Australian Government
Australian Pesticides and
Veterinary Medicines Authority

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# APVMA GUIDE FOR DEMONSTRATING EFFICACY OF POOL AND SPA SANITISERS

## 1. Introduction

For many years, disinfection of swimming pools and spa pools has relied mainly on chemical sanitisers based principally on chlorine and bromine. The efficacy of these traditional sanitisers is well established with regard to different kinds of pathogenic microorganisms. New types of chemical sanitisers however, which lack that established record, must be shown to be effective against pathogenic microorganisms under conditions found in swimming pools and spas before they can be approved for use.

This document is a guide setting out how applicants could demonstrate that a proposed new pool and spa sanitiser would satisfy the APVMA's efficacy criteria as stated below in section 2. While meeting the performance characteristics set out in sections 3 and 4 can be expected to satisfy the APVMA's efficacy requirements, the APVMA is prepared to consider alternative scientific information and argument aimed at satisfying the efficacy criteria.

Note that in addition to efficacy criteria, a new sanitiser must also meet the APVMA's safety criteria relating to human health and to the environment. For example, there should be no adverse impact on bathers or toxic effect from the sanitiser or its by-products that exceeds health standards as a result of either short term or extended immersion in water treated with the sanitiser. Information on toxicology data requirements and other of the APVMA's requirements can be found on its website at -
http://www.apvma.gov.au/MORAG_ag/vol_3/part_3_toxicology.html

## 2. Efficacy Criteria for Pool and Spa Sanitisers

Applicants must be able to establish that the proposed new sanitiser is 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 and viruses. As a general guide, applicants must be able to establish that the new product is equivalent in efficacy to registered sanitisers based on hypochlorous acid/hypochlorite against these classes of microorgansisms.

In addition to efficacy equivalent to hypochlorous acid/hypochlorite as demonstrated in laboratory and field tests (see Table 1 in Section 3 – 'Guide for Laboratory Testing Phase' and Section 4 – 'Guide for Field Testing Phase in a Full Size Swimming or Spa Pool'), a swimming pool or spa pool sanitiser or disinfectant process must have the following general performance features or properties.

- An effective residual concentration of sanitiser can be maintained in the body of the pool to provide continuous disinfection within the water at all times.
- The concentration of the residual sanitiser (or its principal components if there is more than one active constituent) is capable of being measured using a field test kit or other simple method that can be properly managed by an average home pool owner.
- The sanitiser is capable of supplementary dosing if measured levels are found to be below the recommended effective concentration.
- A known safety margin of efficacy can be established for normal operating concentrations.
- For sanitisers containing more than one active constituent, the relative contributions of each principal active constituent to the overall efficacy have been identified.
- The sanitiser has adequate algaecide properties of its own (demonstrated separately from this guide) or else is compatible with one or more registered algaecide products.

It is the responsibility of the applicant to prove through independent assessment that a disinfectant or disinfecting process can meet these criteria.

## 3. Guide for Laboratory Testing Phase

As a first step, a sanitiser or disinfecting process must 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 in Section 3(m) below).

In testing a new pool and spa sanitiser, there is no specific APVMA requirement for a parallel chlorine standard (control) to be incorporated into a test protocol. However, the performance characteristics of the sanitiser being tested need to be shown to be essentially equivalent in performance to recognized hypochlorous acid/hypochlorite antimicrobial efficacy characteristics. These established characteristics are detailed in Table 1 of Section 3(m) below.

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 AOAC Official Method 965.13[1] can be used as a guide. A free chlorine starting concentration of 1 mg/litre should be used as indicted in Table 1. As outlined in AOAC



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965.13, a ratio of 199:1 for the chlorine test solution to the test organism suspension should provide sufficient reserve free chlorine during the test period. Free chlorine concentration should not drop below approximately 0.7 mg/litre by the end of the exposure period.

Satisfactory efficacy under laboratory conditions can be demonstrated by following the test design principles below.

**a) Standards of Testing Laboratory Used**

- Tests, including preparation of materials and analysis of test samples, are to be carried out by a National Association of Testing Authorities (NATA) registered or similarly accredited laboratory having no affiliation with or commercial connection to the applicant. Assay methods for each type of test should be well established and reproducible by the host laboratory.

**b) Test Conditions Should Simulate Use Conditions**

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

- During disinfection testing, no chemical with disinfecting properties other than the test sanitiser (which may be a mixture of two or more active constituents) is to be present in the water.

**c) Establishing a Safety Margin**

- The sanitiser needs to remain effective against pathogens at 50% of its recommended operating concentration. This efficacy margin can be established sufficiently by testing against the single species *Pseudomonas aeruginosa* according to the performance characteristics indicated in Table 1 of Section 3(m).

- In relation to bather health, the sanitiser must have been independently demonstrated to be safe for bathers at two times the highest recommended concentration of the active(s). (Refer to the APVMA's toxicology data requirements at - http://www.apvma.gov.au/MORAG_ag/vol_3/part_3_toxicology.html)

**d) Establishing Relative Contributions of Active Components**

- For products with more than one claimed active constituent having different modes of action (for example, metal ions and accompanying oxidizers) the independent contributions of the principal components to overall efficacy need to be demonstrated. (Only formulation components shown to contribute to efficacy can be



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acknowledged as active ingredients on the product label.) For an example test protocol, see Table 2 in Section 3(o) - 'Special instructions for testing silver and copper ion based sanitisers'.

**e) Test Organisms**

- The test organisms used in any testing must be recognized, standard strains for the species and be derived from a recognized culture collection. The reference identity number of the culture and its source must be included within the test report. Suggested test species are identified below in Table 1 of Section 3(m).

**f) Contact times**

- The test contact times evaluated for specific indicator organisms should be in keeping with the recommended performance criteria in Table 1 - Section 3(m) of this guide. Where a product is shown to be slower acting than free chlorine, it may still be acceptable provided that the difference is not too great and that other features are equal to or better than comparable features of chlorine. Judgments will be made on a case-by-case basis.

**g) Test Volume to Inoculum Volume Ratio**

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

- A ratio of 199:1 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 sanitiser.

**h) Neutralisation of Antimicrobial**

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

- The neutralization broth must not exert any toxicity or antimicrobial or antiviral properties toward the test organisms.

**i) Maintenance of Active(s) Concentration**

- The actives must be measured at the beginning and at the end of the biocidal test period as confirmation that the concentration of actives has been maintained within



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the correct concentration range for the duration of the experiment as would occur for the actives in a swimming or spa pool under normal use conditions.

- If chlorine is utilized as a comparative control, the concentration of free chlorine must be determined at the beginning and end of the test contact period. A method is described in AOAC 965.13.

**j) 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 of Section 3(m) 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 mL in the test volume is suitable and of such density as to minimize inoculum effects.

**k) Inoculum Preparation**

- Inoculum suspensions need to be in a carrier that will maintain viability of the organisms but one that does not contain solutes that interfere with the action of the sanitiser 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, exposed virions need to be tested in order to make valid comparisons. Therefore virus suspensions need to be treated prior to testing to ensure virions are disaggregated. A nominated method of purification/disaggregation should be confirmed with the APVMA. A suitable method for adenovirus can be found in Thurston-Enriquez et al[2]. A method for rotavirus can be found in Vaughn et al[3].

**l) Replicates**

- The test protocol shall incorporate at least duplicate trials for each set of conditions being evaluated for the product under test. The recovery counts of the test organisms within each trial should be performed at least in duplicate.

- Appropriate controls must be incorporated into each trial.

**m) Target Performance Characteristics**

- The performance characteristics of an effective sanitiser against the recommended test organisms are shown below in Table 1. Note that the performance characteristics of 1 mg/litre of free chlorine (from hypochlorous acid/hypochlorite) have been



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demonstrated in the scientific literature to be equivalent to the performance characteristics shown in Table 1.

**Table 1**

| **Test Organism** | **Number of $log_{10}$ reductions to be achieved** | **Time of exposure to test sanitiser at normal concentration during which reduction is to be achieved** |
|---|---|---|
| **Bacteria** | | |
| **– (for swimming & spa pools)** | | |
| *Escherichia coli* | 4 | 30 seconds |
| *Enterococcus faecium* | 4 | 2 minutes |
| **– (and for spa pools only)** | | |
| *Pseudomonas aeruginosa* | 4 | 2 minutes |
| *Legionella pneumophila* | 4 | 2 minutes |
| **Viruses – (swimming & spa)** | | |
| Adenovirus (disaggregated)* | 3 | 10 minutes |
| Rotavirus (disaggregated)* | 3 | 2 minutes |
| **Protozoa – (swimming & spa)** | | |
| *Naegleria fowleri* - (cysts) | 4 | 30 minutes |
| *Giardia duodenalis‡ or muris†* - (cysts) | 3 | 45 minutes |

* Prior to the test exposure, virus suspensions need to be treated to disassociate aggregated clusters of virus particles. Refer to section 3(k) above.
‡ Older synonyms in the literature for this species are *G. lamblia* and *G. intestinalis.*
† The animal pathogen *Giardia muris* can be used as a surrogate for the human pathogen.

**n) General Comments**

- Results from other efficacy studies with other indicator organisms may be accepted by the APVMA provided that additional scientific information and argument can satisfy the APVMA that those studies prove the product meets the efficacy criteria in section 2.

- Note that a fee will apply for the evaluation of the laboratory test phase by the APVMA. (Contact the APVMA for more information.)

**o) Special instructions for testing silver and copper ion based sanitisers**

- Phosphate buffers should not be used in disinfection tests since phosphate complexes with copper ions and would interfere with test results.

- Disinfection test periods should not be terminated by using chelating agents to sequester copper and silver ions because test results could be invalidated. Chelating agents are not sufficiently specific for copper or silver and would react with other metal ions as well. Removal of calcium ions, for example, is known to interfere with



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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 at least a 100 fold dilution method with appropriate culture medium be used to terminate disinfection test periods and that the sample be progressed as quickly as possible to the plating and incubation stage to further dilute the concentration of metal ions. Additional options might be 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 would also be considered.

- Copper and silver ion based sanitisers are necessarily used in conjunction with oxidizers, usually either chlorine or one or more of the peroxygen compounds. It is necessary to establish how much of the overall efficacy is contributed by the metal ions and how much by the oxidizer. In addition, it is necessary to establish that the sanitiser is still effective at half its recommended operating concentration. These questions can be answered to the APVMA's satisfaction 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 oxidizer are M and O respectively, a suitable trial design is shown below in Table 2.

**Table 2**

| **Metal Ion Series** | **Oxidizer series** | | **Efficacy Threshold Series** |
|---|---|---|---|
| Nil M with O | Nil O with M | | 0.4 of [M with O]* |
| 0.2 M with O | 0.2 O with M | | 0.5 of [M with O]* |
| 0.4 M with O | 0.4 O with M | | 0.6 of [M with O]* |
| 0.6 M with O | 0.6 O with M | | – |
| 0.8 M with O | 0.8 O with M | | – |
| M with O | O with M | | – |
| Control (Nil M & O) | Control (Nil M & O) | | Control (Nil M & O) |

* i.e. 0.2 or 0.4 etc. times the recommended operating concentrations of metal ions (M) and oxidizer (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 2 minutes of exposure to the sanitiser.

- Note that 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. In the same way, if a mixture of oxidizers is formulated or



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recommended for the final product, the same mixture as intended for the marketed product must be used as the "oxidizer" in the tests.

## 4. Guide for Field Testing Phase in a Full Size Swimming or Spa Pool

After performance in the laboratory efficacy testing phase has been accepted as adequate by the APVMA and after the APVMA has been satisfied that water containing the sanitiser at its recommended concentration is safe for human exposure during swimming and bathing, the proposed new sanitiser needs to be tested in a field situation in a full size swimming pool (or spa pool if applying to be registered for spa pool sanitation) that has a significant bather load. A busy public pool and/or spa are preferred for these field tests.

The trials should be conducted by an independent agency accredited by the Joint Accreditation System of Australia and New Zealand (JAS-ANZ) or equivalent organization with which JAS-ANZ has a memorandum of understanding. Results should be analyzed and reported without intervention by the applicant.

The aim of the field test is to demonstrate the efficacy of the swimming pool or spa pool sanitiser or disinfection process under actual use conditions. The applicant should design a suitable test protocol of not less than six months duration on the type of pool/spa in which the sanitiser or disinfecting process is to be used. The protocol should be designed to provide an accumulation of evidence that clearly shows compliance with relevant guidelines for control of swimming pool and spa pathogenic microorganisms under field conditions.

Because field studies such as these can be strongly affected by a pool's location and use pattern, it is recommended that the applicant discuss the design of a field trial with the APVMA before committing to a particular test site and protocol.

See Table 3 below for guidance on effective sanitiser performance characteristics during field testing.

**Table 3**

| **Test Organisms** | **Test Method** | **Maximum Count Allowable** |
|---|---|---|
| Heterotrophic Colony Count | Pour plate method. Incubation for 48 hours at 35ºC following Australian Standard Method AS4276.3.1 – 1995 | 100 Colony Forming Units (CFU) per mL |
| Thermotolerant coliforms | Australian Standard Method AS 4276.6 – 1995 (MPN Method) or AS 4276.7 – 1995 (Membrane Filtration Method) | Nil per 100 mL |



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| | | |
|---|---|---|
| *Pseudomonas aeruginosa* | Australian Standard Method AS 4276.12 – 1995 (MPN Method) or AS 4276.13 – 1995 (Membrane Filtration Method) | Nil per 100 mL |

The following minimum methodology and features should be incorporated into the trial design and should be found to be satisfactory by the APVMA prior to commencement of the trial.

Note that fees will apply to approval of the test protocol and to issuance of a permit for the field trial. (Contact the APVMA for more information.)

**a) Features of the Trial to be included**

- 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 sanitiser (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 Material Safety Data Sheets for active constituents handled as concentrates

**b) Test Protocol aspects to be included**

- 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 should be recorded throughout the test
- bather load for the one hour period prior to sampling – note that at least 50% of the total number of samples taken will need to be associated with significant bather loads.

  A “significant bather load” for this purpose is the number of bathers that would constitute 25 to 30% of the instantaneous maximum bathing load according to a



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guideline from the UK Pool Water Treatment Advisory Group (PWTAG)[4]. This part of the guideline for determining maximum bather load can be summarized as follows.

| Pool Depth | Pool Surface Area |
|---|---|
| Shallow water (under 1m depth) | 1 bather per 2.2m$^2$ |
| Standing depth water (1 – 1.5m depth) | 1 bather per 2.7m$^2$ |
| Deep water (over 1.5m depth) | 1 bather per 4m$^2$ |

- concentration of sanitiser 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 sanitiser using redox potential

## REFERENCES

1. *Official Methods of Analysis of AOAC INTERNATIONAL* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **965.13**.

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3. Vaughn, J. M., Y. Chen, and M. Z. Thomas. 1986. Inactivation of human and simian rotaviruses by chlorine. Appl. Environ. Microbiol. **51:**391-394.

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