and cool stores.	ASTM D 2020-03 Samples of untreated material should demonstrate a high susceptibility to fungal growth in the test. Treated samples should be free of growth.
Biocide with fungicidal and bactericidal properties is used to protect PVC sheet materials from spoilage and degradation in service	9	PVC sheet flooring used on solid floors can become colonised by bacteria and fungi on its under surface. This causes staining, cracking and detachment from the substrate.	ISO 846 Parts A and C. Samples of untreated material should support bacterial and fungal growth. Treated material should be free of growth.
	10		

5.5.7.3 PT 7 and PT 9 Material protection

Uses within PT 7 and 9 often overlap. Sometimes, PT 7 and 9 differ only in the manner of application: the biocide can be applied as a coating layer onto the material or it can be incorporated into the material. Thus, the described requirements and principles apply in the same way to both PTs.

When selecting the appropriate method, consideration must be given to the release mode characteristics of a particular biocide/material combination. Some biocides have a very low solubility in water and hence are emitted at a very low rate from a matrix. This may be sufficient to protect a material that is inherently highly susceptible and which microorganisms may penetrate and colonise. However, if a test (*e.g.* ISO 16869) relies on the emission of the biocide from the matrix into an agar layer to measure the effect, it would indicate that such a biocide has no function. Other materials, which are damaged by growth on their surface (especially where soiling is present) due to the production of extracellular enzymes, may fail to be protected by a biocide with such a low emission rate. Thus, the choice of method will be highly dependent on the characteristics of the material as well as the biocide. The applicant should justify this for the product under evaluation.

5.5.7.3.1 Simulation Tests

The ideal test method would present a material to a consortium of relevant test organisms under conditions that simulate real life realistically. This would produce effects that are identical to those observed in practice and allow a treatment to be identified with precision. There are methods that come closer to this ideal than others. For example, BS 3900 Part G6 (Appendix 7) exposes painted panels that have been inoculated with a mixture of spores of fungi known to colonise paints to humid conditions, free of external nutrients (although these can be added with the inoculum if necessary) for up to 12 weeks (see Figure 7). The resulting growth on untreated coatings has a visual appearance very similar to that observed in practice and pre-

exposure, leaching or artificial weathering can be used to help explore service life. A comparison can be made between treated and untreated variants of a formulation. A similar test, that forms the basis of many of the military standards and specifications, is EN 60068-2-10:2005/BS 2011-2.1J: 1989 (see Appendix 7); this test is applicable to a wider range of materials. Again, samples are inoculated and incubated under conditions intended to simulate real life or at least be optimal for fungal growth.

Figure 7: Example of a Simulated Growth Test

BS 3900 Part G6, Method Overview:

Replicate sub-samples of both treated and untreated variants of each coating are sprayed with a suspension of spores of a range of fungi known to colonise surface coatings. The samples are then transferred to a humid chamber and incubated for up to 12 weeks. The extent of growth is assessed using a rating scale and this, as well as photographs of the panels, are presented as the results.

Rating scale: 0 = no growth, 1 = trace to 1% cover, 2 = 1 - 10% cover, 3 = 10 - 30% cover, 4 = 30 - 70% cover and 5 = > 70% cover

There is no pass/fail criterion in the standard but many workers in the coatings industry consider that growth represented by a rating of 2 is the maximum that would normally be tolerated. An example of growth on an untreated coating is shown on the left.

Example for growth level 5.

Modifications of these methods have been made to allow them to study the effects on algae (the IBRG algal test method for surface coatings) and, less commonly, bacteria. Effectiveness is assessed in these tests by visual appearance, measuring loss of weight or determining changes in the physical properties of the material (*e.g.* resistance to bending or extension under load). As with all biological tests, some degree of replication will be essential and tests should employ, as a minimum, three replicate sub-samples of each variant. Simulation tests are indeed very useful and provide valuable information especially for specific material/biocide combinations and can be correlated in some cases to service expectations. However, they can take a long time to perform and, in many cases, need to be adapted in some manner to accommodate a specific material.

5.5.7.3.2 Tests based on artificial growth media

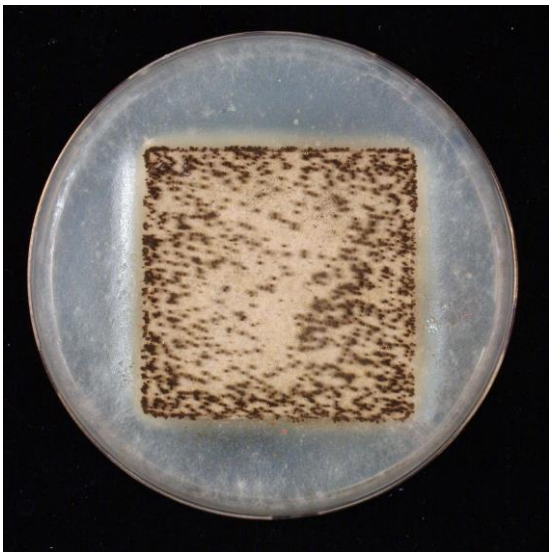
By far the most commonly used methods for studying the performance of biocides intended to protect materials are those based on artificial growth media such as agar plates. For example, both ISO 846: 1997 and ASTM G21-09 are used widely in the plastics industry to measure the performance of fungicides in formulations (also ISO 16869: 2008, although its adoption has been relatively slow to date). ISO 846 allows for studies into the susceptibility of plastic formulations to fungal and bacterial deterioration by attempting to make the plastic the sole source of nutrients for the organisms used, as well as providing a variant that provides an external source. It also includes a service life simulation test variant in which samples are buried in soil and then examined for loss of weight and strength (extremely useful in industries manufacturing pipes and cables). Although making the plastic the sole source of nutrients might seem like the ideal way to examine the ability of a biocide to protect the material, in many instances it is the presence of soiling that leads to colonisation and subsequent damage to the polymer (sometimes referred to as bio-corrosion). Thus, for certain polymers, the presence of external nutrients is essential in determining the efficacy of a biocide. In many instances a consortium of organisms is required to effect colonisation and deterioration of the material and, in general, methods that employ consortia should be selected.

Similar testing technologies as those used for plastics exist for certain textiles, paper and surface coatings. The most common are listed in Appendix 7 and a description of the basic principles involved is given in Figure 8 using ASTM G21 as an example.

The huge disadvantage of agar-plate based tests is the interference of the growth medium with the biocide. The biocide can diffuse into the agar, demonstrating an effect there but at the same time be diluted in the original matrix. A less soluble substance, which does not diffuse into the agar, may in contrast show a false negative effect. For these reasons, a simulation test is always to be preferred over an agar-plate based test.

Figure 8: An Example of an Agar Plate Based Test

ASTM G21, Method Outline:



Replicate samples of both treated and untreated material are embedded in a mineral salts-based agar medium. The sample and surrounding agar are then inoculated with the spores of a mixture of fungal species known to colonise plastics. The plates are then placed into chambers in which the humidity is maintained at > 85% RH for up to 28 days. The samples are then inspected for the presence of fungal growth. Typical growth on an untreated material is shown in the plate on the left.

Growth on Untreated Plastic

5.5.7.3.3 Tier 2 Testing

Depending on the intended use, pre-exposure, leaching or artificial weathering can be used to help explore service life. The relevance of the chosen parameters should be explained. There are no special tests or designs available for tier 2 testing. Basically, the same methods as in tier 1 can be applied except that the tested material undergoes pre-treatment. In some cases, ageing norms can be employed (e.g. adaptations of EN 73:2014[7], EN 84:1997[8], which are both developed for treated wood). In other cases, variations of the tier 1 methods can be used (as for example the soil burial variant of ISO 846 as described above). It is particularly important to show growth or damage on the untreated material under service-life conditions.

In some cases it may not be necessary to use an artificial inoculum for tier 2 tests. It may be possible to use a test medium colonised naturally so that it is representative of the organisms that are typically encountered during the use of the product. It may be valid to use lower levels of contamination such as those encountered in practice. In some cases there may be a need to include application-related test-organisms in addition to standard test-organisms. In any case, the applicant should provide a rationale as to why the test organisms are relevant for the respective application/s of the preservative.

[7] Accelerated ageing test of treated wood prior to biological testing. Evaporative ageing procedure
[8] Accelerated ageing tests of treated wood prior to biological testing. Leaching procedure

5.5.7.3.4 Tier 3 Testing

In some cases, tier 3 testing might be needed to support specific claims. These can be field tests where treated materials are compared to untreated materials in use. For example, treated house facades could be compared to untreated house facades in the same area and the time until re-painting is needed could be measured. Likewise, the replacement time for untreated buried cables compared to treated ones can be studied in a field test. Care has to be taken that the conditions for the treated and untreated materials are the same or at least comparable and that other parameters than the parameters observed are not influencing the results. The validity of the conclusions may need to be reinforced by statistical analysis etc, especially if any differences observed are small.

Table 10: Basic Requirements for a Valid Test Protection

The following summary provides a guide to the basic requirements for a valid test:

i. A relevant model matrix should be chosen to represent the material(s) which shall be protected
ii. Relevant use conditions should be chosen in terms of humidity and temperature
iii. An untreated variant of the test material must be included and show the pattern of growth/deterioration that the biocide is intended to prevent at the end of the test.
iv. The test should employ organisms that are relevant to the material/problem being addressed.
v. Tests that employ a consortium of organisms should be favoured over those that use single species.
vi. A minimum of three replicate test pieces of both treated and untreated materials should be employed.
vii. The final data should include either some indication of the impact of service conditions on the performance of the treated material/article or data from an ageing.

5.5.7.4 PT 9: Prevention of Odour by odour-producing microorganisms

With most of the biocidal functions within PT 7 and 9, test conditions simulate in-use conditions rather well and the effects of microbial growth or activity can be observed quite easily. With the control of odour, this is much harder to achieve in a laboratory test, as odour often cannot be measured in a simple manner.

Laboratory tests to simulate odour production are currently not available, though some work is done to develop such tests (for example a test to inhibit the bioconversion of L-leucine to iso-valeric acid, representing a dominant compound of foot-odour). Thus, at present, the prevention of odour is in most cases measured indirectly by measuring microbial inhibition.

There are two major types of test that have traditionally been used with textiles (and related materials). The first major group employs agar plates and the other major group uses suspension in an aqueous medium. In both cases, the impact of a treated textile on populations of (usually) bacteria are studied. An overview is given in Appendix 8.

5.5.7.4.1 Agar plate-based tests

Agar plate-based tests are not recommended. These tests have almost no useful utility in measuring effects intended to control odour in textiles. Such tests rely on the biocide migrating from the textile into the agar medium at sufficient concentration to inhibit the growth of bacteria either seeded into the agar or placed onto it (see Figure 8). The

diffusion characteristics vary hugely from one biocide to another and from one textile to another and the growth medium itself presents a large soiling load to be overcome by the biocide. Larger areas clear of growth are often associated with more potent effects but they could be attributed equally to differences in the leaching rate of a biocide from a material.

5.5.7.4.2 Suspension tests

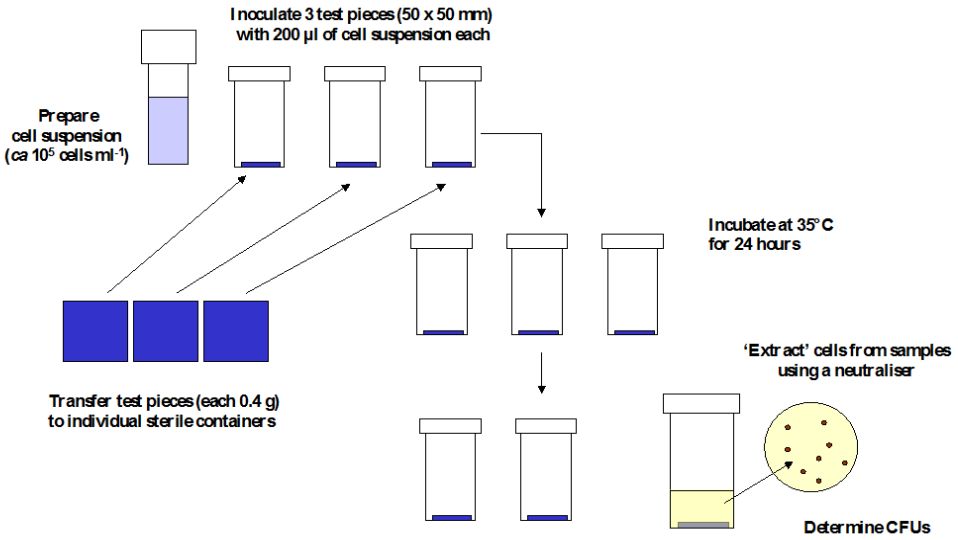
The second major group, the suspension tests, measure changes in the size of a population following contact with a treated textile. A number of protocols are described in Appendix 8. However, most employ relatively high concentrations of nutrients in the suspending medium so that their application, like the agar diffusion methods, can lead to over-treatment of textiles. Thus, these methods should not be used. By using lower concentrations of nutrients in the suspending medium and using pre-treatments such as laundering, these methods can be adapted for use in measuring effects on odour. Such an adaptation has been applied in the OECD Tier 1 method for treated articles (porous materials[9]) and the IBRG Textile Method[10]. These are described schematically in Figure 9 and are based on the 'germ' count or absorption phase of ISO 20743: 2007 (Appendix 8) where the amount of nutrients present in the cell suspension has been reduced substantially.

Many treated materials would certainly be capable of demonstrating activity in a suspension test. Activity against a consortium of bacteria (e.g. against a range of Gram Positive and Gram Negative bacterial species such as *Staphylococcus epidermidis*, *Corynebacterium xerosis*, *Proteus vulgaris, Escherichia coli, etc.*) would probably inhibit the production of odour. However, excess exposure of the skin of the wearer should be minimized as far as possible. Therefore, tests adapted to textile treatments such as the OECD Tier 1 method and the IBRG Textile method (Figure 9) are preferable.

[9] OECD (OECD ENV/JM/MONO(2014)18: Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials.

[10] IBRG, International Biodeterioration Research Group (2013): Quantitative Method for Evaluating Bactericidal Activity of Textiles and Porous Materials and Articles. IBRG TEX/13/005 (www.ibrg.org).

Figure 9: OECD/IBRG Tier 1 Textile Test



5.5.7.4.3 Tier 2 testing

In many cases, a large fraction of the active substance incorporated in a textile is lost during laundering, either through emission of loosely or only partially bound material or associated with loss of fibres (lint). This also means that there is potential for active substances to be transferred from treated materials to non-treated materials when laundered together. In general, the emission rate is rarely continuous either to the environment or to the wearer. Moreover, other chemicals from the textile treatment as well as chemicals used in the laundering process might interfere with the function of the biocide.

In general, the effects required to prevent the formation of odour in shoes and apparel are subtle. The greatest demand on them is usually in maintaining activity following multiple laundering cycles. Therefore, simulation of service life conditions by laundering and ageing are essential. Care must be taken to maintain the functionality and to minimise excess exposure of the environment through emissions of the biocide in use, during cleaning and at the time of disposal. The method described in Figure 9 (as well as

chemical analysis) in combination with laundering cycles can be useful in measuring the maintenance of efficacy in service.

An active substance or a biocidal product is often intended to treat a wide range and mix of textile types with a wide variety of anticipated demands and expectations of durability. It might be difficult to address every potential combination and garment type. However, studies on typical textile blends could be used to provide appropriate efficacy. Some examples are given in Table 2 below.

5.5.7.4.4 Tier 3 testing

At present the only truly reliable methods for demonstrating anti-odour functionality is through replicated and statistically designed wearing trials. Tier 1 and 2 tests described above can provide useful data related to durability *etc.* but care must be taken when interpreting the data they produce. For example, a treatment may be applied to only certain parts of a garment or shoe or it may be present on only a certain number of filaments in the weave of a textile. In the bioassay, the inoculum is dispersed throughout the whole of the sub-sample of textile and any active substance released would be able to migrate throughout that inoculum whereas in use, this may not occur. The humidity produced by bodily excretions might trigger less release of the biocide than the liquid suspension the textile is covered with in the test. The bacterial populations present on the skin might be less affected by the biocide as compared to the testing consortium employed. Consequently, to reliably prove anti-odour effects, user trials have to be carried out. A standard with human assessors which could possibly be adapted to test anti-odour claims is EN 13725.

Table 11: Odour: Example Claims, Problems and Testing Approaches

Claim	PT	Proof Required	Example Method
Carpet is treated to prevent odours caused by mould growth.	9	Data should show that the treated carpet does not support fungal growth whereas the untreated one does.	A method such as AATCC 174 can be used to demonstrate resistance to fungal growth. For active substances that do not migrate from the fibres/backing a cabinet-based simulation test may be more appropriate.
		The effect should be shown to be sufficiently durable.	Activity should be shown to persist following simulated ageing.

A sports vest is treated to inhibit the production of odour.	9	Data from a field trial should show that odour is reduced in treated sports shirts when compared with untreated ones. The effect should be shown to be of sufficient durability during service life to match any claim made.	Wearing trial or scientifically valid odour based simulation study. A comparison of the effectiveness both before and after simulated ageing/washing should be performed. This could be performed either through field trials, simulation tests or the use of a test such as the OECD Tier 1 method. The latter could be used to demonstrate that sufficient activity is still present after washing/ageing to elicit an antimicrobial effect.

1

2

5.5.8 PT6

The text for this section is under development and will be added at a future update.

5.5.9 PT7

The text for this section is under development and will be added at a future update.

5.5.10 PT8

NOTE FOR PEG CONSULTATION

The text for this section has been published as a Transitional Guidance (March 2015). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_effifacy_wood_preservatives_pt8_en.pdf]

5.5.11 PT9

The text for this section is under development and will be added at a future update.

5.5.12 PT10

The text for this section is under development and will be added at a future update.

5.5.13 PT11

The text for this section is under development and will be added at a future update.

5.5.14 PT12

The text for this section is under development and will be added at a future update.

5.5.15 PT13

The text for this section is under development and will be added at a future update.

5.6 Pest Control

5.6.1 General

The text for this section is under development and will be added at a future update.

5.6.2 PT14

NOTE FOR PEG CONSULTATION

The text for this section is under development and will be published as a Transitional Guidance (foreseen for November/December 2016). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

5.6.3 PTs 15, 16 & 17

The text for this section is under development and will be added at a future update.

5.6.4 PT18 + PT 19 on arthropods

NOTE FOR PEG CONSULTATION

The text for this section is ready for publication as a Transitional Guidance (September 2016). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The document will be available on the ECHA website under Transitional Guidance: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/transitional-guidance]

5.6.5 PT19 other non-arthropods

The text for this section is under development and will be added at a future update.

5.6.6 PT20

The text for this section is under development and will be added at a future update.

5.7 Other biocidal products

5.7.1 PT21

NOTE FOR PEG CONSULTATION

The text for this section has been published as a Transitional Guidance (May 2014). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_pt_21_en.pdf]

5.7.2 PT22

NOTE FOR PEG CONSULTATION

The text for this section has been published as a Transitional Guidance (August 2014). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_pt_22_en.pdf]

NOTE FOR PEG CONSULTATION

The text for Appendices 1-5 is published in a Transitional Guidance (May 2016). The text is out of scope of this consultation. The published text will be incorporated into this document after the PEG consultation.

The TG document is available on the ECHA website: [https://echa.europa.eu/documents/10162/15623299/biocides_transitional_guidance_efficacy_disinfectants_pt1-5_en.pdf]

Appendix 1. Claims Matrices

Add appendix 1 from TG PT1-5

Appendix 2. Standards and testing methods for efficacy-testing of disinfectant biocidal products (PT 1-5)

Add appendix 2 from TG PT1-5

Appendix 3. Table of Reference Test Organisms

Add appendix 3 from TG PT1-5

Appendix 4. Overview of standards, test conditions and pass criteria

Add appendix 4 from TG PT1-5

Appendix 5. Examples of viruses sorted according to their presence in the human body in case of virus infection

Add appendix 5 from TG PT1-5

Appendix 6. Methods to Measure the Effects of Preservative/Curative Action in Liquid Matrices

Reference	Title	Description	PT
ASTM D2574-06	Standard Test Method for Resistance of Emulsion Paints in the Container to Attack by Microorganisms	This test method covers the determination of the relative resistance of emulsion paints to attack in the container by microorganisms.	6
ASTM D4783-01e1	Standard Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi	Determination of the resistance of liquid adhesive preparations to microbial attack in the container by challenging adhesive specimens with cultures of bacteria, yeast, or fungi, and checking for their ability to return to sterility. These test methods return qualitative results.	6
ASTM E1259-05	Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C	The procedure should be used to evaluate the relative efficacy of microbicides in liquid fuels boiling below 390°C. The effect of environmental conditions, such as a variety of fuel additives, metal surfaces, and climatology, are variables that can be included in specific tests using this protocol.	6
SABS 1102 (1987)	Bacterial efficacy of biocides used in water-based emulsion paints	Efficacy test for in can preservatives in paints (emulsion) against bacteria.	6
NF X41-520 March 1968	Protection. Testing method for resistance of paints to microorganisms and their protective power.		6
ASTM E2275-03e1 (replaces D3946 and E686)	Standard Practice for Evaluating Water-Miscible Metalworking Fluid. Bioresistance and Antimicrobial Pesticide Performance	Laboratory procedures for rating the relative inherent bioresistance of water-miscible metalworking fluids, the bioresistance attributable to augmentation with antimicrobial pesticides or both, for determining the need for microbicide addition prior to or during fluid use in metalworking systems and for evaluating microbicide performance. Relative bioresistance is determined by challenging metalworking fluids with a biological inoculum that may either be characterized (comprised of one or more known biological cultures) or uncharacterized (comprised of biologically contaminated metalworking fluid or one or more unidentified isolates from deteriorated metalworking fluid). Challenged fluid bioresistance is defined in terms of resistance to biomass increase, viable cell recovery increase, chemical property change, physical	13

Reference	Title	Description	PT
		property change or some combination thereof. This practice is applicable to antimicrobial agents that are incorporated into either the metalworking fluid concentrate or end-use dilution. It is also applicable to metalworking fluids that are formulated using non-microbicidal, inherently bioresistant components. The results of tests completed in accordance with this practice should be used only to compare the relative performance of products or microbicide treatments included in a test series. Results should not be construed as predicting actual field performance.	
ASTM E979-91(2004)	Standard Test Method for Evaluation of Antimicrobial Agents as Preservatives for Invert Emulsion and Other Water Containing Hydraulic Fluids	This laboratory test method is designed to utility and effectiveness of antimicrobial ag to control microbial growth in invert emulsi water containing hydraulic fluids.	13
ASTM WK8252	New Standard Test Method for Determining Resistance of Aqueous Metalworking Fluids towards Non-Tuberculous, Environmental Mycobacteria	Determines the relative bioresistance of aqueous metalworking fluids towards non-tuberculous (NTM), rapidly growing (RGM), environmental mycobacteria by challenging them with a mycobacterial inoculum isolated from actual spoiled metalworking fluid field samples from the user/s site. In order to simulate field conditions, another challenge inoculum consisting of a mixture of common metalworking fluid spoilage microorganisms originating from actual MWF field samples is also used	13
SABS 1435-1987	South African standard specification for biocides for use in emulsions of aqueous metal working fluid and aqueous hydraulic fluid.		13
Rawlinson and Shennan, 1987.	A recirculating test rig for the investigation of metal-working fluid spoilage. In Industrial microbiological testing 1987 pp. 227-231. Edited by Hopton and, J.W.; Hill, E.C.	The method described, which attempts to simulate the conditions under which a metal working fluid will be used in service, has been used extensively for the testing of new product formulations and the evaluation of biocides.	13
UK MOD 91-70 issue (1990)	Cutting fluid, soluble, biostable joint service designation ZX-9		13

1

2

Appendix 7. Methods to Measure the Effects of Protecting Material

Table I: Methods used to Examine the Resistance of Porous Materials to Biodeterioration: Textiles

Reference	Title	Description	Major Principle/Use
EN 14119:2003	Testing of textiles –Evaluation of the action of microfungi	The test is designed to determine the susceptibility of textiles to fungal growth. Assessment is by visual rating and measurement of tensile strength.	Agar plate test
AATCC 30-2004	Antifungal activity, Assessment on textile materials: mildew and rot resistance of textile materials	The two purposes of the test are to determine the susceptibility of textiles to microfungi and to evaluate the efficacy of fungicides on textiles.	Agar plate test
DIN 53931	Testing of textiles; determination of resistance of textiles to mildew; growth test	The test determines the efficacy of treatments for prevention of fungal growth on/in textiles. It also allows the performance testing of a treatment after UV irradiation , leaching *etc*.	Agar plate test
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of that material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 6085 :1992	Determination of the resistance of textiles to microbial deterioration	The purpose of the method is to assess the extent to which a material will support fungal/bacterial growth and how performance of the material is affected by such growth. Visual Assessment and measurement of tensile strength.	a) soil burial test; b) agar plate test, c) humid chamber test
EN ISO 11721-1 (2001)	Textiles - Determination of resistance of cellulose-containing textiles to micro-organisms: Soil burial test Part 1: Assessment of rot retarding finishing	The test is designed to determine the susceptibility of cellulose containing textiles against deterioration by soil micro-organisms. Preserved and unpreserved textiles are compared. Visual Assessment and measurement of tensile strength.	Soil burial test

EN ISO 11721-2 (2003)	Textiles - Determination of resistance of cellulose-containing textiles to micro-organisms: Soil burial test Part 2: Identification of long-term resistance of a rot retardant finish	The test identifies the long-term resistance of a rot-retardant finish against the attack of soil inhabiting micro-organisms. It allows to make a distinction between regular long-term resistance and increased long-term resistance. Visual Assessment and measurement of tensile strength	Soil burial test
BS 2011 : Part 2.1J (IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards colonization by fungi.	Humid chamber test (90 to 99% humidity)
AS 1157.2 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 1 - Resistance to Surface Mould Growth.	Test specimens are inoculated with a suspension of spores of *Aspergillus niger* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Specimens are examined for the presence of surface mould growth.	Agar plate test
AS 1157.4 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 2 - Resistance to Cellulolytic Fungi.	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.3 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Cordage and Yarns to Fungal Growth.	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test.	Agar plate test (other vessels containing media are employed for large specimens).

Table II: Methods used to Examine the Resistance to Biodeterioration: Geotextile			
Reference	**Title**	**Description**	**Major Principle**
EN 12225:2000	Geotextiles and Geotextiles-related products - Method for determining the microbiological resistance by a soil burial test	The test is designed to determine the susceptibility of geotextiles and related products to deterioration by soil micro-organisms. Visual Assessment and measurement of tensile strength.	Soil burial test

1

Table III: Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Paper *etc.*			
Reference	**Title**	**Description**	**Major Principle**
DIN EN 1104 - 05	Paper and board intended to come into contact with foodstuffs Determination of transfer of antimicrobic constituents	A minimum of 20 replicate sub-samples (each 10 - 15 mm in diameter) taken from 10 samples of a batch of paper are placed in intimate contact with nutrient agar plates inoculated with either *Bacillus subtilis* or *Aspergillus niger* and incubated at 30° C for 7 days and at 25° C for 8 - 10 days respectively.	Zone Diffusion Assay.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Direct Inoculation	Replicate samples (3) are inoculated with a suspension of fungal spores and then incubated on the surface of a minimal mineral-salts medium to determine if they support fungal growth.	Biodeterioration Test.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Soil Burial	Replicate samples (5) are buried in soil for 14 days and then examined for the deterioration compared with unburied samples for both physical deterioration and loss of tensile strength.	Biodeterioration/ Biodegredadation Test.
AS 1157.7 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Papers and Paper Products to	Test specimens are placed on the surface of a mineral-salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test

	Fungal Growth.		
AS 1157.5 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 5: Resistance of Timber to Fungal Growth.	Test specimens are placed on the surface of a mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test
AS 1157.6 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Leather and Wet 'Blue' Hides to Fungal Growth.	Test specimens are placed on the surface of a mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Growth on specimens is assessed. Sucrose containing media is employed where true controls cannot be obtained.	Agar plate test

1

Table IV: Methods used to Examine the Resistance to Biodeterioration: Plastics

Reference	Title	Description	Major Principle
ASTM D 5338 - 92	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Biodegradability test
ASTM E 1428 - 99	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Agar plate test
ASTM G 22 - 76	Agar plate test	Agar plate test	Agar plate test
ASTM G 21 - 96	Agar plate test	Agar plate test	Agar plate test
ASTM G 29 - 96	Agar plate test	Agar plate test	Biofouling test
EN 14047:2002	Agar plate test	Agar plate test	Biodegradability test
EN 14048:2002	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Biodegradability test

ISO 846:1997	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Agar plate test; soil burial test
EUROCAE ED-14B/ RTCA DO 160B	Agar plate test	Agar plate test	Humid chamber test (90 to 99% humidity)
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of the material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 2011 : Part 2.1J (identical with IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards the colonization by fungi.	Humid chamber test (90 to 99% humidity)
ISO 16869:2008	Plastics - Assessment of the effectiveness of fungistatic compounds in plastics formulations	A specimen is placed on a nutrient-salt- agar (without additional carbon source) in a petri dish and overlayed with the same agar containing fungal spores. Rate of growth on the specimen is visually assessed.	Agar plate test
AS 1157.4 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 4: Resistance of Coated Fabrics and Electronic Boards to Fungal Growth.	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 1: Resistance to Growth	Test specimens are inoculated with a suspension of spores of a range of fungi and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test

AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 2: Fungistatic Properties	Test specimens are placed on the surface of a sucrose, mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Growth on both the specimen and inhibition of growth on the surrounding agar are assessed.	Agar plate test

1

Table V: Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Surface Coatings & Adhesives

Reference	Title	Description	Major Principle
BS3900 Part G6	Assessment of resistance to fungal growth	Replicate test panels coated with the test coating are inoculate with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth (23 ± 2°C and high humidity/surface condensation). In the published standard, condensation on the test panels is achieved by increasing the temperature in a water bath below the samples for short periods of time. Revisions are in progress which may obviate this step. The method is validated if fungal growth/germination of spores is observed after two weeks on a standard coating known to be susceptible to fungal growth. After incubation growth is rated in accordance with a scale related to the percent cover with fungal growth (following visual and microscopical examination). A natural and artificial soiling are described in the method which can be employed when appropriate.	Biodeterioration Test
ASTM D3273-12	Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under	Biodeterioration Test

	in an Environmental Chamber	conditions suitable to support fungal growth.	
WK4201	Standard Test Method for Resistance to Mold Growth on Building Products in an Environmental Chamber	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth.	Biodeterioration Test
ASTM D5590-94	Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay		Agar Plate Test
SS345 Appendix 7	Formal Title Missing at Present	The bottom of glass petri dishes are coated with paint. After drying, a culture of algae in a suitable growth liquid medium is placed into the dish and incubated under conditions suitable for algal growth.	Biodeterioration Test.
EN 15457:2007	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against fungi	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of 4 fungal species selected from a list of 10. The plates are then incubated at 24°C for 21 days and then assessed for growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPD. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay
AS 1157.10 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 10:	Test materials coated onto glass microscope slides are inoculated with a suspension of spores of a range of fungal species and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth.	Agar plate test

	Resistance of Dried or Cured Adhesives to Fungal Growth		
EN 15458:2007	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against algae	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of 3 algal species selected from a list of 5. The plates are then incubated at 23°C under illumination (16 hour day length, 1000 Lux) for 35 days and then assessed for growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPD. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay
VdL RL06	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of *A niger* and *Penicillium funiculosum*. The plates are then incubated at 28°C for 3 weeks and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and 'wet' applications are leached in water prior to testing.	Zone Diffusion Assay/Humid Chamber Test
VdL RL07	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of *Scenedesmus vacuolaris and Stichococcus bacillaris*. The plates are then incubated at 23°C for 3 weeks under illumination (16 hour day length, 1000 Lux) and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and 'wet' applications are leached in water prior to testing.	Zone Diffusion Assay/Humid Chamber Test

1

Table VI: Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile/wadding)

Reference	Title	Description	Major Principle
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Three replicate samples of fabric, yarn or pile/wadding are placed in intimate contact with the surface of agar plates that have been inoculated with a cell suspension of either *Staph aureus* or *K. pneumoniae* and incubated at 37° C for 24 - 48 hours. The presence of and size of any zone of inhibition around the samples is then recorded.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Replicate samples of fabric (6 of the control and 3 of the treated) are inoculated with individual bacterial species (*e.g. Staph. aureus* and *K. pneumoniae*) suspended in a heavily diluted nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is assessed by comparing the size of the initial population in the control with that present following incubation. No neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
EN ISO 20645 - 2004	Textile Fabrics - Determination of the antibacterial activity - Agar plate test (ISO/FDIS 20645:2004)	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *Staph. aureus*, *Escherichia coli* or *K. pneumoniae*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed for growth based on either the presence of a zone of inhibition of > 1 mm or the absence/strength of the growth in the media overlaying the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN 195920	Examination of the Antibacterial Effect of Impregnated Textiles by the Agar Diffusion Method	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *Staph. aureus*	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		or *E coli*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in BS EN ISO 20645 above.	
SN195924	Textile Fabrics - Determination of the Antibacterial Activity: Colony Plate Count Method	Fifteen replicate samples (each replicate is comprised of sufficient specimens of 25 ± 5 mm to absorb 1 ml of test inoculum) are inoculated with cells of either *E. coli* or *Staph. aureus* suspended in a liquid nutrient medium and incubated in sealed bottles for up to 24 hours at 27° C. After 0, 6 and 24 hours, 5 replicate samples are analysed for the size of the viable population present. A neutraliser is employed. An increase of 2 orders of magnitude of the population exposed to a control sample is required to validate the test. The method defines a textile as antibacterial if no more than a specified minimum level of growth is observed after 24 hours in 4 of the 5 replicate groups of samples.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN195921	Textile Fabrics - Determination of Antimycotic Activity: Agar Diffusion Plate Test	Replicate (4) samples of sterilised fabric (25 ± 5 mm diameter) are placed in intimate contact with a solid nutrient medium in a petri dish. Each petri dish has been prepared as a double layer. The first layer consists of 10 ml nutrient agar, the second layer of another 10 ml of the same nutrient agar to which 0.1 ml spore suspension (10^7 ml^{-1}) of either *Candida albicans*, *Aspergillus niger*, *Cladosporium sphaerospermum* or *Trichophyton mentagrophytes* had been added. The plates are then incubated at 28° C either 2 days (*C. albicans*) or 7 days (*A. niger*, *C. sphaerospermum* and *T. mentagrophytes*). The test is valid when control specimens of the same material without biocide, or of a biocide-free standard specified cotton material are fully overgrown. Good antimycotic efficacy is considered to be demonstrated when the specimens show no fungal growth on their surface. The test specifies that both sides of a material have to be tested.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption method	Replicate (6) samples of textile are inoculated with a standardised broth culture of either *Staph. aureus* or *K. pneumoniae* in individual tubes and then incubated at 37° C for 18 - 24 hours in closed containers. Samples are analysed for the presence of viable bacteria both before and after incubation by either total viable count or the determination of total ATP. Samples are sterilised prior to testing and a neutraliser is employed during recovery. The test is validated by growth of ˆ1 order of magnitude during the incubation period.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer method	Replicate (6) samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *Staph. aureus* and *K. pneumoniae* using a 200 g weight for 1 minute. The samples are then removed. Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation under humid conditions at 37° C for 24 hours. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either growth of ˆ 1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	Replicate (6) samples of test material are either *Staph. aureus* and *K. pneumoniae* by 'printing' cells collected on a membrane filter onto their surface in a standardised manner. The samples are then incubated under humid conditions for 18 - 24 hours at 20° C for a specified contact time(s). Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either determining the survival of the inoculum on the control material.	'Dry' inoculum intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.

ISO/FDIS 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 1 - Luminescence Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the ATP concentration associated with the samples. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced concentrations of ATP associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.
ISO/WD 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 2 - Plate Count Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the number of colony forming units. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced numbers of colony forming units associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.

1

Table VII: Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Qualitative Antibacterial Activity	Petri dishes with nutrient media are inoculated with a single, diagonal streak (approx.7.5 cm) of either *Staph. aureus* or *K. pneumoniae*. An unsterilized test specimen (25 mm x 50 mm) is placed in intimate contact and transversely across the inoculum on the agar surface. The plates are then inoculated at 37° C for 18 - 24 hours. The front and back of the carpet are tested separately. After incubation, the plates are inspected for the presence of growth both below the specimens and for any zone of inhibition surrounding the specimens. The test can also be used to test the effect of cleaning	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a simulation of final use of a treated material.

		regimes. An untreated control is optional.	
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antibacterial Activity	Unsterilized specimens of carpet are pre-wetted with either sterile water or a wetting agent before being inoculated with individual suspensions of either *Staph. aureus* or *K. pneumoniae* in either a low or a high nutrient solution. The samples are then incubated in a tightly closed jar at 37° C for a specified contact time. Cells are recovered in 100 ml of a neutraliser after 0 and 6 - 24 hours of incubation. Activity is assessed by comparing the size of the initial population in the control (if used) with that present following incubation. A control is optional. When not employed, viable counts following incubation of the treated specimens alone are considered. The test can also be used to test the effect of cleaning regimes.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabouraud Dextrose Agar are inoculated with 1 ml of a spore suspension of *Aspergillus niger*. Immediately afterwards, specimens (38 mm diameter) of unsterile test material are placed into intimate contact with the agar. An additional 0.2 ml of the same spore suspension is also employed to inoculate the test pieces directly. The samples are then incubated at 28°C for 7 days. The back and front of the discs of carpet are tested in separate dishes. The zone of inhibition and the growth of fungus on the upper surface of the specimens are reported (no growth, microscopic growth, macroscopic growth). The test can also be used to test the effect of cleaning regimes.	Zone diffusion test/surface growth test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
WIRA Test F	Test Method for Assessing the Survival of Test Organisms on Floor Coverings	Specimens (850 mm x 350 mm) are conditioned at 20°C and 65% RH before being subjected to 2 wet and 2 dry passes using a commercial spray extraction machine or a test rig. After 24 h drying, 12 specimens (each 60 mm diameter) are cut from the carpet. An aliquot (1 ml) of a suspension of cells of *E. coli* in nutrient broth is poured onto filter paper (7 cm	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

		diameter). The filter paper is then pressed for 1 min onto the surface of the carpet using a 1 kg weight. The filter paper is then discarded. After 0, 6 and 24 hours incubation at a specified temperature the carpet´s surface is pressed onto contact plates of McConkey agar. After 24h replicate (3) plugs (10 mm) are taken from each specimen and suspended in 10 ml nutrient broth for 30 seconds and then analysed for the presence of *E. coli* by total viable count.	

1

Table VIII: Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2000	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate sample (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either *E. coli* or *Staph. aureus* in a highly diluted nutrient broth. The cell suspension is then held in intimate contact with the surface by the use of a sterile polyethylene film (usually 40 mm x 40 mm) for 24 hours at 35° C under humid conditions. The size of the population on the treated surface is then compared with the size on the control surface both prior to and after incubation. A neutraliser for certain biocide types is employed. Antibacterial activity is certified if the difference between the Log_{10} of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 22196:2011	Plastics - Measurement of antibacterial activity on plastics surfaces.	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan. Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes -	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *Staph. aureus* and *K. pneumoniae*	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of

	Caractérisation et mesure de l'activité antibactérienne	using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	a treated material.
ASTM E2180-07	Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials	Replicate (3) samples of material are inoculated with cells of either *Staph. aureus* or *K. pneummoniae* suspended in molten semi-solid isotonic saline/agar. This attempts for form an 'artificial biofilm' which holds the suspension in intimate contact with the test surface of inherently hydrophobic materials. Samples are then incubated at a temperature similar to that intended for the final use for a specified period (usually 24 hours) under humid conditions. The size of the viable bacterial populations on the control and treated surfaces is then determined using a dilution plate count. Any effect is recorded using percent reduction calculated from the geometric means of the data. A neutraliser may be employed and sonication is used to separate the 'biofilm' from the test surfaces and suspend the agar gel. Subsequent imprinting of the test surface onto solid nutrient media can be performed to look for the presence of adherent viable cells.	Immobilised cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial agents from treated material into the cell suspension or due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.

1

2

Appendix 8. Methods to Measure Antimicrobial Activity

Table VI: Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile/wadding)

Reference	Title	Description	Major Principle
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial from treated material into the cell suspension. Some activity may be due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 147-2011	Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method	Agar plates are inoculated with 5 parallel streaks (60 mm long) of either *Staphylococcus aureus* or *K. pneumoniae.* A textile sample is then placed over the streaks and in intimate contact with the surface of the agar and incubated. Activity is assessed based on either the mean zone of inhibition over the 5 streaks or the absence of growth behind the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 100-2012	Antibacterial Finishes on Textile Materials: Assessment of.	Replicate samples (sufficient to absorb 1 ml of test inoculum) of fabric are inoculated with individual bacterial species (*e.g. Staph. aureus* and *K. pneumoniae*) suspended in a nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is assessed by comparing the size of the initial population with that present following incubation. A neutraliser is employed recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *Staph. aureus* and *K. pneumoniae* using a 200 g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37° C for 24 hours. A	Cell suspension intimate contact test. The transfer method of inoculation could be adapted to provide some

	antibactérienne	neutraliser is employed during cell recovery.	simulation data.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Three replicate samples of fabric, yarn or pile/wadding are placed in intimate contact with the surface of agar plates that have been inoculated with a cell suspension of either *Staph aureus* or *K. pneumoniae* and incubated at 37° C for 24 - 48 hours. The presence of and size of any zone of inhibition around the samples is then recorded.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Replicate samples of fabric (6 of the control and 3 of the treated) are inoculated with individual bacterial species (*e.g. Staph. aureus* and *K. pneumoniae*) suspended in a heavily diluted nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is assessed by comparing the size of the initial population in the control with that present following incubation. No neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
EN ISO 20645 - 2004	Textile Fabrics - Determination of the antibacterial activity - Agar plate test (ISO/FDIS 20645:2004)	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *Staph. aureus*, *Escherichia coli* or *K. pneumoniae*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed for growth based on either the presence of a zone of inhibition of > 1 mm or the absence/strength of the growth in the media overlaying the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN 195920	Examination of the Antibacterial Effect of Impregnated Textiles by the Agar Diffusion Method	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *Staph. aureus* or *E coli*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in BS EN ISO 20645 above.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN195924	Textile Fabrics - Determination of the Antibacterial Activity: Colony Plate Count Method	Fifteen replicate samples (each replicate is comprised of sufficient specimens of 25 ± 5 mm to absorb 1 ml of test inoculum) are inoculated with cells of either *E. coli* or *Staph. aureus* suspended in a liquid nutrient medium and incubated in sealed bottles for up to 24 hours at 27° C. After 0, 6 and 24 hours, 5 replicate samples are analysed for the size of the viable population present. A neutraliser is employed. An increase of 2 orders of magnitude of the population exposed to a control sample is required to validate the test. The method defines a textile as antibacterial if no more than a specified minimum level of growth is	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		observed after 24 hours in 4 of the 5 replicate groups of samples.	
SN195921	Textile Fabrics - Determination of Antimycotic Activity: Agar Diffusion Plate Test	Replicate (4) samples of sterilised fabric (25 ± 5 mm diameter) are placed in intimate contact with a solid nutrient medium in a petri dish. Each petri dish has been prepared as a double layer. The first layer consists of 10 ml nutrient agar, the second layer of another 10 ml of the same nutrient agar to which 0.1 ml spore suspension (10^7 ml^{-1}) of either *Candida albicans*, *Aspergillus niger*, *Cladosporium sphaerospermum* or *Trichophyton mentagrophytes* had been added. The plates are then incubated at 28° C either 2 days (*C. albicans*) or 7 days (*A. niger*, *C. sphaerospermum* and *T. mentagrophytes*). The test is valid when control specimens of the same material without biocide, or of a biocide-free standard specified cotton material are fully overgrown. Good antimycotic efficacy is considered to be demonstrated when the specimens show no fungal growth on their surface. The test specifies that both sides of a material have to be tested.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption method	Replicate (6) samples of textile are inoculated with a standardised broth culture of either *Staph. aureus* or *K. pneumoniae* in individual tubes and then incubated at 37° C for 18 - 24 hours in closed containers. Samples are analysed for the presence of viable bacteria both before and after incubation by either total viable count or the determination of total ATP. Samples are sterilised prior to testing and a neutraliser is employed during recovery. The test is validated by growth of ˆ1 order of magnitude during the incubation period.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer method	Replicate (6) samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *Staph. aureus* and *K. pneumoniae* using a 200 g weight for 1 minute. The samples are then removed. Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation under humid conditions at 37° C for 24 hours. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either growth of ˆ 1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products:	Replicate (6) samples of test material are either *Staph. aureus* and *K. pneumoniae* by 'printing' cells collected on a membrane filter onto their surface in a standardised manner. The samples are then incubated under humid conditions for 18 - 24 hours at 20° C for a specified contact time(s). Replicate (3) samples are analysed for either the	'Dry' inoculum intimate contact test. The transfer method of inoculation could

	Printing method	number of viable bacteria or the total ATM content both before and after incubation. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either determining the survival of the inoculum on the control material.	be adapted to provide some simulation data.
ISO/FDIS 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 1 - Luminescence Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the ATP concentration associated with the samples. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced concentrations of ATP associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.
ISO/WD 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 2 - Plate Count Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the number of colony forming units. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced numbers of colony forming units associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.

1

Table VII: Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Qualitative Antibacterial Activity	Petri dishes with nutrient media are inoculated with a single, diagonal streak (approx.7.5 cm) of either *Staph. aureus* or *K. pneumoniae*. An unsterilized test specimen (25 mm x 50 mm) is placed in intimate contact and transversely across the inoculum on the agar surface. The plates are then inoculated at 37° C for 18 - 24 hours. The front and back of the carpet are tested separately. After incubation, the plates are inspected for the presence of growth both below the specimens and for any zone of inhibition surrounding the specimens. The test can also be used to test the effect of cleaning	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a simulation of final use of a treated material.

		regimes. An untreated control is optional.	
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antibacterial Activity	Unsterilized specimens of carpet are pre-wetted with either sterile water or a wetting agent before being inoculated with individual suspensions of either *Staph. aureus* or *K. pneumoniae* in either a low or a high nutrient solution. The samples are then incubated in a tightly closed jar at 37° C for a specified contact time. Cells are recovered in 100 ml of a neutraliser after 0 and 6 - 24 hours of incubation. Activity is assessed by comparing the size of the initial population in the control (if used) with that present following incubation. A control is optional. When not employed, viable counts following incubation of the treated specimens alone are considered. The test can also be used to test the effect of cleaning regimes.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabouraud Dextrose Agar are inoculated with 1 ml of a spore suspension of *Aspergillus niger*. Immediately afterwards, specimens (38 mm diameter) of unsterile test material are placed into intimate contact with the agar. An additional 0.2 ml of the same spore suspension is also employed to inoculate the test pieces directly. The samples are then incubated at 28°C for 7 days. The back and front of the discs of carpet are tested in separate dishes. The zone of inhibition and the growth of fungus on the upper surface of the specimens are reported (no growth, microscopic growth, macroscopic growth). The test can also be used to test the effect of cleaning regimes.	Zone diffusion test/surface growth test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
WIRA Test F	Test Method for Assessing the Survival of Test Organisms on Floor Coverings	Specimens (850 mm x 350 mm) are conditioned at 20°C and 65% RH before being subjected to 2 wet and 2 dry passes using a commercial spray extraction machine or a test rig. After 24 h drying, 12 specimens (each 60 mm diameter) are cut from the carpet. An aliquot (1 ml) of a suspension of cells of *E. coli* in nutrient broth is poured onto filter paper (7 cm	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

		diameter). The filter paper is then pressed for 1 min onto the surface of the carpet using a 1 kg weight. The filter paper is then discarded. After 0, 6 and 24 hours incubation at a specified temperature the carpet´s surface is pressed onto contact plates of McConkey agar. After 24h replicate (3) plugs (10 mm) are taken from each specimen and suspended in 10 ml nutrient broth for 30 seconds and then analysed for the presence of *E. coli* by total viable count.	

1

Table VIII: Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2000	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate sample (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either *E. coli* or *Staph. aureus* in a highly diluted nutrient broth. The cell suspension is then held in intimate contact with the surface by the use of a sterile polyethylene film (usually 40 mm x 40 mm) for 24 hours at 35° C under humid conditions. The size of the population on the treated surface is then compared with the size on the control surface both prior to and after incubation. A neutraliser for certain biocide types is employed. Antibacterial activity is certified if the difference between the Log_{10} of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 22196:2011	Plastics - Measurement of antibacterial activity on plastics surfaces.	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan. Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes -	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *Staph. aureus* and *K. pneumoniae*	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of

	Caractérisation et mesure de l'activité antibactérienne	using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	a treated material.
ASTM E2180-07	Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials	Replicate (3) samples of material are inoculated with cells of either *Staph. aureus* or *K. pneummoniae* suspended in molten semi-solid isotonic saline/agar. This attempts for form an 'artificial biofilm' which holds the suspension in intimate contact with the test surface of inherently hydrophobic materials. Samples are then incubated at a temperature similar to that intended for the final use for a specified period (usually 24 hours) under humid conditions. The size of the viable bacterial populations on the control and treated surfaces is then determined using a dilution plate count. Any effect is recorded using percent reduction calculated from the geometric means of the data. A neutraliser may be employed and sonication is used to separate the 'biofilm' from the test surfaces and suspend the agar gel. Subsequent imprinting of the test surface onto solid nutrient media can be performed to look for the presence of adherent viable cells.	Immobilised cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial agents from treated material into the cell suspension or due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.

1

2

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