Broad-Spectrum Microbicidal Activity, Toxicological Assessment And Materials Compatibility Of A New Generation Of Accelerated Hydrogen Peroxide (AHP)-Based Environmental Surface Disinfectant

N.Omidbakhsh, B.SC
Virox Technologies, Inc., Canada

ABSTRACT

Background:
Concerns on human and environmental safety and the unfavorable toxicological profile of many microbicides point to the need for safer, faster-acting and broad-spectrum substitutes. ACCEL TB, 0.5% accelerated H_2O_2 (AHP)-based disinfectant described here combines excellent antimicrobial performance with a more favorable toxicological profile.

Objective:
To test the formulation for its broad-spectrum microbicidal activity, safety and materials compatibility using internationally-accepted protocols.

Materials and Methods:
Activity against bacteria (*Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, VRE, MRSA* and *Salmonella choleraesuis*) was tested with the AOAC use-dilution method and the first tier of a quantitative carrier test (QCT-1). Mycobactericidal activity was tested against *Mycobacterium bovis* and *M. terrae* using a quantitative tuberculocidal suspension test (QTB) and QCT-1, respectively. Fungicidal activity (*Trichophyton mentagrophytes)* was determined with the AOAC test and QCT-1. Activity against several enveloped and non-enveloped viruses was evaluated using ASTM method #E-1053. Sanitizing action was tested against seven types of vegetative bacteria with AOAC method #961.02. All microbicidal tests contained an added soil load. The methods to test for acute oral, dermal, inhalation toxicities, and dermal and eye irritation as well as skin sensitization complied with the requirements of the Organization for Economic Co-op. & Develop. and U.S. EPA (OPPTS 870). ASTM standards G-1 and G-31 were used to test compatibility with metals. Also, for plastic compatibility testing, a similar method to that of metals was used.

Results:
At 20°C, the ready-to-use product was bactericidal and virucidal in 1 min, and mycobactericidal and fungicidal in 5 min. It was non-irritating to skin and eyes and it was non-toxic. It was compatible with most plastics and metals.

Conclusions:
The tested formulation showed a favorable safety and materials compatibility profile in addition to being a fast-acting and broad-spectrum microbicide

INTRODUCTION

Chemical disinfectants are widely used in infection control (1). Our reliance on them is increasing further as preventive strategy due to rampant antibiotic resistance and mounting threats from emerging and reemerging pathogens. This, in turn, is forcing a closer look at label claims of such products as well as human health and environmental safety of chemicals in them (2). Therefore, safer and better substitutes are urgently needed. This poster presents one such formulation with broad-spectrum and fast microbicidal action, low toxicity and high materials compatibility.

MATERIALS AND METHODS

Formulation tested: The product tested in this study, Accel TB, is a newly developed, AHP-based intermediate level disinfectant.
Accel TB is a blend of 0.5% hydrogen peroxide, anionic surfactants, non-ionic surfactants and stabilizers. It is a clear, colorless liquid, odorless and has a pH of 2.5-3.0. It is free from nonyl phenol ethoxylates (NPEs) and alkyl phenol ethoxylates (APEs). The formulation is registered for sale in Canada, and in the U.S. Accel TB was tested for its antimicrobial activity, stability, toxicity, dermal and eye irritancy, using well-recognized protocols.
Stability Tests: were performed in compliance with OPPTS 830.317 as well as the paragraph C.01.062 in the Food and Drugs Act, wherein the concentration of medicinal active in a drug product cannot lie outside of a band defined by 90% to 110% of the nominal concentration.
Antimicrobial Tests: Three lots of the solution were tested using A.O.A.C methods as well as QCT-I methods against bacteria, viruses, fungi, and mycobacteria.
Materials Compatibility: ASTM standards G-1 (10) and G-31 (11) were used to test the compatibility of the formulation with commonly used plastics and metals**.** The plastics tested were HEDP, LDPE, Teflon, PP, acetyl polystyrene, poly acrylate, polycarbonate, ABS, polyester, polyvinyl chloride and polysulfone. The metals tested were mild steel, aluminum and stainless steel.
Toxicity tests: The solution was tested for Acute Eye Irritation/Corrosion, Acute Dermal Irritation/Corrosion, Acute Oral Toxicity, Acute Dermal Toxicity, Acute Inhalation, and Skin Sensitization. The studies were performed using OPPTS test methods.

RESULTS

Stability Tests: (2 year shelf life)

The solutions had about 8% loss for hydrogen peroxide in one year at room temperature. Based on these results and reasoning, the product is stable for a period of 2 years

Microbicidal tests (Table 1)

Table 1. Microbicidal activity of the test formulation against different microorganisms.

Test Organisms	Test Method	Contact time
Bactericide		
S.aureus, S.choleraesuis, P. aeruginosa *E. coli 0157, MRSA, VRE, Acinobacter baumanni*	AOAC Use-dilution/QCT, 3 lots	1 min
Sanitizer		
S.aureus, K.pneumoniae *S. choleraesuis, P. aeruginosa, E. coli, MRSA, VRE*	AOAC 961.02 non-food sanitizer test method modified to meet EPA requirements, 3 lots	30 sec
Mycobactericide (Tuberculocide)		
M.Bovis	Quantitative Tuberculocidal Suspension Test method, QCT	5 min
Fungicide		
Tricophytom mentagrophytes	AOAC Fungicidal test method, QCT	10 min
Virucide		
Poliovirus type 1, Feline Calicivirus, Coronavirus, Influenza A, Rhinovirus, Rotavirus, Herpes Simplex type 1 and 2, HIV, HCV	ASTM E1053/97	1 min

All tests have been done under 5% soil load at 20°C

Compatibility tests

Accel TB was compatible with all plastics and metals tested herein.

MATERIALS AND METHODS CONTINUED

Toxicity Tests (Non-toxic)

Table 2: Toxicity Test Results for Accel TB:

Test method	Results
Acute oral toxicity study (UDP)	There was no morality in the study. LD50>5000 mg/kg
Acute dermal toxicity study	There was no morality in the study. LD50>5050 mg/kg
Acute inhalation toxicity study	There was no morality in the study. LC50>2.59mg/l
Acute eye irritation study	Non-irritating (the product is assigned to toxicity category IV)
Acute dermal irritation study	Slightly irritating, (the product is assigned to toxicity category IV)
Skin sensitization (local lymph node assay)	The product is not a sentisizer.

DISCUSSIONS AND CONCLUDING REMARKS

Hydrogen peroxide is among the oldest microbicides known and it is generated naturally in many settings. However, it is relatively unstable by its very nature and somewhat slow-acting when used on its own (3). Both of these weaknesses have now been addressed in that it is now possible to produce highly stabilized forms of H_2O_2 in solution and also speed-up its microbicidal action. Further, its corrosivity has been tamed, thus widening its materials compatibility. The AHP technology has combined these developments to enhance the potential of H_2O_2 as a microbicide, resulting in formulations for a variety of applications (4,5). The stabilizers, corrosion inhibitors and other excipients in these formulations have a high safety and biodegradability profile and free from aquatic toxicants such as nonyl phenol ethoxylates (NPEs) or alkyl phenyl ethoxylates (APEs). The findings summarized here further substantiate the desirable attributes of the AHP-based formulation evaluated.
Its microbicidal activity was assessed using relevant AOAC and ASTM protocols and in the case of fungicidal and mycobactericidal activities the findings with the suspension test were reconfirmed with the more stringent QCT-1. As shown in Table 1, the vegetative bacteria tested represented a wide array of nosocomial pathogens. The viruses tested also included important nosocomial pathogens or their accepted surrogates (6). While certain of the enveloped viruses tested are not known to spread through contaminated environmental surfaces, they were included to ensure that ACCEL TB, based on a relatively new technology is successful in dealing with both non-enveloped and enveloped viruses. ASTM protocol #E-1053 was used for this purpose because it is currently the only protocol accepted by the U.S. Environmental Protection Agency for submission of data for virucidal activity.
All evaluations for microbicidal activity were performed with an added soil load in test microbial suspensions. The soil load was either 5% serum or the tripartite mixture of proteins. Such soil loads not only simulate the presence of body fluids but also adds to the level of stringency of the methods for added confidence in the test data (7).
In summary, the AHP-based environmental surface disinfectant tested in this study proved to be a broad-spectrum and fast-acting microbicide with high safety and materials compatibility profiles. It, therefore, addresses many of the concerns relating to other types of actives in wide use in infection control today (2,8,9).

REFERENCES

1. Block, S.S. *Disinfection, Sterilization & Preservation.* 5th ed., Block, S.S. (ed.), 2000. Lippincott Williams & Wilkins, Philadelphia, PA.
2. Sattar, S.A. (2004). Current issues in testing, selection and use of microbicides in infection control: a critical review. Aust. Infect. Control 9: 84-100.
3. Best, M., Springthorpe, V.S. & Sattar, S.A. (1994). Feasibility of a combined car-rier test for disinfectants: Studies with a mixture of five types of microorganisms. Am. J. In-fect. Contr. 22:152-162.
4. Sattar, S.A., Adegbunrin, O. & Ramirez, J. (2002). Combined use of simulated reuse & quantitative carrier tests to assess high-level disinfection: experiments with an accelerated hydrogen peroxide-based formulation. Am. J. Infect. Control. 30:449-457.
5. Sattar, S.A., Springthorpe, V.S. & Rochon, M. (1998). A product based on accelerated & stabilized hydrogen peroxide: evidence for broad-spectrum germicidal activity. Can J. Infect. Control 13: 123-130.
6. ASTM International. Standard quantitative disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal & sporicidal activities of liquid chemical germicides (E-2197). Vol. 11.05 2005; ASTM, Conshohocken, PA.
7. Springthorpe, V.S. & Sattar, S.A. (2005).Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. J. AOAC International 88: 182-201.
8. Dringen R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. J Neurosci Res. 2005;79:157-165.
9. Daschner, F. The hospital and pollution: role of the hospital epidemiologist in protecting the environment. In *Prevention and Control of Nosocomial Infections.*