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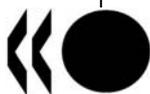
English - Or. English

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**GUIDANCE DOCUMENT ON THE EVALUATION OF THE EFFICACY OF ANTIMICROBIAL
TREATED ARTICLES WITH CLAIMS FOR EXTERNAL EFFECTS**

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OECD Environment, Health and Safety Publications

Series on Biocides

No. 1

**GUIDANCE DOCUMENT ON THE EVALUATION
OF THE EFFICACY OF ANTIMICROBIAL
TREATED ARTICLES WITH CLAIMS FOR
EXTERNAL EFFECTS**

IOMC

**INTER-ORGANISATION PROGRAMME FOR THE
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Paris 2008**

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The Pesticide Programme was created in 1992 within the OECD's Environmental Health and Safety Division to help OECD countries:

- harmonise their pesticide review procedures,
- share the work of evaluating pesticides, and
- reduce risks associated with pesticide use.

The Pesticide Programme is directed by the Working Group on Pesticides, composed primarily of delegates from OECD Member countries, but also including representatives from the European Commission and other international organisations (*e.g.* United Nations Food and Agriculture Organization, United Nations Environment Programme, World Health Organization, Council of Europe), and observers from the pesticide industry and public interest organisations (NGOs).

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This publication was produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC). It was approved for derestriction by the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, the governing body of the Environment, Health and Safety Division.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organization. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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PREAMBLE

Efficacy of the biocide incorporated in a treated article against target organisms in the intended use must be demonstrated as one component of an application dossier. A complete dossier also includes a toxicological profile for humans including dermal effects. It should be noted that treated articles contain biocide formulations designed for incorporation and already authorized by competent authorities for uses in specific product types. Those authorizations mean a full dossier on the biocide formulation has been evaluated for hazard and risk. Governments also have authority to take all actions necessary to ensure that risk of resistance is minimised before granting authorization for treated articles and chemical biocides alike.

Any efficacy testing for the subsequent treated article dossier would be done with that background knowledge and therefore minimize potential for unanticipated effects.

All test methods should be reproducible, and have to show that both the methodical procedure and the reagents used are not influencing the results. Therefore, beside the test agent, also a negative and/or positive control should be run.

SCOPE

This guidance document focuses on microbiological efficacy testing only. It covers efficacy testing of articles treated with antimicrobials in the manufacturing process with the intention of the achieving an external effect. Also included are articles which have been in some way modified during service so as to exert an antimicrobial effect.

Target organism in this document refers only to the range of bacteria for which the referenced test methods are suitable.

INTRODUCTION

The development of harmonized data requirements for biocides is a goal of the Organization for Economic Cooperation and Development (OECD) Task Force on Biocides (TFB). In April 2002, the US Environmental Protection Agency (EPA) and the OECD Steering Committee under the TFB sponsored an Efficacy Workshop on Certain Antimicrobial Biocides in Washington, DC to which representatives from member countries, academia and industry were invited. The goal of the workshop was to set a foundation for harmonizing efficacy test methods and selecting two as priority projects for development into OECD Harmonized Methods by a Steering Committee under the TFB. Standardizing test methods makes it easier for companies to submit registration applications to many countries and allows the regulatory agencies to benefit from each other's reviews.

One of the topics selected was "treated articles" (see definitions Appendix 1) which can be distinguished into two categories; 1) articles impregnated with a biocide, usually as a preservative against bio-deterioration, and 2) those impregnated with a biocide to achieve an 'external' effect against organisms that are not harmful to the article itself, e.g. effects against human pathogens. Recent technological product innovations can have the potential for ongoing pathogen control on the surface of the treated article. The articles may be designed to address a specific use setting with high risk for pathogen transmission (e.g. long term care facilities for the chronically ill, food service/processing common areas) where limiting the growth of pathogens may reduce the risk of infection and thus contribute to an overall hygiene program. As a result, many could be represented as having a health benefit to the user. As

technology advances, it may be possible to have a continuously disinfecting surface; certain research is in progress. It is therefore necessary to agree on methods to measure such effects in a quantitative way.

The present state of the regulations in the US EPA consider treated articles as pesticides. According to the regulation in 40CFR152.25(a), treated articles are exempt from requiring registration as long as the claims are restricted to materials preservation, and the incorporated biocide formulation is registered by EPA for that use. Claims to control pathogens require the article be registered as a pesticide. Similarly, the European Biocidal Products Directive (BPD) 98/8/EC regulates both indirectly through the authorisation of the biocide formulation incorporated into the article and requires authorization of treated articles with claims for external effects. Recognizing that there are no established efficacy methods or performance standards for government regulation of such health claim related, external effects, the Treated Articles Work Group which considered the situation during the above mentioned workshop made the following recommendations to the OECD and its member countries:

- OECD member states should use agreed upon terms and harmonize the terms and label claims. More specifically, the term "antibacterial" becomes a subdivision (or set) of "antimicrobial".
- Acknowledgement that treated materials may be part of overall hygienic practices rather than substitutes for sanitizers, disinfectants & sterilants. Accordingly, different performance standards are necessary for showing a benefit for treated materials. Member states may harmonize performance standards in relation to claims.
- That antimicrobial claims for treated materials must be supported by scientifically sound quantitative efficacy data.
- A tiered approach to testing is necessary in order to substantiate the range of efficacy claims for treated materials
- The methods to be used in tier 1 testing must include critical parameters identified by this group as appropriate.
- For a tiered testing approach, the critical parameters identified for tier 1 testing should be adapted for subsequent testing.

It was considered that a tiered testing system would consist of a) Tier 1 - basic test, proof of principle b) Tier 2 - laboratory simulated tests to support specific applications and label claims and c) Tier 3 - field studies (in some cases field studies may not be possible and a rigorously argued case may suffice).

A steering committee was formed under the TFB to lead the harmonization efforts. The OECD Efficacy Steering Committee was formed in 2003 from the Workshop Participants in the two antimicrobial projects selected (Hard Surface Disinfectants and Treated Articles). In 2004 a contract was placed to survey member countries and industries for existing test methods and performance standards. From the identified methods, the final report was to recommend the best example method(s) for ongoing development into possible OECD Guideline Test Methods. The final report recommendation was for a tiered testing approach using methods "typified by Japanese Industry Standard (JIS) Z 2801 (non-porous articles)[Figure 1] and American Association of Textile Chemists and Colorists (AATCC) Method 100¹ (absorbent porous articles) [Figure 2]. The tiered test approach would add test conditions to simulate proposed use conditions as appropriate to substantiate the intended claim(s).

The report specifies that other methods may be suitable as long as they are similarly scientifically sound and quantitative. The realm of treated articles is broad, and there may be matrices or target

organisms (filamentous fungi or algae, for example) for which the 2 selected methods may not be suitable. This document will provide guidance on both how such methods may be altered to simulate use conditions which should be applicable to any equally sound methods and how to approach the generation on data intended to support a label-claim. The present focus is on bacteriological efficacy methods for porous and non-porous articles which at this time are the most prevalent in food contact or health care settings where there is a need to control pathogenic organisms.

Initial Considerations

Articles treated with biocides which are intended to have external effects present situations and use conditions unlike traditional biocides. In general, the biocide concentration on the surface must be sufficiently high at all times to maintain the efficacy over long periods of time in widely varying environments, depending on the intended use for the article. As with any developing field, there is a need to establish different ways to evaluate performance and describe the biocidal function in a way that is clearly understood and not misleading to the user. This guidance document is intended to establish a basis on which regulatory decisions regarding efficacy can be made.

Key to that basis is the proposition that efficacy be evaluated under the proposed use conditions taking regard of the claim being made (or implied). In order to function, the biocide must come in contact with the target organism and be sufficiently efficacious during the available contact time. Biocide migration from within a matrix can vary with conditions such as temperature, pH and humidity. It is also important to know when the biocide level has dropped below an efficacious level or is no longer migrating from the substrate. Testing under simulated use conditions will more accurately depict the biocide functionality. Repeating the tests over time, much like a shelf life stability study will predict the useful life of the biocide and/or article when used as directed and with normal maintenance (e.g. cleaning).

During the 2002 Antimicrobial Efficacy Workshop, critical parameters were identified for treated article test methods (Table 1). It can be seen that these fall into several categories and a number of these parameters can be considered fundamental. For example, if data is used to support the claim that a treated article prevents the growth of bacteria on its surface, it is obviously essential to demonstrate that bacteria would grow on an equivalent but unmodified substrate under equivalent conditions. The principle of the appropriate control is a key feature. Similarly, it is important that the physical/chemical conditions presented to the test population should be suitable to support the growth/survival of that population providing that is relevant to the end use. The test should be capable of accommodating species that are relevant to the claim being made and the data generated should be sufficient to support the claim being made in a statistically valid manner.

Critical Test Method Parameters

Category	Parameter	Impact
Test Sample	Relevant Control / Standard	Validity of claim / need for effect
	Preparation <i>e.g.</i> Sterilisation, cleaning, ageing	Interference
	Size, weight, shape, surface texture	Interaction of inoculum with surface
	Hydrophobicity / absorbency / stability	
	Number of replicates samples	Measurement of effect
Inoculum	Range of test organisms	Relevance to claim
	Selection of strains	
	Maintenance of strains	Vigour of test strains / maintenance of any 'special' characteristics.
	Preparation of inoculum	Vigour / susceptibility of inoculum
	Size of bioburden	Scale of effect required
Exposure Conditions	Suspension / delivery medium	Detection of biocidal or biostatic effect. Effect on susceptibility of population to effect.
	Delivery mechanism (spray, drip, dip <i>etc</i>)	
	pH of system	Relationship of inoculum with surface and vigour of inoculum
	Presence of Soiling agents	Growth / survival of test species & relevance to end use
	Exposure temperature	
	Duration of exposure	Effect on inoculum and effect on mechanism of claimed action.
	Humidity during exposure	
	Surface area : volume ratio	
	Are conditions static or dynamic?	
Recovery Mechanism	Recovery fluid	Effect on inoculum
	Use of Neutraliser	Interaction with effect mechanism
	Volume of recovery medium	Limit of detection / efficacy of recovery
	Method of recovery	Effect on inoculum
	Efficiency of recovery method	Limit of detection and size of effect claimed
	Measurement of recovered population	

Before testing for the efficacy of antimicrobially-treated articles with claims for external effects, the substances must have undergone hazard and risk assessment. It must further be determined that uptake by the skin causing systemic effects, including sensitization is minimized. Treated clothes especially must be proved not to pose a risk to the wearer. Detrimental effects on the normal dermal micro-organism flora should be avoided, as this is of importance for a well-functioning skin. Development of cross-resistance must also be avoided since it could obstruct the use of antimicrobial substances for clinical purposes.

In-Use-Evaluation should only be performed after a risk assessment has been carried out and found not to indicate any risk for the persons that will participate in the testing taking due consideration of exposure from other sources. The testing should be performed i) under normal use conditions with the amount of antimicrobials being below a dose considered of low concern, based on a long term animal study, and ii) along approved ethical guidelines.

PRINCIPLES OF THE TEST DESIGN

All data submitted must be scientifically sound, quantitative and be statistically valid.

A tiered testing protocol series is recommended for treated articles.

Depending on the type of claim desired, demonstration of efficacy would be achieved through a cascade of testing, possibly using the same method but adding service related conditions appropriate to substantiate the claims. If biocidal claims are made, a permanent reduction in the size of a microbial population must be demonstrated. A biostatic claim would require demonstration of the prevention of growth/metabolism of the target species. There may be claims for which it may be sufficient to demonstrate that growth on the treated article is either slower or reaches a lower level than that on an equivalent control material (e.g. the control of odour in garments). The important criterion is that the effect demonstrated substantiates the claim made.

In all cases it is vital that the performance of a treated article be compared with the performance of an appropriately similar material that has not been treated. All test procedures, including variations on the Tier 1 test protocols, should be fully validated to ensure that no artefacts arise nor interactions occur that could influence the outcome of the tests. The test protocols for Tier 2 and above must be demonstrated to be relevant to the claim being made and the benefit being sought.

Tier 1 Proof of Principle. Tier one tests should document the biocidal efficacy of the incorporated biocide in the treated article against the target organism(s). Laboratory test conditions should be in the manner of those described in current versions of JIS Z 2801 / (ISO 22196) or AATCC-100 / 100 / JIS L 1902 (Germ Count) / SN195924 / ISO 20743 (Cell Suspension Test). See figures 1 and 2 for a description of the most common methodology used. Other methods may also be able to demonstrate the basic effect e.g. the net onset inhibition method described in the Numetrika protocol. Test microorganisms selected should be relevant to the application supported. Representative test materials (plaques, swatches, painted tiles, etc) with incorporated biocide and controls of the same materials without biocide would be challenged with test organisms per the referenced methods to substantiate an efficacy claim. A relevant difference between the effects observed on the treated and untreated material must be demonstrated. In some instances the treated articles may itself represent a unique material and so no unfortified version may exist. In such situations a similar and functionally relevant non-biocidal should be used as a control. The size / speed of the effect measured should be relevant to the range of uses intended for the material and is not associated with a prescribed set of pass / fail criteria.

Absorbant Article Example Claim: "Fabric inhibits the growth of mildew/bacteria in the shirt". A textile contains a biocide to help inhibit the growth of microorganisms (Bacteriostatic / Fungistatic). Pieces

of the textile, without any preconditioning, are tested according to AATCC 100. Under these conditions, increased numbers of the test organisms would be observed on the untreated, control textile while the results for the treated textile should show no increase in size or a statistically insignificant reduction in viable test organisms compared with the initial population (the claim is for a biostatic effect and so a biocidal effect should not occur).

Rigid Polymer Example Claim: “Antibacterial coating for a more hygienic surface”. A polymer film cast from a water dispersed emulsion is fortified with an antibacterial agent to enable it to be used to coat items intended to exhibit enhanced hygienic properties. Plaques of an appropriate inert substrate (e.g. polyethylene) are coated with either fortified or unfortified material and cured/dried according to manufacturer’s directions. Without any preconditioning, the plaques are tested according to JIS Z 2801. After incubation for 24 hours at 35°C, populations are eluted from the plaques coated with the treated polymer film using a suitable neutraliser and demonstrate a sizeable (in this example, maybe 4 orders of magnitude) and statistically significant difference from the populations eluted from the plaques coated with the unfortified film and bactericidal activity is demonstrated. It should be noted that the actual size of the difference does not need to be fixed with a pass / fail criterion as relevance to claim (which is supported by additional data) is the more important factor. Please refer to paragraph 32 for a discussion on the development of standards and terminology in this emerging field of application.

Tier 2 Simulated Use. In both of the examples above, an effect is demonstrated in a material fortified with an antimicrobial agent. The purpose of the effect is not described specifically and the ability of the effect measured to support a claim has not been made. Further tests are required to link these. Tier 2 tests would be conducted in the laboratory using test conditions that attempt to simulate realistic conditions of use and simulate and support the claim being made. For example, in addition to the conditions appropriate to Tier 1, realistic “In Use” preconditioning might be included, where appropriate. For instance, if the article is intended for re-use, the article & control should be cycled through simulated wear conditions (anticipated conditions of use) and maintenance (cleaning) cycles. At various time points during the wear cycling, the test materials would be tested through the length of time manufacturers intend it to be used (per the claim).

Absorbent Article Example Claim: “Fresh Shirt resists the growth of odour-causing bacteria in the shirt through 10 launderings”. A shirt manufactured using a treated textile remains ‘fresh-smelling’ during summer months. The textile itself has been shown to inhibit bacterial growth during tests at Tier 1 and it is considered likely therefore that this would inhibit the transformation of compounds in sweat absorbed by the shirt into odour compounds during normal wear cycle. The effect is intended to be long-lived and so must survive a realistic number of laundering cycles. Samples of the treated textile are inoculated with a mixture of artificial sweat and bacteria capable of transforming secreted fatty acids and proteins to odour compounds. The volume of inoculum *etc.* is manipulated such that it simulates sweating (*i.e.* the ration of inoculum to textile does not result in the saturation conditions employed during the Tier 1 test). The inoculated textile is then incubated for an interval intended to simulate a normal use cycle (e.g. 8 – 10 hours at 30°C in humid conditions – *i.e.* intending to simulate the conditions in the under-arm area) and then analysed for both effects on the microbial population and the presence of odour compounds. Clearly, the textile fortified with the antimicrobial agent should demonstrate the appropriate difference from the untreated material. This difference should be maintained even following an appropriate number of laundering cycles.

Polymer Film Example Hypothetical Claim: “Floor coating may help control bacterial surface contamination between routine maintenance cycles”. In the Tier 1 example, an antibacterial effect was demonstrated using JIS Z 2801. However, the final use for the film is to coat vinyl sheet flooring to provide it with antimicrobial properties relevant to a proposed setting (e.g. bathroom or kitchen). In the JIS Z 2801 protocol, the inoculum is held in intimate contact with the surface of the test specimens for 24

hours at 35°C under conditions of high humidity: constant wetness is maintained. When used on flooring, water will rarely be present except when the floor is being cleaned and the ambient temperature is likely to be between 20 – 24°C. The effect itself is delivered using silver ions and these require free water to be both released and to interact with microbial cells. There is clearly a significant disparity between the method used to demonstrate potential activity and the normal exposure scenario. Also, activity will probably only be present under certain exposure scenarios and so thought needs to be given as to the nature of the claim that is being made. An exposure scenario that results in a risk of contamination transfer results from small splashes of infected material reaching the floor and being transferred to another location. The splashes of concern are too small to result in local cleaning and disinfection outside of normal maintenance: they have gone unobserved. The faster the population that results from such contamination events is reduced in size, the lower the associated risk would be. The claim therefore is that the presence of the coating results in a reduction in the size / elimination of the population resulting from such splashes faster than on an uncoated floor (or a floor coated with a non-antibacterial polymer coating). The claim is now sufficiently well described for a simulation to be produced. The polymer is intended to be applied once per week and must demonstrate its effect after being cleaned daily with a detergent / sodium hypochlorite based cleaning / disinfection regime. Multiple sub-samples of vinyl sheet flooring either coated with a polymer film fortified with an antibacterial agent or without and uncoated flooring are inoculated with a suspension of cells of relevant test organisms such as *E coli*, *E. faecium*, *S. aureus* (and / or other microorganisms for which claims are desired) in a dilute protein-based soiling agent (e.g. bovine serum albumin). The sub-samples are incubated at 20°C and 50% relative humidity for 24 hours (the inoculum will dry under these conditions and the size of the population will be likely to decline as a result). Replicate sub-samples are analysed at intervals (e.g. 1, 3, 6, 12, and 24 hours) to measure the size of the populations present on the various surfaces. The data shows that the inoculated species population survives two times longer on coated vinyl sheet flooring than un-coated flooring but is reduced to below the limit of detection after 24 hours. In contrast, the population exposed to the vinyl sheet flooring coated with a polymer film fortified with an antibacterial agent is reduced to below the limit of detection after 1 hour. A significant difference has been demonstrated. After 7 repeat daily washing / disinfecting cycles, intended to simulate normal practice, the rate of reduction is reduced to 3 hours but persists. A claim that the coating reduces risks associated with unobserved, accidental splashes of certain contaminated liquids can be supported.

Tier 3 In-Use Evaluation. To either substantiate direct health benefit claims or to support marketing initiatives, treated and untreated articles would be tested via statistically designed use trials by a representative user group. Study observation of the test subjects should document a lower incidence of the microorganisms for which claims are desired.

Absorbent Article Example Claim: “9 of 10 ‘Fresh Shirt’ wearers reported reduced odours”. Control and treated groups of volunteers are given shirts to use during the summer months. A blind trial is designed and participants are selected such that groups or shirts are exposed to similar environments (e.g. non-air conditioned offices in warm summer weather). Uses are given questionnaires to complete to describe their experience with the shirts and at randomly selected intervals, unlaundered shirts are returned to experienced odour specialists for evaluation. Care is taken to ensure the identity of any treatments is not revealed. The data is analysed statistically to determine whether the effects seen in the simulation studies are experienced in practice. The data could be exploited commercially in the ‘nine out of ten users reported...’ form as well as being used, when required by a regulatory agency, to support the claims made.

Polymer Film Example Claim: “Floor Finish reduces the incidence of cross-contamination from room to room”. Trials of the polymer coating are performed in a number of areas likely to have high bacterial contamination on the floor, such as public restrooms in high traffic areas. Using a statistically designed double blind trial design, flooring in selected areas is analyzed to investigate the impact of the treatment on both ambient microbial populations and on the presence of certain organisms on flooring outside the restroom and in rooms elsewhere in the building. In parallel, data is collected on the incidence

of contamination in the non-restroom areas under study and analysis of the data is used to investigate whether an attempt to enhance the hygienic performance of floor coverings has had an effect on the relative incidence of contamination agents.

Treated Articles Example Claims

Surveys conducted in preparation for the Harmonization Workshop and subsequently as part of the Efficacy Methods Steering Committee project summarized the types of commercial treated articles with a range of associated claims. These surveys solicited examples of treated articles and their associated claims from manufacturers, agencies, as well as public print and electronic media. Many product claims restrict biocide activity to protection of the article from microbial degradation. Those were not included in the examples provided in Appendix 2. Claims examples herein represent those for which claims extend to benefits outside of microbial degradation and promote, for instance, aesthetic properties or health benefits.

The most important criterion is that the effect demonstrated through efficacy testing, substantiates the claim, or claims made for the treated article. Product claims found in commerce range broadly and substantiation for most examples will require testing conducted using method modifications appropriate to Tiers 2 and 3. Many claims as stated would require user surveys to define use conditions in order to design a representative test protocol. It is suggested that applicants for authorization/approval of a specific treated article should obtain regulatory agency approval for the claims and proposed substantiation test protocol prior to test commencement.

Data Handling

Normally data should be obtained via statistically designed investigations and presented as raw data plus after statistical analysis.

Examples of important test parameters re: data handling are given in the table below.

Category	Parameter	Impact
Handling of Data	Validation of initial population	Validity of test / claim
	Measurement of variation	Measurement of size of effect
	Calculation of effect	Support of claim
	Statistical validity of effect	Validity of claim
	Biological validity of effect	
	Comparison with claim made	

Performance standards typically associated with biocides used on hard surfaces (sanitize, disinfect) may not necessarily be the only ones appropriate for articles. This guidance document takes the approach that the efficacy test substantiate the claims made rather than force-fitting performance terms at this embryonic stage of regulation. In this manner, claims should be factual, accurate, and substantiated statements. As acknowledged in the recommendations from the OECD Efficacy Workshop, treated articles that demonstrate a degree of efficacy in controlling pathogenic micro-organisms may play a role in overall hygienic practices. With time and experience, terms or symbols such as the JAFET marks discussed below

may become more commonly adopted by other countries in which case they can be added to the definitions. See Appendix 1 definitions for example terms that may be employed in claims for articles.

There are a number of significant scientific challenges in establishing valid test methods, and relevant performance standards and label claims for these products. In addition, different types of product require different performance levels. For example, to claim 18 hours of effective odour control on an athletic shirt, biostatic efficacy may be enough to control offensive odours. However, if a public health term such as “self-disinfects for 24 hours” was to be used, the same log reduction rate would be required as that for hard surface disinfectants. Test material would be challenged repeatedly over a 24 hour period with conditions and frequency of cycles simulating what would be anticipated as the typical use conditions for that article.

At present the only performance standards specifically for treated articles are those used in Japan. They are not regulated by law, but several voluntary schemes exist to cover their applications. These include the Japanese Association for Functional Evaluation of Textiles (JAFET) and the Society of Industrial-Technology for Antimicrobial Articles (SIAA).

Under **JAFET** there are three classes of treated product, depending on the intended use:

- Blue mark: Articles used in the home to improve consumer comfort and well-being.
- Orange mark: Articles used in public areas such as restaurants, hotels and bars.
- Red mark: Medical articles.

The three marks have well defined efficacy (using Japanese Industry Standard JIS L 1902-2002) and toxicology requirements. Those products which meet the requirements receive certification and label issued by JAFET.

SIAA employs Japanese Standard: JIS Z 2801 as the base for their efficacy certification / approval system; however, these are aimed at supporting a brand-mark rather than describing efficacy. Following modification this test method has been published as ISO 22196.

DESCRIPTION OF TIER 1 TESTS - PROOF OF PRINCIPLE

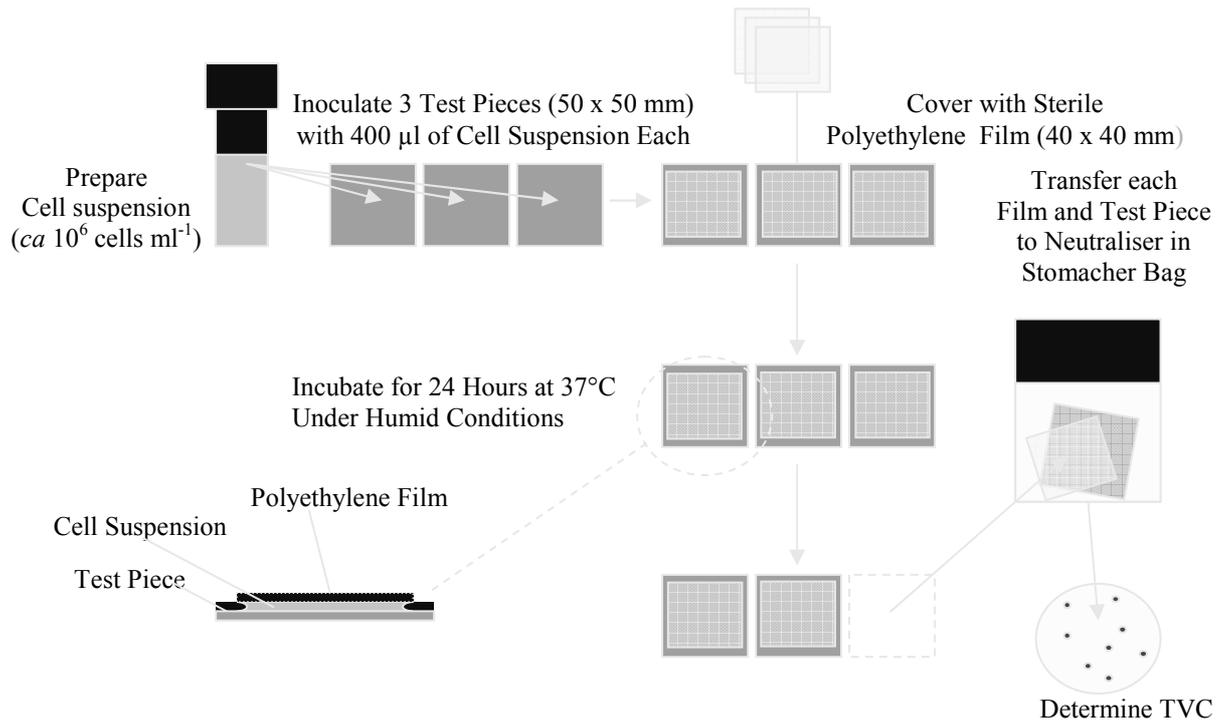
JIS Z 2801: 2000 – Brief Summary of Method.

An aliquot (typically 400 µl) of a cell suspension of organisms typical to the proposed use of the article, usually either *Escherichia coli* ($1.5 - 5 \times 10^5$ cells ml⁻¹; ATCC 8739) or *Staphylococcus aureus* ($1.5 - 5 \times 10^5$ cells ml⁻¹; ATCC 6538p) in a 1 : 499 dilution of a nutrient broth are held in intimate contact with each of 3 replicates of a test surfaces supplied using a 40 x 40 mm polyethylene film (cut from a sterile Stomacher bag) for 24 hours at 35°C (the size of film used should be adjusted in proportion is a different volume of inoculum is employed). The size of the surviving population is determined by immersing the test specimens in individual aliquots (typically 10 ml) of a neutraliser validated for the antimicrobial agent employed (e.g. SCDLP for Ag⁺). The colony forming units in the resulting suspension are enumerated by a suitable dilution plate count method. An additional 3 replicates of the unfortified surfaces are also inoculated in the manner described above but then analysed immediately to determine the size of microbial population present prior to incubation. The method is described schematically in Figure 1 below.

Figure 1 – Schematic of JIS Z 2801

NON-POROUS MATERIALS / COATINGS

JIS Z 2801 - TYPE



AATCC-100: Brief Summary of Method.

The test requires that replicate groups of swatches of textile be inoculated with and completely absorb 1 ml of inoculum therefore, prior to testing, the moisture holding capacity of the textile must be determined. Replicate (typically 3) groups of sub-samples of each textile, sufficient to just absorb 1 ml of inoculum are cut from randomly selected areas on the samples supplied such that small stacks of test specimens are produced.

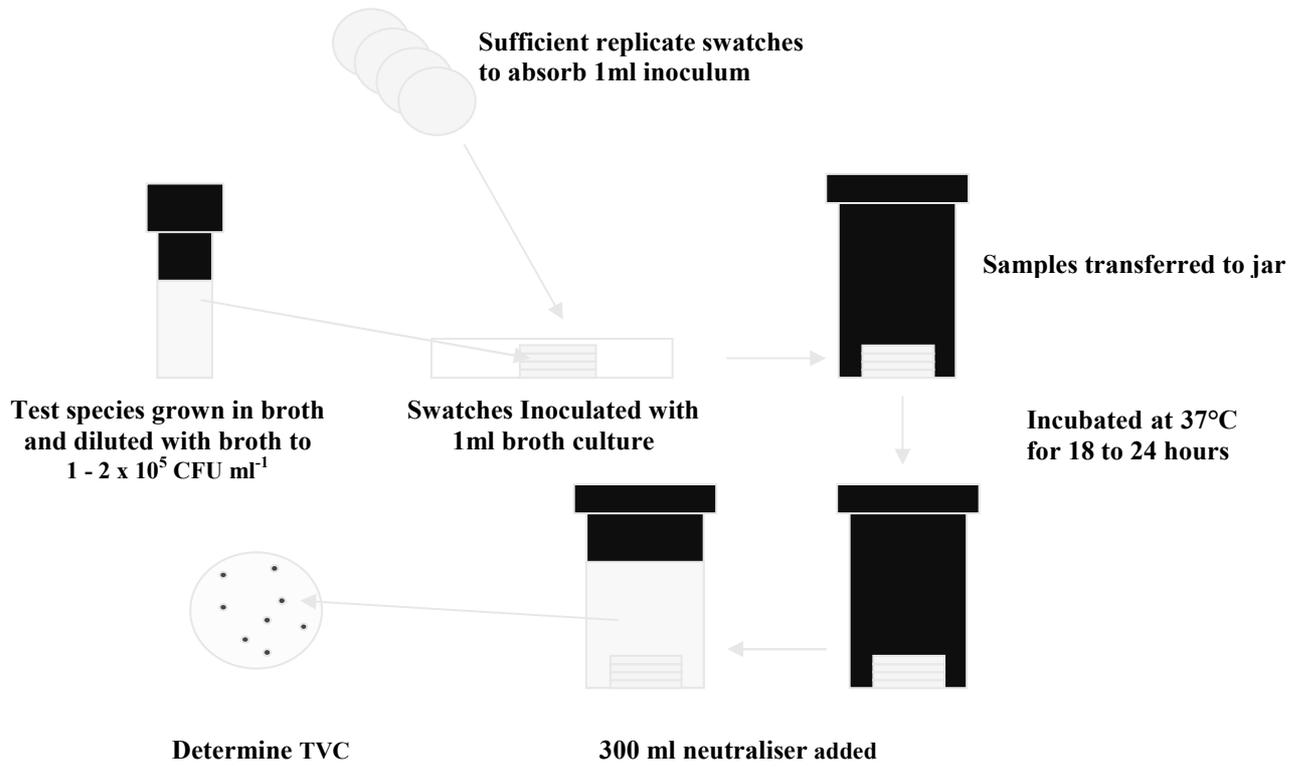
An aliquot (1ml) of a log phase cell suspension diluted in Brain-Heart Infusion Broth of either *Klebsiella pneumoniae* (ca 1×10^5 cells ml⁻¹; NCIMB 10204) or *Staphylococcus aureus* (ca 1×10^5 cells ml⁻¹; AATCC 6538p) or other microorganisms expected to be typical to the proposed use scenario for the article are used to inoculate the 3 replicate groups of sub-samples of the test materials supplied. The inoculated sub-samples are then incubated for 18 hours at 37°C. The size of the surviving population is determined by immersing the test specimens in individual aliquots (typically 100 ml) of a neutraliser validated for the antimicrobial agent employed. The colony forming units in the resulting suspension are enumerated by a suitable dilution plate count method. An additional 3 replicates of the unfortified material are also inoculated in the manner described above but then analysed immediately to determine the size of microbial population present prior to incubation. The method is described schematically in Figure 2 below.

It should be noted that in many instances the soiling presented by the use of a full strength broth in the inoculum is unrealistic and can overwhelm many antimicrobial technologies (e.g. Ag⁺ donors). Variations of the method that employ a more realistic 'challenge' medium (e.g. as used in JIS Z 2801) are, therefore, often employed.

Figure 2 – Schematic of AATCC 100

POROUS MATERIALS

AATCC 100 TYPE



REFERENCES

OECD (2002) *OECD Efficacy Workshop on Certain Antimicrobial Biocides (Washington, USA)*, OECD, Paris, France. <http://www.oecd.org/dataoecd/28/7/2494153.pdf>

OECD (2007) *Analysis and Assessment of Current Protocols to Develop Harmonized Test Methods and Performance Standards for the Efficacy Testing of Treated Articles/Treated Materials*
ENV/JM/MONO(2007)4

Shouji Suzuki, Shigeo Imai, & Hiroki Kourai (2006) *Background and Evidence Leading to the Establishment of the JIS Standard for Antimicrobial Products*. *Biocontrol Science* Vol. 11 No. 3
March 2006

APPENDICES

Appendix 1

Definitions

1. Treated article - a plastic, textile or other pre-formed article pre-treated with biocide before the first use and intended to have either an internal or an external effect.
2. Treated material - is an intermediate product (e.g. plastic pellets, fibers, textile) with an incorporated biocide that is intended to function (internally or externally) in the treated article into which it will be formed.
3. Porous / Absorbent Articles – articles with a porous/absorbent nature such as carpets, paper, sponges, textiles and wipes.
4. Non-Porous - articles with solid, semi-rigid or flexible polymeric surfaces which are essentially non-absorbent.
5. Coatings - a biocide treated film applied to the surface of an article designed to transform the functional face of the article into a treated article.
6. Antibacterial - a product or process which either kills bacteria or inhibits their growth.
7. Antimicrobial - a product or process which either kills microorganisms or inhibits their growth.
8. Biocide - the effect is limited to a reduction in the size of a population of microorganisms
9. Bacteriocide - the claim infers kill and therefore an irreversible reduction in the numbers of viable bacteria from the original inoculum
10. Bacteriostatis - the effect is limited to the prevention of growth/metabolism of bacteria and possibly the germination of bacterial endospores and other resting structures.
11. Fungicide - the effect is limited to fungi. This effect may be attributed to activity against vegetative growth, spores/resting structures or both and may require clarification on the intended use of the product.
12. Fungistatis - the effect is limited to the prevention of growth of fungi and possibly the germination of fungal spores and other resting structures.
13. Algicide - the effect exhibited against algae and their resting stages.
14. Algistatis - the effect is limited to growth prevention of algae and possibly the germination of resting structures.
15. Protisticide - the effect is exhibited against protozoa and their resting structures.
16. Sporicide - the effect is against the spores/resting structures of bacteria
17. Virucide - the effect is limited to virus particles.

18. Clean Surface, Visibly clean surface - a surface which shows no evidence of visible dirt.
19. Hygienically Clean Surface* - a surface which does not constitute a threat to health as a result of the presence of micro-organisms.
20. Hygienic Cleaning* - a procedure that removes soil or organic material from an object and also reduces the number of micro-organisms on that surface to a level where there is no longer a threat to health by transmission of micro-organisms. The reduction in the number of micro-organisms is achieved by removal of the micro-organisms by detergent-based cleaning followed by rinsing, by the action of an agent which has a bactericidal, virucidal or fungicidal activity, or by a combination of both processes.
21. Hygiene* - a procedure or system of procedures or activities used to reduce microbial contamination on environmental sites and surfaces etc. in order to prevent the transmission of infectious disease.
22. Hygiene Procedure* - a procedure that is applied to reduce the number of viable organisms to a level which is considered safe for its intended use. This may be achieved by a process of removal of the microbes, or by inactivation in situ using heat or a disinfectant. A combination of both processes may also be used.
23. Hygienic Surface* - A surface on which the number of microbes has been reduced to a level which is considered microbiologically safe for its intended use.

NOTE:

Definitions 1 -17 were taken from the glossary of terms document prepared for the April 2002 OECD Efficacy Workshop On Certain Antimicrobial Biocides.

* Definitions 18-23 were taken from an International Scientific Forum on Home Hygiene entitled "Guidelines for Prevention of Infection and Cross Infection in the Domestic Environment".

For some hygiene processes referred to in this document, there is no agreed definition or the definitions are currently under discussion within ISO (International Standards Organisation) or CEN (Comité European de Normalisation) bodies.

Some definitions are different from those used in the EU Directive 98/8/EC (known as the Biocidal Product Directive).

Appendix 2

Treated articles and associated claims

Product	Type	Claims Made
Antibacterial Fabric	Textile	Effective control and prevention of growth of a wide range of bacteria
Anti bacterial Masks	Fabric	Antibacterial shield is permanent and will continue killing microorganisms for the life of the mask as they contact the treated surface...even with repeated washings
Socks & Sport socks	Textile	Improved freshness, reduces odour, inhibits bacterial growth Hygienic Helps keep your feet fresh and odour-free Antibacterial finish for long lasting hygienic protection and odour free feet Protection shield against foot odor. The self-disinfecting sock Inhibits the growth of odour-causing bacteria Silver fiber kills and prevents bacteria that causes foul foot odor Control odours & Athlete's Foot Fungus, powerful antibacterial locked into the sock fibers won't wear off or wash out, even after repeated laundering, 99.9% effective in preventing growth of Athlete's Foot Fungus on socks Socks prevent the growth of Athlete's Foot Fungus while fighting sock odour for one full year
Tights (Hosiery)	Textile	Combats the growth of yeast and fungi that cause 'Thrush' and 'Athlete's Foot'. Don't share your clothes with microbes
Bathroom Towel	Textile	Prevents bacterial and fungal growth, keeping your towel hygienically clean
Kitchen Sponge	Polymeric Foam Cellulose	Stays fresher for longer Helps reduce growth of bacteria in the sponge, Helps prevent the spread of germs. Antimicrobial additive provides continuous protection against bacteria Resists odors, bacterial odors Inhibits growth of stain and odor causing microorganisms

Antimicrobial Fibers	Textiles	Eliminates odor causing bacteria and athlete's foot fungi Eliminates 99.9% of bacteria in less than one hour exposure Anti-odor - Inhibits the growth of bacteria and fungi Studies prove when worn, fiber is so effective that sweat actually becomes antimicrobial Products using the [brand] yarn have clinically shown elimination of 99.9% of bacteria in less than one hour of exposure including mrsa and vmrsa
Necktie	Textile	Ties inoculated with E coli and Salmonella showed greater than 99.9% reduction in bacterial growth compared to standard 100% silk neckties inoculated with the same concentration of bacteria.
Kitchen Wipes Floor Wipes Multipurpose Household Wipes	Textiles	Hygienic, built-in protection against bacteria. Antibacterial protection Built-in protection against bacteria Microfiber technology allows our cloths to remove 99.94% of bacteria using only water as a cleaning agent Testing showed that the bacteria in the microfiber itself were reduced by more than 99.99% after 24 hours (effective against E coli, Klebsiella pneumoniae, SARS, MRSA, H5N1 [bird flu virus]) Helps prevent the transfer of germs to your hands while cleaning and prevents cross contamination
Antibacterial Impregnated Tissue	Nonwoven Textile	Kills germs in the tissue
Medicated Strong Toilet Tissue	Nonwoven textile	Fine strong toilet tissue for hygiene cleansing which helps kill bacteria and germs, and better family hygiene
Mattress Cover	Textile	Treated with anti-mite to prevent the action of house mites outside the cover (in the mattress)
Sleeping Bag	Textile	Treated to resist the growth of mould and mildew
Digital Thermometer Sleeve	Polymer	Storage case inhibits growth of pathogenic microorganisms on the sleeve. Minimizes cross-contamination of foods
Lavatory Brush	Rigid / Flexible Polymer	Helps prevent growth of bacteria on the body of the brush.
Flooring	Flexible Polymer	Effective / lifetime antimicrobial protection ensuring that the floor remains free of bacteria between cleaning cycles.
Hygienic Coated Steel	Polymer	Neutralises the ability of bacteria to function, grow and reproduce.

Wall coating	Synthetic Paint	Prevents spread of germs and reduces risk of infection, provides protection against harmful bacteria.
Tile Finish	Coating	The coating has an antibacterial effect: by means of the so-called photocatalysis, activated oxygen is produced which decomposes microorganisms such as bacteria, fungi, algae, moss and germs without any chemical products. Moreover, the formation of new pathogens is prevented. In the case of other methods based on the addition of soluble additives, the effect decreases. In the case of [Brand], on the other hand, the antibacterial effect is reactivated by light again and again.
Multi-surface Coating	Polymeric dispersion	Kills microorganisms in contact within 4 hours.
Repellent Mosquito Net	Fabric	For additional protection against all biting insects this model is pre-treated with permethrin
Surface Coating	Polymer	Bactericide layer uses a concept which permanently destroys germs without contaminating the environment and without the development of resistant pathogens
Toilet Seat	Rigid Polymer	Antibacterial toilet seat kills germs on the seat
Hiking Boot Keyboard, Mouse	Coatings for Textiles & Polymers	Footbed wrapped with fibers that inhibit the growth of microbes. This helps reduce odor-causing bacteria. Keyboard and mouse incorporate [brand] antimicrobial compound providing protection to prevent the growth of a broad range of bacteria, mould and mildew
Sheet Protectors, Binders, Pens	Flexible and rigid Polymers	...all from companies who have partnered with [co. name] to create germ-fighting surfaces
Vinyl Gloves	Vinyl	Light-activated antimicrobial gloves begin to kill deadly bacteria in seconds In minutes decontaminate virtually all bacteria on outside and inside of the glove Control bacterial cross-contamination
Rubber Band/Binder	Rubber	Antimicrobial compound protects the product and helps reduce the risk of cross contamination Prevent cross-contamination
Insect Repellent Treated Apparel (USEPA Registered)	Textiles	Apparel will continue to repel insects through 70 washings Repels mosquitoes including those that can carry West Nile virus, and ticks, including those that can carry Lyme disease [brand] apparel provides effective and convenient protection against mosquitoes, ticks, ants, flies, chiggers and midges