



# agriculture, forestry & fisheries

---

Department:  
Agriculture, forestry & fisheries  
**REPUBLIC OF SOUTH AFRICA**

## **GUIDELINES FOR REGISTRATION OF SWIMMING POOL AND SPA POOL REMEDIES**

**Issued by the Registrar: Act No. 36 of 1947, Private Bag  
X343, Pretoria 0001**

**Republic of South Africa**

**Tel. (\*\*27 12) 319 7000 / Fax (\*\*27 12) 319 7179**

**2016**

## TABLE OF CONTENTS

1. INTRODUCTION .....	3
2. EFFICACY TRIAL REQUIREMENTS FOR REGISTRATION OF SWIMMING POOL AND SPA POOL REMEDIES .....	3
2.1 GENERAL CONSIDERATIONS.....	3
2.2 LABORATORY TESTING PHASE.....	4
2.3 FIELD TESTING IN A FULL-SIZE SWIMMING POOL OR SPA POOL.....	8
3. CHEMISTRY DATA REQUIREMENTS.....	10
4. REFERENCES .....	11

## 1. INTRODUCTION

Swimming pool and spa pool remedies are chemicals used to control micro-organisms in pool water. Before they are released into the marketplace they have to comply with certain criteria that relate to their safety, efficacy, trade and labelling and they must be registered under Act 36 of 1947, regardless of whether they are novel or traditional remedies with a long history of safe use. Registrants of the products are responsible for generating and providing sufficient data which proves that the product meets requirements for registration. The aim of this document is to outline data requirements for registration of swimming pool and spa pool remedies. In compiling this guideline document, the OECD Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants in Laboratory and Field Testing as well as the APVMA Guide for Demonstrating Efficacy of Pool and Spa Sanitisers were used for reference. This document must be read in conjunction with all other relevant guidelines related to pesticide registration requirements under Act No. 36 of 1947. Applicants are advised to seek for advice from the office of the Registrar of Act 36 of 1947 where necessary. This document is effective as from 1<sup>st</sup> August 2016.

## 2. EFFICACY TRIAL REQUIREMENTS FOR REGISTRATION OF SWIMMING POOL AND SPA POOL REMEDIES

### 2.1 GENERAL CONSIDERATIONS

In order to satisfy the efficacy criteria, the proposed new remedy product should be effective against the key pathogens in the major classes of human pathogenic microorganisms commonly found in swimming-pool and spa-pool water, namely bacteria, protozoa, algae and viruses. The new product should have equivalent efficacy to a registered generic remedy. Applicants are required to submit information from a combination of laboratory and field trials. Laboratory trials demonstrate efficacy against important human pathogens under a range of controlled conditions whilst field trials demonstrate that the product works as expected under actual use conditions. Both trial components are important in proving efficacy of the chemical.

Applicants are encouraged to read guidelines published by the office of the Registrar of Act 36 of 1947 (data requirements, adjuvant, chemical equivalence and DOH tox evaluation guidelines) before or while planning laboratory and field trials to ensure that the efficacy against the most important pathogens will be addressed, and that field tests are planned at appropriate locations and under appropriate conditions. It is advisable to submit trial protocols for approval by the Registrar prior to commencement of trials if the applicant is not clear about the methodology to be used. Results from other efficacy studies with other indicator organisms may be accepted, provided that additional scientific information and argument is provided to justify that those studies prove the product meets the efficacy criteria. A permit is required for the conduct of a field trial with an unregistered product because supply of an unregistered product without a permit is an offence under Act 36 of 1947.

Field data must be generated within South Africa. Data from overseas and local laboratories trials should be accompanied by an accreditation certificate (ISO17025/OECD GLP). An application to register swimming-pool or spa-pool remedy should address the following criteria:

- (i) An effective residual concentration of remedy should be maintained in the body of the pool to provide continuous control within the water at all times.
- (ii) The concentration of the residual remedy (or its principal components if there is more than one active constituent) should be capable of being measured using a field test kit or another simple method that can be properly managed by an average home pool owner.
- (iii) The remedy should be capable of supplementary dosing if measured levels are found to be below the recommended effective concentration.

- (iv) A known safety margin of efficacy should be established for normal operating concentrations.
- (v) For remedies containing more than one active constituent, applicants should identify the relative contributions of each principal active constituent to the overall efficacy of the product.
- (vi) If the remedy is to be used applied in combination with other products e.g. water conditioners/pH softeners/pH buffers, registrants should provide efficacy results which demonstrates that the two products are compatible and that their use together will not have any adverse effects on the performance of either product. The combination products/adjuvants must have been registered under Act 36 of 1947.

## **2.2 LABORATORY TESTING PHASE**

As a first step, a remedy should be shown to be effective under defined laboratory conditions against key indicator organisms within the major classes of pathogenic microorganisms associated with swimming and spa pools (see Table 1).

While there is no specific requirement for a parallel chlorine standard (control) to be incorporated into a laboratory test protocol, in testing a new pool or spa remedy, the performance characteristics of the remedy being tested must be essentially equivalent to recognised hypochlorous acid or hypochlorite antimicrobial efficacy characteristics. These established characteristics are detailed in Table 1.

If a parallel chlorine test is incorporated into the experimental protocol, the chlorine-testing methodology should follow established principles of controlling chlorine demand and verifying free chlorine concentration at the beginning and end of the exposure period. The Association of Official Analytical Chemists (AOAC) Official Method 965.13 can be used as a guide. A free chlorine starting concentration of one milligram per litre should be used (see also Target performance characteristics). As outlined in AOAC 965.13, a ratio of 199 to 1 for the chlorine test solution to the test organism suspension should provide a sufficient reserve of free chlorine during the test period. Free chlorine concentration should not drop below approximately 0.6 milligrams per litre by the end of the exposure period.

Satisfactory efficacy under laboratory conditions can be demonstrated by following the test design principles below. Design principles incorporated into laboratory testing and any associated validation results should be included in the final trial report.

### **2.2.1 Standards for testing**

Laboratory tests should be carried out by an ISO 17025/OECD GLP accredited laboratory.

### **2.2.2 Test conditions should simulate use conditions**

Tests should be carried out at 25–30 °C for swimming pools and 34–36 °C for spa pools and at a pH that is both consistent with good efficacy of the remedy being tested and acceptable for the comfort and safety of bathers. If parallel hypochlorite controls are incorporated into the testing protocol, these chlorine controls should be carried out at pH of 7.2 to 7.3.

During efficacy testing, no chemical with efficacious properties other than the test remedy (which may be a mixture of two or more active constituents) should be present in the water.

### **2.2.3 Establishing a safety margin**

The remedy needs to remain effective against pathogens at 50 per cent of its recommended operating concentration. This efficacy margin can be established by testing against the single species *Pseudomonas aeruginosa*, according to the performance characteristics indicated in Table 1. In relation to bather health, the remedy must have been independently demonstrated to be safe for bathers also at 2x the highest recommended concentration of the active constituent(s).

#### **2.2.4 Establishing relative contributions of active constituents**

All constituents that contribute to the efficacious properties of a product are included on the product label as 'active constituents'. For products that have more than one active constituent with different modes of action (for example, metal ions and accompanying oxidisers) the independent contributions of the active constituents to overall efficacy should be demonstrated in satisfying the efficacy criterion. For an example test protocol, see Table 2.

#### **2.2.5 Test organisms**

The test organisms used in any testing should be recognised, standard strains of the species and should be derived from a recognised culture collection. The reference identity number of the culture and its source should be included in the test report. The suggested test species are identified in Table 1.

#### **2.2.6 Contact times**

The test contact times evaluated for specific indicator organisms should be in keeping with the recommended performance criteria in Table 1. Where a product is shown to be slower acting than free chlorine, it may still be acceptable, provided the data demonstrates that the difference in activity is not significant and efficacy against key indicator organisms is equivalent to or better than comparable features of chlorine.

#### **2.2.7 Test volume to inoculum volume ratio**

The test volume should have the capacity to act as a sufficient reservoir to maintain the recommended concentration of active constituent(s) when the volume of test inoculum is added. The inoculum volume and its concentration of excipients should not overwhelm the test system in such a way that the recommended concentration of active constituent(s) is substantially altered.

A test volume to inoculum volume ratio of 199 to 1, as described in AOAC 965.13, is satisfactory in most cases where the disinfectant demand of the system has been measured and accounted for. Inoculum suspensions may need to be checked for solutes that could interfere with the remedy.

#### **2.2.8 Neutralisation of antimicrobial**

The test protocol should incorporate a neutralisation step for the active constituent(s). At the end of each contact test period, aliquots of the test mixture intended for survival counts should be added immediately to a neutralisation diluent. You should validate the effectiveness of the neutralisation with appropriate controls or a separate test protocol.

The neutralisation broth should not exert any toxicity or antimicrobial or antiviral effects against the test organisms. Details of the neutralisation steps, including results from validation tests and broth analysis, should be included in the trial report.

#### **2.2.9 Maintenance of active constituent(s) concentration**

The active constituent(s) should be measured at the beginning and end of the biocidal test period to confirm that the concentration of active constituent(s) has been maintained within the correct concentration range for the duration of the experiment, as would occur in a swimming or spa pool under normal use conditions.

If chlorine is used as a comparative control, you should also determine the concentration of free chlorine at the beginning and end of the test contact period. One method is described in AOAC 965.13.

### **2.2.10 Inoculum density**

The inoculum density of the test organism in the test mixture should be such that the appropriate kill factors presented in Table 1 can be measured. A microorganism density in the test mixture that is 100 times higher than the log reduction number (kill factor) being measured is usually practical. For example, with bacteria, a test organism count of  $10^6$  per millilitre in the test volume is suitable and of a density that will minimise inoculum effects.

### **2.2.11 Inoculum preparation**

Inoculum suspensions need to be in a carrier that will maintain the viability of the organisms, but one that does not contain solutes that interfere with the action of the remedy being tested.

In relation to virus suspensions, virus particles are often clustered and associated with cellular debris. Such clustering can protect some of the particles from exposure to the biocide being tested. Since the degree of aggregation and amount of debris cannot be precisely controlled from one test series to another, disaggregated and exposed virions may need to be tested in order to make valid comparisons. Therefore, you may need to treat virus suspensions prior to testing to ensure virions are disaggregated. A nominated method of purification/disaggregation should be confirmed with our office. Suitable methods for disaggregating adenovirus and rotavirus can be found in Thurston-Enriquez et al. (2003) and Vaughn et al. (1986), respectively.

### **2.2.12 Replicates**

The test protocol should incorporate three field and one laboratory trials for each set of conditions being evaluated for the product under test, in order to demonstrate that the results can be replicated. The recovery counts of the test organisms within each trial should also be performed at least in duplicate.

Appropriate controls should be incorporated into each trial to demonstrate the effect of the treatment compared with the absence of treatment.

### **2.2.13 Target performance characteristics**

Table 1 shows the performance characteristics of an effective remedy against the recommended test organisms. The performance characteristics of one milligram per litre of free chlorine (from hypochlorous acid or hypochlorite) have been demonstrated in the scientific literature to be equivalent to the performance characteristics shown in Table 1.

Table 1: Performance characteristics of effective sanitisers against the recommended test organisms of swimming pools and spa pools

Test organism	Number log <sub>10</sub> reductions to be achieved	Duration of exposure to test remedy (at normal concentration) in which reduction is to be achieved
<b>Bacteria</b>		
<i>Escherichia coli</i>	4	30 seconds
<i>Enterococcus faecium</i>	4	2 minutes
<i>Pseudomonas aeruginosa</i> (for spa pools only)	4	2 minutes
<i>Legionella pneumophila</i> (for spa pools only)	4	2 minutes
<i>Staphylococcus aureus</i>	4	2 minutes
<b>Viruses</b>		
Adenovirus (disaggregated) <sup>1</sup>	3	10 minutes
Rotavirus (disaggregated) <sup>1</sup>	3	2 minutes
<b>Protozoa</b>		
<i>Naegleria fowleri</i> (cysts)	4	30 minutes
<i>Giardia duodenalis</i> <sup>2</sup> (cysts) or <i>G. muris</i> <sup>3</sup> (cysts)	3	45 minutes

Notes:

1 Prior to the test exposure, virus suspensions need to be treated to disassociate aggregated clusters of virus particles.

2 Older synonyms in the literature for this species are *G. lamblia* and *G. intestinalis*.

3 The animal pathogen *G. muris* can be used as a surrogate for the human pathogen.

## 2.2.14 General comments

Results from other efficacy studies with other indicator organisms will be accepted as additional information.

## 2.2.15 Special instructions for testing silver- and copper-ion based remedies

Phosphate buffers should not be used in efficacy tests, since phosphate complexes with copper ions will interfere with test results.

Efficacy test periods should not be terminated by using chelating agents to sequester copper and silver ions because this could cause test results to be invalidated. Chelating agents are not specific enough for copper or silver and will also react with other metal ions. Removing calcium ions, for example, is known to interfere with the infectivity of some viruses (including rotavirus), and there is evidence that *Naegleria fowleri* is adversely affected by chelating agents. As an alternative, it is recommended that you use at least a 100-fold dilution method with appropriate culture medium to terminate disinfection test periods, and that you progress the sample to the plating and incubation stage as quickly as possible to further dilute the concentration of metal ions. Other options are the use of a fresh, rapid-flow gel exclusion

column for each sample of the longer test periods or centrifugation through sucrose cushions. Other scientifically valid procedures will also be considered.

Copper- and silver-ion-based remedies are necessarily used in conjunction with oxidisers, usually either chlorine or one or more of the peroxygen compounds. It is important to establish how much of the overall efficacy is contributed by the metal ions and how much by the oxidiser. In addition, it is important to demonstrate that the remedy is still effective at half its recommended operating concentration. This can be achieved by a series of experiments on *Pseudomonas aeruginosa* that test different ratios of the combined active constituents and different concentrations of the intended ratio of the active constituents. For example, if the proposed operating concentrations of the metal ions and oxidiser are M and O respectively, a suitable trial design is shown below in Table 2.

Table 2 : Suitable Trial designs for using different ratios when active constituents are a combination of metal ions and oxidiser

<b>Metal ion series</b>	<b>Oxidiser series</b>	<b>Efficacy threshold series</b>
Nil M with O	Nil O with M	N/A
0.2 M with O	0.2 O with M	0.2 of (M with O)*
0.4 M with O	0.4 O with M	0.4 of (M with O)*
0.6 M with O	0.6 O with M	0.6 of (M with O)*
0.8 M with O	0.8 O with M	0.8 of (M with O)*
M with O	O with M	N/A
Control (nil M and O)	Control (nil M and O)	Control (nil M and O)

N/A = not applicable

\* ie 0.2 or 0.4 etc. times the recommended operating concentrations of metal ions (M) and oxidiser (O)

For the trials suggested in Table 2, it may be necessary to complete a preliminary range-finding experiment to determine how many cells should be used for each test sample so that all are not killed and a reportable value is obtained. The reported value for each sample should be the log reduction in viable *Pseudomonas aeruginosa* cells after two minutes of exposure to the remedy.

When more than one type of metal ion is used in the system (for example, copper, silver and zinc), it is not necessary to test each metal ion separately. However, the mixture of metal ions in the intended ratio of the marketed product must be used. Similarly, if a mixture of oxidisers is formulated for the marketed product, the same mixture as intended for the marketed product must be used as the oxidiser in the tests.

## 2.3 FIELD TESTING IN A FULL-SIZED SWIMMING POOL OR SPA POOL

Field testing of swimming- and spa-pool remedies should be conducted after laboratory trials have been successfully completed. The aim of the field test is to demonstrate the efficacy of the swimming-pool or spa-pool remedy or disinfection process under actual use conditions. As no test microorganisms are added to the swimming pool water in the field testing phase samples should be taken at places microorganisms are likely to be found, i.e. swimming pool corners and (near) holes (air tubes etc.) because a biofilm could be present. For air holes a



swab could be taken to see the level of efficacy on the biofilm. Samples should be collected by a trained person (to avoid cross contamination) using appropriate sampling bottles (which may contain biocide neutralising agent).

It is critical that human beings are not exposed to potential microbial infection or chemical health risks during the field testing phase of any new product. It is essential therefore that a full size pool test of a new product is not undertaken until that product has at least passed human health and environmental safety criteria and has been clearly shown to be effective under laboratory conditions. A full-scale pool test can only proceed after performance in the laboratory efficacy testing phase has been accepted as adequate by the regulator and after the regulator has been satisfied that water containing the product at its recommended concentration is safe for human exposure during swimming and bathing.

A suitable test protocol of not less than three months' duration is to be used. The protocol should be designed to provide an accumulation of evidence that clearly shows compliance with these guidelines or OECD efficacy test guidelines. Pools to be used for these trials should have a significant and variable bather load to ensure that the efficacy of the product is adequately tested.

Because field studies such as these can be strongly affected by a pool's location and use pattern, we recommend that applicants must discuss the design of a field trial with us before committing to a particular test site and protocol. Table 3 provides guidance on effective remedy performance characteristics during field testing.

Table 3: Guidance on effective sanitizer performance characteristics during field testing

Test organisms	Test method	Maximum count allowable (colony forming units)
Heterotrophic colony count	ISO 6222:1999: Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium	100CFU/mL
Thermotolerant coliforms	ISO 9308-1: Detection and enumeration of E.Coli and coliforms – Part 2: Membrane filtration method	Nil CFU/100mL
<i>Pseudomonas aeruginosa</i>	ISO 16266: Detection and enumeration of <i>Pseudomonas aeruginosa</i> – Method by membrane filtration	Nil CFU/100mL

CFU/mL = colony forming units per millilitre

The following minimum methodology and features should be incorporated into the trial design and reported in an applicant's submission.

Features of the trial to be included and reported:

- pool design specifications—dimensions, volume and location (indoor or outdoor)
- water distribution and circulation pattern
- turnover rates of the pool(s) under test, and for spa pools, details of water dumping schedule and refill
- balance tank details

- method of dosing of the remedy (and if chlorine is part of the system, whether chlorine is stabilised or unstabilised)
- details of other chemicals used
- filtration, flocculation and backwashing details
- details of rainfall events (for outdoor pools)
- details of laboratories used
- methodology for all microorganism efficacy tests and key chemical assays
- appropriate safety data sheets for active constituents handled as concentrates.

Test protocol aspects to be included and reported:

- water sampling location(s) for microorganisms and chemicals, sample replication and transport methodology
- sampling design and strategy (note that the number of samples planned per nominated time period and the number for the overall study should be clearly stated)
- details of other relevant parameters at sampling (such as water temperature and clarity)
- daily bather loads throughout the test
- bather load for the one-hour period prior to sampling (note that at least 50 per cent of the total number of samples taken will need to be associated with operational bather loads). An operational bather load for this purpose is the number of bathers that would constitute 25–30 per cent of the instantaneous maximum bathing load, multiplied by 12 (as per the United Kingdom Pool Water Treatment Advisory Group). A guideline for determining maximum bather load is shown in Table 4.
- concentration of remedy at time of sampling
- measurement of pH at time of sampling
- measurement of reserve (total) alkalinity
- concentration of any other relevant chemical
- millivolt equivalence of disinfection agent if it is proposed to control the remedy using redox potential.

Table 4: Guideline for determining maximum bather load

Pool depth	Surface water area per person (m <sup>2</sup> )
Shallow water (less than 1 metre deep)	2
Standing-depth water (1–1.5 metres deep)	3
Deep water (more than 1.5 metres deep)	4

### 3. CHEMISTRY DATA REQUIREMENTS

A letter of supply from the manufacture or source of the active constituent with the detail of the active constituent or minimum purity %, the manufacturing process, physical and chemical characteristics, certificates of analysis (COAs) and declaration of composition must be provided with the application together with technical specifications of the active constituent detailing the minimum content and the maximum levels (% w/w, g/l or g/kg). The names used in the technical specification should be in accordance with IUPAC, ELINCS/EINECS and or CAS nomenclature.

A full formulation composition from the formulator of the product must be provided. This should provide details on the physical and chemical characteristics, chemical composition, concentration (% w/w, g/l or g/kg), purpose of all constituents in the formulated product. If the

formulation contains more than one active constituents, a complete formulation details must be provided.

Formulation storage stability studies should be provided and can be established by accelerated testing, ambient testing and cold stability testing. Samples should be analysed before (initial analysis at the start of the trial) and after testing using the same batch. Analysis of samples should be done on active constituents and form. The packaging should be based upon that in which the product is sold and the packaging used in the study should be assessed for interaction of the formulation.

Accelerated testing should be done at 54°C over a period of 2 weeks or 4 weeks at 50 °C; 6 weeks at 45 °C; 8 weeks at 40 °C; 12 weeks at 35 °C; 18 weeks at 30 °C). The tests of the above mentioned accelerated conditions are equivalent to 2 years. Ambient testing is used to demonstrate the storage stability of a formulation under “true” storage conditions usually over a period of 2 years, i.e. representative of its expected shelf life under normal use. The tests should be conducted at ambient temperature or, 20 °C, 25 °C or 30 °C dependent on the final area of use. For long-term studies, intermediate analysis (3 months intervals) should be carried out also.

Cold stability testing should be provided for liquid formulations and should be done at 0°C over a period of 7days. If a product is intended to be stored under refrigerated conditions and/or it is considered that the active substance or preparation may crystallize, or where phase separation could occur, a cold stability test should be carried out.

#### **4. REFERENCES:**

1. Guidelines published by the Organization of Economic Co-operation and Development (OECD) <http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf>
2. Guidelines published by the Australian Pesticides and Veterinary Medicines Authority (APVMA) <http://www.apvma.gov.au/node/1039>