Evaluation Manual for the Authorisation of plant protection products and biocides

EU part

Biocides

Chapter 7 Efficacy

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Board
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Chapter 7 Efficacy of Disinfectants

Category: biocides
Main group 1 : Disinfectants and general biocidal products
Product type 1 : Human hygiene biocidal products
Product type 2 : Private area and public health area disinfectants and other biocidal products
Product type 3 : Veterinary hygiene biocidal products
Product type 4 : Food and feed area disinfectants
Product type 5 : Drinking water disinfectants

GENERAL INTRODUCTION

This chapter describes the data requirements for the assessement of the efficacy of a biocide and the active substance within PT 1 to 5, and which evaluation methodologies are applied for the EU framework.

Note: This guidance is currently under revision. A revised version will be probably be endorsed and opened for comments in March 2013. For more recent information on data requirements see NL chapters and EU website (http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/guidance-documents).

1. EU FRAMEWORK

1.1. Introduction

The purpose of this chapter to give guidance to the assessment of the efficacy of disinfectants.

The main Group Disinfectants and general biocides, better known as disinfectants, covers 5 product types:

Product type 1	:	Human hygiene biocidal products
Product type 2	:	Private area and public health area disinfectants and other biocidal products
Product type 3	:	Veterinary hygiene biocidal products
Product type 4	:	Food and feed area disinfectants
Product type 5	:	Drinking water disinfectants

Most products in this main group are meant for the control of bacteria (including vegetative cells, spores and mycobacteria), fungi (including yeasts), and viruses (bacteriophages); the control of algae is important for applications in swimming pools. Control may be carried out on surfaces or in liquids.
The most important fields of use are: medical, veterinary, food or drinking water sector. Applications in public areas and industries, insofar as applications without direct contact with food are concerned, come under Product type 2. If contact with food is possible, applications come under Product type 4.

Cleaning products not intended as biocide, including liquid detergents, washing powders etc, do not come under these product types.

Efficacy is assessed on the basis of the TNsG on Product Evaluation [2]. It should be noted that the TNsG on Product Evaluation is based on so-called basic efficacy whereas for national evaluations of products, as laid down in the Biocides Directive, a sufficient efficacy is required. Additional requirements are therefore sometimes laid down for national evaluation (see NL chapter on PT 1 to 5).

The following aspects are relevant for evaluation of disinfectants:
1. The label claim (see Data requirements 1.2),
2. Efficacy of the product
3. The possible occurrence of resistance, cross resistance or tolerance.

1.2. Data requirements

Active substance

The efficacy of an active substance may be demonstrated by a “screening” laboratory test using either the undiluted active substance, the active substance in a solvent carrier or the active substance presented in a simple formulation. Test data are designed and intended to establish

the innate biocidal efficacy of the substance against specific organisms under carefully controlled and reproducible conditions and may include dose-response tests. For example, such tests could include:

- Rate of Kill tests or Minimum Cidal Concentration tests (suspension tests) to demonstrate bactericidal activity against gram negative bacteria (such as *Escherichia coli*, *Pseudomonas aeruginosa*) and gram positive bacteria (such as *Staphylococcus aureus* and *Bacillus subtilis*) at a range of dose levels and exposure times.

Product

The data requirements for disinfectants are discussed in Appendix to chapter 7 of the TNsG on Product Evaluation [2]. Below you can find the verbatim text of this TNsG (grey frames). Numbering of the study corresponds with the numbering of the TNsG on Product Evaluation. Where relevant, extra information has been added.

This appendix is currently under revision (project started by NL) and a new version will be available end of 2010.

1.2.1. Label claim

1.1 Spectrum of biological activity (including target organisms)

A disinfectant product may claim one or more of a number of types of efficacy. The types of efficacy claims that a disinfectant may make depend upon, among other things, the types of microorganisms, the disinfectant targets (e.g. vegetative bacteria, tuberculosis or a specific virus) and the disinfectant's intended level of activity (e.g. a reduction in the level of the microorganism or kill). Label claims and recommendations must be supported by the results of bactericidal, fungicidal, etc. tests appropriate to the area of application.

Applicants must clearly indicate on the product label the spectrum of activity claimed for the proposed product.

A disinfectant product may claim one or more of a number of types of efficacy. The types of efficacy claims that a disinfectant may make depend upon, among other things, the types of microorganisms, the disinfectant targets (e.g. vegetative bacteria, tuberculosis or a specific virus) and the disinfectant's intended level of activity (e.g. a reduction in the level of the microorganism or kill). Label claims and recommendations must be supported by the results of bactericidal, fungicidal, etc. tests appropriate to the area of application.

Applicants must clearly indicate on the product label the spectrum of activity claimed for the proposed product.

Examples of *some* of the common claims are presented in Table 1.

Table 1: Examples of *some* common types of effectiveness claimed for disinfectant products (see also Glossary of Terms at the end of this Annex 1 for further descriptors)

"claim"	**Descriptor**
Sterilants	Sterilants are intended to destroy or eliminate viruses and all living bacteria, fungi and their spores. The claim denotes killing all microorganisms including the highly resistant spore forms.
Disinfectant	Disinfectants are used on inanimate surfaces and objects to destroy infectious fungi, viruses and bacteria, but not necessarily their spores. Disinfectant products can be divided in to a number of broad categories which include, hospital (medical), veterinary; and general use disinfectants. Hospital disinfectants are primarily used on floors,

	walls bed linens, toilet seats and other hospital surfaces and some medical and instruments. Some hospital disinfectants are also effective against the organism that causes tuberculosis. General use disinfectants are used in households, swimming pools and water purifiers.
Bactericide	A product that kills vegetative bacteria.
Bactericidal Activity	The capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions.
Bacteriostat	A product which inhibits growth or spreading of bacteria under defined conditions
Fungicide	A product which kills fungi (vegetative mycelia, budding yeats and/or their spores) under defined conditions.
Fungicidal activity	The capability of a product to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant test organisms under defined conditions.
Fungistatic	A product which inhibits the growth of fungi under defined conditions
Mycobactericide	A product which kills mycobacteria under defined conditions
Tuberculocide	A product which kills *Mycobacterium tuberculosis* under defined conditions
Sporicide	A product which kills dormant bacterial spores under defined conditions
Virucidal	The disinfectant is intended to destroy or inactivate one or more specific viruses under defined conditions.

A “Glossary of Terms” is included as Appendix 1 of this chapter.

1.1.1 Target organisms

The range of target organisms for which claims are made and from which principal organisms representative of the microbial challenge can be selected should be identified on the product label.

Since the claimed microbial efficacy for disinfectant products will encompass a large spectrum of potential target organisms it is not necessary or indeed feasible to include all the possible microorganisms in an efficacy test designed to support a label claim.
Instead for each type of claim one or more indicator(s) or surrogate microorganism(s) relevant to the intended use and claim is recommended.

Efficacy tests usually include strains from the main bacterial groups, bacilli, gram-positive and gram-negative, and mycobacteria in certain standards. Yeast and fungi have to be usedfor fungicidal efficacy testing. It may be useful to include microorganisms of different species involved in specific applications.

Wherever possible strains should be selected from international collections (their genetic stability is checked regularly). The media for preservation procedures must be described with precision. In the case of large-scale use of disinfectant (e.g. in hospitals), it may be necessary to evaluate the sensitivity of bacterial species causing nosocomial infections. These species are often different from the proposed reference species.

When a disinfectant is used against a specific group of microorganisms for example, viruses, bacteria or fungi, a virucidal, bactericidal or fungicidal disinfectant should be used. If the types of microorganisms are unknown, a disinfectant that has broad spectrum of activity (i.e. one that

is capable of effective microbial action against all or the majority of classes of microorganisms) should be used.

The choice of disinfectant therefore depends upon:
- The spectrum of organisms it is required to kill
- The conditions under which it will be used

The information above is not exhaustive. In some cases other factors must also be taken into account in designing and selecting the appropriate efficacy study (see below).

If a biocidal product is intended to be used as a disinfectant in the area of public hygiene and claims use as a disinfectant with a broad spectrum of activity against micro-organisms, then applicants should indicate that efficacy against Gram-negative bacteria (such as *Salmonella choleraesuis*) and Gram-positive bacteria (such as *Staphylococcus aereus*).

If claims for disinfectant products are more expansive then efficacy testing requirements and representative test organisms will be increased as appropriate; for example:
If the product is intended to destroy tuberculosis bacteria (such claims are often used by medical users of disinfectants as an indicator of product strength as tuberculosis bacteria are more difficult to kill than most other types of bacteria) then claims against *Mycobacterium tuberculosis* will need to be substantiated.

Examples of the parameters to be included in the 'label claim'.

Product type	Area of use/site of application	Spectrum of activity[1]	Mode of action/Duration of effect	Application method	Expression of Application /dose rates
Disinfectants	Public health area disinfectants	Bacteria Fungi Virus	Kill	Spray	v/v w/v
	Swimming pools	Bacteria Fungi Virus	Kill	Direct dosing	v/v w/v

[1] May be wide ranging or very specific

Currently available test methods (See Section 2.5) utilise a range of microbial species which are representative species that take into account their relevance to practical use.

1.2 Areas of Use/Sites of Application

1.2.1 Areas of Use

Disinfectants are used almost everywhere people want to kill disease-causing microorganisms. They are used to kill or inactivate bacteria, fungi and viruses in households, hospitals, schools, restaurants, offices, kitchens, bathrooms, dairy farms, on medical and dental instruments, eating utensils and at many other locations.

Although the role of the inanimate environment in transmitting infections has not been completely defined, the use of disinfectants is considered an important part of infection control programmes.

Applicants should clearly indicate on the label the intended area of use for the product e.g. areas of use could include (not exhaustive):

Domestic and institutional use
Food industry
Veterinary/animal health use
Hospital or medical areas
Breweries
Recreational areas

1.2.2 Sites of Application

In addition to the types of efficacy claimed (e.g. bactericidal, fungicidal, tuberculoidal) and the intended area of use, the applicant must specify on the label the use patterns for which the disinfectant is recommended.
Broad examples of use patterns (not exhaustive) could include areas such as:

- Use against microorganisms on hard surfaces, work surfaces, cutting boards etc.
- Use against microorganisms on fabrics or textiles
- Use on toilets, bathroooms, sinks, etc.
- Use in operating theatres, isolation wards, use on medical instruments etc
- Use in food manufacturing, retailing, processing areas etc.
- Use in animal housing and equipment, e.g. pigs, sheep, poultry etc.
- Use against microorganisms associated with human or animal wastes
- Use in air conditioning systems
- Use in swimming pools, spas, aquariums, bathing and other waters
- Use in tanks, pipelines, equipment soak or bottle wash

1.3 Directions for use (Methods of application)

Efficacy data must be developed to substantiate label directions (which should include reference to concentration of the use solution and contact time) and claims in regard to the number of times a prepared use solution of an antimicrobial product can be applied (or re-applied) before a fresh solution must be prepared. Such data must show retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges for the period of time or the number of times specified in the directions for use.

1.2.2. Efficacy

2.1 Laboratory tests

In laboratory testing of disinfectants the ultimate purpose is to establish whether products meet specified requirements under “in use” conditions.

Various laboratory methods have been developed for biocide efficacy testing.
Although these experiments differ in their design and experimental detail, all are based on the principle of adding a test inoculum to disinfectant and removing samples at specified times. The biocide in each sample is neutralised and levels of survival of the organisms assessed. In practice the methods can be classified into 3 groups according to how the end-point of the test is determined:

End-point tests
The sample of biocide treated cells is transferred to nutrient medium and incubated to determine the presence or absence of survivors. The result is expressed as the concentration of biocide producing kill (i.e. no detectable survivors) within a specified contact period, or the time required to achieve kill using a given concentration.

Quantitative tests
Samples of untreated and biocide-treated cells are plated on nutrient medium.
After incubation the number of colony forming units is determined and the log reduction in viable counts determined.

Capacity tests
The biocide is challenged successively with bacteria at defined time intervals.
Following each inoculation, samples are taken after a suitable contact period has elapsed, the biocide is neutralised and the suspension incubated in the medium to determine the presence or absence of detectable survivors. The result is expressed as magnitude of the accumulated inoculum that was required to produce the “failure”.

2.2 Simulated use/Practical tests
Simulated use or practical tests mimicking real-life conditions belong to the second testing stage. After measuring the time-concentration relationship of the disinfectant in an in-vitro test, these practical tests are performed to verify if the proposed use dilution is likely to be adequate in real life conditions.

2.3 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 designated environment. As with standard and non-standard laboratory methods, representative organisms or actual organisms of concern may be used.

2.4 Other considerations and factors

2.4.1 Neutralisation
In trials where the testing organisms are taken from treated samples for further analysis, such as plate count following biocidal treatment, appropriate neutralisers must be used to inactivate the active ingredient. Evidence supporting the effectiveness of the neutraliser against the active ingredient and showing that the neutraliser itself does not have antimicrobial activity must be included in a test report. In such cases:
An effective neutraliser for the test product should be identified and effective neutralisation without toxic effects on surviving organisms should be demonstrated.

Appropriate controls for determining the efficacy of the neutraliser should be performed. This is to provide evidence to eliminate the potential for false-negative results caused by static or microbicidal activity of disinfectant carried over onto the recovery medium.

In lieu of chemical neutralisation it must be documented that appropriate subculture techniques have been employed that preclude residual carry over of active substances.

2.4.2 Hard Water Claims
The degree of hardness of the water used to dilute the disinfectant may affect its performance. Generally the harder the water the less effective is the diluted disinfectant. Therefore it follows that any product that carries label claims for effectiveness in hard water must be tested by the appropriate method in synthetic hard water at the level claimed.

2.4.3 Presence of Interfering Substances
Where disinfectants are applied to either inanimate surfaces or the hands, any number of substances may be present which may affect the disinfectants activity.

Water of Standard Hardness (WSH)
Since there is evidence that the activity of some disinfectants may be affected by the presence of metal ions such as Ca2+ and Mg2+, current test programmes require that products destined for dilution with potable water must, for the purpose of efficacy testing, be diluted in water of standard hardness.

Organic and Inorganic Soiling
The nature, degree and condition of the soiling present will affect the efficacy of a disinfectant. Hard compacted soils are more difficult to disinfect than loose friable soils, and solid soils generally have a greater adverse effect on disinfection than liquid soils.

In many cases, however, residual contamination must be anticipated, and in some situations (e.g. in the treatment of blood spillages) disinfectants are used specifically to decontaminate soil and 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 soils to be encountered. Limescale, milkstone and earth are the most common inorganic soils.

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

When a product is to be recommended for certain patterns of use 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 soil.

In all cases, soiling will reduce the efficacy of the disinfectant, and where soiling is present, longer contact times, higher concentrations, precleaning or a combination of these parameters may be necessary.

2.4.4 Temperature
Generally disinfection performance increases with temperature. This applies to disinfection against all microorganisms though the effect on individual species differs, some being more affected by others.

2.4.5 Contact Time
Within limits, the longer the contact time the more effective is the disinfectant.
Some disinfectants act very quickly, whereas others require an extended contact time to achieve adequate performance. Mycobacteria take longer to kill than most vegetative microorganisms.

2.4.6 pH
The prevailing degree of acidity or alkalinity during disinfection can also affect the performance and choice of disinfectant.

2.4.7 Surfaces
Smooth impervious surfaces are easier to disinfectant (and also to clean) than rough or pitted ones. In some circumstances the microorganisms might be protected from the action of disinfectants by being protected in porous surfaces. Clumps of microorganisms may also be more difficult to kill, as cells inside are protected by dead microorganisms on the outside.

Bacteria and fungi can adhere to surfaces forming biofilms in which the cell surface properties are altered and this makes them more difficult to kill, as penetration can be difficult to achieve.

2.5. Standard Test Methods

Standard test methods have been produced (or are in preparation) by CEN in Europe and by AOAC and ASTM in North America (US EPA and Canada) that address the efficacy testing of disinfectant products.

A list of available efficacy test methods for biocidal products has been collated and referenced by the OECD and this list is available on its website.

Reference: http://www1.oecd.org/ehs/Biocides/efficacy-overview.htm).

2.5.1 European Standard Test Methods

In Europe the European Committee for Standardisation (CEN) Technical Committee (TC 216) was established to produce harmonised European methods for the efficacy testing of disinfectants and antiseptics used in food hygiene, medicine, agriculture and veterinary practices. The standards are largely based on suspension tests (i.e. quantitative tests) although some surface test methods are also included.

Use of these standards in testing to support claims for microbiocidal activity is proposed to follow a matrix of testing ranging from simple innate activity, through simulated use tests to field tests under practical conditions. Three levels of testing are described.
Phase 1 tests determine whether the product diluted in distilled water has a basic level of activity in the absence of any organic or inorganic soiling. Phase 2 tests determine activity in simulated use conditions with an organic load and several test microorganisms, either as a suspension test (step 1) or on surfaces (step 2). Phase 3 tests consist of “in-use” (field) trials.

A summary of this modular approach to testing using and test methodology is outlined below:

PHASE 1 Quantitative Suspension tests for the basic activity of the product to define minimum standards for bactericidal, fungicidal and sporicidal activity. (No specific test conditions).

PHASE 2, STEP 1 Quantitative Suspension tests under conditions representative of practical use. (Specific test conditions related to intended use).

PHASE 2, STEP 2 Other laboratory tests, e.g. handwash, handrub and surface tests simulating practical conditions.

PHASE 3 Field tests under practical conditions.

CEN are currently preparing a guidance document [CEN/TC 216 N 127] which outlines the application and interpretation of European Standards for chemical disinfectants.
This document outlines the various and standards currently available and provides guidance as to the choice of available standards that may be used to verify the effectiveness of disinfectants in particular situations (such as medical, veterinary and food hygiene) and gives guidance for the interpretation of results from such tests in making and supporting efficacy claims.

Whilst the CEN test standards cover the methodology to test for disinfectant products likely to be encompassed within product types 1, 2, 3 and 4 of the Directive, the application areas for disinfectants to water systems such as swimming pools, spas, and drinking water has yet to be addressed. Therefore claims for efficacy of such products will need to be demonstrated through

testing using other test methods where available.

A list of current and standards and those in preparation is given in Annex 2.

2.5.2 North American standard test methods

In the United States, the standard methods for the evaluation of chemical disinfectants are predominately those of the Association of Official Analytical Chemists (AOAC).
These tests are, in the main, end-point tests and are used to determine the optimum use-dilution of a disinfectant product to be used for a specific application and they are also used to satisfy the US EPA requirements for the registration of antimicrobial products. With the use of specified test organisms and, in some instances, representative environmental surfaces, the AOAC methods form the core of the EPA's efficacy data requirements. Some non-AOAC methods are specified by the EPA to demonstrate efficacy against specific microorganisms. These include the EPA virucidal method, or a recently accepted alternative method for the quantitative assessment of tuberculoidal activity.

A list of AOAC test methods is provided in Annex 3 and additionally some ASTM methods in Annex 4.

2.6 Specific Data to Support Label Claims

2.6.1 Basic bactericidal activity

Available data
No chemical substance or preparation can be regarded as a disinfectant if it is not active against vegetative bacteria. Therefore, disinfectant testing should always start with the determination of antibacterial activity.

Of the currently available CEN standards, and 1040 (a Phase 1 test), based on a quantitative suspension test, addresses claims for basic bactericidal activity.

Test species
EN 1040, addresses the activity of a test material against Staphylococcus aureus (ATCC 6538) and Pseudomonas aeruginosa. (ATCC 15442).

Test method and requirements
A test suspension of bacteria is added to a prepared sample of the product under test. The mixture is maintained at 20 ^{o}C. At a specified contact time chosen from one of the following: 1 , 5, 15 , 30 , 45 or 60 minutes, an aliquot is taken. The bactericidal action of this aliquot is immediately neutralised or suppressed by a validated method.
The method of choice is dilution-neutralisation. If a suitable neutraliser cannot be found, membrane filtration is used. The number of surviving bacteria in each sample is determined and the reduction in viable counts calculated.

A criterion for activity by this test method is that the test material should demonstrate at least a 5-log reduction in viable counts of the test organisms in 60 minutes.

2.6.2 Basic Fungicidal activity

Available data
Of the currently available CEN standards, and 1275 (a Phase 1 test) addresses basic claims for fungicidal activity.

Test species

EN 1275 addresses the activity of a test material against Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404).

Test method and requirements
A test suspension of yeast cells or mould spores is added to a prepared sample of the product under test. The mixture is maintained at 20 oC. At a specified contact time chosen from one of the following 5, 15, 30 or 60 minutes, an aliquot is taken;
the fungicide action in this portion is immediately neutralised or suppressed by a validated method. The method of choice is dilution-neutralisation. If a suitable neutraliser cannot be found, membrane filtration is used. The number of surviving yeast cells or mould spores in each sample is determined and the reduction in viable counts calculated.

The criterion for activity by this test is that the test material should demonstrate at least a 4-log reduction in viable counts of the test organisms in 60 minutes.

2.6.3 Basic sporicidal activity

Available data
Of the currently available CEN tests, Pr and 216003 (a Phase 1 test) addresses claims for basic sporicidal activity.

Test organisms
Pr and 216003 addresses the activity of a test material against dormant spores of Bacillus subtilis (ATCC 6633) and Bacillus cereus (ATCC 12826).

Test method and requirements
A prepared sample of the product under test is added to a test suspension of bacterial spores. The mixture is maintained at 20 ^{o}C or any other temperature to be defined.
At a specified contact time chosen from one of the following: 30, 60 and 120 minutes, an aliquot portion is taken and the sporicidal as well as sporistatic action in this portion is neutralised. The method of choice is dilution-neutralisation. The number of surviving bacterial spores is determined in parallel and the reduction in viable counts calculated. The effectiveness of neutralisation is controlled in the test.

The criterion for activity by this test is that the test material should demonstrate at least a 4-log reduction in viable counts of the test organisms in 120 minutes.

2.6.4 Virucidal action

A basic test is not considered to be appropriate.

2.6.5 Claims for disinfectants intended for specified or defined purposes

Disinfectant products to be recommended for a defined purpose will require a further level of testing which is more complex and extensive in design and is intended to simulate conditions more relevant to practical conditions.

Using the CEN methodology as an example this would include testing by Phase 2 suspension and surface tests, selected to be relevant to the area of intended product use.

Suspension tests (PHASE 2, Step 1)
The suspension tests in this situation would follow the procedure of the basic Phase 1 tests but include additional test strains (e.g. Proteus mirabilis and Enterococcus faecium), product diluents (water of standard hardness), organic soil (e.g. 0.3 % or 1 % w/v albumin), contact times (5, 30 or 60 minutes) and temperatures as appropriate to intended use.

Surface tests (PHASE 2, Step 2)
Currently CEN are drafting a series of quantitative surface tests for the evaluation of disinfectants used in the medical and veterinary fields and in food, industrial, domestic and institutional areas.

Surface tests in this situation consider a test suspension of bacteria or fungi in a solution of interfering substances which is inoculated onto a test stainless steel surface and dried. A prepared sample of the product under test is applied in a manner which covers the dried film. The surface is maintained at a specified temperature for a defined period of time. The surface is transferred to a previously validated neutralisation medium so that the action of the disinfectant is immediately neutralised. The number of surviving organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria, fungi etc. on a surface treated with hard water in place of the disinfectant is also determined and the reduction in viable counts calculated by difference.

Each treated surface is transferred into the counting medium in order to check the efficiency of the recovery of the test organisms.

Additional Phase 2, Step 2 test methods
Additional Phase 2, Step 2 test methods have been prepared (or are in preparation) to consider the efficacy of disinfectants in the medical areas for use on instruments, for use as surgical hand disinfectants, hygienic hand washes and hygienic hand rubs.

2.6.6 Claims against specific named organisms

Where label claims for antimicrobial activity against specific target organisms (e.g. a specific virus such as poliovirus 1) are made then such claims must be supported by efficacy data generated using tests that include the specific organism(s). Where available, strains from cultured collections should be used for these tests. The nature and extent of laboratory testing should be equivalent to that of the Phase 1 and Phase 2 suspension and surface tests, wherever possible.

2.6.7 Phase 3 tests

In-use or field testing involves the antimicrobial evaluation of the disinfectant under actual conditions of use on specified surfaces or materials. As with standard and non-standard laboratory methods, representative or the actual organisms of concern are employed.

Such tests can be performed by a variety of procedures. A convenient method involves the sampling of a disinfectant solution following actual use on surfaces by membrane filtration. The recovery of any viable non-spore forming bacteria from these solutions after an appropriate recovery time indicates failure of disinfection.

Another example of a practical or field test involves contact sampling of items after they have been treated with the disinfectant. Again, no vegetative organisms should be recovered.

N.B. CEN TC 216 are intending to prepare a standard “protocol” which specifies how a field trial shall be conducted. This standard is intended to give guidelines on the factors to be taken into account and controlled when carrying out a field trial.

2.7 SUMMARY OF CEN EFFICACY TESTING STRATEGIES FOR DISINFECTANTS

- Label claims and recommendations must be supported by the results of bactericidal, fungicidal, etc. tests appropriate to the area of application
- Ordinarily products should be subjected to a programme of Phase 1 and Phase 2 tests (a number of caveats exist with respect to the modular approach to testing in this way.)
- Label claims and recommendations may be supported by results of Phase 3 (field/in-use tests) as appropriate to the intended area of application

For certain situations it may be that certain tests are considered to be inappropriate for the particular application, e.g. the data available to the Competent Authority may indicate that:

1 Phase 2 suspension tests and surface tests are adequate and that further Phase 1 tests are not relevant
2 Phase 2 suspension tests provide sufficient information and additional Phase 2 surface tests are not relevant
3 Phase 2 surface tests provide sufficient information and additional Phase 2 suspension tests are not relevant

In view of the fact that these are “minimum requirements”, this is sufficient for the EU. Supplementary studies may be required for national evaluations. See NL chapter.

1.2.3. Resistance

The aspect resistance is discussed in the general part of the TNsG on Product evaluation (Chapter 6) and the TNsG on Annex I Inclusion (Chapter 10). This is very general and is discussed in the general chapter.

1.3. Assessment

1.3.1. Efficacy

Efficacy assessment has been subdivided into 3 phases with several steps (see schematic representation in Appendix 2 of this chapter).

- Only Phase 1, step 1 tests are required for active substances with a general disinfectant claim:
  Phase 1, step 1: suspension tests depending on the claim for bacteria, fungi and sporicides.

- Phase 2, step 1 and/or step 2 tests are required for products with a specific claim, depending on the field of use:
  Phase 2, step 1: suspension tests for e.g. *Proteus mirabilis* and *Enterococcus faecium*, where, depending on the intended use, type of water, degree of pollution (0.3% or 1% w/v albumin), contact time (5, 30 or 60 min.) and temperature are taken into account.
  Furthermore, tests must be carried out for specifically claimed target organisms where the best possible agreement should be sought with Phase 1 and 2 CEN tests.

Phase 2, step 2: surface tests: The TNsG on Product Evaluation [2] refers to CEN which has developed a number of surface tests for disinfectants that are used in the medical, veterinary and food sector, and for industrial, domestic and professional use.

- A field test may be requested in a last step
  Phase 3: Field tests. These are tests under field conditions on specific surfaces or materials.

1.3.2. Resistance

Resistance will be assessed on the basis of expert judgement [2].
It should be ascertained that other causes of failing treatment are ruled out. This usually requires supplementary information based on laboratory tests with organisms collected under field conditions.

See also under Point 1.2.3

1.4. Approval

Article 5, 1, b i) and ii) of the Directive of the European Parliament and the Council of 16 February 1998 concerning the placing of biocides on the market (98/8/EG) stipulates that Member States may only authorise a biocide if the product, when used consistent with the authorisation and taking into account:

- all conditions under which the biocide is normally used,
- the way in which material treated with the product can be used,
- the consequences of use and removal,

i) is sufficiently effective
ii) has no unacceptable effects on the target organisms, such as unacceptable resistance or cross-resistance or unnecessary suffering and pain for vertebrates,

1.4.1. Evaluation

General evaluation criteria for biocides (see biocides chapter Efficacy General) have been laid down in the Biocides Directive 98/8/EC (Article 5 and Annex VI 48-52).

Whilst the use of CEN standards {as available and appropriate} are highly recommended for assessment of efficacy of disinfectant products, use of these standards is not mandatory.

It is recognised that some products may be developed for very specific applications and may not pass standard tests that are general in nature. In these instances applicants should present appropriate, repeatable and reproducible data to support their applications.
Competent authorities will consider alternative testing strategies either based on other national or international standard test methods (e.g. BSI, AFNOR, DGHM, EPA etc.) or alternatively non-standard test data provided they are relevant and robust.

A general description of the information to be included in an efficacy testing report is presented in Appendices to chapter 7 of the TNsG on product Evaluation [2].

General criteria and trigger values for biocides (see Biocides chapter Efficacy General) are given in the Biocides Directive 98/8/EC (Article 5 and Appendix VI 48-52). Some specific criteria and trigger values, depending on the label claim, have been laid down for product type 1-5 disinfestation.

Basic claim

For registration as active substance in Annex I, the TNsG on Product Evaluation [2] (Appendices to Chapter 7 product types 1-5, par. 2.6) gives trigger values to be met by substances to be qualified as disinfectant; see Table 1 for the basic claim.

Table 1: Standard phase 1 test methods to be used for the basic disinfectant claim [2]

Basic claim	Test type	Target organisms	Log reduction	Contact time
Bactericide	EN 1040	*Staphylococcus aureus* (ATCC 6538) **and** *Pseudomonas aeruginosa* (ATCC 15442)	5	60 minutes
Fungicid	EN 1275	*Candida albicans* (ATCC 10231)	4	60

e		**and** *Aspergillus niger* (ATCC 16404)		minutes
Sporicide	Pr and 216003	*Bacillus subtilis* (ATCC 6633) **and** *Bacillus cereus* (ATCC 12826)	4	120 minutes
Virucide	Specific test required			

Specific claim
For product registration and national registration, supplementary phase 2, step 1 suspension and phase 2, step 2 tests must be carried out for claims of disinfectants with specific or defined uses, where relevant field conditions that may affect the efficacy, such as water hardness, possibly interfering substances, degree of pollution, temperature, contact time, pH, type of surface (hard, porous) (see Appendix 3 of this chapter) are taken into account.

The TNsG on Product Evaluation [2] (Appendices to Chapter 7 product types 1-5, par. 2.6) gives criteria to be met by substances to be qualified as disinfectant with a specific claim; see Table 2.

Table 2: Standard test methods to be used for the specific disinfectant claim [2]

Claim	Test type	Target organisms	Log reduction	Conditions
Bactericide	Comparable with EN 1040	e.g. *Proteus mirabilis* and *Enterococcus faecium*	5	Water, degree of pollution (0.3% of 1% w/v albumin), contact time (5, 30 or 60 min.) and temperature relevant for envisaged use.
Fungicide	-	-	-	-
Sporicide	-	-	-	-
Virucide	-	-	-	-

- : Not specified in TNsG on product evaluation [2]

Phase 2, step 2 tests
Here, CEN criteria and future new efficacy tests can be used. Label claims against specific organisms should also be tested against these specific species with comparable suspension and/or surface tests.

Phase 3, tests
A method is to sample in the solution that has been used for disinfestation of a surface by means of membrane filtration. Recovery of non-sporulating bacteria after a certain recovery period is an indication of insufficient efficacy.

Another example is contact sampling of surfaces or objects after treatment with the disinfectant. Recovery of vegetative organisms may not occur.

In view of the fact that this concerns “basic efficacy” this is sufficient for the EU. Supplementary criteria are required for national evaluations.
See §2.4.1 Criteria and trigger values.

1.4.2. Decision making
The Biocides Directive 98/8/EC (Annex VI 90-93) stipulates rules for decision making for

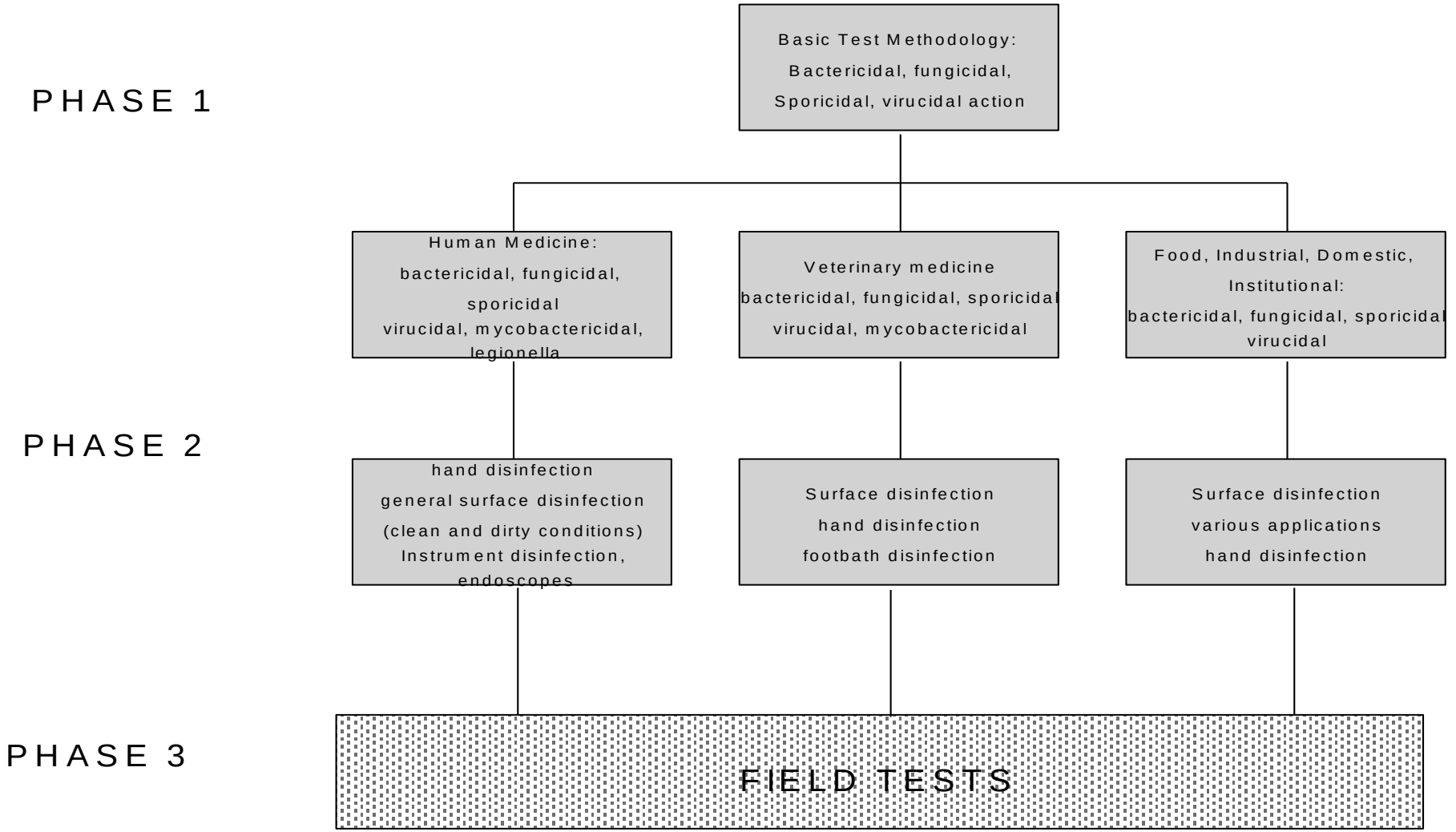
biocides (see Biocides chapter Efficacy General). No specific decision making rules have been laid down for the product type wood preservatives for the aspects unacceptable effects and efficacy.

2. APPENDICES

Appendix 1 Glossary of terms

Antimicrobial product	A product which prevents the growth of/reduces the number of/mitigates the growth of/…….
Bactericide	A product which kills vegetative bacteria under defined conditions
Bactericidal activity	The capability of a product to produce a reduction in the number of viable bacterial cells of relevant test-organisms under defined conditions
Bacteriostat	A product which inhibits the growth or spreading of bacteria under defined conditions
Biofilm	An accumulation of microbial cells immobilised on a substratum and embedded in an organic polymer matrix of microbial origin
Fungicide	A product which kills fungi (vegetative mycelia, budding yeasts and/or their spores) under defined conditions
Fungicidal Activity	The capability of a product to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant test organisms under defined conditions
Fungiostatic activity	The capability of a product to inhibit the growth of fungi under defined conditions
Microbes/microorganisms	Vegetative bacteria (including bacterial spores) or fungi (including fungal spores) or viruses
Mycobactericide	A product which kills mycobacteria under defined conditions
Mycobactericidal activity	The capability of a product 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 microorganisms
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.
Sporicide	A product which kills dormant bacterial spores under defined conditions
Sporicidal Activity	The capability of a product 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
Sterilant	A product that destroys or inactivates all forms of microbial life in the inanimate environment, including all forms of vegetative bacteria, bacterial spores, fungi, fungal spores and viruses.
Tuberculocide	A product which kills Mycobacterium tuberculosis under defined conditions
Tuberculocidal activity	The capability of a product to kill 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 which inactivates virus under defined conditions
Virucidal activity	The capability of a product to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions

Appendix 2 Schematic representation of efficacy testing strategy for disinfectant products [2]



Appendix 3 Examples of additional factors that may contribute to the efficacy of disinfectant biocides

– these and other factors are often incorporated into standard efficacy test methods for these biocidal products

- **Hard water claims**

The degree of hardness of the water (i.e. the presence of Ca^{2+} and Mg^{2+}) used to dilute the disinfectant may affect its performance. Generally the harder the water the less effective is the diluted disinfectant. Any product that carries label claims for effectiveness in hard water must be tested by the appropriate method in synthetic hard water at the level claimed. It is noted that many current test standards require that products tested for dilution with potable water must, for the purpose of efficacy testing, be diluted in water of 'standard hardness'.

- **Presence of interfering substances**

Where disinfectants are applied to either inanimate surfaces, any number of substances may be present which may affect the activity of the products.

- **Organic and inorganic contaminants**

The nature, degree and condition of the contaminant present will affect the efficacy of a disinfectant. Hard compacted contaminants are more difficult to disinfect than loose friable ones, and solid contaminants generally have a greater adverse effect on efficacy than liquid contaminants. In many cases, however, residual contamination must be anticipated, and in some situations (e.g. in the treatment of blood spillages) disinfectants are used specifically to decontaminate 'soil' and 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 contaminants to be encountered. Lime scale, milk stone and earth are the most common inorganic contaminants. Where claims are made for use under 'soiled' or 'dirty' conditions, use concentrations must be determined from tests carried out in the presence of a suitable contaminant. Contaminant materials commonly used in efficacy tests include albumin, serum, blood, yeast and yeast extract. N.B. The interfering substance(s) used in a test method should be selected according to the conditions and intended use pattern for the product.

- **Temperature**

Generally disinfection performance increases with temperature. This applies to disinfection against all microorganisms though the effect on individual species differs, some being more affected than others. However, excessively high temperatures can result in poorer disinfection if the biocide is not stable at elevated temperatures. In balance, temperature may also raise the aggressiveness of targets, generally up to their optimum of survival.

- **Contact time**

Within limits, the longer the contact time the more effective is the disinfectant. Some disinfectants act very quickly, whereas others require an extended contact time to achieve adequate performance. Mycobacteria take longer to kill than most vegetative microorganisms.

- **pH**

The prevailing degree of acidity or alkalinity during disinfection can affect the performance and choice of disinfectant. Generally, biocides are more active as undissociated molecules than when in an ionised form.

- **Surfaces**

Smooth impervious surfaces are easier to disinfect (and also to clean) than rough or pitted ones. In some circumstances the microorganisms might be protected from the action of disinfectants being protected in porous surfaces. Clumps of microorganisms may also be more difficult to kill, as cells inside are protected by dead microorganisms on the outside. Bacteria and fungi can adhere to surfaces forming biofilms in which the cell surface properties are altered and this makes them more difficult to kill, as biocide penetration can be difficult to achieve. When a product is to be recommended for certain patterns of use and where contaminants are present, more potent products, longer contact times, higher concentrations, pre-cleaning or a combination of these parameters may be necessary for the product to be effective.

3. References