GUIDANCE

Guidance on the Biocidal Products Regulation

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

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LEGAL NOTICE

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PREFACE

The Guidance on the Biocidal Products Regulation (BPR) is to be applied to applications for active substance approval and product authorisation as submitted from 1 September 2013, the date of application (DoA) of the Biocidal Product Regulation (the BPR).

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

The scientific guidance provides technical scientific advice on how to fulfil the information requirements set by the BPR (Part A), how to perform the risk assessment and the exposure assessment for the evaluation of the human health and environmental aspects and how to asses and evaluate the efficacy to establish the benefit arising from the use of biocidal products and that it is sufficiently effective (Parts B & C).

In addition to the BPR guidance, the Biocidal Products Directive (BPD) guidance and other related documents are still considered applicable for new submissions under the BPR in the areas where the BPR guidance is under preparation. Furthermore these documents are still valid in relation to the applications for active substance approval or applications for product authorisation under the BPD that may still be under evaluation. Also the Commission has addressed some of the obligations in further detail in the Biocides competent authorities meetings documents which applicants are advised to consult. Please see ECHA Biocides Guidance website for links to these documents: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation].

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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.

List of Abbreviations

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Abbreviation	Explanation
AFNOR	Association française de normalisation; French national organisation for standardisation http://www.afnor.org/
AOAC	Association of Official Analytical Chemists http://www.aoac.org/
ASTM	American Society for Testing and Materials http://www.astm.org/
ATCC	American Type Culture Collection http://www.lgcstandards-atcc.org/
BP	biocidal product
BPD	Biocidal Products Directive 98/8/EC
BPR	Biocidal Products Regulation (EU) No 528/2012
CA/CAs	Competent Authority/Competent Authorities
CEN	Comité Européen de Normalisation; European Committee for Standardisation http://www.cen.eu/
CIP	Cleaning-in-Place
DIN	Deutsches Institut fuer Normung; German national organisation for standardisation http://www.din.de/
DVG	Deutsche Veterinaermedizinische Gesellschaft; German Veterinary Medical Society http://www.dvg.net/
EN	European Standard
EPPO	European and Mediterranean Plant Protection Organization www.eppo.org
ISO	International Organization for Standardisation http://www.iso.org/
MAD	Mutual Acceptance of Data
OECD	Organisation for Economic Co-operation and Development http://www.oecd.org/
prEN	Draft European Standard
PT	product-type
SPC	Summary of Product Characteristics
TC	Technical Committee
TM	Technical Meeting
TNsG	Technical Notes for Guidance

Abbreviation	Explanation
US-EPA	United States Environmental Protection Agency http://www.epa.gov/
VAH	Verbund fuer Angewandte Hygiene; Association for Applied Hygiene http://www.vah-online.de/

Glossary of Terms

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Standard term	Explanation
Activity against enveloped viruses (see also Virucidal activity and Limited spectrum virucidal activity)	A claim for hygienic hand and skin disinfectants with activity against enveloped viruses only.
Algaecide	A product or active substance used to control (inhibit the growth) or kill algae.
Algaecidal activity	The capability of a product or active substance to produce a reduction in the number of viable algae cells under defined conditions.
Antimicrobial product	A product which prevents the growth of/reduces the number of/mitigates the growth of micro-organisms
Bactericide	A product or active substance which irreversibly inactivates vegetative bacteria under defined conditions
Bactericidal activity	The capability of a product or active substance to produce a reduction in the number of viable bacterial cells of relevant test-organisms under defined conditions
Bacteriostatic activity	Capability of a product or active substance to inhibit the growth of bacteria under defined conditions
Biocidal product/ Biocide	BPR Article 3(1)(a): *— any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action,* *— any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.* *A treated article that has a primary biocidal function shall be considered a biocidal product.*

Standard term	Explanation
Biofilm	An accumulation of microbial cells immobilised on a substratum and embedded in an organic polymer matrix of microbial origin
Biostatic product	A product which inhibits the growth of micro-organisms under defined conditions
Curative effect on biofilm	The biocide is added after the biofilm is formed and acts on biofilm stability, facilitating the biocide interaction with cells – it may or may not act as detergent and detach the biofilm from the surface
Disinfectant within PT 2, 3, 4 and 5	A disinfectant is a product that reduces the number of micro-organisms in or on an inanimate matrix- achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Disinfection within PT 2, 3, 4 and 5	disinfection is the reduction of the number of micro-organisms in or on an inanimate matrix- achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Skin disinfection within PT1	Skin disinfection is the reduction of the number of micro-organisms on skin, achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Efficacy	The ability of a product or active substance to produce an effect as described in the label claims made for it, when used under actual use conditions.
Flow condition (for biofilm)	Biofilm is formed on supports of different nature placed along a tube or a chamber where the medium (inoculated and/or fresh) is circulated in a closed (reservoir-pump-tubing) or open (reservoir-pump-tubing-outlet) system
Fungicide	A product or active substance which irreversibly inactivates fungi (vegetative mycelia, budding yeasts and/or their spores) under defined conditions
Fungicidal Activity	The capability of a product or active substance to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant test organisms under defined conditions
Fungistatic activity	The capability of a product or active substance to inhibit the growth of fungi under defined conditions
Hygienic hand disinfectants	A hygienic hand disinfectant is a hygienic handrub disinfectant or a hygienic hand wash disinfectant
Hygienic handrub disinfectant	product used for post-contamination treatment that involves rubbing hands, without the addition of water, which is directed against transiently contaminating microorganisms to prevent their transmission regardless of the resident skin flora
Hygienic handwash disinfectant	product used for post-contamination treatment that involves washing hands with water, which is directed against transiently contaminating microorganisms to prevent their transmission regardless of the resident skin flora

Standard term	Explanation
Limited spectrum virucidal activity (see also Virucidal activity and Activity against enveloped viruses)	Limited spectrum virucidal activity is a claim for hygienic hand and skin disinfectants using Adenovirus and Murine Norovirus as test organisms, thus including activity against the test viruses and all enveloped viruses (see Appendix 5).
Log reduction / log_{10} reduction / lg reduction	Reduction presented in a logarithmic scale. Example 1: when a disinfection reduces 10^8 bacteria to 10^2 bacteria, this is a lg reduction of 6. Example 2: when a disinfection reduces 5.10^7 fungal spores to 8.10^3 fungal spores this is a lg reduction of 3.79.
Microbes/micro-organisms	bacteria (including vegetative cells bacterial spores and mycobacteria) fungi (including yeasts, moulds and fungal spores) algae, viruses (including bacteriophages), protozoa (including cysts and other permanent states), etc.
Mycobactericide	A product or active substance which irreversibly inactivates mycobacteria under defined conditions
Mycobactericidal activity	The capability of a product or active substance to produce a reduction in the number of viable mycobacterial cells of relevant test organisms under defined conditions
Neutraliser	A chemical agent or formulation which suppresses the residual activity of an disinfectant within a test but does not inhibit or inactivate micro-organisms
Performance standard	Regulatory or scientific standard for biocides that is either quantitative or qualitative (that may also be specified in the test method) by which a decision is taken on the acceptability of a claim.
Preventive effect on biofilm	The biocide is present before the biofilm is formed and may act both on cell viability and/or on cell adhesion/biofilm maturation
Product type (PT)	Product types (PT) are defined in BPR annex V
Sporicide	A product or active substance which inactivates dormant bacterial spores under defined conditions
Sporicidal activity	The capability of a product or active substance to produce a reduction in the number of viable bacterial spores of relevant test organisms under defined conditions
Sporistatic activity	The capability of a product to inhibit the germination of dormant bacterial spores under defined conditions
Static condition (for biofilm)	Biofilm is formed on supports such as microplates without agitation after an incubation time that depends on the micro-organism considered

Standard term	Explanation
Surgical hand disinfectants	A surgical hand disinfectant is a surgical handrub disinfectant or a surgical hand wash disinfectant
Surgical handrub disinfectant	Product used for preoperative treatment that involves rubbing hands, without the addition of water, which is directed against the flora of microorganisms on hands to prevent the transmission of microorganisms into the surgical wound
Surgical handwash disinfectant	Product used for preoperative treatment that involves washing hands with water, which is directed against the flora of microorganisms on hands to prevent the transmission of microorganisms into the surgical wound
Treated article	A treated article is any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products
Tuberculocide	A product or active substance which irreversibly inactivates *Mycobacterium tuberculosis* under defined conditions
Tuberculocidal activity	The capability of a product or active substance to irreversibly inactivate Mycobacterium tuberculosis, demonstrated by the capability to produce a reduction in the number of viable cells of the test organism *Mycobacterium terrae* under defined conditions
Virucide	A product or active substance which irreversibly inactivates viruses under defined conditions
Virucidal activity (see also Limited spectrum virucidal activity + Activity against enveloped viruses)	The capability of a product or active substance to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions "Full spectrum" virucidal activity is a claim for biocidal products using relevant test organisms and thus showing activity against the enveloped and non-enveloped viruses.
Yeasticide	A product or active substance which irreversibly inactivates yeast under defined conditions
Yeasticidal activity	The capability of a product or active substance to produce a reduction in the number of viable vegetative yeast cells of relevant test organisms under defined conditions

1. General Introduction

Evaluation and Assessment

The process of evaluation of active substance applications is given in Article 8 (BPR) and the common principles for the evaluation of dossiers for biocidal products (and the active substances) is given in Annex VI (BPR).

The evaluating or receiving CA uses the data submitted in support of an application for active substance approval or authorisation of a biocidal product to make a risk assessment based on the proposed use. The general principles of assessment are given in Annex VI (BPR) from which the evaluating body will base its conclusions on whether or not the active substance or the biocidal product complies with the criteria for authorisation set down in Article 19(1)(b).

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.

These general sections at the beginning of this guidance, (namely sections 1, 2 and 3), provide a general overview for the efficacy evaluation; the more specific requirements for each Product Type (PT), which must be met and should be followed in the first instance, are described in the later sections.

2. 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 (within the product) or the product itself, are supported by suitable efficacy data. A claim is the precondition and base for efficacy testing.

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 should include as a minimum the following information:

- The purpose of the claim (e.g. prevent destruction of material by insect infestations, disinfect surface);
- 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 guidance sections (see later sections).

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, softwood).

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 directions for use

The directions for use and the claims made for the biocidal product are included in a summary of biocidal product characteristics (SPC) in accordance with Article 22(2) (BPR).

A label claim is information which is provided to the user which describes the biocidal effects that will result from using a biocidal 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.). The product label can only include claims that are in line with the authorised uses, as given in the SPC.

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 a product authorisation must include a draft SPC and additionally should 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 claims made for it when used according to the directions for use on the proposed product label (as given in the SPC): 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 studies
- 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 a laboratory according to criteria defined. These tests permit to validate for example a level of mortality during a given time, a knock down (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.

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: however, in some cases it is not possible to include a control sample in field tests.

Screening tests, laboratory studies and simulation tests must always include an untreated control without active substance (i.e. a negative control); it is preferred that this is the formulated product without active substance. However, providing it can be justified, this can be, a control with only the solvent, e.g. water. There are few exceptions to this rule, such as the EN disinfection test, and all exceptions should be justified by the methodology.

Tests should preferably be carried out in accordance with standard protocols, e.g. CEN, ISO, OECD, 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. Ideally, tests are 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 (e.g. tested product composition, conditions of the test temperature, humidity,)
- tested organisms
- 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 standard 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, when appropriate. 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.

When applying for authorisation all the efficacy tests should be summarised in the PAR. The PAR format includes a table. This table should be filled out in a way that it gives an overview of all the efficacy results. When the test is not a standard test a short description of the method should be included. The test column "test system/concentration applied/ exposure time" should include all the relevant information on the test, the test parameter (e.g. contact time, temperature, replicates) in way that it can be compared to the intended use. The results should be specified (e.g. x% mortality, log reduction >x) and not just "test passed". In some cases it might be easier to summarise the results in the text instead of the table (e.g. field trials).

Below the table the tests should be discussed and an explanation should be given on how the test results demonstrated the efficacy of the product for the different uses under use conditions.

3.2 Resistance

The topic of resistance is discussed in the general part of the TNsG on Product Evaluation (Section 6). Information on resistance should be given for active substances and biocidal products. Additionally, in support of the review for each active substance, information on resistance is given in the Competent Authority Report (CAR) of this active substance.

Resistance will be assessed on the basis of expert judgement. This section of the guidance will be updated in the future in the light of experience gained in evaluation of resistance.

4. Active substance approval

4.1 Introduction

According to Article 4 of the BPR, an active substance must 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 of the representative biocidal product are assessed in a relevant matrix. 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. 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 to be effective 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 a representative selection.

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 active substances used in treated articles, see section 4.5 and sub-sections 4.5.2 and 4.5.3.

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 matrix (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 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 the representative product. When only specific biostatic activity (e.g. bacteriostatic, fungistatic) is claimed, an appropriate method should be used.
- For main group 2 (preservatives: PT6, 7, 8, 9, 10, 11, 12 and 13), innate activity is generally a static activity demonstrated in challenge tests on several and relevant target organisms, in the relevant matrix. However, if curative effects are claimed, cidal activity is requested. To demonstrate efficacy against one target organism only could also be acceptable in the case of a strictly defined use relevant for the PT (e.g. 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. Growth in the untreated control is essential to show the validity of the test. If the claim is only for a curative effect, it is sufficient to show that the decline in the microbial population in the treated samples is statistically significantly more than in the untreated control samples.
- 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 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, area of use and the identity of the active substance is described and are relevant. If cited literature is used to support a preserving effect it must also show that untreated test specimens supported growth. When curative effects are claimed the cited literature must demonstrate the efficacy of the active substance according to the requirements per PT. The use of cited literature 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 fulfil 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 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 representative 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 fulfil the minimum requirements mentioned in the active substance part (part A).

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 of active substances not used alone but always in combination with other active substances.

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,.

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 co-formulants (e.g. booster) that is not itself an active substance;
- the active substance is used in combination with another active substance.

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 (specified per use). 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, not 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 level 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 Active substances used to treat materials and 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 previous legislation, BPD.

Guidance on treated articles is addressed in section 5.3.

4.5.1 Efficacy assessment for 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.

4.5.2 Efficacy assessment for active substances in specific PTs

For active substances notified for certain PTs it is obvious that they are mainly, or exclusively used, to treat articles/materials as for example for PTs 6, 7, 8, 9, 10 (Main group 2). Thus, efficacy testing with respect to use to treat articles/materials, 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 (Main group 1) 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; the protection of the material itself is not intended, but a new property is introduced to an article, intended to protect its user. For such 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. At active substance level, the representative product should show the claimed effect(s) in a 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 must 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 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 for the full range of claims made. More test organisms or different uses can be relevant as compared to active substance approval. 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 full 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

(including ageing and weathering if relevant), any claims for residual action, together with any other specific claims.

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 use 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 purpose of the biocide (e.g. prevent destruction of material by insect infestations, decrease of bacterial contamination on surfaces);
- 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);
- Description of the instructions of uses.

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, it is important to categorise possible wide ranges of uses into sets of similar materials and use-conditions. Please see sections 5.3, 5.4.2 and 5.5 for more details.

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 products.[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. However, it is also possible that extra *meta* SPC's should be added because of the efficacy assessment (e.g. some products in the family are not efficacious for some uses). 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

[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

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.

5.2.2 Worst case testing

The BPF concept allows read-across of data between similar products within and across *meta*SPCs. Efficacy tests must be performed 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;
- contact time;
- 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/without pre-cleaning*.

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

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 (i.e. same use in different PTs). 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 product 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).

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: 4%
	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.

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 combined 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 a family

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* SPCs. Another example is explained in Table 6.

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 thisfamily. The worst-case would be a product 5% active substance 1 + 2% active substance 2 . Assuming variation of coformulants have no impact on efficacy, this 'dummy' product should be tested to demonstrate efficacy for this family when it consists of one *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

Whilst ready-to-use 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.

It should be considered that there may be other products on the market which contains a lower concentration of AS and is efficacious for the same intended use.

5.3 Treated articles

NOTE to the reader:
This section concerns treated articles and should be read in conjunction with the CA Note for Guidance "Frequently asked questions on treated articles", CA-Sept13-

Doc.5.1.e, Revision 1 December 2014 [3].

Article 3 Definitions

1. For the purposes of this Regulation, the following definitions shall apply:

(a) 'biocidal product' means

- any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action,

- any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.

A treated article that has a primary biocidal function shall be considered a biocidal product.

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

A treated article according to Article 3(1)(l) of the BPR is any substance, mixture or article which has been treated with or intentionally incorporates one or more biocidal products. A biocidal product, in contrast, is any substance or mixture with a biocidal function. Pursuant to Article 3(1)(a) a treated article with a primary biocidal function is considered a biocidal product.

Liquids fulfil the substance or mixture definition. Consequently, liquids may only be considered as treated articles if they do not intend to control any harmful organism. In contrast, solid treated articles are defined by their shape and function rather than by their chemical composition. Thus, solid treated articles fulfil the definition of a biocidal product if they have a primary biocidal function.

The term "primary biocidal function" is not further defined in the BPR, but in the CA document, it is described as "a biocidal function of first rank, importance, or value compared to other functions of the treated article".

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.

Thus, considering the different product types for PTs 1-4, the following examples would be considered as biocidal products and not treated articles. For PT 1 or 3, disinfecting wipes would be regarded as biocidal products[4]. For PT2, paints and coatings intended to prevent microbial settlement and growth in order to provide a hygienic environment would likewise

[3] CA-Sept13-Doc

[4] See CA document Appendix 1

be regarded as biocidal products[5]. Other PT 2 applications which could fall under either category, depending on their primary function could include for instance textiles, tissues, masks, or other articles or materials in which a biocidal product has been incorporated with the purpose of adding disinfecting properties to these articles and materials. For PT 4, examples are materials or articles which come into contact with food or feed and are treated with or incorporate a biocide; whether such articles are to be regarded as biocidal products again depends on their primary function. PT 5 applications are usually biocidal products. Further product examples are given in Appendix 1 of the CA document.

There are some exemptions in 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 in a liquid matrix. For instance, wet state preservatives (PT 6) or a hand disinfectant (PT 1) are usually both tested in a liquid matrix, the first matrix is a treated article, the latter is a biocidal product; only the performance standards are different in these examples. Specific requirements apply, however, when the efficacy of solid material or articles has to be tested. A test under practical conditions of use (step 3 test) is mandatory. In contrast to preserving claims, where standard materials under certain standard conditions of use can be tested, testing for disinfecting claims has to be specific for every single article. For these types of claims, the specific conditions of use are to be considered when designing the efficacy testing; for example, a polymer coating used for a hospital bedside cabinet has to be tested for the specific contaminating situation of a hospital bedside cabinet, including cleaning schemes and soiling situation; efficacy has to be shown compared to an untreated bedside cabinet. Bacteriocidal effects have to take effect very quickly to show an advantage compared to an untreated cabinet, where droplets of blood or saliva will dry out quickly and not either be contaminating any more. Please read more about how to design such tests in BPR Vol II Efficacy Parts B+C, Section5.3.

Specific requirements apply, however, when the efficacy of biocides in 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.

[5] See CA document Question 8

Treated articles, if not biocidal products, do not require efficacy assessment under the BPR. However, active substances and biocidal products incorporated into treated articles may require assessment of their efficacy in treated articles as part of the active substance approval and biocidal product authorisation processes (if such uses are applied for).

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.

As long as there is no specific EU guidance on efficacy testing of treated articles, the following document should be used:

- Nordic Working Paper "Efficacy Assessment of treated articles: A guidance" concerning data requirements and acceptance criteria for treated articles[6].

Furthermore, there are two OECD test methods available:

- Guidance Document on the Evaluation of the Efficacy of Antimicrobial Treated Articles with Claims for External Effects (OECD Series on Biocides No. 1);
- Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials (OECD Series on Testing and Assessment No. 202 and Series on Biocides No. 8).

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

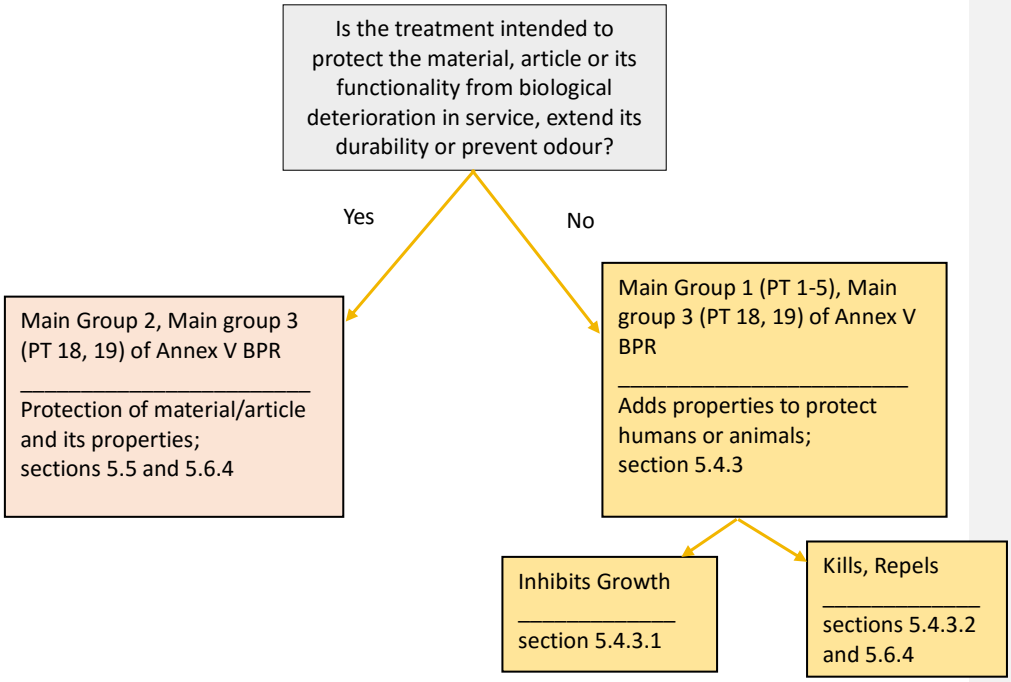
When biocides are incorporated into materials or used in the production of treated articles they are applied with two purposes:

- To protect the materials used in the article or the properties of the article in service. The target organisms have a detrimental or other undesirable effects (e.g. biodegradation, discolouration, odour formation) on the material or article.
- 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:

[6] http://norden.diva-portal.org/smash/get/diva2:702967/FULLTEXT01.pdf

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 4.5.2 and 5.6.4. Guidance for material protection is given in section 5.5.

5.4 Disinfectants (Main group 1)

5.4.0 General

NOTE FOR CA CONSULTATION

The text in the section 5.4.0 has been published in a Transitional Guidance (May 2016). The text (shaded pink) is out of scope of this 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]

5.4.0.1 Introduction

This guidance describes the nature and extent of data which should be available to support the label claims for biocidal products within the Main Group 1: Disinfectants. This group covers 5 product types as described in Annex V of the BPR:

MAIN GROUP 1: Disinfectants

These product-types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.

Product type 1: Human hygiene

Products in this group are biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.

Product type 2: Disinfectants and algaecides not intended for direct application to humans or animals

Products used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuffs.

Usage areas include, *inter alia,* swimming pools, aquariums, bathing and other waters; air-conditioning systems; and walls and floors in private, public, and industrial areas; and in other areas for professional activities.

Products used for disinfection of air[7], water not used for human or animal consumption, chemical toilets, waste water, hospital waste and soil.

Products used as algaecides for treatment of swimming pools, aquariums and other waters and for remedial treatment of construction materials.

Products used to be incorporated in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties.

Product type 3: Veterinary hygiene

[7] This is taken to mean the disinfection of air itself. Disinfectants sprayed or vaporised into the air (e.g. room disinfection by vaporised biocide) are normally for the purpose of disinfecting surfaces and not the air itself. Disinfectants for air conditioning systems disinfect the surfaces or liquids in these systems, not the air coming out of it.

Products used for veterinary hygiene purposes such as disinfectants, disinfecting soaps, oral or corporal hygiene products or with anti-microbial function.

Products used to disinfect the materials and surfaces associated with the housing or transportation of animals

Product type 4: Food and feed area

Products used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food or feed (including drinking water) for humans and animals.

Product type 5: Drinking water

Products used for the disinfection of drinking water for both humans and animals.

Products in this main group are meant for the control of micro-organisms, such as bacteria (including vegetative cells, spores and mycobacteria), fungi (including moulds and yeasts), and viruses (including bacteriophages), algae and protozoa. Control may be carried out on inanimate surfaces or skin or in liquids. Note that the term "disinfectant" used for main group 1 should be read as a generic term and not according to the definition in the glossary of terms. This means that next to disinfectants it can also include products with biostatic activity.

The most important fields of use include medical, veterinary, food, feed and drinking water sectors. Applications in public, commercial and industrial areas, where application is to inanimate surfaces without direct contact with food, are included in Product type 2. If contact between disinfected inanimate surfaces and food is possible (e.g. food industry, private and restaurant kitchens), applications are included in Product type 4.

Disinfectants for medical instruments and medical equipment that are considered medical devices are covered under the Medical Device Directive 93/42/EEC (see 3.9.1 for more information). More borderline cases with other Directives or Regulations are noted elsewhere in this Guidance Document and are defined or described in other legislation or guidance.

Cleaning products which are not intended as biocides, including liquid detergents, washing powders etc. are excluded from these product types and thus this guidance is not applicable (Annex V of BPR).

Treated articles with claimed disinfecting properties or function can also fall within PTs 1 to 5: when such articles have a primary biocidal function they are considered biocidal products (see Competent Authority (CA) document [8]). These articles can include a wide variety of goods, with different applications, matrices etc. This guidance deals mainly with efficacy testing of (liquid) biocidal products; the methodology for testing (solid) treated articles can be quite different. See section 5.3 of this Guidance for details of available guidance.

A "Glossary of Terms" is at the beginning of the document.

5.4.0.2 Dossier requirements

The following aspects are relevant for the evaluation of the efficacy of biocidal products within PT1-5:

1. The label claim and instructions for use

[8] CA-Sept13-Doc

2. Efficacy data of the product
3. The possible occurrence of resistance, cross-resistance or tolerance.

5.4.0.3 Label claim

For each product, clear label claims should be provided. When the label itself cannot contain all the necessary information, any accompanying leaflet should also be considered. To simplify the text only the term "label claim" will be used below.

The types of efficacy claims made for a disinfectant/ biocidal product depend upon, among other things, the types of micro-organisms the disinfectant targets (e.g. fungal spores, yeasts, mycobacteria, bacteria or bacterial spores) and the disinfectant's intended use (e.g. in hospitals, in contact with food, in animal houses, in homes). Label claims and recommendations for use, including concentration and contact time, must be supported by the results of bactericidal, fungicidal, etc. tests appropriate to the area of application, which are normally performed on the basis of the specific standards. Complete instructions for use are an integral part of the label.

The information on the product label should fully correspond with the uses pre-defined at the authorisation stage and reflected in the corresponding version of the SPC[9]. Applicants must indicate clearly on the product's label the spectrum of antimicrobial activity claimed.

Examples of the common fields of applications are presented in the claims matrices which are a set of tables linked to this guidance document (see Appendix 1 for more information). The Claim Matrices are not intended to be exhaustive, but the majority of uses are included.

5.4.0.3.1 Target Organisms

The target organisms for which claims are made should be specified on the product label.

As the claimed antimicrobial efficacy for disinfectant products will encompass a large spectrum of potential target organisms, it is not necessary or indeed feasible to include all possible micro-organisms in an efficacy test designed to support a label claim. Instead the types of target organism the product is intended for are mentioned, for example, fungal spores, yeasts, viruses, algae, protozoa, (myco)bacteria or bacterial spores.

Specific species are mentioned on the label where they are the only or most relevant organisms, or where they have a different susceptibility to biocides than the rest of the group. For instance, mycobacteria are less susceptible than other bacteria and it is only relevant to control them in certain situations such as tuberculosis wards.

In general it is not possible to claim against specific single species without claiming (and demonstrating) efficacy against the group of organisms (e.g. no claim against *Mycobacterium tuberculosis* without also making a general bactericidal claim, no claims against HIV without a general claim against enveloped viruses). However, there are some cases in which it can be justified that a claim only for a single or a small number of species is made (such as bacteriophages in the milk industry, or fungi *Aspergillus fumigatus* in poultry housing.).

Claims against specific organisms or groups of organisms should not be made, if they imply a false impression of superiority of a product; for example, a claim against MRSA should not be made for a bactericidal product, because MRSA do not present a specific challenge for disinfectants.

[9] Details on how to fill out the SPC are available in the ECHA Technical Guide and SPC Editor.

Standard test methods normally specify one or more representative species that should be tested per group of organisms for which the claim is made. For instance, a bactericidal product should be tested on gram-positive and gram-negative bacteria, a fungicidal product should be tested on yeasts and fungal spores. The species used are representative species that take into account their relevance to practical use, susceptibility for disinfectants and adequacy for laboratory testing.

The test organisms and strains which should be used are normally stated in standard efficacy test methods, i.e. according to EN 14885 or OECD-guidance.

When it is not possible to use standard test methods for efficacy testing and other tests are used instead, the test organisms listed in Appendix 3 should be employed. If test organisms other than those listed in Appendix 3 are used, their relevance should be justified.

Wherever possible strains should be selected from international collections (their genetic stability should be checked regularly). The preservation procedures must be clearly described (EN 12353).

Other test organisms, in addition to those specified in the test standards, can also be tested. When efficacy against specific additional species is claimed, efficacy tests with those species should also be performed. In general, claims should not be made against the specific reference species used in a standard test as this can give a misleading impression that the product shows activity beyond that covered by the general (e.g. bactericidal, fungicidal) claim.

Mentioning specific organisms on the label is still a subject of discussion between Member States. The above sections reflect the position at the time that this guidance is written.

For some areas of use there are minimum requirements for the groups of organisms for which efficacy should be demonstrated. For instance, for products used for animal transport vehicles efficacy against bacteria, yeasts and viruses should be demonstrated. For these products it is obligatory to test all required organisms. Per section, a sub-section on test organisms provides information on the minimum requirements for that use.

5.4.0.3.2 Areas of Use

Disinfectants are used almost everywhere that people want to "eliminate" or inhibit (for static products) micro-organisms. They are used to kill or irreversibly inactivate or inhibit bacteria, fungi and viruses on animate and inanimate surfaces and matrices, in hospitals, households, schools, restaurants, offices, swimming pools, kitchens, bathrooms, dairy farms, on medical and dental equipment, eating utensils and at many other locations.

In some cases biostatic products are used which only inhibit microorganisms (see section 5.4.0.5.3 of this guidance).

Applicants should clearly indicate the intended areas of use for the product on the label, for example, areas of use could include (not exhaustive):

- Hospital and other medical areas;
- Domestic use;
- Institutional use (offices, schools etc.);
- Industrial applications, e.g. food, cosmetic, pharmaceutical industry etc.;
- Restaurants and large-scale/canteen kitchens;
- Veterinary areas (animal housing, animal health care, teat or hoof disinfection etc.);
- Recreational areas.

5.4.0.3.3 Sites of Application

In addition to the types of efficacy claimed (e.g. bactericidal, fungicidal, tuberculocidal) and the intended area of use, the applicant must specify the use patterns for which the disinfectant is recommended on the label.

Broad examples of use patterns (not exhaustive) could include areas such as:

- Use on intact skin;
- Use in hospitals, operating theatres, isolation wards, etc.;
- Use in food manufacturing, retailing, processing areas etc.;
- Use in animal housing and equipment, e.g. pigs, sheep, poultry etc.;
- Use on work surfaces, cutting boards etc.;
- Use on fabrics or textiles;
- Use on toilets, bathrooms, sinks, etc.;
- Use against micro-organisms associated with human or animal waste;
- Use in air conditioning systems;
- Use in swimming pools, spas, aquariums and bathing waters;
- Use in tanks, pipelines, equipment soak or bottle wash.

5.4.0.3.4 Directions for use (Methods of application)

The label claim must specify the application method of the product. For disinfectants there is a broad range of application methods (e.g. wiping, aerosol, spraying). The in-use concentration of the solution and the contact time, which are essential for safe and effective use, should be described on the label. Any other directions for use should also be specified, such as whether the surface should be cleaned first, and claims regarding the number of times a prepared use solution can be used (or re-used) before a fresh solution must be prepared.

The application method can have a strong influence on the efficacy of a product, therefore the testing of a product should be appropriate for the application method. If specific equipment is used for application of the product (e.g. vaporisers) this should be taken into account when testing the product for efficacy. Equipment used in laboratory tests or small scale tests may (of necessity) be different from that employed in practice. This is especially the case when biocidal active substances are generated *in situ* using large scale equipment, such as electrolysis. In cases where small scale tests cannot be extrapolated to actual use conditions a large scale test with the equipment should be done.

5.4.0.3.5 Other interfering parameters

Any other circumstances that can influence the efficacy of a product should be mentioned on the label (e.g. temperature or pH requirements). For example, when a surface should be cleaned before applying the biocide and a no rinsing step is involved, or that alkaline cleaning fluids should not be used with acidic biocides, and *vice versa*.

5.4.0.4 Efficacy testing

For efficacy testing of disinfectants in general only quantitative tests methods should be used.

5.4.0.4.1 Tiered approach

For efficacy testing of disinfectants a tiered approach is recommended. The following tiers can be distinguished (in accordance with EN 14885):

- Phase 1 tests are quantitative suspension tests to establish that a product (or an active substance) has bactericidal, fungicidal etc. activity without regard to specific conditions of intended use. Phase 1 tests cannot be used for any product claim.
- Phase 2 comprises two steps:
  - Phase 2, step 1 tests are quantitative suspension tests to establish that a product has bactericidal, fungicidal, virucidal etc. activity, simulating practical conditions appropriate to its intended use.
  - Phase 2, step 2 tests are quantitative laboratory tests, often using carriers or living tissues with dried-on micro-organisms, simulating practical conditions to establish that the product has bactericidal, fungicidal, virucidal etc. activity.
- Phase 3 tests are field tests under practical conditions.

Phase 1

Phase 1 tests are laboratory suspension tests to establish the basic activity of the product or active substance. These tests may be used during the development of the product, but are not accepted for product authorisation. However, a phase 1 test can be used to demonstrate that a co-formulant does not have any biocidal activity in the product.

Phase 2, step 1

Phase 2, step 1 tests are laboratory suspension tests in which the ultimate purpose is to establish at what concentrations the product meets specified requirements under "in-use" conditions. In these tests, in-use conditions (e.g. temperature, contact time, interfering substances) are considered in the test method.

Various laboratory methods have been developed for biocide activity testing. Although these experiments differ in their design and experimental detail, they are all based on the principle of adding a test inoculum to the disinfectant (or vice versa) and taking samples at specified times. The biocide in each sample is then neutralised and the survival of the organisms assessed. In practice, the methods can be classified into two groups, according to how the end-point of the test is determined:

Quantitative tests

Samples of untreated and biocide-treated cells are plated on nutrient medium after neutralisation. After incubation, the number of colony forming units is determined and the $\log_{10}$ reduction in viable counts is determined.

Capacity tests

The biocide is challenged successively with the test organism at defined time intervals. This type of test can be used for instance for swimming pools and toilet disinfectants which are challenged by new bacteria periodically. Following each inoculation, samples are taken, and after a suitable contact period has elapsed, the biocide is neutralised and the sample incubated in a suitable growth medium to determine the surviving micro-organisms. The result is expressed as the amount of the accumulated inoculum that was required to produce the "failure".

Phase 2, step 2

Phase 2, step 2 tests are simulated use or practical tests, performed under rigorous conditions within the laboratory, which mimic real-life conditions, for instance by pre-drying the micro-organisms onto surfaces. These tests are used in a second testing stage. After

measuring the time-concentration relationship of the disinfectant in an in-vitro test (phase 2, step 1), these practical tests are performed to verify that the proposed use dilution is likely to be adequate in real-life conditions. For several uses standardised, simulated use tests exist (surface disinfection, hand wash or rub, instrument disinfection) but there are no standard tests available for many others.

Longer-lasting activity is claimed for some products. When these products are applied to surfaces, it is common that they will not be completely removed or rinsed off after application. This might lead to longer-lasting activity of the biocide on the surface. Likewise, some products are used for maintenance via continued release of low levels of biocidal product. Both effects can be determined by appropriate efficacy tests.

Phase 3 Field or in-use tests

In-use testing involves the antimicrobial evaluation of the product under actual conditions of use on specified surfaces or materials in a specified environment. As with standard and non-standard laboratory methods, representative organisms or actual organisms of concern may be used.

Validated methodologies for these types of tests are currently not available, although some are in development.

The practical use conditions under which a product can be used can be very variable and are therefore difficult to standardise. Field tests, although not standardised, can however give valuable additional information on the efficacy of the product, provided that the studies are scientifically robust, well reported and provide a clear answer to the question. In these types of test, a control treatment without biocide should be included. Where this is not possible, efficacy should be judged on a comparison of the situation before and after application.

Until validated standards are prepared, the responsibility for determining the acceptability of data derived from field trials in support of the claim will lie with the CA, taking into account the guidance given in EN 14885.

5.4.0.4.2 Standard test methods

Ideally, data should be generated using internationally or nationally recognised testing methods (CEN, OECD, ISO, etc.). Several international standard test methods currently exist for disinfectant products. Recommended standard tests are presented in Appendices 2 and referenced in Appendix 4 to this guidance document.

If there are no guidelines available for the specific use of a product, or guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well reported and provide a clear answer to the question. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible. EN 14485 provides guidance on modification of standards (EN 14485, section 4.2 version 2014).

At the time of publication of this guidance document, a broad range of CEN methods are available. OECD has several phase 2/step 2 test methods developed for the efficacy testing of disinfectants to be used on hard surfaces which have been published as Guidance Documents. Available tests are presented in Appendix 2 and referenced in Appendix 4. The use of CEN test methods is highly recommended, where these are available and relevant. However it should be noted that although this Guidance is mainly based on EN standards, there are some cases where there are discrepancies compared to the EN tests. In such cases

the ECHA Guidance should be followed as the leading guidance. OECD test methods may be used if, for example no CEN standard is available.
These methods, described below, typically give a standard set of test parameters, test organisms and pass criteria. Where specific conditions apply for a field of use, such as high/low level soiling, high/low temperatures, relevant contact times etc. these conditions should be included in the efficacy tests.

CEN Standard Test Methods

A Technical Committee (TC 216) was established in the European Committee for Standardisation (CEN), to produce harmonised European methods for testing the activity of disinfectants used in medical, veterinary, food, industrial, domestic and institutional areas. The standards are based on suspension tests (phase 1 and phase 2, step 1) and some simulated use tests like surface tests (phase 2, step 2).

European standard EN 14885 gives information on the application and interpretation of European Standards for the testing of chemical disinfectants within product types 1, 2, 3 and 4 of the Directive / Regulation.

This document outlines the various standards currently available and provides guidance as to the choice of available standards that may be used to demonstrate the effectiveness of disinfectants in particular situations (such as medical, veterinary and food hygiene) and on the interpretation of results from such tests in making and supporting efficacy claims.

In EN 14885 products intended for domestic use are grouped with products for use in food and industrial areas, and therefore the tests specified are not always relevant to domestic areas. For instance, the virus test EN 13610 only tests against bacteriophages. In these cases the test from the medical area should be used where relevant. In cases where no test method is available for one area of use (e.g. sporicidal test in medical area), a test from another area can be used instead, provided that the test parameters (soiling, temperature, etc.) are adapted to the intended use area (for further guidance on adaption of tests see EN 14885 section 4.2).

The application of disinfectants to water systems such as swimming pools, spas, and drinking water is not addressed in EN 14885. For the evaluation of activity against *Legionella* in aqueous systems (water used in cooling towers and water for general purposes, like spas, pools, showers and other uses) a quantitative suspension test is available (EN 13623).

EN 14885 includes guidance on how a phase 3 field trial should be conducted. This guidance is intended to advise on the factors to be taken into account and controlled when performing a field trial.

The use of CEN test methods is highly recommended, provided that the methods are applicable for the use of a product. In some cases, the method can be adapted (other contact times, soiling, etc.) to fit the use conditions. Any deviation from a standard must be clearly described and a justification for any deviations provided.

OECD Standard Test Methods

The OECD publishes practical test methods (comparable to phase 2, step 2 tests (1.4.1.3) or phase 3 (1.4.1.4)) for testing the efficacy of disinfectants on non-porous surfaces within the "Series on Testing and Assessment" or the "Series on Biocides", respectively. Currently, all available methods have been issued as OECD Guidance Documents. Guidance Documents are, however, not covered by the Mutual Acceptance of Data (MAD) principle and are advisory in nature. Further developed OECD Test Guidelines might become available in the future. As European Standards are not available for all types of applications yet, the use of

OECD methods is recommended provided that the methods are appropriately reflecting the use of a product. Again, the methods can be adapted (other contact time, soiling, etc.) to better fit the use conditions, provided that any deviations from the standard are clearly described and justified.

Please note that in the OECD Guidance Documents on disinfectants, the volume of disinfectant solution added to the surface is very high compared to what is normally done in practice. This test protocol can only be used for uses where the volume of disinfectant solution per surface area is similar to the intended use (e.g. flooding).

Other Standard Test Methods

While CEN standards and, in case no CEN standard is available, OECD methods are highly recommended, there are circumstances in which these tests cannot be applied, i.e. they are not available, or relevant to a particular product or use pattern. In those cases other test methods can be used.

Other test methods, for example VAH (former DGHM), DVG, AFNOR, US-EPA, AOAC or ASTM methods, are available and might be used when no international standard is available for a specific application. Where these methods lack predefined test parameters, target organisms or pass criteria, the applicant has to provide evidence why the chosen parameters are appropriate for the intended application.

Where no standard tests are available, suitable test protocols may be designed (and justified) by the applicant, but these should be discussed with and agreed by the CA before testing takes place.

5.4.0.4.3 Data requirements

Label claims and recommendations must be supported by the results of tests appropriate to the area of application.

In each test the composition of the product to be tested should be clearly described, including the identity and function of the active substances specifying quality and quantity in the formulation. In addition, because the co-formulants can affect the efficacy if the product, they must also be clearly described including identity and function. Alternatively, the formulation can be identified by a retrievable reference name or number. In such cases (i.e. it may only state a code for the product for the purposes of confidentiality), the composition of the tested product should be provided separately. As the formulation may affect the efficacy of the product, the composition of the product tested should be the same as the product under consideration. If not, justifications should be provided for any differences, and these will be assessed on a case-by-case basis.

As phase 1 tests do not take practical use conditions into account, they are not considered acceptable to support claims during product authorisation. In general, phase 1 tests are used during the development of the product, for inclusion of active substances on the "Union list of approved substances" under the BPR or to prove that a co-formulant has no biocidal activity.

In general, at least phase 2, step 1 and step 2 tests are required to support label claims during product authorisation. The phase 2, step 1 test will provide basic information on the efficacy of the product (in a standard test), while phase 2, step 2 tests investigate the effects of more in-use factors (such as drying of target organisms). The combination of phase 2, step 1 and step 2 tests will generally provide a robust data package to demonstrate the efficacy of a product. Deviations from the tiered approach should be justified.

In some cases, for example when disinfection is done in suspension under real use conditions (because the target organisms are suspended in a liquid already or will be suspended during the process due to mechanical action, for example, in CIP), a phase 2, step 1 test is sufficient on its own, as this already simulates practical conditions.

In other cases a phase 2, step 2 test may be replaced by a phase 3 test where a phase 2, step 2 tests is not appropriate. In general, a phase 3 test will be done in combination with a standard phase 2, step 1 test, as phase 3 tests are often variable.

Where in-use conditions cannot be simulated, phase 3 tests are required (e.g. drinking water disinfection with ionisation equipment).

If more than one test method is available and applicable in phase 2, step 2 to substantiate a label claim for efficacy, it is sufficient to provide data from only one of the test methods. The test method selected should be one which best represents the way in which the product is used. For example, in the case of a disinfectant used for "hard, non-porous surfaces by spraying", the test method should be one for such surfaces without mechanical action and with representative conditions of use, such as contact time, soiling, temperature and test organisms.

It is not mandatory to perform the tests under obligatory test conditions of the standards if the claimed use conditions of the products are different from these obligatory tests conditions.

Tests have to be performed with relevant target organisms, which are selected in accordance with the standard and the intended use of the product. This is further discussed in Section 1.3.1 of this Guidance. A list of standard test organisms is given in Appendix 3.

The concentrations used in testing should be selected to demonstrate the threshold of product efficacy. Suspension tests should be performed with several dose rates, including at least one rate lower than the effective rate. Competent Authorities (CAs) will evaluate dose response data generated in these tests in order to assess if the recommended dose is appropriate (i.e. the concentration is not too high, or at the minimum) to achieve the desired effect.

For biocidal products which claim a biostatic effect (bacteriostatic, fungistatic, etc. i.e. the ability to inhibit growth of bacteria, fungi etc. without killing them) the efficacy should be shown by suspension tests and simulated use tests (e.g. surface tests). The suspension test and simulated use test should be performed with and without neutralisation and with a water control (where water is tested instead of the product). The results from this testing should show that the product prevents growth of the test micro-organism (i.e. a lower level of test organism compared to the water control) but does not necessarily inactivate them (the micro-organisms survive in the test without neutralisation).

Biocidal products that claim a biostatic effect bear the risk of development of organisms with temporary or permanent reduced susceptibility (resistance). For this reason, efficacy of these types of products has to be examined carefully.

In case of *in situ* production of the active substance or when an apparatus is used to dose the active substance in the right amount to the water, the report should contain information on safety measurements concerning over and under dosing.

Other products, which do not have biocidal or biostatic activity, might fall within the scope of the BPR, Article 3 1 (a) "*with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action"* . No EU standards are

available for these types of product yet, so applicants should provide a method following the principles of this guidance and based on scientific evidence. During development of new tests, or when an applicant is considering using a non-standard test or using novel testing methods, they should discuss this with the CA as to the acceptability and applicability of the test.

In the following sections, guidance on the requirements per product type and use will be given.

Detailed but non-exhaustive lists of the most relevant product applications and uses of biocides, together with the required test methodology, are given in the claims matrices which are a set of tables linked to this guidance document (see Appendix 1 for more information).

For uses and claims that are not specifically mentioned in this document the requirements will be set on a case by case basis by the CA.

5.4.0.4.4 Relevant factors of the test procedure

Formulation of the tested product

A product authorisation is given to a single biocidal product with a defined composition or to a group of products making up a biocidal product family (BPF) and having similar uses, the same active substances, similar composition with specified variations and similar levels of risk and efficacy.

With respect to a single product the efficacy of its specific formulation should be demonstrated. Therefore it is important that the formulation tested is clearly reported in each test report (or provided alongside the test report with a statement that it is the formulation which has been tested). The formulation details should specify the active substances and co-formulants present, together with their respective concentrations, and should confirm that all tested formulations contain the same co-formulants and concentrations. Any deviations should be mentioned and justified in a statement or in the relevant efficacy reports. Where there are deviations in the formulation from that in the product for which authorisation is sought, the tests will only be considered relevant where it is evident that the deviations have no effect on efficacy. In cases where this is not evident, a confirmatory study with the organisms that is most difficult to control should be proposed.

Within the BPF the minimum level of efficacy over the whole potential range of products should be demonstrated and the permitted variations in composition and intended uses should be explicitly identified.

The test formulations should be chosen in such a way that they cover the whole potential range of products. The test formulations should include at least a product with the lowest concentration of active substance. A justification should be given whether co-formulants influence the efficacy. When co-formulants might influence the efficacy, the worst case concentration of co-formulants (i.e. low concentration of a co-formulant that might have a positive effect on efficacy, high concentration of a co-formulant that might have a negative effect on efficacy) should be tested. See also 1.5.7 for more information on testing BPF.

Hard Water Claims

The degree of hardness of the water used to dilute the disinfectant may affect its performance (by the presence of metal ions such as Ca^{2+} and Mg^{2+}). Generally the harder the water is, the less effective the diluted disinfectant will be. Therefore, test programmes which require that products are diluted with potable water must be diluted in water of

standard hardness as defined in the corresponding test standard, for the purpose of efficacy testing.

It follows that any product that carries label claims for effectiveness in hard water must be tested by the appropriate method in water with defined hardness at the level claimed.

Presence of Interfering Substances

Where disinfectants are applied to either inanimate surfaces or skin or liquids, substances may be present on the surface or in the liquid, which may affect the disinfectant's activity.

The nature, amount and condition of the soiling present will affect the efficacy of a disinfectant.

In many cases residual contamination must be expected and in some situations (e.g. in the treatment of blood spillages) disinfectants are specifically used to decontaminate soiling, to prevent infection transfer and to assist in safe disposal.

Blood, urine, faeces, food debris, fats and oils, dust and proteinaceous materials are the most likely organic soilings to be encountered. Limescale, milkstone and soil are the most common inorganic soilings.

Where claims are made for use under soiled or dirty conditions, the use concentrations of the product must be determined from tests performed in the presence of suitable soiling materials. Soiling materials commonly used in efficacy test methods include albumin serum, blood, yeast and yeast extract.

In practice, with exception of a few situations (e.g. clean rooms), the presence of soiling on surfaces or in liquids to be disinfected cannot be ruled out. For this reason, a small amount of interfering substance should always be included during the testing of the product. In the CEN methods this is called "under clean conditions". Tests under clean conditions can be used when the surface is clean before disinfection. This is for instance the case when the label states that cleaning prior to disinfection is necessary. When a product claims combined cleaning and disinfection, the product should be tested under dirty conditions (see Appendix 4 for more information). Also, where the label only states excessive dirt should be remove, and the surface is still soiled after that (e.g. in the meat industry), soiling for dirty conditions should be used. Please note that in some cases EN 14885 is not always sufficient to meet BPR requirements.

When a product is to be recommended for certain uses where the soiling is of a specific type (such as soap film residue or hard water scum), the product must be tested in the presence of that specific soiling type. If more soiling types are relevant for the use of the product (e.g. a product must be used in the beverage industry, in meat industry or in kitchens), pre-testing should be done to determine the most challenging soiling type. Extended testing with the most challenging soiling type will be sufficient to cover all the others.

As an exception to the rule, products to be used in cleanrooms do not require additional soiling in the test. A cleanroom has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. The soiling level in cleanrooms is so low that even testing under clean conditions for the EN tests is still over-dosing of soiling compared to cleanrooms. For these uses the high load of test organisms can be seen as soiling. Tests without soiling will only be accepted when the label states the specific use in clean rooms which are classified according to ISO 14644-1 in class 1 to 9 or according to GMP EU classification in Grade A to D.

Generally, soiling will reduce the efficacy of the disinfectant, and where soiling is present, longer contact times, higher concentrations, pre-cleaning or a combination of these elements may be necessary.

Temperature

Generally, disinfection performance increases with temperature, although this depends on the active substances and the effect on individual species may vary depending on the specific properties. Therefore, the test temperature should be representative of those encountered during the intended use of the product (e.g. low temperature in animal housing, higher temperature in CIP). Some biocides are used in chemothermal disinfection, for instance, some CIP treatments are done under temperatures of 60-80ºC. Also for these uses the products should be tested at the use temperature.

If products (PT 2-4) are tested with high temperatures above 40ºC heat resistant reference test organisms must be used. *Enterococcus faecium* must be used as the only test organism for claiming bactericidal activity. For a virucidal claim the only test organism must be Murine Parvovirus. For a sporicidal claim the test organism can be spores of, for example, *Bacillus cereus* or *Clostridium sporogenes*.

For mycobacteria, yeasts and fungal spores no relevant test organisms for high temperatures are available. Most yeasts and fungal spores are already irreversibly inactivated by high temperature (>40 ºC) in the control without active substance. However, ascospores of several fungi can become heat resistant and can cause problems in, for instance, the food industry.

When standard tests with relevant temperature resistant strains become available for mycobacteria, yeasts and fungal spores, these should be used.

When efficacy against mycobacteria, yeasts and fungal spores is claimed and no temperature resistant strains are available, the standard test organisms should be tested at the maximum temperatures for which the test is validated.

For specific claims against heat resistant species (e.g. *Talaromyces flavus*) efficacy tests with these organisms should be provided. In these tests a control without biocide should be included which shows survival of the test organisms at the high test temperature.

It is possible that the concentration needed to pass the test is higher for the organisms tested at low temperature than for the temperature resistant organisms tested at higher temperature. In that case a justification should be given on how the test results reflect the use concentration in the use instruction on the label.

Contact Time

The contact time of a product on a surface etc. is an important aspect in the evaluation of the efficacy of disinfectants. In general, the longer the contact time, the more effective the disinfectant is. In trials where test organisms are taken from treated samples for further analysis, the contact time between the biocide and the test organisms should be stopped. Neutralisers, membrane filtration or subculture techniques are used to prevent residual carry-over of active substances. Neutralisation is discussed further in section 1.4.4.6 of this Guidance.

Some disinfectants act very quickly, whereas others require an extended contact time to achieve adequate performance. Mycobacteria, bacterial spores, fungal spores and non-enveloped viruses take longer to be irreversibly inactivated than most vegetative micro-organisms.

The contact time that is practical in real life use should be taken into consideration when testing. In phase 2 and phase 3 tests the product should pass the test at the contact time recommended on the product label.

Neutralisation

Neutralisers are used to stop the product's activity in trials where the test organisms are taken from treated samples for further analysis, such as plate count following biocidal treatment. An effective neutraliser for the test product should be identified, and evidence demonstrating the effectiveness of the neutraliser against the product under test, and showing that the neutraliser itself does not have antimicrobial activity, must be included in a test report. Membrane filtration or subculture techniques can be used to stop the product's activity, in combination with or instead of chemical neutralisation. These other methods are covered by the term "neutralisation" as used in this guidance.

Appropriate controls for determining the efficacy of the procedure to stop the product's activity after the contact time should be performed.

pH

The prevailing degree of acidity or alkalinity during disinfection can also affect the performance and choice of disinfectant. Therefore, the pH of the product at the use concentration (diluted) as used in the test must be included in the test report.

Texture of Surfaces

Smooth impervious surfaces are easier to disinfect (and also to clean) than rough or pitted ones. In some circumstances the micro-organisms might be protected from the action of disinfectants by being protected in porous surfaces. Clumps of micro-organisms may also be more difficult to inactivate, as cells inside are protected by dead micro-organisms on the outside. Recently porous surface tests have been developed (CEN) to test under these conditions.

Bacteria and fungi can adhere to surfaces forming biofilms. In biofilms susceptibility is decreased (the bacteria are in a different physical state) and penetration of biocide can be difficult to achieve due to the matrix surrounding the bacteria. This makes bacteria in a biofilm more difficult to inactivate.

Repetition

In general test results become more reliable when the tests are done in replicates (e.g. repeated in time, in more test objects). Replicates should be performed as required in the appropriate EN standards and where appropriate, internal standards or reference substances should be included.

EN14885 section 5 (parts b, c and d) state the following information on precision of the test methods (repetitions):

- For standardised tests, or adaptation of a standard test, it is recommended to repeat the test and/or include an internal standard and/or performing the test in a second and/or third laboratory. When doing the latter the second laboratory (and any further laboratory) might only repeat the test which is regarded as the most relevant one with the least susceptible test organism(s). If results from two or more laboratories are used, each laboratory has to specify one result, e.g. "R = > 5.2 lg (EN 13727-instrument disinfection)". Then the mean of the results of all laboratories is calculated assuming each laboratory's result as equivalent. Results with lg "more than" are set as this figure, e.g. "> 5.2 lg" is used for calculation as "5.2 lg". All lg values are

converted to real numbers, e.g. 5,2 lg to about 158.000. The mean is the arithmetic mean of these converted numbers. If one of the testing laboratories obtains a result less than the required lg reduction, the product must pass if further tests by three other laboratories demonstrate a pass. The calculations above cannot be done with tests where pass criteria are not expressed as lg reduction.

- In case of repetition of the test it is unnecessary to repeat the test with all test-organisms but only with the least susceptible to the product under test.
- If two or more tests are carried out to support a claim of performance (e.g. phase 2, step 1 and phase 2, step 2) and the ensuing recommendation for use, the tests may be ranked according to their order of relevance, i.e. their ability to predict the product's performance under real life conditions. In case of a ranking only the result of the most relevant test may be repeated taking into account advice 3). If a ranking is not possible only the results of the test showing the highest minimum active concentration should be repeated.

5.4.0.5 General data requirements

5.4.0.5.1 Test range

Tests (phase 2, step 1) should be performed at a range of concentrations in order to verify that the use concentration is suitable for the desired effect (e.g. not too high or not at the minimum effective level).

5.4.0.5.2 Claim for several areas of use

In cases where the product is intended for several areas, it is usually acceptable to perform the tests from only one area, as long as the test is performed with the worst case test conditions (temperature, log reduction, interfering substances, etc.) and the test with the highest/most stringent pass criteria is used. In case the strains are different between the PTs all the strains must be tested.

5.4.0.5.3 Biocidal products with biostatic effect

For biocidal products with a biostatic effect (bacteriostatic, fungistatic, etc.), the efficacy should be shown by suspension tests and simulated use tests (e.g. surface tests). The suspension test and simulated use tests should be performed with and without neutralisation. The results from these tests should show that the product prevents growth of the test organism (no increase in numbers compared to the negative control) but does not necessarily inactivate them (survival of the test organism in the test without neutralisation).

5.4.0.5.4 Malodour control

There are specific requirements for products claiming control of organisms that cause malodour. Phase 2, step1 and step 2 tests should be performed with odour producing micro-organisms. A justification for which bacteria, fungi, etc. are relevant to the intended use should be provided. Along with these laboratory tests, an odour test should be performed. The CA will decide on a case-by-case basis whether the product will receive authorisation.

5.4.0.5.5 Changes in ingredients [10]

When small changes are made to the non-active ingredients in a product, it is not always necessary to repeat all the tests with the new formulation. The applicant may provide a description of the changes and the effects that they have on the efficacy of the product. In

[10] For this section, the product family concept of the BPR is not yet taken into account.

the case of a minor change, a robust justification might be sufficient (to be decided by the CA). In other cases, new efficacy tests will have to be provided. This can be either a full set of efficacy tests or a test with the least susceptible organism in the former test.

5.4.0.5.6 Treated articles

5.4.0.5.7 See Section 5.3 for guidance on Treated Articles. Biocidal Product Families

When authorisation is requested for a product family, efficacy should be demonstrated for the whole group but not necessarily of each product. More information is available in Vol II Efficacy Parts B+C, Section 5.2 Product Families [11].

5.4.0.6 Resistance

See section 3.2 for guidance on resistance. .

5.4.0.7 Assessment of application for authorisation

5.4.0.7.1 Decision making

Biocidal Product Regulation 528/2012 (Annex VI) stipulates rules for decision making for biocides.

The test results must meet the requirements of the standards or other criteria for acceptance which are described below per type of use. Where a product does not conform to these criteria, the applicant should provide a justification in the application as to why the product should still be recommended for authorisation. The CA will decide on a case-by-case basis whether the product will receive authorisation.

5.4.0.7.2 Assessment

The CA assessor/expert assesses the performance of the product as demonstrated in the submitted efficacy tests against the label claims made for the product and the above criteria. If the product is judged to be sufficiently effective in laboratory (and, where relevant, field) tests, the product will be recommended for authorisation as far as efficacy is concerned.

In exceptional cases the applicant may provide justification as to why the specified acceptance criteria are not met but the product is still acceptable. The CA will evaluate the justification on a case-by-case basis, possibly in consultation with the other CAs, and decide whether it is acceptable or not.

The following sections give more specific dossier requirements per type of disinfectant.

[11] See footnote 10

NOTE FOR CA CONSULTATION

The text for the following sections 5.4.1 – 5.4.5 (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 at the end of the consultation procedure. 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]

5.4.1 PT1

5.4.2 PT2

5.4.3 PT3

5.4.4 PT4

5.4.5 PT5

5.4.6 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 must 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 biocidal product in the relevant matrix 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 relevant matrix under real-life conditions (e.g. way of contamination, cleaning regimes,time to take effect) and the duration of the effect.

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.

5.4.6.1 Determining the purpose of the Treatment

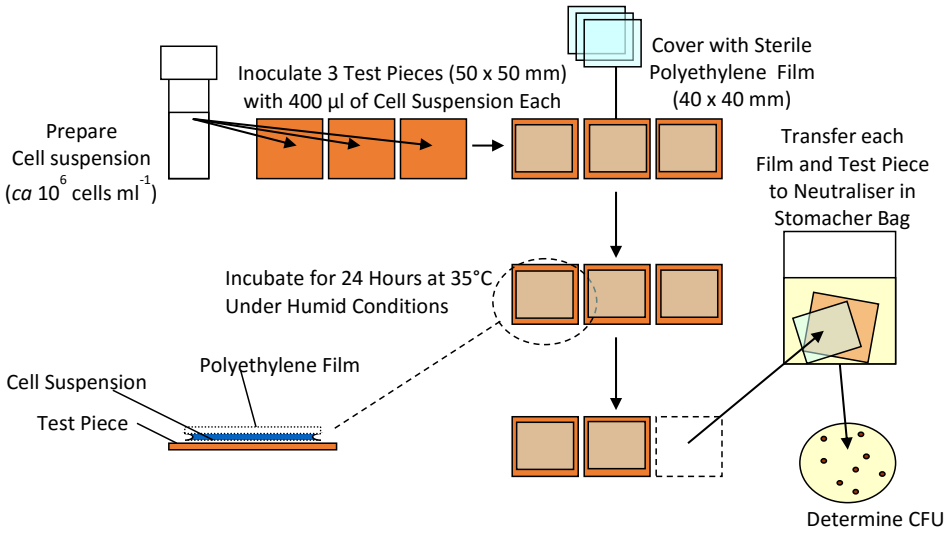
The effects of articles with a disinfection claim cannot be detected by changes in appearance, mechanical properties or odour. The precondition for demonstrating efficacy is a clear description of the purpose of the treatment. Often, claims are unclear about whether the treatment prevents growth or kills 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 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.

5.4.6.2 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, unless otherwise justified. 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). 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.

5.4.6.3 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 using protocols such as those given in Figures 3, 4 and 5 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.

5.4.6.4 Acceptance Criteria

The performance criteria for treated articles can be found in the Claims Matrix for treated articles (Appendix 1). For choosing test organisms please refer to the liquid disinfectants (Appendix 3). As the performance criteria for treated articles are 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

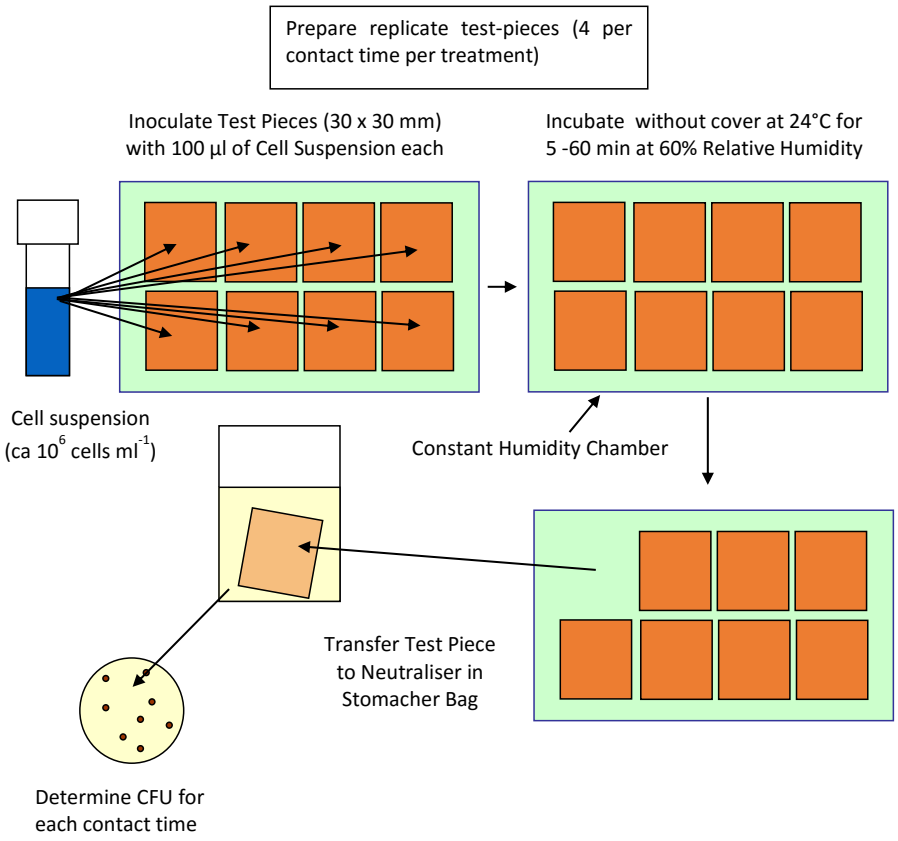


Figure 4: Simulated Splash Model Porous Materials

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

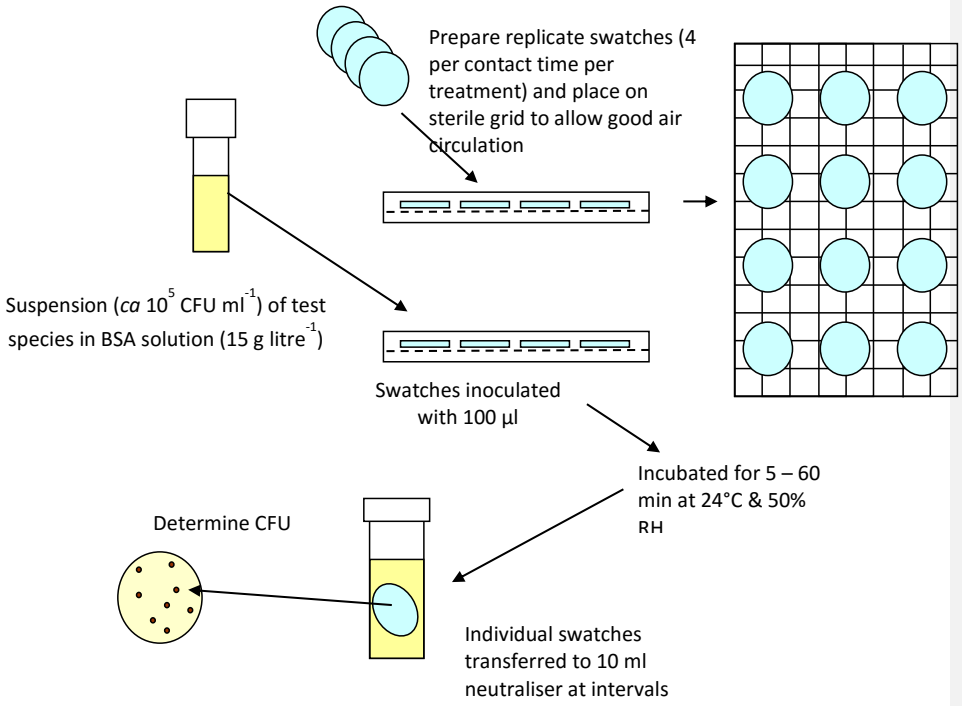


Figure 5: Printing Model

TVC= total viable count
CFU= colony forming units

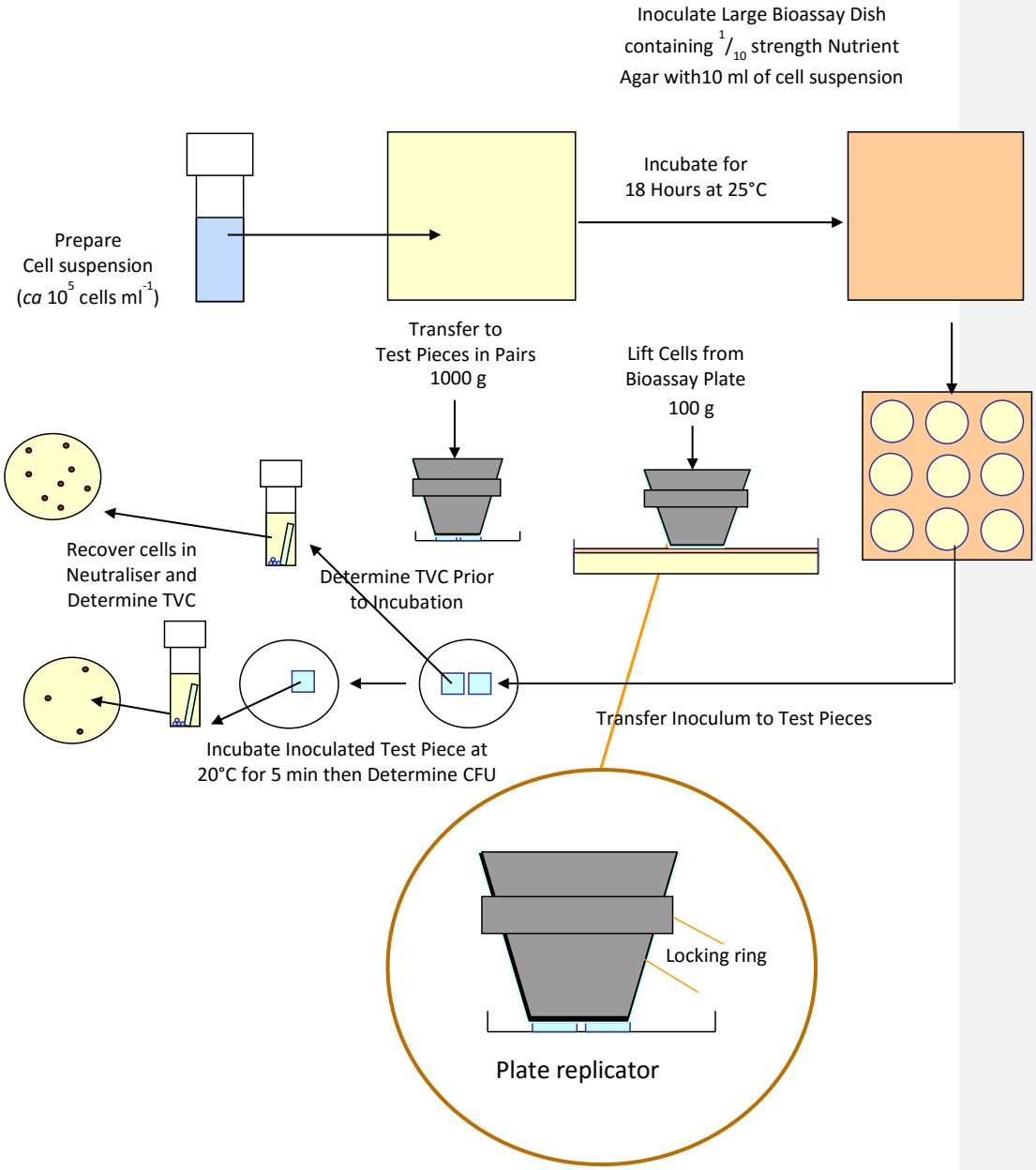


Table 7: Protection of Humans or Animals – Example Claims, Problems and Testing Approaches

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. Minimum of three replicate test pieces of both treated and untreated materials should be employed (unless justified).
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.

5.5 Preservatives (Main group 2)

NOTE FOR CA 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]

General

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.

This section covers the group of preservatives (PT6 to PT13) and the following sections (5.5.1-5.5.3) apply to all PTs (or as indicated in the headings). For PT8, the guidance is more developed and includes standard tests, which is not the case for the other PTs: PT8 is the exception and section 5.5.9 is dedicated to PT8.

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[12]. 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).

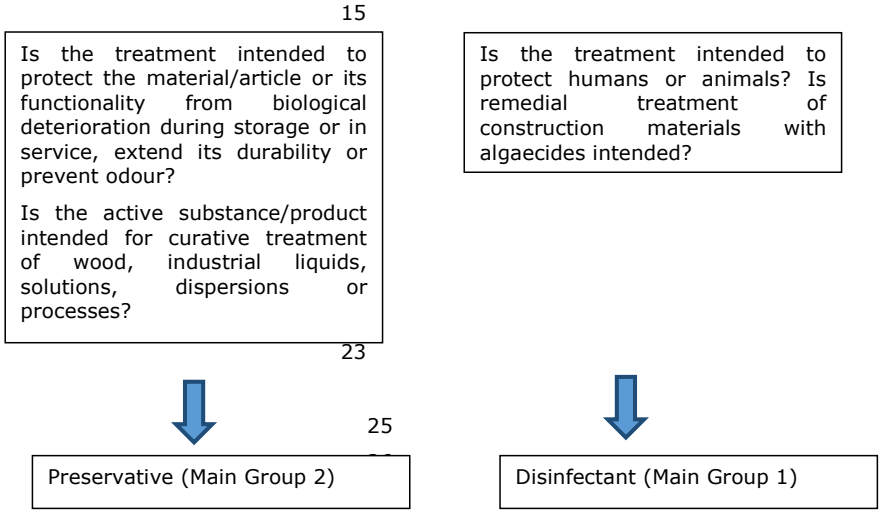
[12] See CA-Sept15-Doc.8.3 – Curative use of preservatives

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
- 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).

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



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 (with the exception of some PT8 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 5.5.5.2 and 5.5.9.

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. Thus 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. 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.

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 for example 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 for example 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. Mixing of bacteria and fungi should generally be avoided 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, or, alternatively, provides growth of microorganisms relevant for the group of organisms which have a negative impact on the stability and/or functionality of the material. 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 and is generally for specific uses in case of specific claims. 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 commonly used standard test methods can be found in Appendices 6, 7 and 8; however, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor as to when and where these tests can be meaningful to prove /support a claim and when they aren't. In contrast to disinfection, there are no specific tests allocated to the different tiers, with the exception for PT8 where standard-tests are available and tiered testing is defined, (see section 5.5.9 for more information). 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. The number of replicates required by the methodology is not necessarily 3 replicates; in such cases this needs to be explained and justified.

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 must 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 biocidal product 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 must have no biocide included. The study must include replicate sub-samples for each treatment (minimum of 3; if less than 3 replicates, then explain and justify).

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 is an important factor in such tests. The number of replicates required

by the methodology is not necessarily 3 which is the usual minimum; in such cases this needs to be explained and justified.

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 Testing conditions for specific states

5.5.5.1 Wet-state preservation and curative treatments (PT 6, 13)

Preservation

Challenge tests are generally employed for preservatives which must preserve liquid matrices, dispersions or fluids used in systems. The inoculum used and the strength of the inoculum depends on which claim must 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 8, however, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor as to when and where these tests can be meaningful to prove /support a claim and when they aren't.

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.

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 must 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 must be employed (minimum of 3, but if the number of replicates required by the methodology is not 3 this needs to be explained and justified) 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.

5.5.5.2 Protection of solid material: PT 7, 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 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 must 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.

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 in any case to support the claims made.

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, odour producing 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). In exceptional cases, it can be acceptable to use individual species when justified, however, using consortia of microorganisms can be a good option to reflect realistic use conditions but the use of individual species is also acceptable. 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 causing stains by 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 should be free of it.
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.
Growth inhibition of moulds occurring on the plasters and walling in building structures	10	Surfaces of walls exposed to weather can be infected by saprophytic molds	Field tests : moulds growth should be shown on untreated material. Treated material should be free of moulds growth.

5.5.6 PT6 Preservatives for products during storage

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 in 5.5.2 and 5.5.5.1. 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 [13]that follow these principles are listed below. Other test methods which are commonly used for PT 6 can be found in Appendix 8. However, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't:

i. A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Polymer Dispersions, IBRG PDG 16-001;

ii. A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Paints, (IBRG2 P 16-001;

[13] Link for IBRG website for test protocols: http://ibrg.org/Methods.aspx

iii. Tier 1 Basic Efficacy Method for Biocidal Active Substances used to Preserve Aqueous-Based Products, (IBRG2, IBRG PDG 16-007.

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. 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) deal 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 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.

5.5.7 PT 7 Film preservatives (and PT 9 Fibre, rubber and polymerised materials preservatives)

Uses within PT 7 (film preservatives) and PT 9 (fiber, leather, rubber and polymerized material preservatives) 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, the test 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.

5.5.7.1 Simulation Tests (Tier 1 testing)

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 6) 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 For Tier 2 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 BS EN 60068-2-10:2005 (see Appendix 6); 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.

5.5.7.2 Tests based on artificial growth media (Tier 1 testing)

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). 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

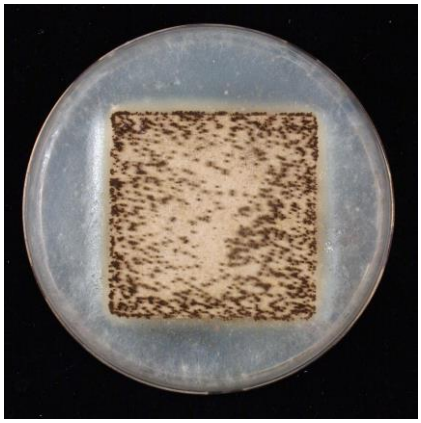
external nutrients is essential in determining the efficacy of a biocide. In many instances a consortium of organisms is required to effect colonisation and deterioration of the material and, in general, methods that employ consortia should be selected.

Similar testing technologies as those used for plastics exist for certain textiles, paper and surface coatings. The most commonly used are listed in Appendix 9; Howwever, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't. A description of the basic principles of tests on artificial growth media 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:



Growth on Untreated Plastic

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.

5.5.7.3 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[14], EN 84:1997[15], 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

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

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. Representatives for all claimed organisms should be tested

5.5.7.4 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 must be protected;
ii. Relevant use conditions should be chosen in terms of humidity temperature and soiling;
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.

5.5.7.5 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 10; however please note that the test methods listed are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't.

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.

Suspension tests

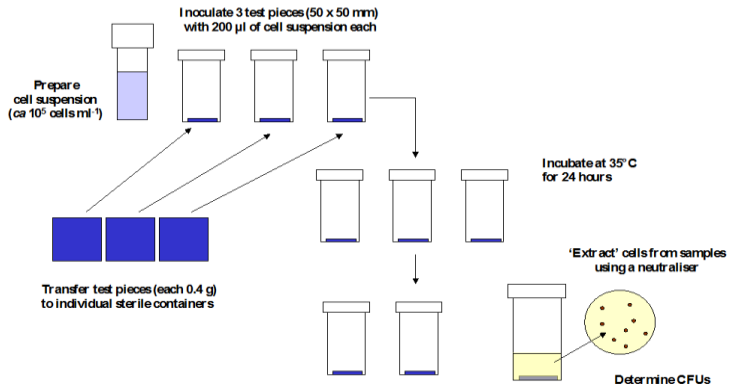
The second major group, the suspension tests, measure changes in the size of a population following contact with a treated textile. A number of protocols are described in Appendix 10. However, most employ relatively high concentrations of nutrients in the suspending medium so that their application, 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[16]) and the IBRG Textile Method[17]. These are described schematically in Figure 9 and are based on the 'germ' count or absorption phase of ISO 20743: 2007 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.

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

[17] 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



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 11 below.

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, user trials are proposed as reliable methods to prove anti-odour effects, especially in case of textiles, but also suitable microbiological studies with relevant odour-causing microorganisms can be acceptable ways to prove anti-odour claims. 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.	Wearing trial or scientifically valid odour based simulation study.
		The effect should be shown to be of sufficient durability during service life to match any claim made.	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.

5.5.8 PT8 Wood preservatives

NOTE FOR CA CONSULTATION

The text in this section (and Appendix 11) will supercede the published Transitional Guidance on Efficacy Assessment of PT8. 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]

General Introduction

This document deals with the evaluation methodology of efficacy tests for wood preservatives biocidal products that are applicable in the frame of the EU Biocidal Products Regulations (BPR) for the authorisation of biocidal products (BPR Annex VI).

The document is not intended to replace standards, standardized methods or other methods used as reference for developing the required data. It is considered as scientific guidance and the reader is advised to refer to the standards themselves or appropriate literature in case details should require further clarification.

The aim of this document is to provide a common base for the assessment of the efficacy for the biocidal product authorization for PT8 products for the applicants and the Competent Authorities (CAs).

Although alternative test methods could be taken into account, this document is mainly based on the EN 599-1 standard for preventive uses and on the EN 14128 standard for curative uses.

This document covers the products used for the preventive treatments of wood (including the saw-mill stage), by the control of wood-destroying or wood-disfiguring organisms (temporary treatments of logs in the sawmill or log yards, temporary treatments of green sawn timber, treatments of sawn timber including round timber, treatments of wood based panel) and products used for the curative treatments of sawn timber in service.

For product already on the market before entering into force of the standards (in 1990 for EN 599 and in 2004 for EN 14128):

- Efficacy data on the product should be provided.
- The assessment of the product efficacy should be based on expert judgement;
- Some data taken from the literature or used in certification could be accepted on case by case basis.

When the data are not enough robust to demonstrate the efficacy of the product, new tests according to EN 599 and/or EN 14128 will be required.

At the review time of this document, it has been chosen to include the catalogue of uses in the Chapter 7 of the Technical notes for guidance (TNsG) on product evaluation (PT8). The inclusion of the catalogue of uses to this document is to provide a common basis to harmonize the claims of the product. It will facilitate in a second time the mutual recognition by listing the elements of the claim in the same order and using the same terminology. On the label, the categories related to the product should be presented as described in the following paragraphs. The codes increase the readability of this document and are not expected on the label.

Concerning the updating of this document, it should be considered as a living document and will be reviewed on a regular basis and updated if necessary, under ECHA's procedures.

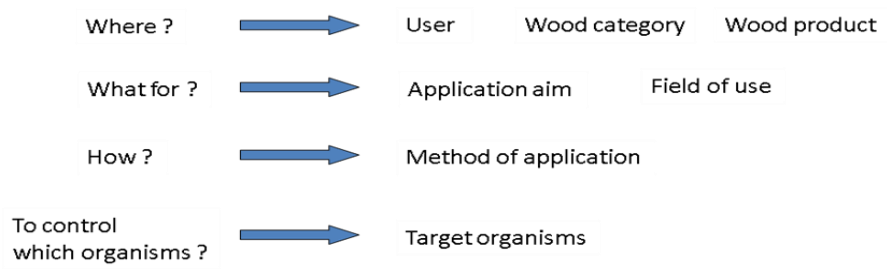
The tests should be performed according to the current version in force of this document. Any tests initiated before the endorsement of the new version remain acceptable.

5.5.8.1 Label claims

In order to harmonize the efficacy issues, it is proposed that the different uses of the product are presented following the proposal below. This should follow the order of the categories listed below.

The aim of this categorisation is to have an explicit answer on the following questions:

- Where is the product used?
- What is the product used for?
- How is the product used? To control which organisms?



The data which support the efficacy should also follow this format.

The main categories that should be present on the label are listed in Table 12 and are detailed in the following paragraphs.

Table 12: Different categories and the related product codes

Categories	Code for product
User category	A.xx
Wood category	B.xx
Wood product	C.xx
Application aim & Field of use	D.xx & E.xx
Method of application and rate	F.xx
Target organisms	G.xx

5.5.8.1.1.1 User Category (Code for Product A.xx)

Information on the intended users of the product has to be presented on the label, the different user categories are presented in Table 13.

Table 13: User categories

User Category	Example	Product Code
Non-professional/general public	Product used at home by consumers	A.10
Industrial	Industrial applicator	A.20
Professional	Pest control operator	A.30

5.5.8.1.2 Wood Category (Code for product B.xx)

This section deals with the wood category and not the use classes as defined in EN 335 standard. From an efficacy point of view, in EN 599-1, annex D the wood timbers are divided into two categories: softwood and hardwood.

Softwood and hardwood species of timber react differently to the degree and the type of attack by certain biological agents.

In most cases, the tests are performed with softwood. In some cases it is acceptable for this data to be read across to hardwoods, but in other cases specific testing against hardwoods is required. (see EN 599-1)

Table 14: Wood categories

Wood Category	Product Code
Softwood	B.10
Hardwood	B.20

5.5.8.1.3 Wood Product (Code for product C.xx)

Table 15 below describes the types of wood products that are used as building materials or in the manufacture of furniture. Wood products are divided in two main categories: solid wood and wood based panels. Based on European standards, wood based panels are divided in four categories: plywood (EN 636), OSB (EN 300), Particles (EN 309 & EN 312) and Fibers (EN 622).

Table 15: Wood product categories

Wood Category	Product Code
Solid wood	C.10
Reconstituted solid wood *Engineered solid wood products produced by processes involving pressure, adhesives and binders*	C.11
Panels	C.20

Wood Category	Product Code
Plywood panels	C.21
OSB panels	C.22
Particles panels	C.23
Fibers panels	C.24

5.5.8.1.4 Application aim and field of use

5.5.8.1.4.1 Application aim (code for product D.xx)

A preventive treatment is used to prevent sound wood from being infected by wood destroying agents and/or disfiguring fungi. The curative treatment is used to kill infective organisms that have already attacked the wood, to prevent them from spreading in the rest of the wood.

The preventive treatments are most of the time used during the manufacturing process but can also be done when the wood is in its service situation (e.g. framework of the building, a bridge.).

According to the fact that a product can be used in wood preventive treatments, in curative treatments and sometimes both, and according to the fact that wood preservative and curative treatments are not covered by the same treatments, it is proposed to split the application aims as presented in Table 16.

The aim of this classification is to ensure having the same classification throughout the EU.

Table 16: Application aim

Application Aim	Kind of Treatment	Product Code
Preventive	Temporary preventive treatment / logs	D.10
	Temporary preventive treatment / green sawn timber	D.20
	Preventive treatment / blue stain in service	D.30
	Preventive treatment-use class (cf. the following section for the field of use – code E)	D.40
Curative	Curative treatment / wood in service	D.50
Preventive	Other (for e.g. pole maintenance)	D.60

5.5.8.1.4.2 Field Of uses (Code For Product E.xx)

The use classes described in EN 335:2013 are defined in terms of service conditions, with reference to the generalised moisture content and the prevailing biological agents of deterioration. The different classes (and their related application codes) are presented in Table 17.

- Use class 1: situation in which the wood or wood based product is inside a construction, not exposed to the weather and wetting;
- Use class 2: situation in which the wood or wood-based product is under cover and not exposed to the weather (particularly rain and driven rain) but where occasional, but not persistent, wetting can occur;
- Use class 3: situation in which the wood or wood-based product is above ground and exposed to the weather (particularly rain);
- Use class 4: situation in which the wood or wood-based product is in direct contact with ground or fresh water;
- Use class 5: situation in which the wood or wood based product is permanently or regularly submerged in salt water (i.e. sea water and brackish water).

Use class 3 is split into two sub-classes:

- 3.1: wood and wood based products will not remain wet for long periods. Water will not accumulate;
- 3.2: wood and wood-based products will remain wet for long periods. Water may accumulate.

The use classes 4.1 and 4.2 described in the former version of the EN 335 standard (2009) have been merged into a single use class 4, including both wood in exterior, in ground and/or fresh water contact.

Table 17: Different field of uses

Field of Uses	Product Code
Use class 1	E.10
Use class 2	E.20
Use class 3*	E30
Use class 3.1	E.31
Use class 3.2	E.32
Use class 4	E.40
Use class 5	E.50

* includes use class 3.1 and use class 3.2

5.5.8.1.5 Method of application and application rate (Code for product F.xx):

The various methods available can be broadly split into three groups:

- **Superficial treatments**

Such non-pressure processes include brush, spray, roller, pad application and immersion (dipping) processes (where the wood can be in contact for preservative for periods of time ranging from a few minutes to several hours). The application rates are commonly expressed in g/m^2, ml/m^2.

- **Penetrating treatments**

Such processes include the vacuum pressure, alternating oscillating pressure, double vacuum and non-pressure processes such as diffusion treatments. The application rates are commonly expressed in kg/m^3.

- **Other treatment methods**

For application methods different from those described above (fumigation, injection), either specifically relevant data or some justification for non-inclusion of data (i.e. details on penetrability/retention, etc.) will need to be provided to the CA for consideration.

Some PT 8 products are designed to be used with a top coat, e.g. primers for window framing. If a top coat is needed according to the manufacturer, this must be applied with the product. When a more general use is envisaged, generic coating materials can be used according to the norms performed.

Table 18: Method of application

Method of application	Product Code
Superficial application / brush/roller/pad treatment	F.10
Superficial application / spray treatment	F.11
Superficial application / flow coat /aspersion	F.12
Superficial application / foam treatment	F.13
Superficial application / dipping treatment	F.14
Injection	F.20
Pressure process	F.30
Pressure process / vacuum pressure impregnation	F.31
Pressure process / double vacuum	F.32
Fumigation	F.40
Fumigation bubble	F.41
Pole in services fumigation	F.42
Mixing with glue and mortar	F.50
Diffusion	F.60
Solid pellets / rods	F.61
Pole bandage / wrapping / pad application	F.62
Other application methods	F.70

5.5.8.1.6 Target organisms (Code for product G.xx)

This section describes the main categories of target organisms, in relation to the claimed uses of the product, either for treatments to prevent biological attack, or for curative treatments to disinfest or to eradicate existing attack.

Appendix 11 gives more information on the principle target organisms.

There are a number of possible effects on target organisms resulting from the proposed use of a wood preservative product. The efficacy data for a wood preservative must be suitable to demonstrate the efficacy of products applied as either pre-treatments to prevent biological attack, or as curative treatments to disinfest or to eradicate existing attack. These may be in a variety of forms; they may yield toxic values, mortality values, subjectively derived ratings or effective retention values.

On the claimed matrix, the target organisms against which an efficacy is claimed must be clearly described. For the purpose of harmonisation, it is proposed that the target organism presented in Table **19**19 should be used, although these should not be considered as an exhaustive list. The species presented below are the species being representative of wood attacking organisms. For specific claims, efficacy data against each named target pest will be required.

Table 19: Examples of target organisms for wood preservatives (N.B. these examples are not intended to be exhaustive with respect to target organisms or prescriptive with respect to data to be generated).

Target organisms				
Common English term	Code F forproduct	Target organisms according to EN 1001	Classification	Scientific name
Fungi			Fungi	
Wood rotting fungi				
Wood rotting basidiomycetes	G.10	Brown rot fungi	Basidiomycetes	e.g. *Gloeophyllum trabeum*
	G.11	White rot fungi	Basidiomycetes	e.g. *Coriolus versicolor*
Soft rot fungi	G.12	Soft rot fungi	Ascomycetes, Deuteromycetes	e.g. *Chaetomium globosum*
Wood discolouring fungi	G.21.1	Sapstain fungi (bluestain mainly)	Ascomycetes, Deuteromycetes	e.g. *Ophiostoma piliferum (Ceratocystis pilifera)*
	G.21.2	Bluestain in service	Ascomycetes, Deuteromytcetes	e.g. *Aureobasidium pullulans*
	G.22	Mould fungi	Ascomycetes, Deuteromycetes,	e.g. *Aspergilus niger*
Insects			Insecta	
Beetles	G.30	Wood boring beetles	Coleoptera	
	G.31	House longhorn beetle		e.g. *Hylotrupes bajulus.*
	G.32	Common furniture beetle		e.g. *Anobium punctatum*
	G.33	Powder post beetles		e.g. *Lyctus brunneus*
	G.40	Fresh wood insect	Coleoptera	e.g. *Scolytus spp*
Termites	G.50	Termites (genus claimed)	Isoptera	
	G.51	Subterranean termites (genus claimed)		e.g. *Reticulitermes spp,* e.g. *Coptotermes spp*
	G.52	Drywood termites (genus claimed)		e.g. *Cryptotermes spp*
	G.53	Tree termites (genus claimed)		e.g. *Nasutitermes spp*
Wood destroying marine organisms	G.60	Marine borers (genus claimed)		
	G.61	Mussels	*Teneridae, Pholadidae*	e.g. *Toredo sp, Martesia sp*
	G.62	Crustaceans	*Isopoda, Amphipoda*	e.g. *Limnoria spp, Chelura spp*

5.5.8.1.7 Examples of a claimed matrix

To illustrate the previous sections described, the following table gives an example of claimed matrix based on the categories from the catalogue of uses. This framework should be followed for the efficacy claim's part of the label. Only the categories and the matrix wordings (not the code) are expected to be listed on the label.

This matrix allows a harmonisation of the efficacy elements presented in the dossier for product authorization. Elements in the claimed matrix must be present on the physical label.

Table 20: Examples of claim matrix based on the application codes for product

Label 1:

Categories	Matrix Wording	Code for Product
User category	Industrial	A.20
Wood category	softwood and hardwood	B.10; B.20
Wood product	solid wood	C.10
Application aim and field of use	preventive treatment - use class 3.2	D.40; E.32
Method of application and rate	superficial application/dipping treatment application rate: 100 g/m² in the analytical zone a top coat must be applied.	F.14
	pressure process/vacuum impregnation application rate: 50 kg/m³ in the analytical zone	F.31
Target organisms	wood boring beetles	G.30
	termites (genus *Reticulitermes*)	G40
	brown rot fungi	G.10
	white rot fungi	G.11

Label 2:

Categories	Matrix Wording	Code for Product
User category	Industrial	A.20
Wood category	softwood and hardwood	B.10; B.20.
Wood product	solid wood	C.10
Application aim and field of use	preventive treatment - use classes 2, 3 and 4	D.40 - E.20; E.30; E.40

Categories	Matrix Wording	Code for Product
Method of application and rate	superficial application/dipping treatment application rate in the analytical zone: UC 2: 80 - 120 g/m² UC3 (coated): 100 – 160 g/m² pressure process/vacuum pressure impregnation application rate in the analytical zone: UC2: 30 kg/m³ UC3: 40 - 70 kg/m³ UC4 (softwood): 80 – 150 kg/m³ UC4 (hardwood): 100 – 150 kg/m³	F.14 F.31
Target organisms	brown rot fungi	G.10
	white rot fungi	G.11
	soft rot fungi	G.12
	wood boring beetles	G.30
	termites (genus *Reticulitermes*)	G.40

1 **Label 3:**

Categories	Matrix Wording	Code for Product
User category	Industrial	A.20
Wood category	softwood	B.10.
Wood product	solid wood	C.10
Application aim and field of use	temporary preventive treatment - use class 1	D.20 E.10
Method of application and rate	superficial application / dipping treatment application rate 100 g/m² in the analytical zone	F.14
Target organisms	sapstain	G.21.1
	mould fungi	G.22

2

5.5.8.2 Available data

5.5.8.2.1 Standard test mthods

When considering the overall evaluation of proposed claims, CAs should ensure that the test methods (data, method of application and application/dose rates used in the tests, product tested) are appropriate to demonstrate the efficacy claimed on the label for the product.

Many standard protocols currently exist to test wood preservatives; the lists of standards for the efficacy assessment of wood preservatives are available on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy]. . For PT8, the CEN standards are highly recommended.

Two main categories of treatment are described:

- Preventive treatments, which are covered by EN 599-1;
- Curative treatments, which are covered by EN 14128.

Some other treatments (C.20: green sawn timber) are covered by other standards (e.g. CEN TS 15082).

It is highly recommended to perform the studies according to these standards. If the standards are not applicable or suitable, the applicant may adapt the methodology or use another method (including his own method). When a standard is modified or when a non CEN standard is used, a robust justification and description have to be provided. For very specific cases, tests or ageing procedures could be waived with a robust justification. The study submitted has to provide a clear answer to the issue.

In the general part of the TNsG on data requirements it is mentioned that the test (and the data generated) should be based on sound scientific principles and practices. Compliance with quality standards is highly recommended.

In the TNsG on product evaluation, it is mentioned that for efficacy testing, the principles of Good Laboratory Practice (GLP) are not required by the legislation. However this guidance indicates that the spirit of such principles should be applied for the testing of efficacy.

Particular attention should be paid to:

- what information is needed to substantiate a 'claim matrix';
- the Quality Assurance procedures which should be adopted (cf. ISO 17025 for testing and certification);
- the overall evaluation of the data package when the completeness and adequacy of the data are compared with the label claim.

For products intended for application as solids, pastes or encapsulated forms and those intended for curative (in-situ) use, modification of the relevant protocols/testing strategies may be done or other direct evidence may be submitted on their potential efficacy against the claimed target organisms (e.g. for pastes such evidence could be in the form of penetrability and retention characteristics).

The test methods used to provide data should be relevant to the target organisms and application processes claimed on the label (see EN 599-1 and individual test standards).

It has to be noted that in some cases, a different formulation from which an authorization is sought could be tested. The results could be accepted by the RMS in a case by case approach (see section 5.5.8.3 of this guidance and Annex A of the EN 599-1 and EN

14128). A full composition of the tested product and a robust justification why the test is relevant should be provided.

For EN113, where the protocol states that several organisms have to be tested in order to fulfil the efficacy criteria, it is recommended that all testing is done in the same laboratory at the same time. The sponsor must have the right to provide his rational for justification why the simultaneous testing may have not been followed. Derogation (inter alia) is acceptable i.e. in the following cases:

- where the test was performed with limited organisms and later completed with additional organisms which could be tested in another laboratory (extension of claim);
- where the laboratory cannot run the test with specific targets;
- where the laboratory has ceased to provide services;
- in the case where a 'simultaneous test' is not available, but valid tests (according to the criteria in the standard) are available.

Table 21 and Table 22 below are informative for the test methods used. The user should also refer to EN 599-1 or EN 14128 depending on the claims.

1 **Table 21: Preventive treatments: List of available standards and others methods used in wood preservation**

Organisms	Code for product	Temporary treatment of logs	Temporary treatment	Treatment of solid wood (List of standards mentioned in the tables 1 to 5 of EN 599-1) Note 1: In some conditions, ageing tests (EN 84, EN 73) or natural weathering are required (see EN 599-1) Note 2: It is highly recommended to refer to EN 599-1 to determine the tests to be done in accordance with table 1 to 5 of EN 599-1					Treatment of wood based panels[18]
				Use Class 1	Use Class 2	Use Class 3	Use Class 4	Use Class 5	
Brown rot fungi	G.10				EN 113	EN 113 EN 839 EN 330	EN 113 EN 252	EN113	ENV 12038
White rot fungi	G.11					EN 113 EN 839 EN 330	EN 113 EN 252	EN113	ENV 12038
Soft rot fungi	G.12						ENV 807 EN 252	ENV 807	
Sapstain fungi	G.21.1	No CEN standard*	No CEN standard*						
Bluestain fungi	G.21.2		No CEN standard*		EN 152	EN 152	EN 152	EN 152	
Mould fungi	G.22		No CEN standard*			No CEN standard			
Wood boring beetles	G.30			EN 46 EN 47 EN 49-1	EN 46 EN 47 EN 49-1	EN 46 EN 47 EN 49-1	EN 47 EN 49-2 EN 20-2	EN 47 EN 49-2 EN 20-2	

[18] For wood based panels, the reader is aware that standards can be adapted in specific cases (e.g. CEN/TS 15083-2 for soft rot fungi, EN 20-2 for powder post-beetle and EN 117 and EN 118 for termites)

Organisms	Code for product	Temporary treatment of logs	Temporary treatment	Treatment of solid wood (List of standards mentioned in the tables 1 to 5 of EN 599-1) Note 1: In some conditions, ageing tests (EN 84, EN 73) or natural weathering are required (see EN 599-1) Note 2: It is highly recommended to refer to EN 599-1 to determine the tests to be done in accordance with table 1 to 5 of EN 599-1					Treatment of wood based panels[18]
				Use Class 1	**Use Class 2**	**Use Class 3**	**Use Class 4**	**Use Class 5**	
				EN 49-2 EN 20-1 EN 20-2	EN 49-2 EN 20-1 EN 20-2	EN 49-2 EN 20-1 EN 20-2			
House longhorn beetle	G.31			EN 46 EN 47	EN 46 EN 47	EN 46 EN 47	EN 47	EN 47	
Common furniture beetle	G.32			EN 49-1 EN 49-2	EN 49-1 EN 49-2	EN 49-1 EN 49-2	EN 49-2	EN 49-2	
Powder post-beetle	G.33			EN 20-1 EN 20-2	EN 20-1 EN 20-2	EN 20-1 EN 20-2	EN 20-2	EN 20-2	
Fresh wood insect	G.40	No CEN standard*							
Termites	G.50			EN 118 EN 117	EN 118 EN 117	EN 118 EN 117	EN 117 EN 252	EN 117	
Marine borers	G.60							EN 275	

1 Blank cell: Not applicable;

2 * National standards available (see the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-
3 are/biocidal-products-committee/working-groups/efficacy]).

4)

Table 22: Curative treatments: List of available standards used in wood curative treatments (based on EN 14128)

Organisms	Code for Product	Curative treatment
Brown rot fungi	G.10	
White rot fungi	G.11	
Soft rot fungi	G.12	
Sapstain fungi	G.21.1	
Blue stain fungi	G.21.2	
Mould fungi	G.22	
Wood boring beetles	G.30	
House longhorn beetle	G.31	ENV 1390
Common furniture beetle	G.32	EN 48 or EN 370
Powder post beetles	G.33	No CEN standard available
Fresh wood insect	G.40	
Termites (genus claimed)	G.50	No CEN standard available
Marine borers (genus claimed)	G.60	

*Blank cell: Not applicable

5.5.8.2.2 Preventive treatments

Most of the available data are laboratory generated and related to the organisms for which biocidal efficacy is claimed.

Field tests, although desirable in cases where the product is intended for use in the more severe service environments (e.g. in ground contact (use class 3, 4 and 5)) are considered mandatory to fulfil the minimum performance criteria, according to the tests required in the paragraphs related to the use classes As this could lead to a significant delay before a new product could be introduced to the market, literature, monitoring or other methods provided to support the derived application rate could be accepted in case by case by the CAs (see also notes in sections 5.5.8.2.2.3 and 5.5.8.2.2.4).

The assessment of the preventive efficacy of wood preservative formulations has to be made from values derived from a relevant biological test. These values are either the actual quantitative amounts of the product established in the test as causing the appropriate level of mortality of the target organism, or they represent the threshold limits, the so-called 'toxic values'. These toxic values are two concentrations in the series

used in the test, the first which just permits continued attack and the second which just prevents it.

5.5.8.2.2.1 Temporary treatments of logs (in the sawmill or in storage area)

This kind of treatment is used to prevent the degradation of logs which do not immediately have their bark removed. Indeed, some microscopic fungi (e.g. stain) infect the wood and/or some species of insects belonging to the family of *Scolytidae* and *Bostrychidae* (named "Fresh wood insect" in Table 1919) lay their eggs between the bark and the wood.

To prevent these damages, the logs may be treated with a biocidal product.

As the treatment is temporary, use class is not relevant in this case.

5.5.8.2.2.2 Temporary treatment of green timber

This kind of treatment is used for the protection of freshly felled green lumber against colonization by blue stain and other discolouring micro-organisms (often named 'sapstain' as there are more than 200 fungi which can caused discoloration of the sapwood) and surface mould.

A technical specification (CEN/TS 15082) is available.

1 blue stain fungi and other discolouring sapwood fungi

Blue stain is caused by microscopic fungi that only infect the sapwood. They can cause blue or grey discoloration of the sapwood, but have no impact on its strength. Blue stain reduces the value of the wood.

Typical blue stain fungi are: *Ceratocystis spp, Ophiostoma spp Aureobasidium spp*

Typical other discolouring fungi are: *Stereum spp*

In the final stage of processing in a sawmill, treatment with a biocidal product (commonly applied by dipping to prevent blue stain fungi) may be carried out.

2 moulds growing often on the wood surface

The major problems caused by moulds fungi are discoloration on surfaces, and sometimes health problems. They do not affect the strength properties of wood.

Typical mould fungal genera on wood are: *Alternaria, Aspergillus, Penicillium, Trichoderma*.

A dose rate / dipping time is part of the efficacy assessment. The label claim must mention the dose rate and the dipping time.

5.5.8.2.2.3 Treatments of solid wood (EN 599-1 Standard)

When the purpose is to protect the wood, a preventive treatment is often applied to prevent the degradation of wood by micro-organisms (for example fungi) and/or by insects (for example wood boring insects). The treatment type is related to the organisms against which the wood has to be protected and to the use class. EN 599-1 specifies what test should be done for each use class claimed.

Different target organisms may preferentially attack either softwood or hardwood. Tests must be conducted on softwood and/or on hardwood as appropriate to the target organisms and following the requirements presented in the relevant test procedures.

It must be noted that Use Class 1 requires only insecticide products and, starting from Use Class 2, products are fungicide alone or combine fungicide and insecticide activities.

Use Class 1

Required data

Refer to EN 599 -1 table 1.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against insects as appropriate,.

Data should be presented on test blocks subjected to pre-conditioning by an evaporative ageing process (e.g. EN 73).

Test species

The insect species tested will depend on whether a general or a specific efficacy claim is made. Data should demonstrate activity against one or more of the following specific insects as indicator species: *Hylotrupes bajulus, Anobium punctatum, Lyctus brunneus*, and where appropriate, termites.

Note

CAs should evaluate the available data to determine whether they are sufficient for label claims as follows:

a) for general claims against "wood boring beetles"[19]

All relevant beetle species (*Hylotrupes bajulus, Anobium punctatum* and *Lyctus brunneus*) should be tested except if data (relevant and robust literature data where the materials and methods are detailed; certification data[20] on a case by case basis) are provided which demonstrate that one of the targets is the less sensitive or that the product has an equivalent activity against all beetle species (refer to EN599-1:2014, section 5.2.3)

b) for claims against a specific beetle species

If claims against individual beetle species are detailed on a product label, then suitable efficacy data against those named target pests will be required.

c) for claims against termites

Some data on efficacy against termites will only be required when the product is to be marketed for use as a termiticidal product or where local requirements demand such activity.

For a product claiming activity against termites, suitable data demonstrating preventive efficacy against a European *Reticulitermes* species will be required.

For a product claiming efficacy against overseas tropical termites, suitable data demonstrating preventive efficacy against relevant species will be required.

Use Class 2

Required data

Refer to EN 599-1:2009 table 2.

[19] This correction has been made for an error in drafting and should be considered to be effective immediately and not subject to the standard transitional period of 2 years for new guidance.

[20] This certification ensures that products are fit for purpose and defines a capacity in the use of products taking into account among others the durability in the function (efficiency of the treatment). The efficacy part of the certification scheme is (in France) generated according the requirement of the EN 599.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate.

Test species

The test species used will depend upon the label claims and will include as a minimum the brown rot fungi and insects if appropriate (as in Use Class 1).

Note

The CAs evaluate the available data to determine if they are sufficient for label claims as follows:

a) For claims against wood rotting fungi the following data have to be available:

Suitable laboratory data demonstrating efficacy against brown rot fungi after ageing test in accordance with EN 73.

b) For claims against wood discolouring fungi the following data have to be available:

- Suitable laboratory data on the protective efficacy of the product against blue stain in service after ageing test in accordance with EN 73 or after a natural or artificial weathering cycle as given in EN 152;
- The application process used in the tests (i.e. whether by superficial or penetrative treatment) has to be in accordance with label claims.

c) For claims against insect pests the following data have to be available:

As outlined in Use Class 1.

Use Class 3

Required data

Refer to EN 599-1:2009 table 3a and table 3b.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate.

Test species

The test species used will depend upon the label claims and will include as a minimum the brown rot fungi and insects if appropriate (as in Use Class 1).

Note

The CAs should evaluate the available data to determine if they are sufficient for claims matrix as follows:

a) For claims against wood rotting fungi, the following data have to be available:

- Suitable laboratory tests as outlined for Use Class 2 and in addition, the efficacy will be demonstrated following preconditioning of the treated test blocks by a suitable leaching procedure according to EN 84

b) For claims against wood discolouring fungi the following data have to be available:

- Suitable laboratory data on the protective efficacy of the product against blue stain in service after a natural weathering or an artificial weathering as given in EN 152.
- The application process used in the tests (i.e. whether by superficial or penetrative treatment) should be in accordance with label claims.

c) For claims against insect pests (if relevant) the following data have to be available:

As outlined in Use Class 1, and in addition the efficacy will be demonstrated following pre-conditioning of the treated test blocks by a suitable leaching procedure according to EN 84 if technically possible (i.e. this is not the case for EN 20-1 and 20-2 due to methodological constraints).

According to EN599-1 field test results, ~~according to~~ EN330 may be used by the applicant instead of certain EN 113 test results, after EN 84 leaching test to derive the brown rot fungi. They are not needed to derive the minimum retention requirements.

Moreover EN 330 may be used as an alternative to basidiomycetes laboratory tests (EN 113 + EN 84) for product under coating.

Use Class 4

Required data

Refer to EN 599-1:2009 table 4.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate. In this situation available data should only include application of the preservative by penetrative treatments.

Test species

Test species used will depend upon the label claims and will likely include the following target organisms: brown and white rot fungi, soft rot micro-fungi and if relevant to label claims, blue stain fungi and insects as appropriate.

Note

The CAs should evaluate the available data to determine if they are sufficient for matrix claims as follows:

a) For claims against wood rotting fungi, the following data have to be available

- Suitable laboratory data as outlined for Use Class 3 with the following supplements:
  - all laboratory data should derive from impregnated treated test blocks (i.e. a penetrative treatment) with the test formulation to determine the toxic values against both brown and white rot fungi separately;
  - a suitable laboratory test to determine the toxic efficacy against soft rot fungi and other soil inhabiting microorganisms is required;

b) For claims against wood discolouring fungi, the following data have to be available:

- A suitable laboratory test determining the protective efficacy of the product against blue stain for wood in service as given in EN 152.

c) For claims against insect pests, the following data have to be available:

- As outlined for Use Class 1 and in addition, efficacy will be demonstrated following pre-conditioning of the treated test blocks by a suitable procedure according to EN 73 and to EN 84 separately).

In Use Class 4 data (e.g. EN 252, literature, monitoring or other methods) will be provided to support the derived application rate.

Use Class 5

Required data

Refer to EN 599-1 table 5.

The principal agent of decay in this situation is the marine borers. Therefore in this Use Class available data must include evidence of efficacy in a relevant marine field trial carried out for a minimum of 5 years (e.g. to EN 275 or an equivalent test).

The decay in this situation by basidiomycetes fungi does occur but marine soft rot fungi are more common causing surface softening of timber. Assessment of products against marine fungi is not normally conducted using routinely laboratory tests because of the difficulties for providing conditions which appropriately model the marine environment. There is, at present, not a recognised standard laboratory test for assessment of timber intended for use in salt water.

Test species

Test species used will depend upon the label claims. The principal agent of decay in the marine environment is the marine borers although claims against fungi can also be made.

The CAs evaluate the data to determine if they are sufficient for label claims as follows:

For claims against wood rotting fungi and marine borers, the following data have to be available:

- For fungi available data as outlined in Use Class 4 as a surrogate has to be acceptable.
- For marine borers, a relevant marine field trial data has to be carried out for a minimum of 5 years according to EN 275

5.5.8.2.2.4 Treatments of wood-based panels

The biocidal treatment of wood-based panels is achieved either during or after the manufacturing process.

During the manufacturing process, product can be included into the glue prior to application or directly by wood treatment.

The evaluation of the durability of wood-based panels against brown rot fungi and white rot fungi should be carried out according to the ENV 12038 test method.

There is no specific standardized methodology allowing the evaluation of the resistance of treated wood-based panels against soft rot or insects such as *Lyctus spp.* or termites. However, some of the existing standards usually applied to solid wood can be adapted to the evaluation of wood-based panels: CEN/TS 15083-2 (natural durability to soft rot fungi), EN 20-2 (*Lyctus spp.*), EN 117 and EN 118 (termites).

For post-manufacturing treatment, product can be applied by using a surface application process or pressure process.

In that case, the EN 599-1 is appropriate for determining the retention of post manufacture treatment.

5.5.8.2.2.5 Barrier treatment against *Serpula lacrymans*

The dry rot fungus (Serpula lacrymans = true dry rot fungus) occurs in buildings, causing brown rot in timber. The fungus can develop at relatively low wood moisture contents and is able to penetrate damp masonry over long distances in order to infect further timber or to develop its fruit-bodies.

In general, in case of an infestation of Serpula lacrymans, the infected wood is cut away. To prevent the infection of the new placed wood with fungi coming from the surrounding

masonry, a curative treatment against dry rot in walls (mortar) will result in creating a 'preventive' barrier in / on walls hindering the fungus to grow through.

There is a specific Technical Specification (CEN/TS 12404) for determining the performance of a preservative applied to the upper surface of the mortar in preventing the growth of dry rot through the treated mortar when exposed to the fungus. This method is only applicable to masonry fungicides applied as a true solution of preservative. It is not applicable to rods, pastes and other similar preservative types. This method is applicable to preservatives applied to masonry by brushing, spraying and/or injection techniques or mixed into rendering and plastering mortar for masonry.

5.5.8.2.2.6 Determination of preventive product application rate with regard to service life

The evaluation of PT8 products efficacy is based on the retention of the product as determined in standard test methods, e.g. according to standards listed in EN 599-1. The values determined in this way are critical values (CV's) for a particular formulation. The application rates derived from the CV's are deemed to provide only a baseline efficacy and no conclusion on service life can be made. Indeed, neither is the term service life an absolute measure and no uniform mathematical model exists to derive such from CV's, nor is determination / claim of a distinct service life part of the BPR. Estimation of service life (ESL) is based on the assumption, that different parameters have an impact on the service life of wood. This is explained in ISO 1586-1 and ISO 15686-2.

An estimated service life of wooden products is influenced e.g. by local exposure conditions, maintenance, consumer expectation and long term experiences from field testing or industrial experiences. This can provide justification for setting higher or lower retention rates as derived from CV's only.

Because the concept of ESL is not part of the BPR and claims for a specific service life is consequently solely the applicant's responsibility, the applicant must have the right to apply for lower or higher retentions than just the CV up to the retention rate which is limited by the human health and environmental risk assessments.

In order to support his claim, for UC3 claims, the applicant should submit data from e.g. literature, EN 330. For UC4, the applicant will provide, EN 252 (applicable to UC4 claims) and/or other methods for justification.

Particular specification for use class 4:

The field tests sites (minimum two) or the data extracted from literature must be representative for climatic zones with regards to the markets targeted by the product. The selected sites must allow the evaluation of the product's efficacy on all the biological organisms covered by the label claim.

5.5.8.2.3 Curative treatment

EN 14128 is the lead standard providing detailed insight into the minimum testing requirements for wood preservatives claiming curative activity. It must be noted, that testing standards concerning PT8 products are only available for testing against wood boring insects

It is important to understand that conducting curative treatments may comprise series/combinations of different steps and application methods/techniques in order to achieve the desired result and quite often result in providing preventive and curative efficacy at the same time. .

5.5.8.2.3.1 Wood boring insects

Data required to support label claims for curative efficacy may include some tests generated using existing EN standards for the relevant beetle species or other alternative supporting data.

A number of EN standard tests exist for curative treatments for insecticides against *Hylotrupes bajulus* (ENV 1390) and *Anobium punctatum* (EN 48).The curative activity against *Lyctus* is not tested separately but is derived from results from testing against *Anobium punctatum* and *Hylotrupes bajulus.*

5.5.8.2.3.2 Termites

The control of termites enters into the scope of the PT8 and the PT18 depending of the use of the product. The definition of the product type is related to the use/mode of application of the product.

The reader is also invited to refer to the PT18 efficacy (section 5.6.4).

The curative treatments against termites are designed most of the time to kill the termite colony and prevent degradation of wood.

We can distinguish treatment applied to wood, for example treatment of art furniture, wood rubble from treatment applied to other support than wood for example soil or masonry.

If the product is applied on wood, then this product is covered by the requirement of the PT8. If the product is applied on another support than wood then it is covered by PT18.

We can distinguish three groups of termites:

Drywood termites (Cryptotermes, Kalotermes)

Drywood termites live inside of the wood which is attacked. The curative treatments applied to the wood consequently destroy the entire colony.

Subterranean termites (Reticulitermes, Coptotermes, Heterotermes)

The core of the subterranean termite colony is located in the soil. Termite workers built tunnels to reach wood and destroy it. The treatment applied on infested wood kills the termites present inside of the wood but not the other members of the colony.

Tree termites (*Nasutitermes*)

Tree termites built epigeous (above-ground) nests, frequently on living trees. As a part of the colony has a subterranean location, termites infestations of wood in building may originate either from the nestmates located in the ground or in the epigeous nests. The treatment applied on infested wood kills the termites presents inside the wood but not the others members of the colony.

5.5.8.2.3.3 Fungi

Any claims for curative activity against wood rotting fungi will be supported by suitable efficacy data. No EN standard test protocols presently exist for curative treatments applied to wood. In general, as curative treatment, the infected wood is cut away.

In all cases CAs evaluate the data available to determine if they are sufficient for supporting the label claims.

5.5.8.2.4 Resistance

Information on resistance and the likelihood of its development is required for BPR Annex I inclusion and is also demanded for product authorisation.

At this point, no target organism resistance in field of chemical wood preservatives is known.

More information on resistance can be found in Chapter 6.2 of this TNsG on Product Evaluation, in the Chapter 10 on the TNsG on the BPR Annex I inclusion and on the website of the Insecticide Resistance Action Committee and the Fungicide Resistance Action Committee (FRAC: http://www.frac.info).

5.5.8.3 Biological re-testing after changing the product formulation

While EN599-1 and EN 14128 provide the baseline for the testing requirements of new products, the corresponding annexes to both standards provide guidance on testing requirements when a formulation variation is caused by the addition, the substitution or removal of an active substance. Not all changes are subjected to re-testing and the informative sections of the standards do allow the consideration and taking into account of other data on a case by case expert judgment basis without additional testing. These data sources are not defined in detail but could include:

- Literature data;
- Certification of the product by recognised national quality scheme systems e.g. CTBP+RAL;
- National registrations;
- Others.

For any other changes in the formulation, refer to the informative annex A of EN599-1 and EN 14128. An explanation of Annex A of EN599-1 can be found on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy].

Commented [A3]: EDITORIAL NOTE: Appendix under development: to be available at time of publication

5.5.9 PT9 Fibre, rubber and polymerised materials preservatives

The text for this section is under section 5.5.7 with PT7.

5.5.10 PT10 Construction material preservatives

Please refer to the General sections 1-3 and the Preservatives general sections (i.e. 5.5.1- 5.5.3) of this guidance and the TNsG

5.5.11 PT11 Preservatives for liquid-cooling and processing systems

Please refer to the General sections 1-3 and the Preservatives general sections (i.e. 5.5.1- 5.5.3) of this guidance and the TNsG.

5.5.12 PT12 Slimicides

Please refer to the General sections 1-3 and the Preservatives general sections (i.e. 5.5.1- 5.5.3) of this guidance and the TNsG.

5.5.13 PT13 Working or cutting fluid preservatives

PT13 deals with preservatives for metal working fluids during their use in industrial processes. The general principles for evaluating PT13 products can be found in section 5.5.2 to 5.5.5. IBRG[21] 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. This method should be used, unless it is justified that the method is not relevant for this specific product.

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

5.6 Pest Control (Main group 3)

5.6.1 General

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

Humaneness

According to the BPR (Article 19(1)(b) criterion ii and common principles point 49 and 76 in Annex VI) biocidal products should cause no unacceptable effects on the target organisms, including unnecessary suffering and pain for vertebrates (humaneness). This criterion is relevant for biocides in the Pest Control PTs14, 15, 17, 19 (repelling or attracting vertebrates) and PT20.

For these biocides an assessment must be made to demonstrate that the biocidal product does not cause unnecessary suffering in its effect on target vertebrates. This must include an evaluation of the mechanism by which the effect is obtained and the observed effects on the behaviour and health of the target vertebrates; where the intended effect is to kill the target vertebrate, the time necessary to obtain the death of the target vertebrate and the conditions under which death occurs must be evaluated.

A biocidal product intended to control vertebrates must not normally be regarded as satisfying criterion (ii) under point (b) of Article 19(1) unless:

- death is synchronous with the extinction of consciousness, or
- death occurs immediately, or
- vital functions are reduced gradually without signs of obvious suffering.

For repellent products, the intended effect must be obtained without unnecessary suffering and pain for the target vertebrate.

Guidance on the assessment of humaneness is currently not included in Volume II Efficacy Part B/C: Efficacy Assessment and Evaluation, but some general guidance can be found in the TNsG on Product Evaluation Chapter 6.

5.6.2 PT14 Rodenticides

NOTE FOR CA CONSULTATION

The text for this section has been published as a Transitional Guidance (December 2016). The text is out of scope of this consultation. The published text will be incorporated into this document at the end of the consultation procedure.

The document is available on the ECHA website: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/transitional-guidance]

5.6.3 PTs 15, 16 & 17

. Please refer to the General sections 1-3 of this guidance and the TNsG.

5.6.4 PT18 Insecticide, Acaricides & other Biocidal Products against Arthropods+ PT 19 Repellents & Attractants (arthropods)

NOTE FOR CA CONSULTATION

The text for this section has been published as a Transitional Guidance (September 2016). The text is out of scope of this consultation. The published text will be incorporated into this document at the end of the consultation procedure.

The document is available on the ECHA website: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/transitional-guidance]

5.6.5 PT19 Repellents & Attractants (non-arthropods)

Please refer to the General sections 1-3 of this guidance and the TNsG.

5.6.6 PT20 Other vertebrates

. Please refer to the General sections 1-3 of this guidance and the TNsG.

5.7 Other biocidal products (Main group 4)

5.7.1 PT21 Antifouling products

NOTE FOR CACONSULTATION

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 at the end of the consultation procedure.

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 Embalming and taxidermist fluids

NOTE FOR CACONSULTATION

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 at the end of the consultation procedure.

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

Appendix 1. Claims Matrices

The claims matrices are a set of tables linked to this guidance document: these documents are available on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy.].

The claims matrices linked to this document are intended to cover biocidal products covered under the scope of Product Type 1, 2, 3 and 4

The claims matrix is a tool for the applicant and CAs. It is intended to capture the information that is needed in the authorisation dossier, to adequately describe typical combinations of products, formats of application of the products, as well as target sites. It also includes the claims made and the requirements for testing these claims (in terms of methodology and appropriate performance standards) for a product to be used in this way.

The reader should note that the matrices are not exhaustive in terms of use patterns, scenarios and test methods.

The claims matrix must be used together with the relevant sections within the efficacy guidance document so as to provide both applicants and CAs alike with clear direction as to the nature and extent of the efficacy data required to support a claimed effect. The claims matrix acts as a guide to the information required when compiling an efficacy dataset for a PT1, PT2, PT3 or PT4 biocidal product.

To note:

- Each row (entry) within the matrices is not independent and can be linked to other entries.
- These matrices only address biocidal claims made for these products.
- The claim matrix will be updated regularly according to the state-of-the-art.

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

The methods for testing efficacy referenced within this guidance document are enlisted below. The use of European Standards (Table 23) is highly recommended if available and appropriate for the respective application[22]. Should no European Standard for an application be available yet and an adaption of an existing standard is not possible according to the rules laid down in EN 14885, other test methods and guidance documents (Table 24) may be used. In cases where the below mentioned methods are inappropriate to demonstrate efficacy of a product for special applications, methods from other national or international standardisation bodies may also be employed. These include for example, AOAC, ASTM or ISO methods. It is recommended to agree such testing strategies with the evaluating CA before tests are performed.

Tests should be carried out according to the respective latest edition of a standard. Please check the respective web sites for the latest information.

Table 23: CEN European standards

Reference	Title	PT	Scope/Remarks
EN 1276	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)	1,2,4	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 1499	Chemical disinfectants and antiseptics - Hygienic handwash - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a hygienic handwash product reduces the transmission of transiently contaminating microorganisms when used to wash the artificially contaminated hands of volunteers.
EN 1500	Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a hygienic handrub product reduces the transmission of transiently contaminating microorganisms when rubbed onto the artificially contaminated hands of volunteers.
EN 1650	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)	1,2,4	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

[22] The CEN does not sell or distribute standards or any other deliverable. All European Standards (EN) and drafts (prEN) as well as other approved documents are directly available for purchase from the CEN national standardisation bodies.

Reference	Title	PT	Scope/Remarks
EN 1656	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 1657	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 12353	Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal (including *Legionella*), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity	1,2 ,3, 4,5	This method specifies how to keep test organisms used and defined in European Standards for the determination of bactericidal, mycobactericidal, sporicidal, fungicidal and virucidal (incl. bacteriophages) activity of chemical disinfectants and antiseptics drawn up by CEN/TC 216.
EN 12791	Chemical disinfectants and antiseptics - Surgical hand disinfection - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a product for surgical hand disinfection reduces the transmission of the microbial flora on hands when used for the treatment of clean hands of volunteers.
EN 13610	Chemical disinfectants - Quantitative suspension test for the evaluation of virucidal activity against bacteriophages of chemical disinfectants used in food and industrial areas - Test method and requirements (phase 2, step 1)	4	This European Standard specifies a method for testing virucidal activity against bacteriophages by assessing reduction in the number of infectious bacteriophage particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13623	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity against *Legionella* of chemical disinfectants for aqueous systems - Test method and requirements (phase 2, step 1)	2,4 ,5	This European Standard specifies a method for testing bactericidal activity against *Legionella* by assessing reduction in the number of viable *Legionella* cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13624	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal and yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1)	1,2	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 13697	Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2, step2)	2,4	This European Standard specifies a method for testing bactericidal and/or fungicidal or yeasticidal activity by assessing reduction in the number of viable bacterial cells and/or mould spores and/or yeast cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13704[23]	Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)	4 (1, 2,3)	This European Standard specifies a method for testing sporicidal activity by assessing reduction in the number of viable bacterial endospores in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13727	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)	1,2	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14204	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14347	Chemical disinfectants and antiseptics - Basic sporicidal activity - Test method and requirements (phase 1)	1,2 ,3, 4	This European Standard specifies a method for testing sporicidal activity by assessing reduction in the number of viable bacterial endospores in suspension under defined conditions. The method is declared as a phase 1 test but, but based on its requirements, it can serve as a suspension test (comparable to phase 2, step 1) until revised/additional CEN methodology for testing sporicidal activity becomes available. The approach can be applied to formulated products or to biocidal active substances.
EN 14348	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)	1,2	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells in suspension under defined conditions. The method is also applicable to demonstrate tuberculocidal activity only. The approach can be applied to formulated products or to biocidal active substances.

[23] EN 13704 is under review and the revised standard will include veterinary and human health care areas.

Reference	Title	PT	Scope/Remarks
EN 14349	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14476	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (phase 2, step 1)	1,2 (4)	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14561	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a frosted glass carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14562	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells dried on a frosted glass carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14563	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells dried on a frosted glass carrier under defined conditions. The method is also applicable to demonstrate tuberculocidal activity only. The approach can be applied to formulated products or to biocidal active substances.
EN 14675	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 14885	Chemical disinfectants and antiseptics - Application of European Standards for chemical disinfectants and antiseptics	1,2 ,3, 4,5	This European Standard specifies the European Standards, i.e. test methods, to which products have to conform in order to support the claims for microbicidal activity which are referred to in this document. It also specifies terms and definitions which are used in European Standards. It is applicable to products for which activity is claimed against the following microorganisms: vegetative bacteria (incl. mycobacteria and *Legionella*), bacterial spores, yeasts, fungal spores and viruses (incl. bacteriophages).
EN 16437	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a wood carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 16438	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 16615	Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2)	2 (4)	This European Standard specifies a method for testing bactericidal and/or yeasticidal activity by assessing reduction in the number of viable bacterial and/or yeast cells dried on a PVC carrier under defined conditions. The test applies to products that are used for disinfecting non-porous surfaces by wiping and includes 'ready-to-use wipes' which are impregnated with a microbicidal solution.
EN 16616	Chemical disinfectants and antiseptics - Chemical-thermal textile disinfection - Test method and requirements (phase 2, step 2)	2 (3, 4)	This European Standard specifies a method for testing microbicidal activity of a disinfection process for the treatment of contaminated textile. The procedure is carried out by using a washing machine and microbicidal activity is assessed as the reduction in the number of viable test organisms, such as bacterial, mycobacterial or yeast cells and mould spores, dried on a cotton carrier under defined conditions.

Reference	Title	PT	Scope/Remarks
EN 16777	Chemical disinfectants and antiseptics - Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2, step 2)	2 (4)	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

1 **Table 24: Other test methods and guidance documents**

Reference	Title	PT	Remarks
ASTM E2196	Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with Medium Shear and Continuous Flow Using Rotating Disk Reactor	2,3,4	This test method is used for growing a reproducible *Pseudomonas aeruginosa* biofilm in a continuously stirred tank reactor (CSTR) under medium shear conditions. In addition, the test method describes how to sample and analyse biofilm for viable cells. Available via: http://www.astm.org/Standard/ or the national standardisation bodies
ASTM E2274	Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants	2,3	This test method is designed to evaluate sanitizing/disinfectant laundry detergents/additives for use in top-loading automatic clothes washing operations. This test method is designed predominantly to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.
ASTM E2406	Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations		This test method is designed to evaluate sanitizing/disinfectant laundry detergents/additives for use in high efficiency (HE) automatic clothes washing operations that typically utilize very low wash water volumes. This test method is designed to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.
ASTM E2562	Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor	2,3,4	This test method specifies the operational parameters required to grow a reproducible *Pseudomonas aeruginosa* biofilm under high shear. The resulting biofilm is representative of generalized situations where biofilm exists under high shear rather than being representative of one particular environment. Available via: http://www.astm.org/Standard/ or the national standardisation bodies

Reference	Title	PT	Remarks
DIN SPEC 10534	Food hygiene - Commercial dishwashing - Hygiene requirements, testing	4	This document is a summary of the standards DIN 10510, DIN 10511, DIN 10512 and DIN 10522. It specifies hygiene requirements relating to the design, construction and operation of commercial warewashers and in particular provides information on their hygienic and proper operation, on cleaning and disinfection of wash ware and on care and maintenance of the machinery. It describes the methods for testing hygienic operation. Available via: http://www.beuth.de/en/ or the national standardisation bodies
DVG Guidelines	Guidelines for the testing of disinfection procedures and chemical disinfectants; Original title: Richtlinien für die Pruefung von Desinfektionsverfahren und chemischen Desinfektionsmitteln	3,4	DVG Guidelines specify methods for testing activty of chemical disinfectants against bacteria, yeasts and fungal spores, viruses, and parasites. They apply to the veterinary and the food sector, such as animal husbandry, veterinary practices, meat production/food of animal origin, and large-scale/canteen kitchens (except ward kitchens catering patients). DVG Guidelines are published by the German Veterinary Medical Society (DVG). Available in German via: http://www.desinfektion-dvg.de
ISO/TS 15883-5	Washer-disinfectors - Part 5: Test soils and methods for demonstrating cleaning efficacy	2,3,4	ISO 15883 relates to a series of standards that specify the required performance levels of Washer-Disinfectors. Part 5, the Technical Specification (TS), describes a method to generate biofilm formed by *Pseudomonas aeruginosa*. Available via: http://www.iso.org/iso/home.htm or the national standardisation bodies
NF T72-281	Methods of airborne disinfection of surfaces - Determination of bactericidal, fungicidal, yeasticidal, mycobactericidal, tuberculocidal, sporicidal and virucidal activity, including bacteriophages; Original title: Procédés de désinfection des surfaces par voie aérienne - Détermination de l'activité bactéricide, fongicide, levuricide, mycobactéricide, tuberculocide sporicide et virucide incluant les bactériophages	2,3,4	This French standard specifies a method for testing microbicidal activity of airborne disinfection processes. The tested product is diffused, e.g. in gaseous form or as an aerosol, to reduce the number of relevant test organisms, such as bacteria, bacterial spores, yeasts, and fungal spores. Available in French via: http://www.afnor.org/en or the national standardisation bodies

Reference	Title	PT	Remarks
Nordic Working Paper	Efficacy Assessment of Treated Articles: A guidance	1,2,3,4	The document provides guidance on efficacy testing of biocides used in treated articles. The presence and relevance of existing standard test methods is described and, where they do not exist or where they do not provide sufficient support, the nature of the data required will be described. The document was published by the Nordic Council of Ministers. Open access via: http://www.norden.org/en/publications/publikationer/2014-904/
OECD Series on Biocides No. 1	Guidance Document on the Evaluation of the Efficacy of Antimicrobial Treated Articles with Claims for External Effects		The document guidance on efficacy testing of articles treated with antimicrobials and articles modified to exert an antimicrobial effect. http://www.oecd.org/env/ehs/pesticides-biocides/41692131.pdf
OECD Series on Biocides No. 4	Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants and Field Testing (Series on Testing and Assessment No. 170 and Series on Biocides No. 4)	2	The document provides guidance on setting up a strategy for efficacy testing of pool and spa disinfectants in a laboratory scale testing phase and a field testing phase in a full-size swimming or spa pool. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
OECD Series on Biocides No. 6	Guidance Document on Quantitative Methods for Evaluating the Activity of Microbiocides used on Hard Non-Porous Surfaces (Series on Testing and Assessment No. 187 and Series on Biocides No. 6).	2 (4)	This document describes four quantitative methods for testing bactericidal, mycobactericidal, fungicidal and virucidal activity on steel carriers with high application volumes of liquid products. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
OECD Series on Biocides No. 8	Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials (Series on Testing and Assessment No. 202 and Series on Biocides No. 8)	1,2,3,4	The document provides guidance for testing the basic antibacterial performance of porous (textile) and non-porous (plastic) materials that have been treated with a biocide with the intention of introducing antibacterial/hygienic properties into that material. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
VAH Standard methods	VAH certification of chemical disinfection procedures; Original title: VAH-Zertifizierung chemischer Desinfektionsverfahren	1,2	VAH Standard methods specify methods for testing activty of chemical disinfectants against bacteria (incl. mycobacteria), yeasts, and fungal spores. They apply to testing products used for disinfection in public facilities (medical and other) and, in the event of substantiated medical indications, also in the private home. VAH Standard methods are published by the Association for Applied Hygiene (VAH). Available in German via: http://www.mhp-verlag.de/en/home/

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Appendix 3. Table of Reference Test Organisms (PT 1-5)

This table (Table 25) is given as a general overview of relevant test organisms for testing disinfectants in accordance with the BPR.

This table comprises mainly those reference test organisms that are included in the EN norms covered by EN 14885. Furthermore, strains are listed that are recommended for some uses (e.g. endoparasites from DVG standard).

The reader can check the website of the CEN (European Standardization Organizations): www.cen.eu for new and updated standards.

Since the EN systematics of WG's 1 to 3 does not fit exactly to the BPR PT scheme, in borderline cases an indicated reference test organism might be used for other PTs as well. In cases where there are discrepancies between this ECHA guidance and the guidance in EN 14885, the ECHA Guidance should be followed as the leading guidance. However, EN 14885 can be followed with satisfactory justification to meet the requirements of the BPR.

Tests with test organisms in addition to those mentioned below are acceptable, if adequate scientific evidence is submitted on which the relevance of the test organism to the field of use can be judged.

Table 25: Reference Test Organisms

Key for Table 5:

* X = basic requirement to claim activity against micro-organism;

(X) = basic requirement for specific use as described in the table below in brackets;

O = optional;

** *Aspergillus brasiliensis* is the name of *Aspergillus niger* after reclassification in 2008;

*** for a limited spectrum virus claim in PT1 Poliovirus does not have to be tested.

Micro-organisms	PT1*	PT2*	PT3*	PT4*	PT5*
Bacteria					
Staphylococcus aureus ATCC 6538	X	X	X	X	X
Pseudomonas aeruginosa ATCC 15442 (not for teat disinfection)	X	X	X	X	X
Enterococcus hirae ATCC 10541	X	X	X	X	X
Escherichia coli ATCC 10536 (teat disinfection)		O	(X)	X	X
Escherichia coli K12 NCTC 10538	X	X			O
Salmonella Typhimurium ATCC 13311		O		O	
Lactobacillus brevis DSM6235		O		O	
Enterobacter cloacae DSM 6234		O		O	
Enterococcus faecium ATCC 6057 (for T ≥40°C)		(X)		(X)	
Proteus vulgaris ATCC 13315 (not for teat disinfection)			X		
Streptococcus uberis ATCC 19436 (teat disinfection)			(X)	(X)	
Legionella pneumophila ATCC 33152 (PT2: pools, hot tubs; PT4: drinking water systems, PT5: in collective drinking water systems)		(X) O			X O

Micro-organisms	PT1*	PT2*	PT3*	PT4*	PT5*
Legionella pneumophila ATCC 43108					
Yeasts					
Candida albicans ATCC 10231	X	X	X	X	X
Saccharomyces cerevisiae ATCC 9763 (breweries)				(X)	
Saccharomyces cerevisiae DSM 70487 (breweries)				(X)	
Fungal spores					
*Aspergillus brasiliensis*** ATCC 16404	X	X	X	X	X
Viruses					
Polio virus type 1, LSc-2ab (Picornavirus)	X***	X			
Adenovirus, type 5, strain Adenoid 75, ATCC VR-5.	X	X		X	
Murine norovirus, strain S99 Berlin	X	X		X	
Murine Parvovirus, strain Crawford, ATCC VR-1346 (for T ≥40°C)		(X)		(X)	
Bovine Entrovirus Type 1, ECBO - Virus ATCC VR-248			X		
Enveloped Viruses					
MVA = Modified Vacciniavirus Ankara (teat disinfection)	X		(X)		
Bacteriophages					
Bacteriophage P001 DMS 4262 (milk industry)				X	
Bacteriophage P008 DMS 10567 (milk industry)				X	
Mycobacteria					
Mycobacterium terrae ATCC 15755	X	X			
Mycobacterium avium ATCC 15769	X	X	X		
(PT1 and PT2 claim for mycobactericidal: both, tuberculocidal: *M. terrae* only)					
Bacterial spores					
Spores of *Bacillus cereus* ATCC 12826 (bee hives)		O	(X)	O	
Spores of *Bacillus subtilis* ATCC 6633 (bee hives)		X	O (X)	X	
Spores of *Clostridium sporogenes* ATCC 7955		O		O	
Spores of *Geobacillus stearothermophilus* (for T ≥60°C)		(X)		(X)	
Endoparasites					
Oocysts of *Eimeria tenella* strain Houghton (chicken farms)			(X)		

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Appendix 4. Overview of standards, test conditions and pass criteria (PT 1-5)

The overview is presented in a number of tables which are available on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy.].

These tables provide an overview of available phase 2,1 and 2,2 EN standards which are applicable for testing the efficacy of disinfectant biocides. This overview is not exhaustive. For other or more specific uses and tests other than EN standards, reference should be made to the relevant sections of this guidance.

It should be noted that although this Guidance is mainly based on EN standards, there are some cases where there are discrepancies amongst the EN tests and in such cases the ECHA Guidance should be followed as the leading guidance. Where noted these are identified in the table.

The reader is strongly advised to check whether there are new versions of the standards on the website of the CEN: www.cen.eu.

It should be noted that if tests other than CEN standards (notably when no CEN tests are available) are used, and pass criteria are available, these should be met (unless stated differently in this guidance). When the test does not provide pass criteria, the criteria in this table can be taken into account as guidance for what level of reduction is normally required.

In all cases, deviations from these standards are possible but should be justified in the application.

Regarding the table for PT05, it should be noted that the text in Section 6 (PT 5)of this Guidance document is only "preliminary draft text" and has not been reviewed or revised to address written PEG comments received and the section is currently under review within the "Disinfectants Project". In the meantime, the "preliminary draft text" is available to readers for information and it is for this reason that a table for PT05 is included, but this will be reviewed when Section 6 of the Guidance is reviewed.

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

These viruses may contaminate hands, instruments, other surfaces and textiles.

NOTE 1 This list is not exhaustive.

NOTE 2 Enveloped viruses are in **bold**.

Table 26: Examples of viruses

Blood	
Enterovirus	**Hepatitis C virus (HCV)**
Filoviridae	**Hepatitis Delta virus (HDV)**
Flavivirus	**Human Immunodeficiency Virus (HIV)**
Herpesviridae	**Human T Cell Leukaemia Virus (HTLV)**
Hepatitis A Virus (HAV)	Parvovirus B 19
Hepatitis B virus (HBV)	
Respiratory tract	
Adenovirus (Mast-)	**Influenza Virus**
Coronavirus	**Paramyxoviridae**
Enterovirus	Rhinovirus
Herpesviridae	**Rubella Virus**
Neuronal tissue, ear,nose & eye	
Adenovirus (Mast-)	**Human Immunodeficiency Virus (HIV)**
Enterovirus	Polyomavirus
Herpesviridae	**Rabies Virus**
Measles Virus	**Rubella Virus**
Gastro-intestinal	
Adenovirus(Mast-)	Enterovirus
Caliciviridae	Hepatitis A Virus (HAV)
Coronavirus	Hepatitis E Virus (HEV)
Astrovirus	Rotavirus
Skin, breast and/or milk	
Enterovirus	**Human T Cell Leukaemia Virus (HTLV)**
Herpesviridae	Papillomavirus
Human Immunodeficiency Virus (HIV)	**Poxviridae**

Spleen and lymph nodes (see also blood)	
Human T Cell Leukaemia Virus (HTLV)	
Human Immunodeficiency Virus (HIV)	
Dental procedure	
Adenovirus(Mast-)	**Hepatitis C Virus (HCV)**
Enterovirus	**Hepatitis Delta Virus (HDV)**
Herpesviridae	**Human Immunodeficiency Virus (HIV)**
Hepatitis B virus (HBV)	
Urogenital tract	
Hepatitis B Virus (HBV)	**Human T Cell Leukaemia Virus (HTLV)**
Herpesviridae	Papillomavirus
Human Immunodeficiency Virus (HIV)	Polyomavirus

Reference:

Van Regenmortel MHV et al.,Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000

Appendix 6. Selection of recommended tests for solid materials (excluding wood-preservatives)[24]

Standard Method + section reference	Title	Description	Possible application area
ISO 22196, Section 5.4.2.2	Measurement of antibacterial activity on plastics and other non-porous surfaces	Test to measure inhibition of bacterial growth on plastic material used in wet or humid conditions.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by inhibition of bacterial growth.
Section 5.4.2.3, Figure 3	Simulated Splash Model Non-Porous Materials	Test to measure killing on contact for non-porous material when the contaminant is spread by splashes. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
Section 5.4.2.3, Figure 4	Simulated Splash Model Porous Materials	Test to measure killing on contact for porous material when the contaminant is spread by splashes. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
Section 5.4.2.3, Figure 5	Printing Model	Test to measure killing on contact for non-porous material when the contaminant is spread by e.g. hand-contact. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
BS 3900 Part G6, Section 5.5.8.1	Methods of test for paints. Part G6: Assessment of resistance to fungal growth	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	PT 7

[24] These tests are not necessarily appropriate for all claims and materials. Tests have to be chosen depending on the claim made, the materials used and the conditions of use foreseen for the treated material/article.

Standard Method + section reference	Title	Description	Possible application area
		should be free of it.	
ASTM G21-09, Section 5.5.8.2	Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi	The synthetic polymer portion of plastic materials is usually fungus-resistant in that it does not serve as a carbon source for the growth of fungi. It is generally the other components, such as plasticizers, cellulosics, lubricants, stabilizers, and colorants, that are responsible for fungus attack on plastic materials.	PT 7, 9
ISO 846: 1997, Section 5.5.8.2	Plastics - Evaluation of the action of microorganisms	Method for determining the deterioration of plastics due to the action of fungi and soil microorganisms by visual appearance, changes in mass or changes in physical properties. The aim is not to determine the biodegradability of plastics. Includes even a soil burial variant. Note: the section covering bacteria is not considered to be useful.	PT 7, 9
ISO 16869:2008, Section 5.5.8.2	Plastics - Assessment of the effectiveness of fungistatic compounds in plastics formulations	Method for determining the effectiveness of fungistatic compounds in protecting susceptible ingredients like plasticizers, stabilizers, etc., in plastics formulations. A minimum diffusion of the fungicide out of the matrix is necessary as the spores are added in an agar-layer. Evaluation by visual examination.	PT 7, 9
BS EN 60068-2-10:2005, Section 5.5.8.1	Environmental testing. Tests. Test J and guidance: Mold growth	Test for fungal and microbial resistance applicable to a wider range of materials	PT 7, 9

Standard Method + section reference	Title	Description	Possible application area
OECD (OECD ENV/JM/MONO(2014)18 Section 5.5.8.5.2	Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials.	Method for measuring the inhibition of bacterial growth or metabolism of porous and non-porous materials that have been treated with a biocide.	Anti-odour testing for textiles, PT 9
IBRG TEX13-005.4, Section 5.5.8.5.2	Tier 1 Textile Method Antibacterial Properties	Method to determine the basic antibacterial properties of textiles and porous materials and articles treated with a biocide.	Anti-odour testing for textiles, PT 9

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Appendix 7. Selection of recommended tests for liquid materials[25]

Reference + section reference	Title	Description	Possible application area
IBRG P 16-001.2, Section 5.5.7	Tier 1 Wet State Paint Method	A Method for Determining the Basic Efficacy of Biocidal Active Substances in aqueous based paints.	PT 6
IBRG PDG 16-001.2, Section 5.5.7	Tier 1 Polymer dispersion Method	A Method for Determining the Basic Efficacy of Biocidal Active Substances used in polymer dispersions.	PT 6
IBRG PDG 16-007.2, Section 5.5.7	Tier 1 Basic Efficacy Method for Biocidal Active Substances used to Preserve Aqueous-Based Products	Method for determining the basic efficacy of biocidal active substances for in-can preservation in aqueous based products	PT 6
IBRG FFG 16-001.4, Section 5.5.13	Tier 1 Metal Working Fluids Method	Method for determining the basic efficacy of biocidal active substances in aqueous based metalworking fluids.	PT 13

[25] These tests are not necessarily appropriate for all claims and materials. Tests have to be chosen depending on the claim made, the materials used and the conditions of use foreseen for the treated material/article.

Appendix 8. Commonly Used Methods to Measure the Effects of Preservative/Curative Action in Liquid Matrices[26]

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	13

[26] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

Reference	Title	Description	PT
		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 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 emuls 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-	Cutting fluid, soluble, biostable		13

Reference	Title	Description	PT
70 issue (1990)	joint service designation ZX-9		

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Appendix 9. Commonly Used Methods to Measure the Effects of Protecting Material[27]

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	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

[27] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

	to micro-organisms: Soil burial test Part 1: Assessment of rot retarding finishing		
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	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based	Agar plate test (other vessels containing

	Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Cordage and Yarns to Fungal Growth.	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.	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

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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/ Biodegredadatio n Test.
AS 1157.7 - 1999	Australian Standard - Methods of	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	Agar plate test

	Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Papers and Paper Products to Fungal Growth.	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.	
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

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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 -	Agar plate test	Agar plate test	Biofouling test

96			
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	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	Agar plate test

	Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 1: Resistance to Growth	rings are employed to hold the specimens in intimate contact with agar when necessary.	
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

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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	Biodeterioration Test

		natural and artificial soiling are described in the method which can be employed when appropriate.	
ASTM D3273-12	Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings 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
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 9	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	Zone Diffusion Assay

		of surface coatings.	
AS 1157.10 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 10: Resistance of Dried or Cured Adhesives to Fungal Growth	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
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	Zone Diffusion Assay/Humid Chamber Test

		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.	

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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	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		in the media overlaying the test specimen.	
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 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	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		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.	
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	'Dry' inoculum intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.

		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.	
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.

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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	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	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a

	Antibacterial Activity	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 regimes. An untreated control is optional.	simulation of final use of a treated material.
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 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.	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

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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	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan.	Cell suspension intimate contact test.

	activity on plastics surfaces.	Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	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 antibactérienne	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 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.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of 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.

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Appendix 10. Commonly Used Methods to Measure Antimicrobial Activity[28]

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	Four replicate samples of test material are placed	Cell suspension

[28] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

	étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	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 neutraliser is employed during cell recovery.	intimate contact test. The transfer method of inoculation could be adapted to provide some 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	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	Cell suspension intimate contact test. Basic efficacy test that has

	Count Method	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.	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.

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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	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.

		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 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	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	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an

	Floor Coverings	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 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.	antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

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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 - Caractérisation et mesure de l'activité antibactérienne	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 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.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of 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.

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Appendix 11. Information on the principle target organisms outlined in the document

Fungi

Wood rotting fungi

White rot/ brown rot fungi (*Basidiomycetes):*

Fungi responsible for brown rot (e.g. *Serpula lacrymans, Coniophora puteana*) and white rot (e.g. *Coriolus versicolor, Donkioporia expansa*)

Soft rot fungi (mainly *Ascomycetes, Deuteromycetes*)*:*

Fungi responsible for a type of rot characterised by surface softening of the wood although they also cause rot at depth *(e.g. Chaetomium globosum).* They are specifically significant for wood in ground contact.

Wood discolouring fungi

Sapstain*:*

The blue-black and brown discolouration of freshly felled logs or sawn timber have an economic importance. Sapstain causing fungi can only colonise wood as long as the sap wood contains enough water to provide solved sugars as a nutrient for these fungi ("green" wood). Therefore, these fungi can be controlled by rapid drying of the wood after felling, chemical treatments are sometimes used.

Common sapstain species include e.g. *Stereum spp,* blue staining species.

Blue stain cause blue to black permanent colour of variable intensity and depth mainly in the sapwood depending on the wood species. This does not result in appreciable alteration of the mechanical properties but can increase the permeability of the wood and thereby makes it more susceptible to fungal degradation.

Common blue staining species include e.g. *Aureobasidium* spp*, Ceratocystis* spp

Mould fungi:

Fungi, e.g. *Aspergilus spp*, *Penicillium spp* being evident as spots of various colours on the surface of moist wood. (for instance, as a result of high relative humidity or of condensation of water vapour). They do not significantly alter the mechanical properties of the wood but have a special significance for wood in service if discoloration is undesirable or unacceptable.

For green sawn timber, the moulds are covered by the CEN TS 15082 standard. But for the preservation of solid wood against mould, the EN 152 does not cover mould and no CEN standard is available. In that case the applicant is invited to submit relevant data (in house method, literature data...) which could be accepted by expert judgement.

Insects

Fresh wood insects

A number of insects bore and tunnel into fresh logs after they are cut and debarked. These fresh wood insects feed upon the starch reserves and can cause damages to the wood. Most of them belong to the families of Scolytidae (genus *Scolytus*), Cerambycidae (genus *Phematodes*), Lyctidae (genus *Lyctus*), Anobiidae (genus *Anobium*), Bostrychidae (genus *Bostrychus*).

Some other groups, belonging to the Scolytidae family, bore the fresh logs and introduce 'Ambrosia' fungi inside the gallery, resulting in wood staining (as a consequence of the development of the dark hyphae).

Wood boring beetles (Coleoptera)

Insects which lay their eggs in wood pores or cracks and whose larvae feed upon wood. They are present throughout Europe but the risk of attack varies greatly and is ranged from high to insignificant. The most important are *Hylotrupes bajulus*, *Anobium punctatum* and *Lyctus brunneus*.

Hylotrupes bajulus (House longhorn beetle)

This beetle attacks many softwood species and can cause significant structural damage. Many softwood species are affected, whereas hardwoods are not attacked. Larvae damage both the sapwood and the heartwood of non durable species.

This insect occurs throughout Europe, but is of less importance in the north and north-west of Europe. The vitality and longevity of larvae depend principally on ambient temperature and the wood moisture content.

Anobium punctatum (Common furniture beetle)

The larvae attack the sapwood of certain softwood and hardwood species. The damage can extend to the heartwood in some wood species and can have occasionally a structural significance impact. Its presence is particularly noted in coastal climates and where damp conditions prevail.

Lyctus brunneus (Powder post beetle)

The larvae attack sapwood of certain starch-containing hardwoods and have a significant impact throughout Europe for both European and imported hardwood timbers.

Termites (Isoptera)

Termites belong to the order Isoptera. In Europe and in the European tropical overseas regions there are three main termite families; subterranean termites (Rhinotermitidae), drywood termites (Kalotermitidae) and tree termites (Nasutitermitidae):

- *Reticulitermes* is the most common genus encountered from the Rhinotermitidae family in Europe. The main species registered are: *R. flavipes* (former *R. santonensis*), *R. grassei, R. lucifugus*, *R. banyulensis, R. balkanensis, R. urbis.*

They are widespread around the Mediterranean basin (Spain, France, Italy, Portugal, Balkans, and Greece) and Black Sea (Turkey, Romania), though some termite spots in the UK or Germany have been reported. Several unanswered questions remain about the origin of these termites. While some *Reticulitermes* are native to Europe, others may be related to species from eastern North America and the Middle East (Israel, Asian Turkey, etc.).

Coptotermes and *Heterotermes* are the main two genera belonging also to the Rhinotermitidae family located in the European tropical overseas regions.

- *Kalotermes flavicollis* and *Cryptotermes brevis* are the main two species of drywood termites present in Europe (especially in the coastal areas of Mediterranean countries and Canary Islands). *Cryptotermes* is a main genus belonging to drywood termites encountered in the European tropical overseas regions.

- *Nasutitermes* is the main genus belonging to the Termitidae family (tree termites) encountered in the European tropical overseas regions.

Marine borers

This term is applied to marine invertebrates such as *Limnoria* spp *and Teredo* spp which need a certain salinity of water and which hollow out extensive tunnels and cavities in wood. These organisms can cause serious damage to fixed or floating structures.

In European waters the most common marine borers are shipworm (*Teredo navalis*) and gribble (*Limnoria spp.*). Shipworm is a bivalve mollusc related to the sea snails and mussels. It is a soft, worm like animal with its shell modified into hard grinding jaws. The larvae are part of the microscopic zooplankton and swim freely in the sea until they settle on timber. They develop a shell with which they bore into the wood and lodge there, growing into large worms in holes up to 5 mm in diameter. They destroy the wood by making a massive network of galleries throughout the timber. Gribble is a small shrimp-like crustacean about 4 mm in length. It bores into the surface of the wood and lodges near the surface making numerous side burrows. The combination of this boring and wave action causes rapid erosion of marine timbers.

NOTE FOR CA CONSULTATION

Appendices 2 and 3 in the published Transitional Guidance on Efficacy Assessment for PT8, will move to the ECHA Biocides Efficacy Working Group webpages. Cross references have been revised accordingly

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

Appendix 2 Informative list of standards for efficacy assessment of wood preservatives

Appendix 3 Efficacy criteria in biological tests

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