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GUIDANCE DOCUMENT FOR DEMONSTRATING EFFICACY OF POOL AND SPA DISINFECTANTS IN LABORATORY AND FIELD TESTING

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FOREWORD

This Guidance Document for demonstrating efficacy of pool and spa disinfectants in laboratory and field *testing* has been developed under the auspices of the Task Force on Biocides (TFB).

A first draft document was developed by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and discussed at a TFB meeting in 2008. It was further reviewed and revised; there were three commenting rounds through the TFB and the Working Group of National Co-ordinators of the Test Guidelines Programme (WNT) between 2009 and 2011. The draft document was approved at the 24th WNT Meeting in April 2012. The Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 5 July, 2012.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

GUIDANCE DOCUMENT FOR DEMONSTRATING EFFICACY OF POOL AND SPA DISINFECTANTS IN LABORATORY AND FIELD TESTING

INTRODUCTION

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

2. This Guidance Document describes how applicants could demonstrate that a proposed new pool and spa disinfectant would satisfy the regulator's efficacy criteria as stated below in section 3. While meeting the performance characteristics set out in sections 4 and 5 can be expected to satisfy the regulator's efficacy requirements, the regulator may choose to consider alternative scientific information and argument aimed at satisfying the efficacy criteria.

3. Applicants should note that as a prerequisite to demonstrating efficacy criteria, a new disinfectant should also meet the regulator's safety criteria relating to human health and to the environment. For example, valid scientific evidence should show that there is no adverse health impact on bathers or toxic effect from the disinfectant or its by-products that exceeds health standards as a result of either short term or extended immersion in water treated with the disinfectant. Information on toxicology data requirements and environmental safety data requirements should be sourced from the relevant member country.

IMPORTANT INFORMATION ABOUT TESTING

4. Before any biocide efficacy testing is conducted involving exposure of human volunteers, the substances should have undergone risk assessment (see paragraphs 15-22).

5. When in-use evaluation is required, 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 biocide product, taking due consideration of exposure from other sources. It is essential therefore that a full size field test of a new biocidal substance is not undertaken until that substance has at least passed human health and environmental safety criteria and has been clearly shown to be an effective biocide under laboratory conditions. A full-scale field 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 using the biocidal substance at its recommended concentration is safe for human exposure. Guidance by an appropriate ethical committee may be required by the relevant member country. In any case, a field test should be performed according to the proposed label instructions (intended use) of the products.

Resistance Issues

6. This Guidance Document is designed to assess the efficacy of a test substance under specified conditions. The Guidance is not intended to address the possible development of resistance. It is recommended that the possible development of resistance be considered in the overall assessment of the substance.

EFFICACY CRITERIA FOR POOL AND SPA DISINFECTANTS

7. Applicants should be able to establish that the proposed new disinfectant is effective against suitable indicator species of pathogens in the major classes (bacteria, protozoa and viruses) of human pathogenic microorganisms commonly found in swimming pool and spa pool water. As a general guide, applicants should be able to establish that the new product is equivalent in efficacy to the performance of hypochlorous acid/hypochlorite against these classes of microorganisms.

8. In addition to efficacy equivalent to hypochlorous acid/hypochlorite as demonstrated in laboratory and field tests (see Table 1 in section on "*Guide for Laboratory Testing Phase*"), a swimming pool or spa pool disinfectant or disinfectant process should have the following general performance features or properties.

- An effective residual concentration of disinfectant can be maintained in the body of the pool to provide continuous disinfection within the water at all times. An automatic dosing system for the disinfectant is required for public baths.
- Efficacy can be maintained in a pH range consistent with bather safety¹ and comfort and in the presence of ion and other solute concentrations commonly found in pool water where appropriate water quality maintenance is practiced.
- A practical method for measuring the concentration of the disinfectant should be available. If the disinfectant is intended to be used in home pools, the concentration of the residual disinfectant (or its principal components if there is more than one active constituent) should be 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 disinfectant should be capable of supplementary dosing if measured concentrations are found to be below the recommended effective concentration or if accidental microbiological contamination requires remedial treatment.
- A known safety margin of efficacy can be established for normal operating concentrations.
- For disinfectants containing more than one active constituent, the relative contributions of each principal active constituent to the overall efficacy should have been identified.
- The disinfectant has adequate algaecide properties of its own (demonstrated separately from this guide) or else is compatible with one or more registered algaecide products.

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

¹ It is difficult to suggest a pH range for the purpose of this guidance. On the one hand, the recommended safe pH range of 7.2 - 7.8 for maintenance of swimming pools is not optimal for testing disinfectant efficacy, as it has been set to be compatible with the disinfection efficacy of hypochlorous acid so that enough free chlorine is present in solution. On the other hand, drinking water guidelines cite a safe range of pH as between 6.5 and 8.5. In addition, human health data related to the definition of a safe pH range should be taken into account as well.

GUIDE FOR LABORATORY TESTING PHASE

10. As a first step, a disinfectant or disinfecting process 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. Testing 'surrogate' micro-organisms is common and acceptable for laboratory testing phase. In any case, the competent regulatory authority should be consulted prior to conducting research with a surrogate. Table 1 in Section 4(m) below provides a set of performance characteristics of hypochlorous acid/hypochlorite; meeting these performance characteristics within the requirements set out below will satisfy the laboratory test phase.

11. Batch tests are most often used in test protocols because they are easy to perform in laboratories.²

12. There is no specific regulatory requirement for an additional parallel chlorine control to be incorporated into a test protocol. However, carrying out a parallel chlorine control will ensure that the test design and test strains are working properly in the laboratory.

13. If a parallel chlorine test is incorporated into the experimental protocol, the chlorine testing methodology should follow established principles of controlling free 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 indicated in Table 1. As outlined in AOAC 965.13 (1), 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 0.6 mg/litre by the end of the exposure period.

14. To demonstrate efficacy under laboratory conditions, applicants should follow the test design principles set out below.

a) Standards of Testing Laboratory Used

• Tests, including preparation of materials and analysis of test samples, are to be carried out by a nationally accredited laboratory that has 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 25° to 30°C for swimming pools and 34 to 36°C for spa pools and at a pH that is consistent both with good efficacy of the chemical disinfectant being tested and acceptable for the comfort and safety of bathers (see footnote 1). 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.
- Simulated pool water should be used that reflects typical pool source water and good pool water maintenance practices and compatible with pool equipment (*e.g.* not acidic as it corrodes metal). In this way, the lowest effective concentration identified for the disinfectant will be compatible with real use conditions. Each member country may choose to specify test water quality characteristics according to prevailing local conditions.

² Tests can also be carried out in flow-through mode design experiments which provide a better control of the test conditions (6).

- During disinfection testing, no chemical with disinfecting properties other than the test disinfectant (which may be a mixture of two or more active constituents) is to be present in the water.
- Bather load, i.e. an artificial organic content consistent with a well-maintained pool. It is recommended to perform the test in the presence of interfering substances *e.g.* 0.3 g/l of bovine serum albumin that is representative of 'clean conditions' (7)

c) Establishing a Safety Margin

- The disinfectant should remain effective against pathogens at 50% of its recommended operating concentration to accommodate inevitable lapses of proper user maintenance, dosing errors or occasional failure of automatic dosing systems. This efficacy margin can be established sufficiently by testing against the single species *Pseudomonas aeruginosa* according to the performance characteristics indicated in Table 1 in this section under point (m).
- In relation to bather health, the toxicological risk assessment should also take into account reasonable foreseeable dosing errors. This should be comparable with existing chlorine/ bromine based disinfection techniques, where excess dosing of two times the highest recommended operating concentration of the disinfectant would be unlikely to result in adverse health effects.

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 acknowledged as active ingredients on the product label). An example test protocol is given in Table 2 in this section under point (o).

e) Test Organisms

• The test organisms used in any testing should 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 should be included within the test report. Preferred test species are identified in Table 1 in this section under point (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 in this section under point (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 by the relevant regulator.

g) 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(s) when the volume of test inoculum is added. The

inoculum volume and its solutes should not overwhelm the test system such that the recommended concentration of the test disinfectant is substantially altered.

• A ratio of 199:1 as described in AOAC 965.13 (1) 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 disinfectant.

h) Neutralisation of Antimicrobial

- The protocol should 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 should be added immediately to a neutralization diluent. The effectiveness of the neutralization should be validated with appropriate controls or a separate test protocol.
- The neutralization broth should not exert any toxicity or antimicrobial or antiviral properties toward the test organisms.

i) Maintenance of Active(s) Concentration

- The disinfectant concentration should be measured at the beginning and at the end of the biocidal test period at least for times of exposure longer than one minute and as far as possible for shorter exposure times (30-60 seconds) as confirmation that the concentration of actives has been maintained within 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 should be determined at the beginning and end of the test contact period. A method is described in AOAC 965.13 (1).

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 in this section under point (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 10⁶ 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 disinfectant 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 adequate exposure to the disinfectant being tested. Since the degree of aggregation and amount of debris is variable and 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 regulator. 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). Also, to limit virus clustering and retain small aggregates, a filtration method $(0.2 \ \mu\text{m})$ may be used.

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 without product should be incorporated into each trial, *e.g.* at least three for each set of conditions.

m) Target Performance Characteristics

• The performance characteristics of an effective disinfectant 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 demonstrated in the scientific literature to be equivalent to the performance characteristics shown in Table 1. The reference value of 1 mg/litre of free chlorine is used for this table to be consistent with references in the scientific literature. It is understood that some member countries recommend a lower concentration of free chlorine for normal pool operation.

Table 1: Target performance characteristics of pool & spa disinfectants against recommended bacteria, virus and protozoa (laboratory testing phase)

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

- a Among virus, *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 need to be treated to disassociate aggregated clusters of virus particles. Refer to section 4(k) above.
- 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 other terms sometimes used in the literature for this species are *Gardia 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.

n) General Comments

• Results from efficacy studies with other indicator organisms might be accepted by the regulator provided that additional scientific information and argument can satisfy the regulator that those studies prove the product meets the efficacy criteria (see paragraphs 7-9).

o) Special instructions for testing silver and copper ion based disinfectants

- Buffers should be chosen in order to avoid forming complexes with copper ions. For this reason phosphate and Tris buffers are not convenient.
- 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 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 alternatively centrifugation through sucrose cushions. Other scientifically valid procedures would also be considered.
- Copper and silver ion based disinfectants are necessarily used in conjunction with oxidizers, usually either chlorine or one or more of the peroxygen compounds. It is usually required 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 disinfectant is still effective at half its recommended operating concentration. These questions can be answered to the regulator'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 metal ions and oxidizer are symbolised as M and O respectively, a suitable trial design is shown below in 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)

Table 2.	Trial	design	for testing	metal-based	disinfectants	in the	presence of oxydizers
1 and 2.	11141	ucsign	ioi itsiing	metai-paseu	uisinicciants	in the	presence or oxyuizers

* 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 log₁₀ reduction in viable *Pseudomonas aeruginosa* cells after 30 seconds of exposure to the disinfectant.
- 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 should be used. In the same way, if a mixture of oxidizers is formulated or recommended for the final product, the same mixture as intended for the marketed product should be used as the "oxidizer" in the tests.

GUIDE FOR FIELD TESTING PHASE IN A FULL-SIZE SWIMMING OR SPA POOL

15. Before being approved by a regulator, the proposed new disinfectant 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 disinfection) that has a significant bather load. A busy public pool and/or spa are preferred for these field tests. A teaching pool may be the best choice for achieving a sufficiently high bather load during the test. Note: the meaning of "full-size swimming pool" is not prescribed exactly but is intended to mean one in the size range of typical public swimming pools.

16. 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 effect of the disinfectant 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).

17. Please note information under section on "*Important information about testing*" (Paragraphs 4-6) of this Guidance Document. 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 biocide product. It is essential therefore that a full size pool test of a new disinfectant is not undertaken until that disinfectant has at least passed human health and environmental safety criteria and has been clearly shown to be an effective disinfectant under laboratory conditions as outlined in paragraphs 10-14. 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 disinfectant at its

recommended concentration is safe for human exposure during swimming and bathing. Guidance by an appropriate ethical committee may be required by the relevant member country.

18. The full-scale trials should be conducted by an independent agency accredited by a recognised accrediting authority in the member country.

19. The aim of the field test is to demonstrate the efficacy of the swimming pool or spa pool disinfectant or disinfection process under actual use conditions. The applicant should design a suitable test protocol of not less than three months duration on the type of pool/spa in which the disinfectant 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.

20. 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 regulator before committing to a particular test site and protocol. Some member countries choose to regulate normal use concentration to the lowest feasible effective concentration (rather than the 2x minimum effective concentration established in paragraph 14 under point (c) as a way of keeping exposure to disinfectants and disinfection by-products as low as possible. For these countries, safeguarding the bather is not only achieved by the disinfectant but by sophisticated bathing water treatment as well. Consequently, for these countries it is essential that the field trial besides pool design, water quality and disinfectant concentration has to take into account a well functioning sophisticated treatment. The treatment's efficacy in removing contaminants has to be shown as well under challenging conditions.

21. Table 3 provides guidance on effective disinfectant performance characteristics against bacteria during field testing.

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	5 8
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 <i>Pseudomonas aeruginosa</i> – Method by membrane filtration	Not detectable in 100 mL

Table 3: Target performance characteristics of pool & spa disinfectants against bacteria (field testing phase)

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

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 disinfectant (and if chlorine is part of the system, whether chlorine is stabilised or not stabilised);
- 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; and
- 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;

<u>Note</u>: 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 guideline from the UK Pool Water Treatment Advisory Group (4); the German DIN 19643 recommends higher bather load $(5)^3$. This part of the guidance for determining maximum bather load can be summarized as shown in Table 4.

Table 4^{4 5}: Determination of maximum bather load

³ The German DIN 19643 recommends a bather load value >75% in order to take into account the fact that an excess of the instantaneous maximum bathing load (>100%) in outdoor swimming pools is common on hot summer days.

⁴ For calculating the maximum bathing load the German DIN uses 2.7 m² per bather at a water depth up to 1.35 m and 4.5 m² at depths >1.35 m. These values have been generated by measurements in experimental set-ups.

⁵ For calculating a maximum bathing load from the given values of "Pool Surface Area" it can be mentioned the related "frequency (number of persons per hour, e.g. 1.0 according DIN 19643).

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

- concentration of disinfectant at time of sampling;
- measurement of pH at time of sampling;
- measurement of reserve (total) alkalinity;
- concentration of any other relevant chemical; and
- millivolt equivalence of disinfection agent if it is proposed to control the disinfectant using redox potential.

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