Evaluation Manual for the Authorisation of plant protection products and biocides

NL part

Biocides

Chapter 7 Efficacy

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Board
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Chapter 7 Efficacy

Category: biocides
Main group 1 : Disinfectants and general biocidal products
Product type 1 : Human hygiene
Product type 2 : Disinfectants and algaecides not intended for direct application to humans or animals
Product type 3 : Veterinary hygiene
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GENERAL INTRODUCTION

This chapter describes the data requirements for the assessment of the efficacy of a biocidal product within PT 1 to 5, and which evaluation methodologies are applied for the NL framework.

NL FRAMEWORK

The NL framework describes the authorisation evaluation of biocides based on existing substances, included in Annex I, and new active substances. A new substance is a substance not authorised in any of the EU Member States on 14 May 2000.
The biocide that contains these substances may be authorised if the testing criteria laid down in the Wgb (Plant protection products and biocides Act) 2006 [1] are met. The product is evaluated according to the Plant Protection Products and Biocides Regulations (RGB) [2]. The evaluation dossiers must meet the conditions of Annex IIA, IIB, IIIA and IIIB of 98/8/EC.
For biocides based on active substances included in Annex I the EU frame work is applicable. There is a lot of similarity between the NL framework evaluation and the EU framework evaluation of disinfectants. The data requirements for efficacy of disinfectants for which specific regulation applies in the NL framework or where the NL testing framework has been elaborated in more detail than the EU framework are marked in grey.

1.1 Introduction

This chapter 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[a]:

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[b], 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*

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.

[a] These definitions are taken from the Biocidal Products Regulation.

[b] This refers to 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.

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.
The most important fields of use include the medical, veterinary, and food and drinking water sectors. Applications in public, commercial and industrial areas, where application is to surfaces without direct contact with food are included in Product type 2. If contact between disinfected 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. However, disinfectants with a broader claim, e.g. disinfection of instruments and surfaces, are under the BPD or BPR.

Cleaning products which are not intended as biocides, including liquid detergents, washing powders etc., are excluded from these product types.

Treated articles with claimed disinfecting or biostatic properties or function also fall within PTs 1 to 5, when they have a primary biocidal function. These articles can include a wide variety of products, with different applications, matrices etc..
There is currently little guidance on data requirements and acceptance criteria available for treated articles. A chapter on treated articles will be included in this guidance at a later date.

1.2 Dossier requirements

The following aspects are relevant for the evaluation of the efficacy of disinfectants:
1. The label claim and instructions for use
2. Efficacy data of the product
3. The possible occurrence of resistance, cross resistance or tolerance.

1.3 Label claim

For each product, clear label claims should be provided. When the label itself cannot contain all the necessary information, any accompanying leaflet containing instructions for use 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 depend upon, among other things, the types of micro-organisms that the disinfectant targets (e.g. fungi, yeasts, (myco)bacteria or bacterial spores) and the intended use of the disinfectant (e.g. in hospitals, in contact with food, in stables). 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 specific standards.

The applicant must clearly indicate the spectrum of antimicrobial activity claimed for the proposed product on the product label.

In NL we have a label with Legal Instructions for Use (LIU, Dutch abbreviation is WG/GA). In the LIU we have a format with standard sentences to describe the intended use. In NL this is the only use that is allowed because this is the only save use that is evaluated. The applicant

should provide a draft LIU and directions for use. After evaluation by all aspects (toxicology, environmental, physical & chemical properties and efficacy) the NL CA prepares a final label with LIU and directions for use. See the general chapter on efficacy for more information on the Dutch label format.

1.3.1 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 the 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, e.g. fungi, yeasts, viruses, algae, protozoa, (myco)bacteria or spore forming bacteria. Specifying the groups of organisms (e.g. bacteria, fungi) is also relevant as products are not normally specific to single species.

Specific species are mentioned on the label where they are the only or most relevant organism, or where they have a different susceptibility to biocides than the rest of the group. For instance, mycobacteria are less susceptible then 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 Rotavirus without a general virucidal claim). However, there are some cases in which it can be justified that a single or a small number of species are relevant (for instance bacteriophages in milk industry).

Standard test methods normally specify one or more representative species that should be tested per group of organisms claimed. For instance, a bactericidal product should be tested on two gram-positive and two gram-negative bacteria, a fungicidal product should be tested on yeast and fungi. 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 and guidelines.
When it is not possible to use standard test methods for efficacy testing and other tests are used, the test organisms listed in Appendix 1 should be employed. If test organisms other than those listed in Appendix 1 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 (EN12353).
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 can 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 subject of discussion between Member States. The above paragraphs reflect the position at the time this guidance is written.

In general in NL efficacy of disinfectants should at least be demonstrated for bacteria **and** yeast. Efficacy tests with these organisms should always be provided. Efficacy tests with all other groups of organisms only have to be provided when these organisms are claimed.

1.3.2 Areas of Use

Disinfectants are used almost everywhere people want to “eliminate” micro-organisms. They are used to kill or irreversibly inactivate bacteria, fungi and viruses on animate and in-animate surfaces and matrices, in hospitals, households, schools, restaurants, offices, swimming pools, kitchens, bathrooms, dairy farms, on medical and dental instruments, eating utensils and in many other locations.

Applicants should clearly indicate the intended areas of use for the product on the label e.g. areas of use could include (not exhaustive):
Hospitals and other medical areas
Institutional use (offices, schools etc.)
Industrial applications, e.g. food, cosmetic, pharmaceutical industry etc.
Veterinary areas (animal housing, animal health care etc.)
Recreational areas
Domestic use

1.3.3 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 skin as hand disinfectant or teat disinfectant
- Use in hospitals, operating theatres, isolation wards, use on instruments etc.
- Use in food manufacturing, retailing, processing areas etc.
- Use in animal housing and equipment, e.g. cattle houses, poultry houses 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

1.3.4 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. aerosol, wiping, 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 of an antimicrobial product 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.

1.3.5 Other interfering parameters

Any other circumstances that can effect 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 no rinsing step is involved, alkaline cleaning fluids should not be used with acidic biocides, and vice versa.

1.4 Efficacy testing of the product

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

1.4.1 Tiered approach

For efficacy testing of disinfectants a tiered approach is recommended. The following tiers can be distinguished (in accordance with EN 14885: 2006):

Phase 1 tests are quantitative suspension tests to establish that a product has bactericidal, fungicidal, virucidal etc. activity without regard to specific conditions of intended use.
These tests are **not** accepted for product authorisation.
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.

For more information on the tiered approach see the EU framework on PT1 to 5.
For product authorisation done under Article 121 of the Wgb it is sometimes acceptable to demonstrate efficacy with phase 2 step 1 tests only. For product-use combinations where this is not acceptable it is mentioned specifically in the sections below. For products in PT01, PT02, PT03 and PT04 for private use always a phase 2 step 1 and a phase 2 step 2 or a phase 3 test is required.

1.4.2 Standard test methods

Ideally, data should be generated using international or national recognised testing methods (CEN, OECD, ISO, etc.). Several international standard test methods currently exist for disinfectant products. A list of recommended standard tests is presented in Appendix 3 to this document. Normally required tests in NL are listed in Appendix 2.
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 revisions to make the guideline more suitable for the specific product or use conditions, is also possible.

At the time of production of this guidance document, a broad range of CEN methods is available, as described in Appendix 2 and 3. 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.
Further, the development of a series of phase 2 / step 2 methods for the disinfection on hard surfaces is under progress by the CEN and OECD. The use of CEN or OECD test methods is highly recommended, where these are available and relevant.

These methods 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.

1.4.3 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 tested product should be clearly described: including the identity of the active substances and co-formulants, and their concentrations in the tested formulation. 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. In cases where the test report does not report the formulation of the test product (e.g. it may only state a code for the product for the purposes of confidentiality with the testing organisation), the full composition of the product should also be provided.

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 an active substances on Annex I of the BPD or "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.
For product authorisation in NL, done under Article 121 of the Wgb, it is sometimes acceptable to demonstrate efficacy with phase 2 step 1 tests only. However, this is not the case for products for private use.
The phase 2 step 1 test will provide basic information on the efficacy of the product (in a standard test) that can be compared to similar biocides, while phase 2 step 2 tests investigate the effects of more in-use factors (such as interfering substances, drying of micro-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, e.g. when disinfection is done in suspension under real use conditions, a phase 2 step 1 test is sufficient on its own, as this already simulates practical conditions.
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.

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. A list of standard test organisms in given in Appendix 1.
In general in NL efficacy of disinfectants should minimal be demonstrated for bacteria and yeast. Efficacy test with these organisms should always be provided. For all other groups of organisms test only have to be provided when the organisms are claimed.

The concentrations used in testing should be selected to demonstrate the threshold of product efficacy. Suspension test should be performed with several dose rates, including at least one rate lower than the effective rate. The Competent Authority 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) both suspension and surface tests should be performed. The suspension 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 micro-organism (i.e. a lower level of test organism compared to the water control) but does not necessarily inactivate them (the micro-organism survive in the test without neutralisation).
Biocidal products that claim a biostatic effect bear the risk of development of resistant organisms. For this reason, efficacy of these types of products has to be examined carefully.

Other products, which do not have biocidal or biostatic activity, might fall within the scope of BPD or BPR, i.e. which “deter, render harmless, prevent the action or otherwise exert a controlling effect on any harmful micro-organism”. 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 Competent Authority 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. A list of product applications and uses of biocides, together with the required test methodology, is given in Appendix 2.

1.4.4 Relevant factors of the test procedure

1.4.4.1 *Formulation of the tested product*

A product authorisation is given to a specific product with a defined composition, and the efficacy of this 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.

1.4.4.2 *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 Ca2+ and Mg2+). 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 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.

1.4.4.3 *Presence of Interfering Substances*

Where disinfectants are applied to either inanimate surfaces or the hands, substances may be present on the surface 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, however, 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 soils to be encountered. Limescale, milkstone and soil are the most common inorganic soils.

Where claims are made for use under soiled or dirty conditions, the use concentrations of the product 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.
In practice, with exception of a few situations (e.g. clean rooms) the presence of soiling on surfaces or in liquids to be disinfected can not be ruled out. For this reason, a small amount of interfering substance should always be added in testing 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. When a product claims combined cleaning and disinfection the product should be tested under dirty conditions.

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 soil.

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.
Appendix 2 shows the test soiling required in NL for disinfectants.

1.4.4.4 *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 the intended use of the product (e.g. low temperature in stables, high temperature in contact with skin).
Appendix 2 shows the test temperature required in NL for disinfectants.

1.4.4.5 *Contact Time*

The contact time of a product on a surface or in a matrix 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 below.

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.
Appendix 2 shows the test contact time required in NL for disinfectants (according to Artikel 3.7a of the RGB where applicable).

1.4.4.6 *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 active ingredient, and showing that the neutraliser itself does not have antimicrobial activity, must be included in a test report. Appropriate controls for determining the efficacy of the neutraliser should be performed.

Membrane filtration or subculture techniques can be used to neutralise the product's activity, in combination with or instead of chemical neutralisation.

1.4.4.7 *pH*

The prevailing degree of acidity or alkalinity during disinfection can also affect the performance and choice of disinfectant, and must be included in the test report.

1.4.4.8 *Texture of Surfaces and Biofilm Formation*

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.

Bacteria and fungi can adhere to surfaces forming biofilms. In biofilms, the cell resistance is increased (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 biofilm more difficult to inactivate.

1.5 Resistance

The topic of resistance is discussed in the general part of the TNsG on Product Evaluation (Chapter 6). Additionally, in support of the review for each active substance information on resistance is given in the Competent Authority Report of this active substance.
Resistance will be assessed on the basis of expert judgement.

1.6 Assessment of authorisation

1.6.1 Decision making

The Biocides Product Directive 98/8/EC (Annex VI 90-93) and Biocidal Product Regulation 528/2012 (Annex VI) stipulates rules for decision making for biocides (see Biocides chapter Efficacy General). A biocide will only be authorised if the biocide is, as laid down in Wgb (Plant protection products and biocides Act) 2006 [1], Art. 49 (1) (b1): "sufficiently effective".

The test results shall meet the requirements of the standards or other criteria for acceptance which are described below per type of use.
Some criteria are laid down in Artikel 3.7a of the RGB.
Where a product does not perform to these criteria, the applicant should provide a justification in the application as to why the product should still be recommended for authorisation.

1.6.2 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 the required laboratory and, where relevant, field tests, the product will be recommended for authorisation as far as efficacy is concerned. In exceptional cases the applicant can provide justification why the specified acceptance criteria are not met but the product is still acceptable. The Competent Authority will evaluate the justification on a case by case basis, possibly in consultation with the other Competent Authorities, and decide whether it is acceptable or not.

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

2 PT 1 HUMAN HYGIENE

2.1 Introduction

Product type 1 contains 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. Some of the products used for skin disinfection are not considered a biocide under the scope of the PT1.
A product applied on human skin could be either a biocidal or a medicinal or a cosmetic product. If the product under investigation is within the scope of either the Medical Product Directive or Cosmetic Product Directive, it is excluded from the BPD. Therefore, for each application for an authorisation of a disinfectant for human skin a specified description of the intended use should be given, to prevent authorisation of biocides for uses that are under the scope of one of the other directives.
Products for disinfection of damaged skin (e.g. wound disinfection) and products with a claim with respect to curing or preventing diseases are always medicinal products (covered under the Human Medicinal Products Directive 2001/83/EC2).
In NL there is a policy agreement between the Ctgb and the Medicines Evaluation Board (MEB, in Dutch: CBG) that products that are used for disinfection of undamaged skin prior to a medical intervention (e.g. surgery or injection) and prior to other interventions where the skin will be opened (e.g. tattooing, piercing, applying permanent makeup) are considered to be medicinal products rather than biocidal products (see website Ctgb.nl, FAQ Biocides, “Distinguishing between biocidal products and medicinal products (human and veterinary)”.
Biocidal products within PT1 are mainly handdisinfectants or hand and arm disinfectants.

2.2 Data requirements PT1

2.2.1 *Test methods*

For efficacy testing of human hygiene biocidal products, the tiered approach as described in section 1.4.1 is preferred. For handwash, handrub and surgical hand disinfection phase2 step 1 and 2 tests are required. For an overview of available EN tests see Appendix 2 and 3.

2.2.2 *Test organisms*

In NL human hygiene products should be at least sufficiently effective against **bacteria and yeasts**. Efficacy test with these organisms should always be provided. For all other groups of organisms test only have to be provided when the organisms are claimed.
The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference organisms is given in Appendix 1.

2.2.3 *Contact time*

It is important that the tests are carried out with the same contact time as claimed on the label. The claimed contact time has to be a realistic value, therefore in NL maximum contact times are set:

1. for handwash and handrub products the contact time should not exceed 30 seconds
2. for surgical hand disinfection products the contact time normally does not exceed 3 minutes but can be up to maximum 10 minutes.

It must be assured that the disinfected parts stay wet during treatment (e.g. by applying enough product or by applying the product several times if the volume necessary is too much to apply at once).

2.2.4 *Soiling*

Phase 2 step 1 tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Dirty conditions are mandatory for handwash applications. For (surgical) handrub clean conditions suffice when the label instructions state that cleaning prior to disinfection is necessary. If this is not stated on the label the test should be done under dirty conditions.
Phase 2 step 2 tests can be carried out without soiling.

Dirty conditions for hospitals and health care:
3 g/L bovine albumin solution plus 3 ml/L erythrocytes
Dirty conditions for other uses:
3 g/L bovine albumin solution
Clean conditions:
0.3 g/L bovine albumin solution

2.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT1 products the required log reductions are summarised in Appendix 2.
Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3 PT 2 DISINFECTANTS AND ALGAECIDES NOT INTENDED FOR DIRECT APPLICATION TO HUMANS OR ANIMALS

3.1 General Introduction PT2

Product type 2 contains disinfectants and algaecides not intended for direct application to humans or animals. This includes *inter alia:*

- 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 like 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[c], 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.

The data requirements (test standards and test organisms) and assessment criteria for the most common uses are specified below. An overview of data requirements for efficacy testing of disinfectant biocides in NL under Article 121 of the Wgb can be found in Appendix 2.
All of the possible uses in this PT cannot be covered in the Appendix. For less common uses, there is often no international standard test available. Where this is the case, the applicant should provide tests that show the efficacy of the product and a justification for the use of these tests. The assessment of these products will be based on expert judgement and will be handled case by case.

3.2 General data requirements PT2

There are some general data requirements which apply to all uses in PT2, and these are described below. There are also specific data requirements which apply to different types of use, and these are described in the sections covering those uses.
The intended uses of the disinfectant determine which tests will be required to support the product. Tests which most closely reproduce the practical application conditions should be selected.

In general it is not known which organisms are present on a surface or matrix to be disinfected. Therefore a disinfectant must have a broad spectrum of activity, in order to control all of the organisms which may be present.

3.2.1 Minimum requirements

In NL for general applications within PT2, products should be at least sufficiently effective against **bacteria and yeasts**. There are few exceptions to this rule: a claim against black mold is possible when only fungicidal efficacy is demonstrated, a claim against algae is possible without bactericidal and yeasticidal efficacy.
Within the EU this is only a requirement for the medical sector (bacteria and yeasts are responsible for most common nosocomial infections).
Additionally efficacy against other organisms can be claimed.

Products intended for surface disinfection shall be tested with a **contact time of maximum 5 min** (Artikel 3.7a of the RGB). In case of immersing instruments or clean in place (CIP) or room disinfection with vaporised biocide it is possible to test with a contact time longer than 5 minutes, as long as this corresponds with the contact time mentioned on the label.

3.2.2 Tuberculosis departments

If the product is to be used on tuberculosis departments, the product should be efficacious as general disinfectant used in health care (efficacy against bacteria and yeast), but efficacy against mycobacteria (representative for *M. tuberculosis*) must also be demonstrated.

3.2.3 Products against viruses

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

Products against viruses must be effective against viruses with and without an “envelope” (protein or lipid mantle). Products can claim virucidal efficacy if efficacy against non-enveloped viruses has been proven. Such products can be regarded as efficacious against enveloped and non-enveloped viruses.
The virus test EN13610, which is recommended in EN14885 for food, domestic and industrial areas, is not relevant for use in domestic areas, as it only tests on phages. Instead, the test specified for the medical area, EN14476, should be used for products against viruses used in domestic areas.

3.2.4 Biocidal products with biostatic effect

For biocidal products with a biostatic effect (bacteriostatic, fungistatic, etc.), both suspension and surface tests should be performed. The suspension test should be performed with and without neutralisation. The results from this test should show that the product prevents growth of the test organism (a reduction in numbers compared to the negative control) but does not kill them (survival of the test organism in the test without neutralisation).

3.2.5 Malodour control

There are specific requirements for products claiming control of organisms which 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. Next to these laboratory tests an odour test can be performed.

3.2.6 Test range

Tests (phase 2, step 1) should be carried out 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).

3.2.7 Changes in ingredients

When small changes are made to the non-active ingredients in a product, it is not always necessary to redo all the tests with the new formulation. The applicant may provide a justification for changes and the effects they have on the efficacy of the product. In case of a minor change, a robust justification might be sufficient (to be decided by the Competent Authority). In other cases, new efficacy tests will have to be provided. This can be a full set of efficacy tests or a test with the most resistant organism in the former test.

3.3 DISINFECTANTS FOR HARD SURFACES

3.3.1 Introduction

Biocides can be used to disinfect hard surfaces in areas such as hospitals, industry, institutions or private homes. These surfaces can be tables, floors, walls, the outsides of machinery and hard furniture, etc.. Products are often wiped or sprayed onto the surfaces, and may be washed or wiped off after a certain contact time.
The testing requirements for some specific uses of hard surface disinfectants are discussed in separate sections e.g. toilets, room disinfection with vaporised biocide, immersion of equipment into the product, etc.. As the areas of use can be as diverse as private homes, operating theatres etc., the test requirements might vary depending on the area of use.

3.3.2 Data requirements

See general data requirements PT2 (section 3.2).
An overview of data requirements for efficacy testing of disinfectant biocides in NL under Article 121 of the Wgb can be found in Appendix 2.

3.3.2.1 *Test methods*

For efficacy testing of hard surface disinfectants, the tiered approach as described in section 1.4.1 is preferred within the EU.

For product authorisation of hard surface disinfectants in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only. For products for private use both phase 2 step 1 and phase 2 step 2 tests have to be provided.

The following tests are normally required for a hard surface disinfectant:
- a quantitative suspension test (phase 2/step 1),
- and a quantitative surface test (phase 2/step 2),

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).
Tests in phase 3 are optional, as no validated test methods are available yet.

Several test methods for testing the efficacy of hard surface disinfectants are available. Appendix 2 gives a list of recommended test methods in NL.

The following documents are recommended for surface disinfection:
- EN 14885: gives an overview of which EN phase2/step1 and step2 tests to use for different uses.
- OECD guidance and guidelines for the testing of chemicals: Quantitative method for evaluating activity of micro-biocides used on hard non-porous surfaces. (These are surface tests which would be considered phase 2/step 2 tests)

The use of the specified tests is strongly recommended where they are relevant and appropriate. Where the tests are not appropriate to the product claim other tests can be used, although a justification for the relevance of the tests used should also be provided.

Preferably, tests should be selected that correspond to the use area of the product (e.g. tests from medical area for use in hospitals and tests for industrial areas for use in cosmetic industry). Where the product is intended for use in several areas it is acceptable to perform the tests specified for only one of the areas, as long as the test with the highest/most stringent pass criteria is used.
A phase 2/step 2 surface test is not yet available for the medical sector. However the surface test for the food/industrial sector can be used, with medical area specific soiling, instead.
Currently only validated surface tests without mechanical action are available (EN and OECD). Validated surface tests with mechanical action are being developed, and should be used for products that are intended to be used with mechanical action when they are available.

Where specific conditions apply for a field of use, such as high/low level soiling, high/low temperatures, relevant contact times etc. (see introduction 1.4.4), these conditions should be included in the efficacy testing.

Disinfectant towels/wipes
For disinfectant towels, the phase 2/1 tests should be done with liquid extracted from the towel. Phase 2/2 tests should be tests with mechanical action (CEN in preparation). Until these tests are available, surface tests can be done with liquid extracted from the towel, although a justification of the volume that is applied per square centimetre will also be required. In addition, a test has to be performed that shows that either the towel will still disinfect if the towel dries out or that the towel stays wet long enough to disinfect according to the claim. Alternatively, the use directions can address these issues, for instance, stating on the label that only wet towels are efficacious, defining the surface area each towel can disinfect (e.g. 0.5 m^2), or giving expiry dates for resealable packages.

3.3.2.2 *Test organisms*

For hard surface disinfectants in NL efficacy should at least be demonstrated for bacteria and yeast. Efficacy test with these organisms should always be provided. For all other groups of organisms tests only have to be provided when the organisms are claimed.
The only exception to this rule is when only efficacy against staining moulds or algae is claimed.

In this case only these organisms have to be tested.
The test organisms used in efficacy tests are normally stated in the applicable standard test methods.
If standard tests are not used (there will normally need to be a justification for this), the test organisms used to support a general claim should be demonstrated to be equivalent to the reference organisms given in Appendix 1.

Tests with test organisms other than those mentioned in Appendix 1 are acceptable, if adequate scientific evidence is submitted on which the relevance of the test organism to the field of use can be judged.

Also see the general data requirements PT2 for specific claims and minimum requirements.

3.3.2.3 *Contact time*

Products intended for surface disinfection shall be tested with a **contact time of maximum 5 min** (Artikel 3.7a of the RGB). The only exception to this rule is when only efficacy against staining moulds is claimed in indoor areas with moist conditions. For this use the maximum contact time is 15 minutes.

3.3.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met.
If the test doesn't provide these criteria, the general criteria for log reduction in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.4 ROOM DISINFECTION WITH VAPORISED BIOCIDE

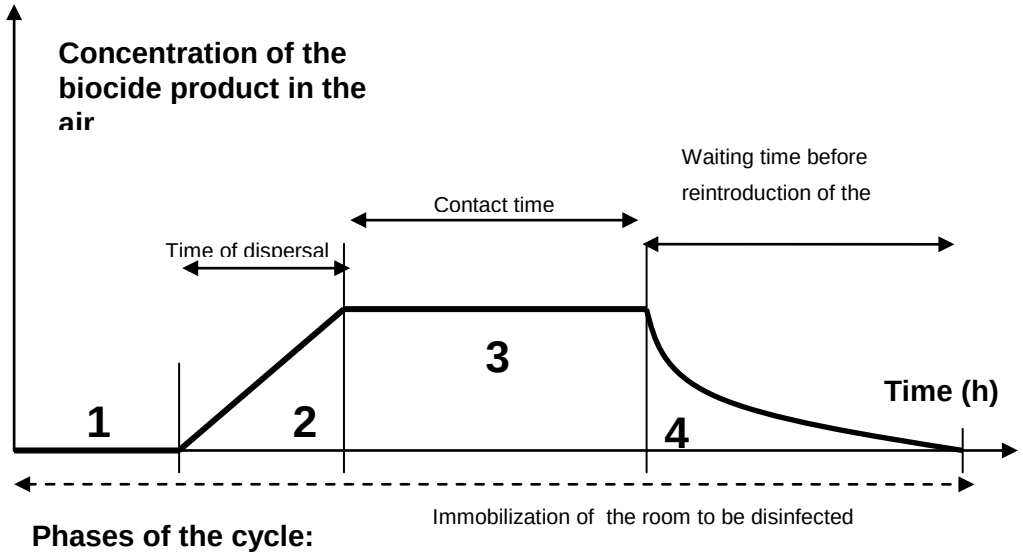
3.4.1 Introduction

Room disinfection involves the reduction and inactivation of micro-organisms on the surfaces of the walls, floor and ceiling of the room, as well as on external surfaces of the furniture and equipment present in the treated room. The product is applied by airborne diffusion of an aerosol, a smoke, a vapour or a gas. The technical characteristics of the diffuser equipment play a central role, ensuring an homogeneous distribution of the biocide product in the volume of the room and reaching all surfaces (including ceilings and the undersides of horizontal surfaces), therefore the diffuser equipment contributes in a decisive way to the efficacy of the product. Manual spraying is not covered in this section, but under hard surface disinfection (section 3.3).

Room disinfection may not disinfect the inside parts of furniture, and will not disinfect the air itself, so these uses are not considered in this chapter. Room disinfection is therefore closely related to surface disinfection without mechanical action. As this causes complications in cases of organic contamination, cleaning of surfaces is necessary prior to room disinfection.

3.4.1.1 *Process:*

The application of the product consists of four phases:
(1) The preparation phase (required depending on type of active substance and application procedure), during which the environmental conditions (relative humidity, temperature) are modified to an optimal level for the product,
(2) The conditioning phase, during which the product is diffused into the room, in order to reach the desired effective concentration,
(3) The disinfection phase, which corresponds to the contact time required to obtain the expected level of efficacy, and
(4) The terminal phase, which includes aeration of the room to remove any disinfectant present in the air, or other procedures for inactivation of the active substance, before access of people or animals into the room can be permitted (figure 5.1).



Phases of the cycle:
1: preconditioning (optional)
2: diffusion
3: phase of contact
4: aeration

Figure 5.1. The various phases of a cycle of disinfection of an automatic process.

Particular attention must given to the dispersal time and contact time. The dispersal time is the time necessary to reach a target concentration of the product on the surfaces to be disinfected in a given volume, while the contact time is the time necessary to reach the expected efficacy.
Note: the various phases of the cycle presented are theoretical and can be adapted according to the process. The maintenance of a concentration of biocide in the atmosphere may be achieved by the regular introduction of additional biocide during the contact phase.

3.4.2 Data requirements

3.4.2.1 *Test methods*

Airborne disinfection differs from direct application of liquids to surfaces. Therefore the EN phase 2/ step 2 standards for surface disinfection are not applicable for room disinfection. The tiered approach is still possible, however, by using different test methods.

The following tests are normally required for a room disinfectants:
- when applicable a quantitative suspension test (phase 2/step 1), (not required in NL)
- semi-field trial such as NF T 72-281 or European standard (EN standard in preparation) for disinfection using airborne application (phase 2/step 2).

The CEN phase 2/step 1 tests are suitable as suspension tests under clean or dirty conditions, although only applicable for products that can be tested in suspension (e.g. not for gasses). These tests are not sufficient on their own, and should be combined with a semi-field trial, such as NF T 72-281 for disinfection using airborne application. Where it is not possible to test the product in a suspension test, the semi-field trial will be sufficient.
NF T 72-281 was developed by AFNOR[d] (the French standardisation body) in 1986, updated in 2009, and proposed as new item within the framework of TC 216 in November 2010. This semi-field method evaluates the efficacy of disinfectants when vaporised in a room (automatic diffusion process) or when sprayed into the direction of a surface (manual application). Only application by vaporisation is discussed in this section. Once this method has been finalised and adopted at European level, any method variations should be taken into account.

3.4.2.1.1 *Basic principles of room disinfection*

Inert and dry carriers infected with a known number of micro-organisms (bacteria, yeast, fungi mycobacteria, or bacterial spores) are placed in a room of defined volume, temperature and relative humidity. The size of the test room should be relevant to the claims for the product. The carriers used are often stainless steel, but other relevant (generally non-porous) materials can also be used, for instance, glass, filter paper, or plastic.
When the disinfection of textiles (curtains etc.) and other materials (e.g. wallpaper. filters in flow cabinets, etc.) is claimed, appropriate carriers should be used to demonstrate efficacy.
The standard NF T 72-281 does not include tests against viruses. Inoculation of carriers with viruses according to the protocols of CEN or OECD surface tests and exposure to the airborne disinfection process as according NF T 72-281 is acceptable.

The inoculated carriers must be placed in a vertical position with inocula facing away from the diffuser. Their distance to diffuser depends on the room dimensions (for instance: see Appendix B of NF T 72-281). The test method defines obligatory conditions for parameters that may influence the success of the disinfection.
This test includes the use of milk as interfering substances in order to maintain viability of the micro-organisms on the carriers during the test. Depending on the area of use, other suitable interfering substances should be tested (e.g. blood for use in hospitals under dirty conditions). Alternatively carriers can be prepared according to EN13697 with bovine albumin as interfering substance.
Similar carriers are placed in a second room nearby, which is not treated with diffused product, as controls.

Additional tests can be performed to simulate specific conditions that are encountered in the practice and to fit with label instructions. In that case, all experimental conditions should be clearly described in the test reports. The standard lists the information that must be included in the final report.

[d] AFNOR : Association Française de Normalisation

3.4.2.1.2 *Diffuser*

As mentioned earlier, the disinfection efficacy is closely related to the technical characteristics of the diffuser. Chapter 5 “intended uses and efficacy” of the “Guidance on data requirements for active substances and biocide products” requires applicants to take into account the technical equipment used together with the product to be authorised.
A detailed description of the equipment and its characteristics must be provided in sufficient detail to distinguish it from other equipment:

- equipment name and model,
- diffusion principles (fogging, vapour, fumigation,…) and particles size distribution of aerosols or powder,
- description of the diffusion performance of the equipment (volume to disinfect, diffusion speed…),
- description of the ambient conditions (humidity, temperature,…) in which the process can be used,
- diffusion time for a specific volume
- precautions for over- and under-dosing.

The product authorisation will only be granted for use with the equipment described in the application. After authorisation, any modification to the equipment should be validated and reported to the Competent Authority for evaluation.
For major modifications which can affect the efficacy (pipe, pump, nozzles,…), it should be demonstrated that the efficacy of the process has not been affected (for example: by a new study with the most resistant organism).
For minor modifications which do not change the efficacy of the process, only a notification of the modifications to the equipment must be provided.

3.4.2.1.3 *Contact time*

As room disinfection may necessitate a long period of treatment, the contact time to be tested is not defined. The testing should demonstrate efficacy at contact time proposed for the intended use. This should be relevant to practical use and depends on substance concentration, volume of room, power of the diffuser equipment, etc... All of these parameters should be stated on the product label or in a technical information sheet.

3.4.2.2 *Test organisms*

Since room diffusion is used to disinfect hard (and soft) surfaces the same organisms should be tested as for hard surface disinfection (section 3.3). Appendix 1 contains a table of reference organisms.
The general data requirements for PT2 for specific claims and minimal requirements also apply for room disinfection with vaporised biocide (NL minimum requirements: bacteria and yeast).

3.4.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests these should be met.
If the test doesn’t provide these criteria, the general criteria in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.4.4 Notes

3.4.4.1 *Limitations*

Any limitations of the procedure should be specified in the application.
Literature has shown that disinfection by vaporised biocide may not be as effective on wet surfaces (lower concentration of the product) or inside closed cupboards and closets (where the vapour cannot penetrate). Therefore carriers should be tested under these conditions, and if efficacy is not proven, the label instructions should provide appropriate information (such as stating that cupboard doors should be opened, surfaces should be dried and wet areas (such as sinks and toilet bowls) should be disinfected with suitable alternative products.
Other factors which may influence the efficacy of the process in the practical use (such as the equipment, furniture, special structures (e.g. bumps on the walls) or special materials (copper in hydrogen peroxide procedures) including environmental conditions (e.g. temperature, relative humidity) which may affect the success of the disinfection also have to be considered. The conditions of a sufficient vaporisation should also be specified.

3.5 SWIMMING POOLS, SPAS AND HOT TUBS

3.5.1 Introduction

Disinfectants can be used to disinfect water in swimming pools, spas and hot tubs. These may be public pools (which may be used by many people daily) or household pools or tubs (which might be used only occasionally). An intermediate situation consists of facilities in hotels, housing complexes or sports clubs, where the bather load may be lower than in a fully public facility, but still high compared to private, domestic facilities.

Disinfectant products can be added to a pool continuously, intermittently, by shock dosing or through generation in situ. Large public facilities may have dedicated staff to maintain the pool using automated control systems, whereas smaller pools may be treated using manual methods by janitorial staff. Private pools may be treated by individual householders, supplemented in some cases by professional pool treatment personnel. Disinfection is only one aspect of pool maintenance and other activities, such as ensuring the correct pH and the removal of pollutants by oxidation, flocculation and filtration, are essential to ensure adequate water quality.

The principal purpose of disinfection is to disinfect the water to prevent the water-borne transmission of pathogens between pool users. Supplementary purposes are to ensure the aesthetic quality of a pool (by ensuring that algae do not result in turbid water or unsightly microbial growth on pool surfaces, such as the floor and walls of the pool) and to prevent microbial slime and biofilm formation in pipework and related equipment.

This section only deals with disinfection of the pool water and the pipework and related equipment containing pool water. The disinfection of hard surfaces surrounding the pool is covered in section 3.3.

3.5.2 Data requirements

See PT2 general data requirements (section 3.2).

3.5.2.1 *Test methods*

For efficacy testing of pool disinfectants the tiered approach as described in section 1.4.1 is preferred.

The following tests are normally required for a pool disinfectant following a tiered approach:
- a quantitative suspension test (phase 2/step 1),
- simulated-use tests with pool water or a surface test (phase 2/step 2)*
- and a field test (phase 3)**,

all simulating practical conditions appropriate to its intended use (temperature, contact time, soiling/bather load etc.).

* A phase 2/step 2 test may be appropriate in cases where a product has a specific use in surface disinfection. Otherwise, a simulated use test is appropriate for products intended to disinfect the water in a pool or spa.

** In some cases the field trial can be waived. The OECD guidance document (described below) is based on experience with hypochlorous acid/hypochlorite. Therefore, it is acceptable that for products based on hypochlorous acid/ hypochlorite the field test is waived and only laboratory test data are provided. In some other cases, waiving the phase 3 test can also be justified.

The OECD ”Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants in Laboratory and Field testing” (OECD Series of Testing and Assessment No 170, version dated 08 October 2012) describes laboratory and field test methods, conditions and criteria needed to demonstrate efficacy of a pool disinfectant. The protocol for field tests should be agreed between the applicant and Competent Authority before a field test is initiated.
For products that are used for specific purposes such as disinfecting pipework, filters and filter media, it may be more appropriate to test using the EN 14885 methods for the disinfection of surfaces in institutional applications.

3.5.2.2 *Test organisms*

Besides bacteria and viruses, protozoa can also be of importance in swimming pools. Fungi may pose a health hazard on wet surfaces surrounding the pool and can cause slime build up in pipework. Table 6.1 lists the organisms that normally should be tested. Although algae and protozoa in pools are in general only a problem when maintenance of the pool is not carried out properly, data against algae and/or protozoa should be provided where claims against these targets are made.

3.5.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

When pass criteria are available in the standard tests these should be met.
The OECD guidance document sets out criteria for laboratory and field tests. Table 3.5.1 and 3.5.2 show these default criteria.

Table 3.5.1 Criteria for laboratory tests

Test Organisms for both swimming & spa pools	Number of log_{10} reductions to be achieved	Time of exposure to test disinfectant at normal concentration during which reduction is to be achieved
Bacteria		
Escherichia coli	4	30 seconds
Enterococcus faecium	4	2 minutes
Pseudomonas aeruginosa	4	30 seconds
Legionella pneumophila	4	30 seconds
Staphylococcus aureus	4	30 seconds
Viruses [a]		
Adenovirus (disaggregated) [b]	3	10 minutes
Rotavirus (disaggregated) [b]	3	2 minutes
Protozoa [c]		
Naegleria fowleri - (cysts)	4	30 minutes
Giardia intestinalis [d] *or Giardia muris* [e] - (cysts)	3	45 minutes
Algae		
None specified [f]		

a Among viruses, *Enterovirus* can be added to the above list, but the performance characteristics against free chlorine are not known.
b Prior to the test exposure, virus suspensions should be treated to disassociate aggregated clusters of virus particles
c Among protozoa, *Cryptosporidium* can be added to the above list, but the performance characteristics against free chlorine are not known
d *Giardia intestinalis* is the human pathogen – in the literature this species may also be referred to as G*ardia lamblia* and the more general mammal parasite *Giardia duodenalis.*
e The rodent pathogen *Giardia muris* can be used as a surrogate for the human pathogen
f There are no European or international standardised test methods for activity against algae; species selected should be representative of those that require control. Algaecidal properties are not covered by the OECD guidance document

Table 3.5.2. Criteria for field tests

Test Organisms	Test Method	Maximum Count Allowable
Culturable micro-organisms colony count (also called „aerobic" colony count or „heterotrophic" colony count)	ISO 6222:1999 – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium.	100 Colony Forming Units (CFU) per ml
Thermotolerant coliforms	ISO 9308-1 – Detection and enumeration of *E. Coli* and coliforms – Part 2: Membrane filtration method.	Not detectable in 100 ml
Pseudomonas aeruginosa	ISO 16266 – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration.	Not detectable in 100 ml

Where these criteria are not met, the applicant can provide a justification as to why the product should still be considered acceptable. However, the Competent Authority will evaluate any justifications on a case-by-case basis, consulting the other Competent Authorities as necessary, and will decide whether it is acceptable or not.
The OECD guidance document contains more details on factors to be considered.

3.6 TOILETS

3.6.1 Introduction

Biocides can be used to disinfect toilet bowl surfaces in diverse environments including; hospitals, industry, institutions or households. Toilet bowl biocides are available in a wide variety of forms, including liquids, foams, powders, and tablets. These products are often applied via pouring around the inside rim of the toilet bowl surfaces, with the area scrubbed after a minimum contact time.
Toilet rim block biocides are used in flush toilets, are attached over the rim of a toilet, hanging down into the bowl. The block slowly dissolves in water as the toilet gets flushed. These biocides also come loose for placement directly in the cistern (water reservoir), and these variants usually dissolve slower with the constant contact with water.
Hard surfaces on the inside of toilets are covered by this section. Surfaces on the outside and toilet seats, lids etc. are covered by section 3.3 “hard surfaces”.

The use of biocides in chemical toilets, most commonly found on airplanes, trains, and in portable toilets is not covered in this section.

3.6.2 Data requirements

See PT2 general data requirements (section 3.2).

3.6.2.1 *Test methods*

For efficacy testing of toilet disinfectants the tiered approach as described in section 1.4.1 is preferred.

The following tests are normally required for a hard surface disinfectant:
- a quantitative suspension test (phase 2/step 1),
- and a quantitative surface test (phase 2/step 2),

both simulating practical conditions appropriate to its intended use (temperature, soiling, contact time, etc.).

Several test methods for quantitative suspension and surface tests are available.
Appendix 3 gives a list of recommended test methods. The following documents are recommended for surface disinfection:

- EN 14885: gives an overview of what EN phase2/step1 and step2 test to use for different uses.
- OECD guidance and guidelines for the testing of chemicals: Quantitative method for evaluating activity of micro-biocides used on hard non-porous surfaces. These are surface tests which would be considered phase 2/step 2 tests.

The use of the specified tests is strongly recommended where they are relevant and appropriate. Where tests are not appropriate to the product other tests can be used, although a justification for the relevance of the tests used should also be provided.

For products intended to be added to the water reservoir or hanging down from to the rim of the bowl, the concentration of the product (or at least the active substance) in the water before, in between and after flushing should be determined. This can be done by an analysis of the water under in-use conditions or, for products were all parameters are defined, by calculation. The laboratory efficacy tests should be performed with these concentrations.
Tests in phase 3 are optional.

3.6.2.2 *Test organisms*

The same test organisms as for hard surfaces should be tested, which is for NL a minimum requirement of bacteria and yeast. See section 3.3.2.2 and Appendix 1.
Fungi are not relevant to target in toilets, so products will normally only target bacteria and yeasts (and optionally viruses).

3.6.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests these should be met.
If the test doesn't provide these criteria, the general criteria in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.7 AIR-CONDITIONING SYSTEMS

3.7.1 Introduction

Disinfection of air-conditioning systems is similar to hard surface disinfection since only the surfaces in the system are disinfected and not the air itself. The difference with general surface disinfection is that the surfaces are mostly hidden inside the system and cannot be reached easily without taking it apart (for instance for air-conditioning systems in cars, dismantling the system would not be desirable).
In general disinfectants for air-conditioning systems are applied by airborne diffusion of an aerosol, a smoke, a vapour or a gas. The biocide is applied to an operating air-conditioning system at the inlet of the system. This way the biocide is sucked into and passes through the whole system.

Preservation of cooling liquids is not covered under PT2 but rather within PT11 (preservatives for liquid cooling and processing systems).

3.7.1.1 *Data requirements*

For products that are applied by airborne diffusion of an aerosol, smoke, vapour or gas the same test methods and test organisms should be used as for room disinfection. Therefore, the same data requirements as for section 3.4 (Room disinfection with vaporised biocide) are applicable here (NL minimum requirements: bacteria and yeast, contact time according to the label claim).

The following tests are normally required for a disinfectant for air-conditioning systems:
- when applicable a quantitative suspension test (phase 2/step 1),
- semi-field trial such as NF T 72-281 for disinfection using airborne application (phase 2/step 2).

See section 3.5 for specifications.

In the semi-field test the carriers with test organisms are placed in the air-conditioning system at the beginning and at the end of the system. When it is not possible to put carriers in the system they should be between the biocide application site and the inlet of the system and at the end of the system, in the out flowing air. If carriers at both sides fulfil the criteria it can be assumed that the surfaces in between are also disinfected sufficiently.

For products that are applied by manual spray the test methods and test organisms should be used as for hard surface disinfection. See section 3.3 (Hard surface disinfection) for data requirements (NL minimum requirements: bacteria and yeast, contact time maximum 5 minutes).
In addition to these data, the applicant should provide a justification that the spray apparatus is capable of reaching all (hidden) surfaces of the air conditioning system.

3.7.2 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

The same pass criteria can be used as for other surface disinfection (section 3.3.3). The criteria in Appendix 2 can be taken as guidance for what level of log reduction is normally required. Deviations from the norm are possible, but have to be justified in the application.

3.8 EQUIPMENT DISINFECTION BY IMMERSION

3.8.1 Introduction

Although instrument or equipment disinfection can be considered equal to hard surface disinfection, it differs from the intended use in section 3.3 because it is mainly applied by immersion of the instruments in the biocide solution or by filling equipment with the solution (disinfection of inner surfaces). The products are intended for instruments used in health care facilities (including dental care and veterinary care), laboratories and industry.

Some of the products used for disinfection of medical instruments, which are to be used specifically for diagnostic and/or therapeutic purposes for human beings, are not under the scope of the BPD. Disinfectants that are specifically used for disinfection of medical devices or a group of medical devices (anaesthetic equipment, endoscopes, surgical instruments, incubators) are covered under the Medical Device Directive 93/42/EEC. However, disinfectants with a broader claim, e.g. disinfection of instruments and surfaces, are under the BPD/BPR. The BPR states that such biocidal products should comply, in addition to the requirements laid down in this Regulation, with the relevant essential requirements set out in Annex I to Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices, Council Directive 93/42/EEC of 14 June 1993 concerning medical devices and Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices.

3.8.2 Data requirements

3.8.2.1 *Test methods*

For efficacy testing of equipment disinfectants the tiered approach as described in section 1.4.1 is preferred.

The following tests are normally required for a instrument disinfectant:
- a quantitative suspension test (phase 2/step 1),
- and a quantitative carrier test (phase 2/step 2),

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Methods for testing efficacy of instrument disinfectants are available.
Appendix 3 gives a list of recommended test methods. The following document is recommended for instrument disinfection:

- EN 14885: gives an overview of which EN phase2/step1 and step2 test to use for different uses.

The use of the specified tests is strongly recommended where they are relevant and appropriate.
For use in industry and institutional areas, no specific tests for instrument disinfection are given in EN14885. While these tests are not available for the phase 2/step 1, either the surface disinfectants test from the industry and institutional areas or the instrument tests for medical areas can be used, by employing area specific soiling. For phase 2/step 2 the instrument tests for medical areas are most appropriate, also with area specific soiling.

3.8.2.2 *Test organisms*

For general disinfection of medical (including dental and veterinary) instruments, efficacy against **bacteria, yeasts, fungi, and viruses** must be demonstrated. For all other uses the test organisms specified for hard surfaces should be tested (NL minimum requirements: bacteria and yeast). See section 3.3.2.2 and Appendix 1.

3.8.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests these should be met.
If the test doesn't provide these criteria, the general criteria in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.9 TEXTILE

3.9.1 Introduction

Biocides can be used to treat textiles and fabrics in hospitals, health care facilities, industry, institutions or private homes, when relevant micro-organisms (pathogenic, spoiling) in the textiles have to be reduced. These products can be in the form of laundry products which combine detergent and biocide or can be specialised products in the form of laundry additives which are added to the wash cycle or as finishing products (e.g. fabric softeners) which are added in the last rinsing step or as pre-treatment.

Typically contaminated clothes, linen or other washable textiles are treated in an appropriate washing machine. The biocide is added in concentrated form and diluted in the machine with water according to the specification of the manufacturer to get a defined concentration in the machine.

The automated chemical-thermal process normally comprises an (optional) initial pre-treatment step for heavily soiled laundry, followed by the main washing step (at a defined temperature and defined contact time) and 3 to 4 rinsing steps with cold water.

In some cases laundry can be treated through a hand-wash process in diluted biocide, which can be as pre-soak (after which machine washing is used), as a hand wash only, or through soaking to disinfect textiles before they are destroyed (for example, in an infectious disease outbreak situation).

Biocidal laundry products, either as combined biocide/detergent/conditioner or as special additives, are available for either targeted pre-treatment of contaminated articles or for whole-wash use.

3.9.2 Data requirements

See PT2 general data requirements.

3.9.2.1 *Test methods*

For efficacy testing of textile disinfectants the tiered approach as described in section 1.4.1 is preferred.

The following tests are normally required for a textile disinfectant:
- a quantitative suspension test (phase 2/step 1),
- a quantitative carrier test involving carriers made of test fabric (cotton, polyester) (phase 2/step 2),

Both should simulate practical conditions relevant to its intended use (concentration of the product, temperature, soiling, different fabrics, contact time, etc.).

At this moment three types of test are available:
- phase 2/step 1 suspension tests as described in EN14885 (see Appendix 2)
- phase 2/ step 2 tests involving test fabrics in a small scale laboratory setting (e.g. ASTM E2406) or a full-scale laundry machine test (as a CEN draft TC 216/N 472, or DGHM).

In the phase 2/step 2 tests fabric is contaminated with test organisms and then exposed to the disinfectant.

The EN tests are strongly recommended where available and appropriate.

3.9.2.1.1 *Test conditions*

For products intended to be added to washing machines, information on the following in-use conditions should be provided:
- the concentration of the product (or at least the active substance) in the water during disinfecting process (i.e. washing or rinsing). The water volume used can differ between wash and rinse cycle and different washing programmes, but also between washing machines
- the water to textile ratio in the test is an important factor that should reflect the in-use conditions
- the temperature during the disinfection process (high when added in wash process, low in rinse process)
- the contact time (differs between various washing programmes and washing machines)

The laboratory tests should be performed under these conditions. The identified conditions of effective disinfection can normally only be carried out in professional washing machines.
If the exact conditions cannot be met, e.g. in household machines, reasonable worst case conditions shall be tested.

Worst case conditions:
- the lowest temperature
- the highest volume of water (i.e. maximum dilution of the product)
- the shortest contact time
- the maximum load of laundry (i.e. smallest water to textile ratio).

When phase 2, step 2 tests involving fabric test carriers are performed, both the micro-organisms remaining on the test carriers, those released into the washing liquid and those transferred to previously uncontaminated control carriers should be assessed.

A control treatment without the active substance should be included. This includes carriers with micro-organisms which are treated identical to the test carriers (washed similarly, addition of soap when applicable, etc.) except for the addition of disinfectant[e].

Manual soaking or pre-soaking can be done at room temperature but for some intended uses the temperature might start high and will cool down during the contact time (e.g. where hot water is used, which cools naturally). This should also be taken into account in the tests.

3.9.2.1.2 *Soiling:*

The interfering substance most appropriate for the in-use conditions should be used. For instance, blood for products used in the medical area and protein for products used in industry, institutional and domestic areas is recommended. The soiling on a domestic product for use in pre-soak (dirty clothes) will be very higher than the soil for a post-wash rinse additive (clean clothes). For products used during pre-soak and wash tests should be done under dirty conditions. For products used during post-wash rinse test should be done under clean conditions.

3.9.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

EN and DGMH tests provide pass criteria.
No acceptance criteria have been specified in the ASTM standards for laundry (ASTM E 2406-04).

If the test doesn't provide pass criteria, the general criteria in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.10 BIOFILM

3.10.1 Introduction

A biofilm is a complex aggregation of micro-organisms distinguished by the excretion of a protective and adhesive matrix attached to a solid surface in contact with a fluid. The matrix may incorporate other components derived from the environment.

[e] This paragraph on the requirements for a control treatment was not (yet) included in the EU TNsG on PT2, however, it is included here since it is in agreement with NL data requirements.

Once the first cell succeeds to attach to the solid surface and biofilm starts to form, growth of the biofilm may become very fast, as subsequent free floating bacteria find it much easier to attach to the developing matrix.
Biofilms can grow in areas such as inside water tanks and distribution pipelines of hospitals, hotels, industries and in general in all those water systems which have adequate temperature and nutrients for the microbial growth.
The consequences of biofilm formation into a water system or facility may be severe depending on environmental conditions and any safety and performance requirements.

In healthcare facilities, biofilm contamination of medical equipment or water systems may increase the risk of nosocomial infections; in industrial facilities biofilm may cause microbial contamination of production (pharmaceuticals, cosmetics, etc.); in other situations biofilms may be responsible for significant reduction of the performance of water systems by obstructing normal water flow or they may induce corrosion of the pipelines.

Several factors may contribute to biofilm formation, with important factors including the chemical composition and roughness characteristics of the pipe, tank or tube circuit.

Bacteria in biofilms are more resistant to disinfection than free floating bacteria of the same species, as the presence of extracellular polymeric substances acts as a physical barrier to the biocide. This matrix may hamper biocidal penetration to the lower layers of the biofilm or may interact with the biocide and neutralise it. Additionally, the physiological state of the bacteria in the biofilm differs from bacteria in suspension, which can also influence the susceptibility of the bacteria to biocides.

Two types of activities of biocides against biofilm can be identified:

a) Prevention of biofilm formation: the biocide acts on biofilm formation (i.e. in this case the biocide is present before the biofilm is formed and may affect the early adhesion of cells to the surface or the viability of the cells).

b) Biofilm disinfection (“curative”): the biocide acts on a mature biofilm (i.e. when the biofilm is already present on a surface and the biocide interacts with the biofilm-embedded cells, with a -cidal effect). Biocidal products of this type may also achieve detachment of the biofilm (possibly in conjunction with physical action).
In case where the biofilm is not removed as a result of the biocide treatment, it should be followed by mechanical removal of the biofilm.

Industry is increasingly developing new technologies for prevention, inactivation and/or detachment of biofilms and/or inactivation of biofilm embedded organisms, for example through the use of UV light, water ionization or impregnated or coated materials and new biocides which claim specific efficacy against biofilms.

3.10.2 Data requirements

There are currently no standard laboratory test available to verify the efficacy of biocides against biofilms. As this is an area in which the science is developing rapidly, the information below should be considered as general guidance reflecting the state of knowledge at the time of writing.

Tests to demonstrate the efficacy of disinfectants according to EN and OECD are based on simpler models than are found in biofilms. The available surface/carrier tests are not representative of biofilm models, as they do not consider the presence of extra cellular polymeric substances which act as a physical barrier to the biocide.

Other characteristics of the biofilm and biocidal product should be taken into account. For example, if biocide impregnated materials claim a preventive effect on biofilm formation, the prevention of biofilm formation should be demonstrated, taking into consideration the half-life of the impregnating substance which may differ depending on the material characteristics. The active substance may be released from the surface and/or may be inactivated by environmental factors.
A standard suspension test can only be used to confirm basic activity of the product against the claimed organisms in a tiered approach.

A suggested general approach could be:

1) a suspension test: any biocide claiming to act on biofilm, has to be first evaluated in standard suspension test (preferably EN)
2) a simulated use efficacy test to demonstrate the ability of the product to exert a controlling effect on the biofilm under either static condition or under flow conditions depending on the use pattern (claim). This controlling effect can be to destroy and detach, inhibit or prevent the formation of a biofilm
3) a field trial, where the biofilm is formed under (simulated) use conditions.

These tests should be performed in sequence to obtain more complete information on the activity of the product on biofilm.

For biofilm disinfection (curative) a suspension test (as for (1) above) and suitable robust data from either a simulated use test (2) or field trial (3) should be performed. If there are no robust data from a simulated use test (2), a field test (3) is mandatory.
For biofilm prevention the approach is different to that for biofilm disinfection, as the biocide is present before the biofilm is formed and may affect the early adhesion of cells to the surface or the viability of the cells. In this case the suspension test (1) may not be useful since the product might not have a –cidal effect.

3.10.2.1 *Test Methodologies*

3.10.2.1.1 *Suspension tests*

The first step in the tiered approach is a suspension test. The CEN phase 2/step 1 tests are suitable as suspension tests. This test is only applicable for products that can be tested in suspension and which have a –cidal effect.

3.10.2.1.2 *Simulated use tests*

Standard laboratory tests to verify the efficacy of biocides against biofilms are not currently available. Therefore, before carrying out a biofilm test, any test methods should be agreed with the Competent Authority.

Applicants should provide a method following the principles in this guidance and based on scientific evidence. During development of the tests Competent Authorities of member states should be consulted to make sure that the tests are acceptable.

Biofilms can be formed and evaluated in static or flow conditions. The way the biofilm is formed has an effect on the susceptibility of the biofilm to biocides: biofilms formed under flow conditions are generally more resistant to biocides than biofilm formed under static conditions. The conditions under which the biocidal products will have to operate should also be taken into account. Under static conditions the disinfectant operates without the aid of the removal effect of a fluid flow or shear stress. Under flow conditions the contact time might be shorter when shock dosing is used.

Static tests are less expensive and easier to standardise, but flow tests are generally closer to the real use scenarios.
In both cases, the reproducibility and repeatability of results over time should be ensured; so a method that allows a series of observations, rather than a single one, should be employed.

In laboratory testing of the efficacy of biofilm disinfectants the critical factors of a real-world environment should be represented.
In cases where only efficacy against biofilm formed under static conditions is claimed (e.g. use in tanks without flow) it is sufficient to only test against these biofilms.

Examples of methods for testing under flow and static conditions are described below, but other protocols are available in literature or may be under development.

3.10.2.2 *Static condition assay*

Standard laboratory tests to verify the efficacy of biocides against biofilms formed under static conditions are not currently available. However, literature describes several methods of how to create a biofilm in the laboratory under static conditions.
An example of a semi-quantitative method for biofilm evaluation is the microplate test, where biofilm is formed in static conditions and the amount of biofilm can be quantified by spectrophotometric measurements. The amount of living cells in the biofilm before and after treatment can also be determined. In this case, the disinfectant operates without the aid of the removal effect of a fluid flow or shear stress.

A positive aspect of such assay is that it is a low cost, easy-to-conduct test, that allows several replicates and/or testing of several conditions (several biocide concentrations, more species, etc) to be carried out, which would provide the basis for a more accurate and closer-to-reality test.

This method consists of the formation of a biofilm by the species of interest on the bottom of 96 well plates (the material and coating of the plates should be specified); the disinfectant may be present before (preventive effect) or after (inhibition/removal effect) the biofilm is formed. The amount of biofilm (biomass) is quantified after staining of the adherent material and spectrophotometric measurement. Detecting agents such as ATP to measure bacterial viability may also be used.

3.10.2.3 *Flow condition assay*

Standard laboratory tests to verify the efficacy of biocides against biofilm formed under flow conditions are not currently available. However, systems to generate a standard biofilm have been developed by CEN (CEN ISO/TS 15883-5:2005 Annex F) and ASTM (ASTM E2196 and ASTM E2562). Using either of these reproducible biofilms, a method for the assessment of prevention and/or elimination of biofilm in terms of viable cells reduction and bacterial biomass reduction can be carried out.

The CEN method consists of the production of a standard *Pseudomonas aeruginosa* biofilm inside a Teflon tube, using a flowing system to simulate a real world situation.
ASTM E2196 and ASTM E2562 standards use biofilm rotating disc reactors, which are especially suited for high shear forces.
The biofilm is then treated with a disinfectant to evaluate the biocidal capacity to remove or to reduce the biofilm.

Other carrier types (e.g. silicon, steel, PVC, etc.) can be selected and used depending on the biofilm development system, and the experimental conditions can be adapted to compare the efficacy of different treatments in preventing biofilm formation.

A reference substance of known activity shall be tested in parallel (e.g. chlorine dioxide, sodium hypochlorite).

3.10.2.4 *Field trials*

As for other situations in which biocides are used, only field tests (phase 3 tests) would be fully representative of the activity of the biocide on biofilms, but these tests are difficult to standardise, and such tests, if used, should be complemented by laboratory suspension or simulated use tests, which have a higher degree of robustness and reproducibility.

A field trail should reproduce the in-use conditions of the worst case situation of the intended uses.
Prevention and/or elimination of biofilm (in terms of viable cells reduction and bacterial biomass reduction) should be demonstrated by sampling before and after disinfection.

A field test can be waived if a suitably robust simulated use test, which adequately mimics the in-use conditions is provided. A robust test could for instance be a complex pipe system, in which natural biofilm formation takes place, either in combination with the addition of standard organisms or not.

3.10.2.5 *Test organisms*

The choice of micro-organisms for a test is relevant, since the use of only one organism per test is limitating and may not be fully representative of the real events leading to micro-organism aggregation (biofilms in settings where disinfectants are used, are normally multi-microbial, i.e. composed by several different species). Moreover contaminants from environmental sources may be embedded in the biofilm matrix which may reduce the disinfectant's efficacy.
Bacteria are not the only inhabitants of biofilms, as both fungi and algae may also inhabit biofilms. Protozoans that consume bacteria may feed on biofilms. Protozoan oocysts and virus particles can become entrapped in a biofilm and later detach, returning to the environment.
In a suspension test, the standard organisms per claimed group (bacteria, fungi, etc.) should be tested.
For a general claim of efficacy against biofilm, as a minimum bacteria should be tested in laboratory biofilm tests. When action against other groups of organisms (e.g. fungi, algae) is claimed these should be tested too.
In suspension tests the standard organisms should be tested (see Appendix 1).
Pseudomonas aeruginosa and *Staphylococcus aureus* are acceptable test organisms for the laboratory biofilm tests. Mixtures of test organisms for producing biofilms are only acceptable as additionally testing, as it is difficult to standardise these tests.
In simulated use or field trials the biofilm may be formed *in vivo* with naturally occurring micro-organisms.

3.10.3 Acceptability criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests these should be met.
If the test doesn't provide these criteria, the general criteria in Appendix 2 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

3.11 SOIL

Disinfection of soil and other substrates (in playgrounds) with biocides is not common (and so far not claimed for Annex I of the BPD). This is more often done for plant protection. Therefore, plant protection guidelines and EPPO standards on soil treatments should be referred to for test methods. The use of the test methods should be justified with the application.

3.12 Treated articles

A treated article within PT2 is any article which has been treated with or intentionally incorporates one or more biocidal products and which claims disinfecting or biostatic properties or function, when they have a primary biocidal function.
For PT2 this includes for instance self-disinfecting surfaces, surfaces which prevent biofilm formation, etc..
There is currently little guidance on data requirements and acceptance criteria available for treated articles, although OECD test methods are in development.
A chapter on treated articles will be included in this guidance at a later date.

3.13 OTHER USES

Several other uses are mentioned in the description of PT2: waste water and hospital waste disinfection, algaecides for swimming pools and indoor/outdoor aquatic area (aquaria / garden ponds). No data requirements and acceptance criteria for these uses are currently available. The general principals for efficacy evaluation in PT2 are applicable.

Guidance on the requirements for testing "clean-in-place" products is not currently included. At this moment CIP products are evaluated as hard surface disinfectants in NL.
The guidelance will be updated when methods are available.

4 PT 3 VETERINARY HYGIENE

4.1 Introduction

Product type 3 contains biocidal 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 are also included.

4.2 Data requirements PT3

4.2.1 *Test methods*

For efficacy testing of veterinary hygiene biocidal products, the tiered approach as described in section 1.4.1 is preferred.
For product authorisation of veterinary hard surface, hoof and teat disinfectants in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only. For products for private use both phase 2 step 1 and phase 2 step 2 have to be provided.

For hoof disinfection information should be provided on how long the efficacy of a hoof bath can be guaranteed (time period, number of animals passing through). This can for instance be done by a field test in which the amount of active substance is measured several times during the test period. Efficacy (suspension) tests should be provided with the concentration of the product (active substance) at the end of the claimed period of use.

For other uses the tiered approach as described in section 1.4.1 is preferred. A phase 2 step 2 test or field test (phase 3) should provide information under in-use conditions. When no (semi) field test are provided this must be justified in the application and will be evaluated on a case by case basis.

When efficacy against biofilm is claimed a simulated-use test or field test has to be provided, next to a phase 2 step 1 test. See chapter 3.10 for test methods.

For an overview of available EN tests see Appendix 2.

4.2.2 *Test organisms*

In NL veterinary hygiene products should be at least sufficiently effective against **bacteria and yeasts**, for product authorisation done under Article 121 of the Wgb. Efficacy tests with these organisms should always be provided.
The only exception to this rule is for products for hoof disinfection. For this use only bactericidal efficacy should be demonstrated.
Products for disinfection of veterinary instruments and/or animal transportation vehicles should not only be effective against bacteria and yeasts but also against **viruses**. For disinfection of animal transportation vehicles efficacy against specific disease causing viruses have to be demonstrated: Classical swine fever, Suid herpes virus (Aujeszky's disease) and Foot-and-mouth disease virus.

For all other groups of organisms tests only have to be provided when the organisms are claimed.
The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference organisms is given in Appendix 1.

4.2.3 *Contact time*

It is important that the tests are carried out with the same contact time as claimed on the label. The claimed contact time has to be a realistic value, therefore in NL maximum contact times are set:

1. for surface disinfection products the contact time is normally 5 minutes but should not exceed 30 minutes
2. for surface disinfection products used in animal transport vehicles the contact time should not exceed 5 minutes
3. for hoof disinfection products the contact time should not exceed 5 minutes
4. for teat disinfection products the contact time is normally 1 minute but should not exceed 5 minutes
5. for disinfection of hatching-eggs the contact time should not exceed 5 minutes

It must be assured that the disinfected parts stay wet during the contact time. When residual efficacy is claimed for dried product this should be demonstrated in efficacy tests.

4.2.4 *Soiling*

Phase 2 step 1 tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Tests under clean conditions only suffice when the label

instructions state that cleaning prior to disinfection is necessary. If this is not stated on the label the test should be done under dirty conditions.

Dirty conditions for surface disinfection, hoof disinfection and disinfection of hatching eggs:
10 g/L bovine albumin solution + 10 g/L yeast extract
Dirty conditions for teat disinfection:
10 g/L skimmed milk
Clean conditions:
3 g/L bovine albumin solution

4.2.5 *Temperature*

Normally PT3 products are tested at **10ºC** since the temperature in animal housings can be low. For teat disinfection and disinfection of hatching-eggs a temperature of respectively 30ºC and 20ºC is acceptable. Deviations from these temperature requirements must be justified in the application and will be evaluated on a case by case basis.

4.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, or when applicable, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required log reductions are summarised in Appendix 2.
Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

5 PT 4 FOOD AND FEED AREA

5.1 Introduction

Product type 4 contains biocidal 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.

When water systems are disinfected in closed circuits, after which the system is washed with clean water, this is disinfection of the pipework and included in PT4. When disinfection is done in the water systems while it is in service also the water is disinfected and this is included in PT5.

5.2 Data requirements PT4

5.2.1 *Test methods*

5.2.1.1 *Disinfection of hard surfaces in food and feed area*

For efficacy testing of food and feed area biocidal products, the tiered approach as described in section 1.4.1 is preferred.
For product authorisation of hard surface disinfectants within PT4 in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only. For products for private use both phase 2 step 1 and phase 2 step 2 have to be provided.

When efficacy against biofilm is claimed a simulated-use test or field test has to be provided, next to a phase 2 step 1 test. See chapter 3.10 for test methods.

When the disinfection is done by room disinfection with vaporised biocide a simulated-use test or a field test has to be provided. See chapter 3.4 for test methods.

For an overview of available EN tests see Appendix 2.

5.2.1.2 *Disinfection of milking equipment*

Combined cleaning/disinfection products, intended for use in **milking equipment** on the farm, should meet the requirements of the standard laboratory tests, and must be sufficiently effective in a **field test**.

When efficacy against biofilm is claimed a simulated-use test or field test has to be provided, next to a phase 2 step 1 test. See chapter 3.10 for test methods.

5.2.1.3 *Disinfection in drinking water systems*

For combined cleaning and disinfecting of **new** and rehabilitated drinking water pipes (e.g. in newly built or renovated houses) efficacy should be demonstrated with a suspension test (phase 2 step 1).

For all other uses the control of *Legionella* in drinking water systems is of major importance. For these uses efficacy against *Legionella* should always be demonstrated in a tiered approach. Next to a suspension test also a field test has to be provided.

When efficacy against biofilm is claimed a simulated-use test or field test has to be provided, next to a phase 2 step 1 test. See chapter 3.10 for biofilm test methods.

5.2.1.3.1 *Laboratory tests*

Basic efficacy of the product should be demonstrated in a suspension test (phase 2 step 1). Studies should show that the product can accomplish a log 5 reduction against bacteria and, where applicable, *Legionella*. This can be done in laboratory tests (e.g. suspension tests EN1276 and EN13623). Laboratory tests against Legionella can be waived when field trials are available in which the concentration of *Legionella* is high enough to show log 5 reduction (min. 10^5 cfu/l).

5.2.1.3.2 *Field trials*

For products with single or intermittent use (pipe disinfection PT4) field trials with the following requirements should be provided:

- Before testing it should be made clear that the installation suffers of high concentrations of *Legionella*. A zero measurement should be performed.
- A field trial should be performed at a minimum of three locations.
- Minimal one of the locations should have 100 or more operational draw-off points (taps and other outlets) downstream of the application spot.
- The amount of sampling points per location depends on the amount of draw-off points in the installation. The table below should be used (taken from appendix 3, part of article 8 of the Regeling legionellapreventie in drinkwater en warm tapwater)

Number of draw-off points (outlets)*	Number of sampling points
10-100	4
101 – 200	6
201 – 400	8
401 – 800	10
801 – 1600	12
> 1600	14

* a draw-off point is a point where drinking water, household water or warm water will be made available for use.

- Because *Legionella* is incorporated in the biofilm it should be demonstrated that the biofilm is disinfected too. This can be done by showing that the system is still free of *Legionella* after 3-4 month (unless claimed for a longer period in the label / WG/GA).
- After treatment none of the sampling points should measure more that 100cfu/L *Legionella*.

5.2.1.4 *Disinfection in veterinary water systems*

For combined cleaning and disinfecting of veterinary drinking water pipes (e.g. water tanks, water in animal housings etc. used as drinking water for animals and for other uses in stables like cleaning, preparing feed, etc.) efficacy should be demonstrated in a tiered approach as described in section 1.4.1. This includes a phase 2 step 1 test.

When efficacy against biofilm is claimed a simulated-use test or field test has to be provided, next to a phase 2 step 1 test. See chapter 3.10 for test methods.

5.2.1.5 *Disinfection in food and feed area other then the above*

For other uses the tiered approach as described in section 1.4.1 is preferred. Next to a phase 2 step 1 test a phase 2 step 2 test or field test (phase 3) should provide information under in-use conditions.

5.2.2 *Test organisms*

In NL food and feed area products should be at least sufficiently effective against **bacteria and yeasts**. Efficacy test with these organisms should always be provided.
An exception to this rule is for products for disinfection of water pipes. For this use only bactericidal efficacy should be demonstrated. For use in veterinary water systems tests with the standard bacteria are sufficient, for human drinking water also tests with ***Legionellla*** spp. should be included.
For specific uses in industry an exception can be made when sound justification is provided. This will be evaluated on a case by case basis.

For all other groups of organisms test only have to be provided when efficacy against the organisms are claimed.
The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference organisms is given in Appendix 1.

5.2.3 *Contact time*

It is important that the tests are carried out with the same contact time as claimed on the label. The claimed contact time has to be a realistic value, therefore in NL maximum contact times are set:

1. for surface disinfection products the contact time should not exceed 5 minutes (Artikel 3.7a of the RGB)
2. for clean-in-place and in case of immersing instruments and materials it is possible to claim a contact time longer than 5 minutes.

It must be assured that the disinfected parts stay wet during the contact time. When residual efficacy is claimed for dried product this should be demonstrated in efficacy tests.

5.2.4 *Soiling*

Phase 2 step 1 tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Test under clean conditions only suffice when the label instructions state that cleaning prior to disinfection is necessary. If this is not stated on the label the test should be done under dirty conditions.

Dirty conditions general:
3 g/L bovine albumin solution
Dirty conditions for milk industry:
1% milk
Dirty conditions breweries:
10 g/L yeast extract
Dirty conditions beverage industry:
10 g/L sucrose
Dirty conditions cosmetic industry:
5 g/L laurylsulfate
Dirty conditions under greasy conditions
grease such as dried whole milk or cooked-dried cooking oil
Clean conditions:
0,3 g/L bovine albumin solution

5.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, or when applicable, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT4 products the required log reductions are summarised in Appendix 2.
Deviations from the pass criteria are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

6 PT 5 DRINKING WATER

6.1 Introduction

Product type 5 contains biocidal products used for the disinfection of drinking water for both humans and animals. Definition of drinking water is according to article 2 of Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. In this chapter the term drinking water for humans is not only used for water that will be consumed directly but also for other uses of water coming out of the plumbing system like showering, cooking, etc..

When disinfection is done in the water systems while it is in service also the water is disinfected and this is included in PT5. When water systems are disinfected in closed circuits, after which the system is washed with clean water, this is disinfection of the pipework and included in PT4.

Disinfectant products can be added to drinking water, intermittently, by shock dosing or continually. The purpose of disinfection is to disinfect the water to prevent transmission of water-borne diseases via drinking water. Water-borne transmitted pathogens can be bacteria, viruses, yeasts, fungi and protozoan parasites. Disinfection is only one aspect of drinking water treatment. Application of drinking water disinfectants is associated with the responsibility to control toxic disinfectant by products. Treatment substances should only be added for specific hygienic or technical reasons, limiting application to the minimum volumes that are absolutely necessarily for achieving the targeted effect (principle of minimisation) and only under conditions optimizing their efficacy.

Disinfection within PT5 can be divided into five groups:

1. Disinfection in drinking water companies
   This is disinfection of water when it enters the drinking water company, transport in between drinking water companies (semi-finished product) and prior to distribution into (part of) the communal piping system (so called “na-desinfectie” or “nooddesinfectie”).
2. Disinfection in collective drinking water systems
   This is disinfection in collective drinking water systems like hospitals and other health care facilities, hotels, penitentiary institutions, etc.. In these large plumbing systems water might become contaminated with *Legionella*. When physical techniques (heating, UV treatment, etc.) are insufficient chemical disinfection is allowed in NL.
3. Disinfection of stationary water in reservoirs
   This is disinfection of water stored in tanks and reservoirs, for instance on ships.
4. Disinfection of undefined water before drinking.
   This is disinfection of for instance ditchwater or other water that might be contaminated in places where no clean drinking water is available.
5. Disinfection of veterinary water
   This is disinfection of water in animal housings used as drinking water for animals and for other uses in stables (cleaning, preparing feed, etc.).

6.2 Data requirements PT5

6.2.1 *Test methods*

For an overview of available EN tests see Appendix 2.

6.2.1.1 *Disinfection in drinking water companies*

For product authorisation of drinking water disinfectants in drinking water companies in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only.

6.2.1.2 *Disinfection in collective drinking water systems*

Because the control of *Legionella* in drinking water systems is of major importance, efficacy against *Legionella* should always be demonstrated.
The following requirements are set for biocides to be used as disinfectant in drinking water systems:

6.2.1.2.1 *Laboratory tests*

Basic efficacy of the product should be demonstrated in a suspension test (phase 2 step 1). Studies should show that the product can accomplish a log reduction of 5 against bacteria and and specifically *Legionella*. This can be done in laboratory tests (e.g. suspension tests EN1276 and EN13623). Laboratory tests can be waived when field trials are available in which the concentration of *Legionella* is high enough to show log reduction of 5 (min. 10^5 cfu/L).

6.2.1.2.2 *Field trials*

For products with long and continuous use (drinking water disinfection PT5) field trials with the following requirements should be provided:
6.2.1.2.2.1 <u>Locations</u>
A field trial should be performed at a minimum of 10 locations. Depending on the complexity of the systems the number of locations can be lowered to a minimum of 5. Locations outside of the Netherlands will only be accepted if the quality of the tested drinking water is specified and if this water is comparable to Dutch drinking water, this to be decided by the Competent Authority.
Only locations with 100 or more operational draw-off points (downstream of the application spot) are acceptable. A location is a collective drinking water system which is treated by the product. Also part of a collective drinking water system, for instance a wing of a building or only

the cold water system can be seen a test location, as long as it contains 100 or more operational draw-off points.

6.2.1.2.2.2 Duration of the test

When the apparatus is in continuous or discontinuous use (so no single applications) the duration of the test is one year per location, starting from the first sampling round after starting the apparatus. When, due to starting problems etc., the first months do not give the required result (see 2.6), the test should be extended to one year starting from the point that a stable situation is reached. In this way at least a year of test results can show that the product is capable of controlling *Legionella*.

6.2.1.2.2.3 Different types of water

It is recommended that the locations are spread over the country, this to ensure that the product is tested on different types of water. For this purpose information should be provided to the Ctgb on the quality of the provided water at the different locations. In principal this information is available through the water company.

6.2.1.2.2.4 Legionella

Before starting a test it should be clear that the installation to be treated is contaminated with Legionella bacteria. For this purpose information should be provided to the Ctgb on (recent) problems with *Legionella*, like results from sampling in the past and performed cleanings, etc..

6.2.1.2.2.5 Sampling points

The amount of sampling points per location depends on the amount of draw-off points (taps and other outlets) in the installation. The table below should be used (taken from appendix G of the Waterleidingbesluit).

Number of draw-off points (outlets)	Number of sampling points
10-100	4
101 – 200	6
201 – 400	8
401 – 800	10
801 – 1600	12
> 1600	14

All sampling points should be unambiguously coded.

At each sampling round two sampling points are sampled each time (standard sampling points), preferably the sampling point next to the apparatus and the sampling point the most far away from it. These sampling points should be clearly described and the code of these points should be stated. All other sampling points should vary at each sampling round. When a sampling point shows elevated values of *Legionella* or one of the other parameters this sampling point should be sampled again the next month. The total amount of sampling points stays the same, according to the table above.

The tuning of the apparatus at the time of sampling should be recorded.

6.2.1.2.2.6 Efficacy

To be able to evaluate the efficacy the following measurements should be performed:

- zero measurement: measurement of *Legionella*, total hardness, pH, and active substances before the apparatus is put into action.
- *Legionella*, monthly sampling, norm value 100 cfu/l (90%-percentile with a maximum of1000 cfu/l);
- total hardness, Ca, Mg; sampling once per four months, depending on the variation a higher frequency might be necessary; also data from the waterworks companies can be collected;

- pH, monthly sampling on both standard sampling points, or data from the waterworks companies can be collected.

6.2.1.2.2.7 Active substances and metabolites (side effects)

To determine the amount of active substance in the water and any harmful metabolites, the relevant products should be measured monthly.

For anodic oxidation the following products are relevant:

- available chlorine, monthly measurements; norm value 0.3 mg/l at the draw-off points (90 %-percentile with a maximum of 0.5 mg/l)

- trihalomethanes: measurement 3 and 9 months after the apparatus is put into action, always at one draw-off point which represents the worst-case situation, normally the draw-off point the most far away from the apparatus. This concerns the parameters trichloromethane (chloroform), tribromomethane (bromoform), broomdichloormethane.and dibroomchloormethane. Norm value: the total of the trihalomethanes 25 µg/l (90%-percentiel, max. 50 µg/l). The concentration broomdichloormethane should not exceed 15 µg/l.
- halogenated acetic acids: measurement 3 and 9 months after the apparatus is put into action, always at one draw-off point which represents the worst-case situation (see trihalomethanes). This concerns the parameters monochloric acid, dichloroacetic acid and trichloroacetic acid. The norm value: the total of the haloacetic acids 25 µg/L.

For copper/silver ionisation the following products are relevant:

- copper, monthly measurements; norm value 2 mg/l;
  Remark: the technique cannot produce the full 2 mg/l considering the contribution of copper from other sources. An increase of the copper value of maximum 1 mg/l is considered acceptable.
- silver, monthly measurements; norm value 50 µg/l (90%-percentiel with a maximum 100 µg/l).

For chloordioxide-generators the following products are relevant:

- chlorite, monthly measurements at all draw-off points; norm value 0.2 mg/l;
- chlorate, monthly measurements at all draw-off points; norm value 0.2 mg/l;
- trihalomethanes: measurement 3 and 9 months after the apparatus is put into action, always at one draw-off point which represents the worst-case situation, normally the draw-off point the most far a way from the apparatus. This concerns the parameters trichloromethane (chloroform), tribromomethane (bromoform), broomdichloormethane.and dibroomchloormethane. Norm value: the total of the trihalomethanes 25 µg/l (90%-percentiel, max. 50 µg/l). The concentration broomdichloormethane should not exceed 15 µg/l.

6.2.1.2.2.8 Evaluation criteria per location

For the evaluation of the results of the measurements the norm values as mentioned in *6.2.1.2.2.6* and *6.2.1.*2.2.7 are used. Per location 90% of the measurements should fulfil the requirements. Over all locations together 90% of the locations should fulfil the requirements.

6.2.1.2.2.9 General requirements for study reports

Every study report should contain a good description of material (location, number of draw-off point, sampling points, history of *Legionella*, etc.), method (starting date, tuning of the apparatus) and results (including 0-measurement). In the study reports of the field tests the results should be interpreted per location. Remarks like for instance high values above the norm, should be mentioned and explained. The report should be closed with a conclusion.

6.2.1.2.3 *Apparatus*

In case 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.

6.2.1.3 *Disinfection of stationary water in reservoirs*

For this use no specific data requirements are set. For product authorisation in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only. In some cases efficacy against biofilm is of importance in this use. For testing efficacy against biofilms see chapter 3.10.

6.2.1.4 *Disinfection of undefined water used as drinking-water*

For this use no specific data requirements are set. For product authorisation in NL, done under Article 121 of the Wgb, it is in most cases acceptable to demonstrate efficacy with phase 2 step 1 tests only.

6.2.1.5 *Disinfection of water for animals*

For efficacy testing of disinfectants for water for animals the tiered approach as described in section 1.4.1 is preferred. Next to a phase 2 step 1 test also a simulated-use test or field test (phase 3) should be performed, to provide information under in-use conditions. In some cases efficacy against biofilm is of importance in this use. For testing efficacy against biofilms see chapter 3.10.

6.2.2 *Test organisms*

In NL PT5 products should be at least sufficiently effective against **bacteria**. Efficacy test with these organisms should always be provided.
An exception to this rule is for products for disinfection of drinking water in collective systems. For this use also efficacy should be demonstrated against *Legionellla* spp..

For all other groups of organisms test only have to be provided when efficacy against the organisms are claimed.
The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference organisms is given in Appendix 1.

6.2.3 *Contact time*

It is important that the tests are carried out with the same contact time as claimed on the label. The claimed contact time has to be a realistic value. For the use as drinking water disinfectant in NL no maximum contact times are set.

6.2.4 *Soiling*

Phase 2 step 1 tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Depending on the water source that has to be disinfected the test should be performed under either clean or dirty (e.g. undefined or pumped up water) conditions.

Dirty conditions:
3 g/L bovine albumin solution
Clean conditions:
0.3 g/L bovine albumin solution

6.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, or when applicable, field tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required log reductions in suspension tests are summarised in Appendix 2. In the drinking water disinfection field tests the aim is to keep the *Legionella* concentration below 100 cfu/L. Ninety percent of all the test samples per location should show a *Legionella* concentration below 100cfu/L while the maximum *Legionella* concentration should not exceed 1000cfu/L. The test should be done on ten locations and 90% of these locations should meet this criterion. Deviations from the pass criteria are possible, but must be justified in the application.

The Competent Authority will evaluate any justification on a case by case basis, consulting the other Competent Authorities where appropriate, and decide whether it is acceptable or not.

7 DEVELOPEMENTS

Within the EU working groups are reviewing and updating the TNsG on Product evaluation for PT 2 and 5. Hopefully in the future also PT1, 3 and 4 will be reviewed. Several EU countries (competent authority and experts) and industry participate in these working groups. As soon as these updates are available NL will imply these evaluation requirements and criteria in the NL product evaluations. The general guidance for disinfectants and the PT2 guidance was recently revised by a working group, endorsed in CA May 2013 and is now open for consultation. This will be available on http://ec.europa.eu/environment/biocides/guidance.htm. In the EU part of this Evaluation Manual the old version is still available.

8 APPENDICES

Appendix 1 Table of Reference Organisms

This table comprises only reference organisms included in EN norms covered by EN 14885:2006. The reader is also strongly advised to check if there is no new version of the standard on the website of the CEN : www.cen.eu.
Further the EN 13623 (*Legionella*) and the draft EN for laundry disinfection were considered as well. Since the EN systematics of WGs 1 to 3 does not fit exactly to the BPD or BPR PT scheme, in borderline cases an indicated reference organism might be used for other PTs as well.

Micro-organisms	**PT1***	**PT2***	**PT3***	**PT4***	**PT5***
Bacteria					
Staphylococcus aureus ATCC 6538	X	X	X	X	X
Pseudomonas aeruginosa ATCC 15442	X	X	X	X	X
Enterococcus hirae ATCC 10541	X	X	X	X	X
Escherichia coli ATCC 10536		X	O	X	X
Escherichia coli K12 NCTC 10538	X	O			
Enterococcus faecium ATCC 6057 (for T ≥60°C)		(X)			
Proteus vulgaris ATCC 13315			X		
Streptococcus uberis ATCC 19436 (teats)			O		
Legionella pneumophila ATCC 33152 (PT2: pools, spa's, hot tubs; PT4: drinking water systems)		(X)		(X)	X
Legionella pneumophila ATCC 43108		O			O
Yeast					
Candida albicans ATCC 10231 (PT5: for animal drinking water)	X	X	X	X	(X)
Saccharomyces cerevisiae ATCC 9763 (breweries)				O	
Saccharomyces cerevisiae DSM 70487 (breweries)				O	
Fungi					
*Aspergillus brasiliensis*** ATCC 16404	X	X	X	X	
Trichophyton mentagrophytes ATCC 9533 (in households)		O			
Virus***					
Polio virus type 1, LSc-2ab (kitchens in hospitals)	X	X		(X)	
Adenovirus, type 5, strain Adenoid 75, ATCC VR-5.	X	X		X	
Bovine Parvovirus, Strain Haden, ATCC VR-767 (for T ≥60°C)		(X)			
Bovine Enterovirus Type 1 ECBO – Virus ATCC VR-248			X		
Classical swine fever (CFS) (transport vehicles)			(X)		
Suid herpes virus SuHV-1 (Aujeszky's disease) (transport vehicles)			(X)		
Foot-and-mouth disease virus (transport vehicles)			(X)		
Bacteriophage P001 DMS 4262 (milk industry)				(X)	
Bacteriophage P008 DMS 10567 (milk industry)				(X)	
Mycobacteria					
Mycobacterium terrae ATCC 15755		X			
Mycobacterium avium ATCC 15769		X	X		
(mycobactericidal: both, tuberculocidal: *M. terrae* only)					
Bacterial spores					
Spores of *Bacillus subtilis* ATCC 6633		X		X	
Spores of *Bacillus cereus* ATCC 12826		O		O	

* X = basic requirement; (X) = basic requirement for specific use mentioned in between brackets; O = optional

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

*** The reference virusses for PT4 are not difined yet. Per intended use relevant viruses should be tested.

Appendix 2 Overview of data requirements for efficacy testing of disinfectant biocides in NL under Article 121 of the Wgb

This overview is not exhaustive. For other, or more specific uses it is referred to the chapters above.

Product type / micro-organism	Requirements[1]	Test required[2]	Maximum contact time[3]	Temp-erature (ºC)	Soiling conditions[4]	Required lg reduction
PT01 handrub						
bacteria	Basic requirement - 2,1 test	EN 13727/ EN 1276[5]	30 sec	20	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 1500	30 sec	skin T	none	> propan-2-ol
yeast	Basic requirement - 2,1 test	EN 13624/ EN 1650[5]	30 sec	20	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	30 sec	20	clean / dirty	4
viruses	Optional - 2,1 test	EN 14476	30 sec	20	clean / dirty	4
fungi	Optional - 2,1 test	EN 13624/ EN 1650[5]	30 sec	20	clean / dirty	4
PT01 handwash						
bacteria	Basic requirement - 2,1 test	EN 13727/ EN 1276[5]	30 sec	20	dirty[6]	5
bacteria	Basic requirement - 2,2 test	EN 1499	30 sec	skin T	none	>control
yeast	Basic requirement - 2,1 test	EN 13624/ EN 1650[5]	30 sec	20	dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	30 sec	20	dirty	4
viruses	Optional - 2,1 test	EN 14476	30 sec	20	dirty	4
fungi	Optional - 2,1 test	EN 13624/ EN 1650[5]	30 sec	20	dirty	4
PT01 surgical hand disinfection						
bacteria	Basic requirement - 2,1 test	EN 13727	3 min[7]	20	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 12791	3 min[7]	skin T	none	>propan-1-ol
yeast	Basic requirement - 2,1 test	EN 13624	3 min[7]	20	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	3 min[7]	20	clean / dirty	4
viruses	Optional - 2,1 test	EN 14476	3 min[7]	20	clean / dirty	4
fungi	Optional - 2,1 test	EN 13624	3 min[7]	20	clean / dirty	4

Product type / micro-organism	Requirements[1]	Test required[2]	Maximum contact time[3]	Temp-erature (ºC)	Soiling conditions[4]	Required lg reduction
PT02 hard surfaces and other uses where EN test are applicable						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[5]	5 min.	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 13624 / EN 1650[5]	5 min.	20	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	5 min.	20	clean / dirty	4
viruses	Optional - 2,1 test	EN 14476	5 min.	20	clean / dirty	4
fungi	Optional - 2,1 test	EN 13624 / EN 1650[5]	5 min.[8]	20	clean / dirty	4
In addition for non-professional use:						
bacteria	Basic requirement - 2,2 test	EN 13697	5 min.	20	clean / dirty	4
yeast	Basic requirement - 2,2 test	EN 13697	5 min.	20	clean / dirty	3
fungi	Optional – 2,2 test	EN 13697	5 min.[8]	20	clean / dirty	3
PT02 instrument disinfection						
bacteria	Basic requirement - 2,1 test	EN 13727	5 min.[10]	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 13624	5 min.[10]	20	clean / dirty	4
viruses	Basic requirement - 2,1 test[9]	EN 14476	5 min.[10]	20	clean / dirty	4
fungi	Basic requirement - 2,1 test[9]	EN 13624	5 min.[10]	20	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	5 min.[10]	20	clean / dirty	4
In addition for non-professional use:						
bacteria	Basic requirement - 2,2 test	EN 14561	5 min.	20	clean / dirty	5
yeast	Basic requirement - 2,2 test	EN 14562	5 min.	20	clean / dirty	4
fungi	Optional – 2,2 test	EN 14562	5 min.	20	clean / dirty	4

Product type / micro-organism	Requirements[1]	Test required[2]	Maximum contact time[3]	Temp. (ºC)	Soiling conditions[4]	Required lg reduction
PT03 hard surfaces						
bacteria	Basic requirement - 2,1 test	EN 1656	5 min.[11]	10	clean / dirty	5
yeast	Basic requirement- 2,1 test	EN 1657	5 min.[11]	10	clean / dirty	4
viruses	Optional - 2,1 test	EN 14675	5 min.[11]	10	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14204	5 min.[11]	10	clean / dirty	5
fungi	Optional - 2,1 test	EN 1657	5 min.[11]	10	clean / dirty	4
In addition for non-professional use:						
bacteria	Basic requirement - 2,2 test	EN 14349	5 min.[11]	10	clean / dirty	4
yeast	Basic requirement - 2,2 test	EN 16438	5 min.[11]	10	clean / dirty	3
fungi	Optional - 2,2 test	EN 16438	5 min.[11]	10	clean / dirty	3
PT03 hard surfaces in transport vehicles						
Bacteria, yeasts, fungi, mycobacteria	As PT03 hard surfaces					
viruses	Basic requirement- 2,1 test + extra test organisms[12]	EN 14675 [12]	5 min.	10	clean / dirty	4

PT03 teat disinfection						
bacteria	Basic requirement - 2,1 test	EN 1656	5 min.[13]	30	clean / dirty	5
yeast	Basic requirement- 2,1 test	EN 1657	5 min.[13]	30	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14204	5 min.[13]	30	clean / dirty	5
fungi	Optional - 2,1 test	EN 1657	5 min.[13]	30	clean / dirty	4
viruses	Optional - 2,1 test	EN 14675	5 min.[13]	30	clean / dirty	4
PT03 hoof disinfection						
bacteria	Basic requirement - 2,1 test	EN 1656	5 min.	10	dirty[14]	5
yeast	Optional - 2,1 test	EN 1657	5 min.	10	dirty[14]	4
viruses	Optional - 2,1 test	EN 14675	5 min.	10	dirty[14]	4
mycobacteria	Optional - 2,1 test	EN 14204	5 min.	10	dirty[14]	5
fungi	Optional - 2,1 test	EN 1657	5 min.	10	dirty[14]	4

Product type / micro-organism	Requirements[1]	Test required[2]	Maximum contact time[3]	Temp. (ºC)	Soiling conditions[4]	Required lg reduction
PT04 hard surfaces						
bacteria	Basic requirement - 2,1 test	EN 1276	5 min.	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 1650	5 min.	20	clean / dirty	4
mycobacteria	Optional - 2,1 test	EN 14348	5 min.	20	clean / dirty	5
viruses	Optional - 2,1 test	EN 14476 / EN 13610[15]	5 min.	20	clean / dirty	4
fungi	Optional - 2,1 test	EN 1650	5 min.	20	clean / dirty	4
bacterial spores	Optional - 2,1 test	EN 13704	5 min.	20	clean / dirty	3
In addition for non-professional use:						
bacteria	Basic requirement - 2,2 test	EN 13697	5 min.	20	clean / dirty	4
yeast	Basic requirement - 2,2 test	EN 13697	5 min.	20	clean / dirty	3
fungi	Optional – 2,2 test	EN 13697	5 min.	20	clean / dirty	3
PT04 milking equipment						
bacteria	Basic requirement - 2,1 test	EN 1276	5 min.	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 1650	5 min.	20	clean / dirty	4
other organisms when claimed	Optional - 2,1 test	As PT4 hard surfaces				
bacteria and yeast	Basic requirement field trial					
PT04 drinking water systems						
bacteria	Basic requirement - 2,1 test	EN 1276	As claimed	20	clean / dirty	5
Legionella	Basic requirement - 2,1 test	EN 13623	as claimed	20	clean/ dirty	5
other organisms when claimed	Optional - 2,1 test	As PT4 hard surfaces				
bacteria and Legionella	Basic requirement field trial	See 5.2.1.3.2				
PT04 veterinary water systems						
bacteria	Basic requirement - 2,1 test	EN 1276	As claimed	20	clean / dirty	5
other organisms when claimed	Optional - 2,1 test	As PT4 hard surfaces				

Product type / micro-organism	Requirements[1]	Test required[2]	Maximum contact time[3]	Temp. (ºC)	Soiling conditions[4]	Required lg reduction
PT05 drinking water for drinking water companies, stationary water in reservoirs and undefined water						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 1650	as claimed	20	clean / dirty	4
other organisms when claimed	Optional - 2,1 test		As PT4 hard surfaces			
PT05 drinking water in collective systems						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	20	clean	5
Legionella	Basic requirement - 2,1 test	EN 13623	as claimed	20	clean	5
Legionella	Basic requirement field trial	See 6.2.1.2.2				
PT05 drinking water for animals						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	20	clean / dirty	5
yeast	Basic requirement - 2,1 test	EN 1650	as claimed	20	clean / dirty	4
other organisms when claimed	Optional - 2,1 test		As PT4 hard surfaces			
claimed organisms	Basic requirement simulated-use or field trial					

[1] Requirements: basic requirements are mandatory and have to be fulfilled for authorisation of a product with this intended use. In addition, other organisms claimed are optional, i.e. if the requirements for these organisms are not fulfilled these organisms will be excluded from the claim.
[2] EN-tests are strongly advised but not mandatory. Other tests carried out according to standard guidelines are acceptable if a clear description of the test procedure (including contact time, soiling, temperature, suitable controls, lg reduction, etc.) and justification is provided.
[3] Contact time: maximum acceptable contact times are stated. If a shorter contact time is stated on the WG/GA efficacy has to be demonstrated at this shorter contact time.
[4] Soiling conditions: low level soiling conditions are acceptable if it is stated on the WG/GA that cleaning prior to disinfection is necessary. Otherwise and in case no prior cleaning is possible dirty conditions have to be included in the tests.

PT 1 and 2 For hospitals and health care:
Dirty 3 g/L bovine albumin + 3 ml/L sheep erythrocytes // Clean 0.3 g/L bovine albumin
PT 1 and 2 other uses:
Dirty 3 g/L bovine albumin // Clean 0.3 g/L bovine albumin
PT 3 general, including hoof disinfection:
Dirty 10 g/L bovine albumin + 10 g/L yeast extract // Clean 3 g/L bovine albumin
PT 3 teat disinfection:
Clean/Dirty 10g/L skimmed milk
PT 4 general:
Dirty 3 g/L bovine albumin // Clean 0.3 g/L bovine albumin
PT 4 milk industry:
Dirty 1 g/L milk
PT 4 breweries:
Dirty 10g/L yeast extract
PT 4 beverage industry:
Dirty 10g/L sucrose
PT 4 cosmetic industry:
Dirty 5g/L laurylsulfate
PT 5 general:
Dirty 3 g/L bovine albumin // Clean 0.3 g/L bovine albumin

[5] For PT 01 or PT02-'medical applications' the required tests differ from PT01 or PT02-'non-medical' applications. The first test is for medical applications the second for non-medical applications. In case both types of applications are claimed only one test has to be carried out in which the relevant worst case test conditions (in general medical test) are included.

[6] For handwash disinfectants it is assumed that hands will not be washed before washing with a disinfectant. Therefore test have to be done under dirty conditions.

[7] The WHO states that for several products scrubbing for 2-3 minutes reduces bacterial counts to acceptable levels. However, in the past longer scrubbing times were accepted. Contact times of longer than 3 minutes, up to 10 minutes, will only be authorised with a sound justification on the necessity of this long scrubbing time.

[8] In general the contact time for fungi is 5 minutes. The only exception to this rule is when only efficacy against staining moulds is claimed in indoor areas with moist conditions. For this use the maximum contact time is 15 minutes.

[9] For medical equipment tests with fungi and viruses are a basic requirement, for all other equipment tests with fungi and viruses are optional

[10] Contact time for instrument disinfection: in case of immersing instruments or CIP it is possible to claim a contact time > 5 minutes.

[11] For surface disinfection in veterinary areas normally the contact time is 5 minute. The maximum contact time is 30 minutes.

[12] Next to Bovine Enterovirus Type 1 required in EN 14675 also tests with classic swine fever virus, Aujesky's disease (Pseudorabies virus), foot and

mouth disease (picorna virus) and, when appropriate, Swine Vescular Disease (SVD) are required for use in transport vehicles

[13] For teat disinfection normally the contact time is 1 minute. The maximum contact time is 5 minutes.

[14] For hoof disinfection it is not to be expected that hoofs will be sufficiently cleaned before disinfection. Therefore only tests under dirty conditions are acceptable.

[15] For uses where only efficacy against bacteriophages is claimed EN 13610 can be used. For all other uses EN14476 should be used.

Appendix 3. List of currently available standard test methods for disinfectant biocides within product type 1 to 5

ACRONYMS AND ABBREVIATIONS

Acronym	Full names	Web page to organisations (if available)
AATCC	American Association of Textile Chemists and Colors	www.aatcc.org/
AFNOR	Association Française de normalisation (NF standards)	www.afnor.fr/
AOAC	Association of Official Analytical Chemists	www.aoac.org/
ASTM	American Society of Testing and Materials	www.astm.org/
ATCC	American Type Culture Collection	
BBA	Federal Biological Research Centre for Agriculture and Forestry (Biologische Bundesanstalt für Land - Und Forstwirtschaft Bundesrepublik Deutschland)	www.bba.de
BP	Biocidal Product	
BPD	Biocidal Product Directive (referring to 98/8/EC)	
BPR	Biocidal Product Regulation (referring to 528/2012)	
BSI	British Standards Institute (BS standards)	www.bsi.org.uk/
CA	Competent Authority	
CEB	Commission Des Essais Biologiques	www.afpp.net/commande/commissions/CEB.htm
CEFIC	European Chemical Industry Council	www.cefic.org
CEN	European Committee for Standardisation	www.cen.eu
CEPE	European council of paint, printing inks and artist's colours industry	www.cepe.org
CSMA	Chemical Specialties Manufactures Association	www.csma.org
CTBA	Centre Technique du Bois et de l'Ameublement, Bordeaux	www.fcba.fr
DGHM		
EBPF	European Biocidal Product Forum	
EPA	United States Environmental Protection Agency	www.epa.gov
EPPO	European and Mediterranean Plant Protection Organization	www.eppo.org
ISO	International Standards Organisation	www.iso.org/iso/home.htm
MAFF	Ministry of Agriculture Fisheries and Foods	
MS	Malaysian Standards	http://msonline.sirim.my/msonline
NF	NF standards, Association Française de normalisation	www.afnor.fr/

OECD	Organisation for Economic Co-operation and Development	www.oecd.org
OPPTS	Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency	www.epa.gov/internet/oppts/
PT	Product Type	
SABS	South African Bureau of Standards	www.sabs.co.za

The following table includes relevant standards from CEN, OECD and some national standards. The table includes only standards that are related to efficacy testing for PT 1 to 5, available at the writing time of this document. This table could be updated.

For ASTM methods, the number after the hyphen is referring to the last revision date of the test; the number in parenthesis is referring to the last re-approval of the test.

Reference list:

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
EN 12353:2006	*Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity*	*1-5*	*General guidance*	
EN 1276:1997	*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)*	*1-5*	*Dilution/Neutralisation. Efficacy determined by a reduction in CFU.*	
EN 12791:2005	*Chemical disinfectants and antiseptics - Surgical hand disinfection - Test method and requirement (phase 2/step 2)*	*1*	*Testing mainly bactericidal activity; in vivo test; phase 2 step 2*	
EN 13610:2002	*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)*	*1-5*		
EN	*Chemical disinfectants and antiseptics -*	*1-5*		

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
13624:2003	*Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants for instruments used in the medical area - Test method and requirements (phase 2, step 1)*			
EN 13697:2001 Under revision	*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)*	*1-5*	*Dilution/Neutralisation Method. Efficacy measured by a reduction in CFU.*	
EN 13704:2002	*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)*	*1-5*		
EN 13727:2012	*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)*	*1-5*	*Efficacy determined by the reduction in CFU counts*	
EN 14204:2004	*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)*	*1-5*	*Dilution/Neutralisation Method. Efficacy measured by reduction in CFU.*	
EN 14347:2005	*Chemical disinfectants and antiseptics - Basic sporicidal activity - Test method and requirements (phase 1)*	*1-5*	*Basic sporicidal activity against dormant spores of Bacillus subtilis (ATCC 6633) and Bacillus cereus (ATCC 12826). A prepared sample of the product under test is added to a test suspension of bacterial spores. The mixture is maintained at 20 °C or any other temperature to be defined. At a specified*	

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
			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. Medical area* *Veterinary area, Food, industrial, domestic and institutional hygiene*	
EN 14348:2005	*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)*	*1-5*	*Efficacy measured by a reduction in CFU counts*	
EN 14349:2007	*Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)*	*1-5*	*Non-porous surface test, bactericidal, for disinfectants used in the veterinary applications Dilution/Neutralisation Method. By one manufacturer, the test material is deemed to have passed the test and be efficacious if it demonstrates a log 4 or more reduction in viable counts under the conditions defined in the test. Reduction in viable microbial counts compared with water controls.*	
EN 14476:2005+A1:2006	*Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2, step 1)*	*1-5*		
EN	*Chemical disinfectants and antiseptics -*	*1-5*		

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
14561:2006	*Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)*			
EN 14562:2006	*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)*	*1-5*		
EN 14675:2006	*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)*	*1-5*		
EN 14885:2006	*Chemical disinfectants and antiseptics - Application of European Standards for chemical disinfectants and antiseptics*	*1-5*	*Overview of CEN tests for disinfectants*	*Strongly recommended*
EN 1499:1997	*Chemical disinfectants and antiseptics - Hygienic handwash - Test method and requirements (phase 2/step 2)*	*1*	*For handwash products to be used in medical and veterinary areas,(food) industry, and non-professional use.*	
EN 1500:1997	*Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2/step 2)*	*1*	*For handwash products to be used in medical and veterinary areas, (food) industry and non-professional use.*	
EN 1650:1997	*Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)*	*1-5*		
EN 1656 : 2000	*Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation*	*3*	*Dilution/Neutralisation Method. Efficacy measured by a reduction in CFU.*	

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
	of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field - Test method and requirements (phase 2/step 1)			
EN 1657:2005/AC: 2007	*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*	*By one manufacturer, the product was to be deemed to have passed the test if it demonstrated a 10E5 logarithmic reduction in viability.* *By one manufacturer a reduction in viable microbial counts is compared with water controls.* *The test material is deemed to have passed the test and be efficacious if it demonstrates a log 4 or more reduction in viable counts under the conditions defined in the test*	
pr NEN-EN 16437	*Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area on* ***porous*** *surfaces without mechanical action - Test method and requirements (phase 2, step 2)*	*1-5*		
pr NEN-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*		
OECD (2009)	*OECD Guidance Document for establishing the efficacy of biocides used in swimming pools and spas*	*2*	*Draft was developed by the Task Force on Biocides*	on going (normally validated at WNT of April 2012)
OECD Draft Test Guidelines (2009):	*OECD Draft Test Guidelines (2009): Quantitative Method for Evaluating Bactericidal Efficacy of Biocides Used on Hard Surfaces*	*2,3,4*	*This test is considered a phase 2 step 2 test.*	on going

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
OECD Draft Test Guidelines (2009):	*OECD Draft Test Guidelines (2009): Quantitative Method for Evaluating Fungicidal Activity of Biocides Used on Hard Surfaces*	*2,3,4*	*This test is considered a phase 2 step 2 test.*	on going
OECD Draft Test Guidelines (2009):	*OECD Draft Test Guidelines (2009): Quantitative Method for Evaluating Virucidal Activity of Biocides Used on Hard Surfaces*	*2,3,4*	*This test is considered a phase 2 step 2 test.*	on going
OECD (2008).	*OECD (2008). Guidance document on the evaluation of efficacy of antimicrobial treated articles with claims for external effects. Env/JM/Mono(2008)27*	*2,3,4,5*	*This test is considered a phase 2 step 2 test.*	on going
prEN 13623	*Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity against Legionella pneumophila of chemical disinfectants for aqueous systems - Test method and requirements (phase 2, step 1)*	*2-5*		
prEN 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)*	*2-4*		
NEN/ISO 22196 2007	*Plastics- Measurement of antibacterial activity on plastics surfaces.*	*Treated articles ?*	*This International Standard specifies a method of evaluating the antibacterial activity of antibacterial-treated plastic products (including intermediate products). It is not intended to be used to evaluate the effects and propagation of bacteria on plastics without antibacterial treatments. ISO 846 [6] describes tests to evaluate the effects and propagation of bacteria on plastics, which are different...*	
NF T72-281 September	*Methods of airborne disinfection of surfaces. Determination of bactericidal, fungicidal and*	*1-5*	*The guideline is actually often used since a lot of products*	French norm on going

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
1986	*sporicidal activity.*		*have been designed for this type of application. The guideline outlines the conditions and the indications that should be given in the report.*	at CEN level
NF F19-601 (December 1991)	Railway Rolling stock – Additive product used in recirculation retention toilets	*2*	French standard related to chemical toilets for trains	
ASTM E2406-04	*Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations*	*2-4*		
AATCC Method 100,	*Assessment of Antibacterial finishes on Textile Materials,*		*This is a quantitative procedure for testing antibacterial activity of textiles.*	
EPA Pesticide Assessment Guidelines Subdivision G 91-51	*Germicidal spray products test*		*Test method for soft furnishings.*	
ISO 20743:2007	*Textiles -- Determination of antibacterial activity of antibacterial finished products*		*This International Standard specifies quantitative test methods to determine the antibacterial activity of antibacterial finished textile products including nonwovens. Based on the intended application and on the environment in which the textile product is to be used, the user can select the most suitable of the following three methods on determination of antibacterial activity: a) absorption method (an evaluation method in which test bacterial suspension is inoculated directly onto samples); b) transfer method (an evaluation method in which test bacteria are placed on an agar plate and transferred onto samples); c) printing method (an evaluation method in which test bacteria are placed on a filter and printed onto samples). The colony plate count method and the ATP (ATP = Adenosine Tri-phosphate) luminescence method are also specified for measuring the enumeration of bacteria.*	
ISO 20645:2004	*Textile fabrics -- Determination of antibacterial activity -- Agar diffusion plate test*		*This document specifies a method for the determination of the effect of antibacterial treatments applied to woven, knitted and*	

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Remarks
			other flat textiles. This method is applicable to testing hygienic finishes of hydrophilic, air-permeable materials or antibacterial products incorporated in the fibre. A minimum diffusion of the antibacterial treatment into the test agar is necessary with this procedure. This method is not suitable for testing textiles treated with antibacterial treatments that react with the agar.	
CEN ISO/TS 15883-5:2005 Annex F		*Biofilm*	*Includes a system to generate a standard biofilm.*	

9 REFERENCES

1 Regeling voor de toelating, het op de markt brengen en het gebruik van gewasbeschermingsmiddelen en biociden (Wet gewasbeschermingsmiddelen en biociden) (Plant protection products and biocides Act, Wgb 2006);
NL acts, decisions, orders, etc. can be obtained via http://wetten.overheid.nl/;

2 Regeling van de Minister van Landbouw, Natuur en Voedselkwaliteit van 26 september 2007, nr. TRCJZ/2007/3100, houdende nadere regels omtrent gewasbeschermingsmiddelen en biociden (Plant Protection Products and Biocides Regulations (RGB), published in the Government Gazette (Staatscourant) 188 of 28 September 2007 came into effect on 17 Oktober 2007;
including
Regeling van 20 oktober 2009 tot wijziging van de Regeling gewasbeschermingsmiddelen en biociden in verband met de aanwijzing van beoordelingsmethoden), published in the Government Gazette (Staatscourant) 16032 of 26 Oktober 2009 came into effect on 1 January 2010;
NL acts, decisions, orders, etc. can be obtained via http://wetten.overheid.nl/