VIROX™ TECHNOLOGIES INC.

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Solutions®

Antimicrobial Biocides in the Healthcare Environment: efficacy, usage, policies, and perceived problems

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Chemical biocides have been used for centuries, originally for food and water preservation, although there are early accounts of their use for wound management. A clear landmark in the use of biocides in the healthcare setting was the advent of antisepsis and the use of chlorine water in the early 19th century. The 20th century witnessed a tremendous increase in the number of active compounds being used for disinfection, sterilization, and preservation, with the development of cationic biocides such as biguinides and quaternary ammonium compounds (QACs), phenolics, aldehydes, and peroxygens.

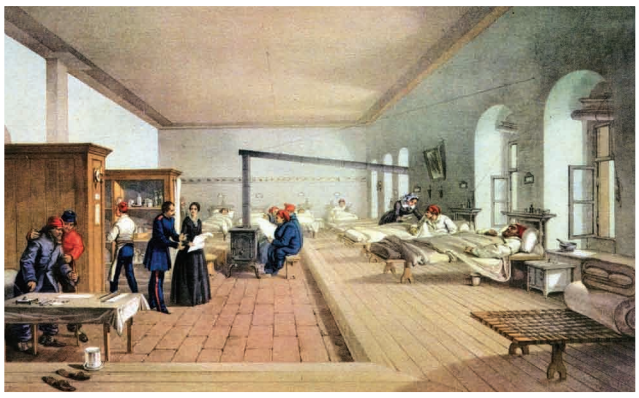
Biocides – usage and policies

Biocides are used extensively in healthcare settings for different applications: the sterilization of medical devices, the disinfection of surfaces and water, skin antisepsis and the preservation of various formulations. In addition, there are now numerous commercialized products containing low concentrations of biocides, the use of which is controversial. Some professionals believe that the indiscriminate usage of biocides in the healthcare environment may not be justified and is detrimental in the long term, for example, by promoting the emergence of bacterial resistance to specific antimicrobials. The use of biocidal products may be more appropriate only in specific situations where the risk of spreading HAIs is high. Some surfaces may only need cleaning and do not require chemical disinfection as they are rarely heavily contaminated, whereas other medical articles need thorough cleaning with detergents and chemical disinfection.

The principles of disinfection policy in healthcare facilities has been described in several reports, by Rutala, Ayliffe, et al, and more recently by Fraise. Disinfection policies should take into account the reasons and purposes for which disinfectants are used, the risk of infection from equipment or the environment, and implementations of such policies (see table). The benefits of the introduction of comprehensive disinfection policies on the reduction of HAIs have been described, although their implementation has sometimes been perceived as unsatisfactory.

Alteration of activity

The activity of a biocide depends upon a number of factors, some inherent to the biocide, some to microorganisms. Among microorganisms most resistant to biocidal exposure are bacterial spores, followed by mycobacteria, Gram-negative, Gram-positive, and fungal microorganisms. The sensitivity of viruses usually depends upon their structure, but notably also depends on whether they possess an envelope, enveloped viruses being more sensitive to disinfection. Although there are exceptions within this summarized classification (eg, some mycobacteria are relatively sensitive to disinfection), this attempt at distinguishing microorganisms according to their susceptibility to biocides gives useful information for the selection of an appropriate biocidal agent. However, it is not always possible to predict which microorganisms will be present on certain surfaces, although the



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Also In This Issue:

"The secret of success is constancy of purpose"

Benjamin Disraeli

Canadian Government to Ban or Restrict Toxic Chemicals

On December 8, 2006, the Canadian government announced a strategy to clean up dangerous chemicals in the environment. A component of the strategy was the phase-out of "legacy chemicals" – those substances that don't dissipate or that have detrimental chronic health impacts. The list of chemicals facing regulation or outright banning includes many proven harmful to animals and suspected to be harmful to human health, includes a chemical commonly used in cleaners and disinfectants.

One Chemical of Note

2-Butoxyethanol is commonly known as ethylene glycol monobutyl ether but has a number of other pseudonyms including Butoxyethanol, Butyl cellosolve, Butyl Oxitol, Ethylene glycol mono-n-butyl ether, Monobutyl ethylene glycol ether, and n-Butoxyethanol. This seemingly ubiquitous chemical is the primary ingredient of such products as white board cleaners, latex paints, cosmetics, dry cleaning solutions, firefighting foam, leather protectors, oil spill dispersants, and many other products including a great many commercial and industrial cleaning products.

Notably, 2-Butoxyethanol is usually found in those disinfectant formulations that combine quaternary ammonium chlorides (aka quats) and alcohol, often called "fifth generation quats". 2-Butoxyethanol is readily absorbed following inhalation, oral or dermal exposure, has moderate acute toxicity and is irritating to the eyes and skin. A risk assessment concluded that chronic exposure could alter blood in ways associated with hemolytic anemia.

Safe and Sustainable

We believe in the need for ongoing research into safe and sustainable cleaning and disinfection products. Significant research went into the selection of chemicals used to create Accelerated Hydrogen Peroxide (AHP) and Stabilized Hydrogen Peroxide (SHP) solutions. Based on this research and subsequent testing conducted on the final products, we feel that when compared to other products on the market Virox Technology's AHP and SHP products are some of the safest to be found.

Accelerated Hydrogen Peroxide and Stabilized Hydrogen Peroxide solutions are formulated through a synergy between hydrogen peroxide, surfactants (detergents), wetting agents (reduces surface tension of a liquid allowing it to more easily penetrate soils), and chelating agents (reduces the impact of hard water). The ingredients are all listed on the EPA and Health Canada "Inert" lists, and the FDA "Generally Regarded as Safe" list. Where possible, the chemicals used to manufacture AHP or SHP solutions have CFR 21 (Code of Federal Regulations) clearance as direct or indirect food additives. In the ready-to-use or use-dilution form, both SHP and AHP have proven to be non-toxic, and not an eye nor skin irritant (data available upon request), they are also free of volatile organic compounds (VOC's), fragrances, and dyes (for the specific reason that these components are commonly found to cause health risks).

With the introduction of the Government of Canada's stronger stance on chemicals, health care facilities currently using quats, phenols, ortho-phthalaldehydes or glutaraldehydes will likely feel pressure in coming months to review the disinfectants and chemicals that they use and investigate safer and sustainable alternatives to legacy chemicals.

Virox Update

Sporicidal Surface Product Registration Expected by Sept.

With the continued *Clostridium difficile* issues our R&D team has been working to create a task oriented product for Environmental Surface cleaning. Two products have been developed, the first product will be available as a Ready-To-Use Liquid and pre-saturated wipe for use as a 10-minute Sporicidal Bathroom Disinfectant. The second product will be a gelled version that has been designed for use as a sporicidal toilet and commode bowel disinfectant. The liquid product has been released without label claims under the name RESCUE and is available for purchase through The Stevens Company. For more information on this product please contact Nicole Kenny at nkenny@virox.com.

1-Minute Intermediate Level Disinfectant launched by Johnson Diversey and The Butchers Company

JohnsonDiversey and The Butchers Company will be show casing their new 1-Minute Intermediate Level Disinfectant at the CHICA-Canada conference in Edmonton. Oxivir Tb (JohnsonDiversey) and Carpe Diem Tb (Butchers) have received DIN registration by Health Canada as well as EPA registration in the United States and carry a 1-minute bactericidal, 1-minute general virucide and 1-mintue tuberculocidal claim.

The formula has been included in two clinical studies and posters will be presented at the CHICA-Canada conference. The first study was a co-sponsored between Suburban Hospital in Bethesda, MD and the FDA and is titled "*Effects of an Environmental Services Professional Training Course and Cleaning Products on the Rates of Infection Seen at Suburban Hospital*". The second study is being conducted by St. Boniface Hospital in Winnipeg and is titled "*The OXIVIRTB Formulation of Accelerated Hydrogen Peroxide (AHP) is Effective for Killing Clostridium difficile Spores on Toilet Seat Surfaces*". Both posters are available at Virox exhibit booths or through www.virox.com.

Partnerships in Paediatric Patient Safety Corporate Sponsor

In our continued support of education, Virox will be the event sponsor for the SickKids Foundation 3rd Annual "Partnerships in Paediatric Patient Safety Symposium: Spreading the Word". This event will be held on Thursday June 7th at SickKids Hospital (Main Auditorium). For more information on this event please contact the Patient Safety Symposium organizers at 416-813-7358 or by email at patientsafety@sickkids.ca.

Two Research Posters to be Presented at the 2007 CHICA Conference

Navid Omidbakhsh, Director of Research & Development at Virox will be attending the CHICA Conference in Edmonton to present two posters. Navid's posters are titled "*A Novel Hydrogen Peroxide-Based Antimicrobial Handwash*" and "*A New Peroxide-Based Fast Acting Surface Sporicide*".

The posters will be presented on Tuesday June 12th from 12:30pm to 1:15pm at poster boards 24 and 25.

2007 CHICA Scholarship Winners

Virox and the Patron Members (JohnsonDiversey, Butchers, Deb, STERIS and Webber Training) would like to congratulate the 2007 Scholarship winners. This years recipients are: Nora Boyd, Laurie Boyer, Nancy Brown, Judi Linden, Suzanne Rhodenizer-Rose, Donna Ronayne, Allyson Shephard, Merlee Steele-Rodway, Virginia Tirilis and Elizabeth Watson.

To date Virox and the Patron Members have contributed $75,000.00 towards the annual scholarship which has provide the opportunity for over 50 Infection Control Practitioners opportunity to attend the annual CHICA-Canada Conference. The scholarship program will continue in 2008 and the application will be posted in the fall on both the CHICA-Canada and Virox Technologies Inc. websites. Be sure not to miss the submission deadline at the end of January, 2008!

Sponsor of the 1st Canadian Symposium on The Hygiene Profession & Science.

The Stanier Institute in partnership with The Faculty of Applied Health Sciences at Brock University is hosting the 1st Canadian Symposium on Hygiene and Public Health at the Hamilton Campus of Brock University on Thursday June 21st. The symposium will cover topics on Food & Water Quality, Toxic Materials in the Environment and Pathogen Trasmission and Prevention. Navid Omidbakhsh, Director of Research & Development will be among the panel of speakers.

Please contact The Stanier Institute at 613-728-2188 or stanier@mco.ca for more information.

Conference & Education
Spring-Summer Schedule

Virox representatives will be participating in the following functions during the upcoming months:

May 4 – ElderHealth Resources Foot Care Conference in Ottawa

May 28 & 29 – JDQ-Montreal Dental Conference in Montreal

May 30 – June 2 – Organization for Safety and Asepsis Procedures (OSAP) in Charlotte, NC

May 31 – HANDIC Education Day in Hamilton

June 1 – CHICA-MB in Winnipeg

June 4 – 5 – AIPI in St. Sauveur

June 8 – Partners in Paediatric Patient Safety Symposium in Toronto

June 9 – 14 – CHICA-Canada in Edmonton

June 19 – TPIC Education Day in Mississauga

June 21 – Stanier Institute Canadian Symposium on the Hygiene Profession & Science in Hamilton

June 24 – 28 - APIC in San Jose, CA

We are very excited about participating in each of these conferences & education days. We wish the best to all of the various organizers and would like to thank them for their dedication and effort in organizing these very important educational opportunities. We look forward to attending and talking to all of the participants.

Bacteria Found 2 Miles Beneath the Earth's Surface

The dark shaft of the Mponeng mine in South Africa. Credit: Duane P. Moser, Desert Research Institute

Scientists found a gold mine of bacteria almost two miles beneath the Earth's surface.

The subterranean microorganisms, a division of Firmicutes bacteria, use radioactive uranium to convert water molecules into useable energy. Uranium is an element contained within the Earth's crust and is an abundant source of energy. The presence of such terrestrial organism raises the potential that bacteria could live beneath the surface of other planets such as Mars.

Researchers found the bacteria when they learned of a water-filled fracture in a South African gold mine close to Johannesburg. Upon sampling the water they noticed something odd. The water contained hydrogen and hydrocarbons that form when water exposed to radiation from rocks containing uranium breaks down. The age of the water and analysis of the microbes revealed that these bacteria parted from their surface relatives some 3 to 25 million years ago.

"We know how isolated the bacteria have been because our analyses show that the water they live in is very old and hasn't been diluted by surface water," said lead author Li-Hung Lin, from National Taiwan University. "In addition, we found that the hydrocarbons in the local environment did not come from living organisms, as is usual, and that the source of the hydrogen needed for their respiration comes from the decomposition of water by radioactive decay of uranium, thorium and potassium."

Scientists still don't know how surface Firmicutes managed to make a home for themselves so deep in the Earth. However, they function similar to photosynthetic organisms that capture sunlight and turn it to energy for other organisms, indicating that Firmicutes could support other microbial communities with the energy acquired from uranium. "It is possible that communities like this can sustain themselves indefinitely, given enough input from geological processes," said study co-author Douglas Rumble, a scientist from the Carnegie Institution. "Time will tell how many more we might find in Earth's crust, but it is especially exciting to ponder whether they exist elsewhere in the solar system."

Reversing Natural Selection

Antibiotic drugs are a well-known test of the idea of natural selection. By killing sensitive bugs, they leave more space and nutrients for resistant ones to thrive. Genes for resistance thus spread through the population unless such drugs are used carefully and sparingly. However, as with other sorts of drug, different types of antibiotic may interact with one another in unexpected ways.

In a paper published in a recent issue of Nature, and highlighted in The Economist, Roy Kishony and his team from Harvard University have just shown that one such interaction has the paradoxical effect, giving the advantage to drug-sensitive bacteria instead of drug-resistant ones.

Dr. Kishony's team studied two strains of E. coli. One of these strains was sensitive to doxycycline, the other was doxycycline-resistant. When the two were grown in cultures containing doxycycline, the resistant strain, as expected, did better. However, when the researchers grew each strain in cultures containing both doxycycline and a second antibiotic, ciprofloxacin, they found the opposite effect. This time it was the doxycycline-sensitive strain that did better even though, in principle, it was not resistant to either drug.

The real test came when Dr. Kishony pitched the two strains against each other. He labeled the doxycycline-resistant strain with a yellow protein and the sensitive with a blue protein. When only docycycline was added to the mixture, the yellow team prevailed. But when both drugs were present, blue bacteria swept the field – or rather, the Petri dish. When faced with a two-pronged attack, therefore, it was the drug-sensitive strain that had the selective advantage.

Exactly how the two drugs interact to produce this result is not yet clear. They work in different ways. Doxycycline gums up the assembly line on which proteins are made, whereas ciprofloxacine stops the DNA message about how to make each protein being read in the first

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A Bacterial Solution to Depression

Nicole Kenny, Virox Technologies Inc.

A chance observation by a research team at Bristol University, published in Neuroscience, led to speculation that a particular sort of bacterium might alleviate clinical depression.

Oncologist Dr. Mary O'Brien, at the Royal Marsden Hospital in London, was trying out an experimental treatment for lung cancer that involved inoculating patients with *Mycobacterium vaccae*. This is a harmless relative of the bugs that cause tuberculosis and leprosy that had been killed before injection. When Dr. O'Brien gave the inoculation, she observed not only fewer symptoms of the cancer, but also an improvement in her patients' emotional health, vitality and general cognitive function.

To find out what was going on, researcher Dr. Craig Lowry turned to mice. His hypothesis was that the immune response to M. vaccae induces the brain to produce serotonin, a neurotransmitter, and one symptom of depression is low levels of it.

Dr. Lowry and his team injected their mice with *M. vaccae* and examined them, looking for a rise in the level of cytokines). As expected, cytokine levels rose. They then looked directly in their animals' brains for the effect of those cytokines.

Cytokines are molecules produced by the immune system that trigger responses in the brain and act on sensory nerves that run to the brain from organs such as the heart and the lungs. That action stimulates a brain structure called the dorsal raphe nucleus. It was this nucleus that Dr. Lowry focused on. He found a group of cells within it that connect directly to the limbic system, the brain's emotion-generating area. These cells release serotonin into the limbic system in response to sensory nerve stimulation.

The consequence of that release is stress-free mice. Dr. Lowry was able to measure their stress by dropping them into a tiny swimming pool. Previous research has shown that unstressed mice enjoy swimming, while stressed ones do not. His mice swam around enthusiastically. This result offers the possibility of treating clinical depression with what is, in effect a vaccination. Besides cancer, and now depression, *M. vaccae* is also being looked at as a way of treating Crohn's disease and rheumatoid arthritis.

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organic load or the extent of microbial contamination, and the presence or not of a biofilm, can be anticipated. An understanding of the factors affecting antimicrobial activity is essential to ensure that a biocidal product/formulation is used properly.

Problems associated with the use of biocides

The emergence of bacterial resistance to biocides and the possible linkage between biocide and antibiotic resistance is a major topic of discussion and concern. The emergence of bacterial resistance to biocides is not a new phenomenon and has been described since the 1950's, particularly with products containing a cationic biocide. More recently, the emergence of bacterial resistance to biocides to low (inhibitory) concentrations has been widely reported, mainly from laboratory studies, but also from environmental investigations.

There is now a better understanding of the overall mechanisms that enable bacteria to withstand exposure to low concentrations of a biocide. As mentioned earlier, some microorganisms are better at surviving a biocidal treatment than others, primarily through their intrinsic properties and impermeability. The impermeability barrier, encountered in spores, but also in vegetative bacteria such as mycobacteria, and to some extent, Gram-negative bacteria, limits the amount of a biocide that penetrates within the cell. The role of specific cell structure, such as lipopolysaccharides (LPS) in Gram-negative bacteria and the mycoylarabinogalactan layer in mycobacteria, in this resistance mechanism has been demonstrated by the use of permeabilizing agents such as ethylenediamine tetraacetic acid, or organic acids, and cell wall inhibitors such as ethambutol. The insusceptibility of Gram-Negative bacteria to biocidal agents can be decreased further by a change in overall hydrophobicity, outer membrane ultrastructure, protein content, and fatty acid composition. Bacteria are also able to decrease the intracellular concentration of toxic compounds by using a range of efflux pumps, which can be divided into five many classes: the small multidrug resistance (SMR) family (now part of the drug metabolite transporter (fuperfamily), the major facilitator superfamily (MFS), the ATP-binding cassette (ABC) family, the resistance-nodulation-division (RND) family and the multidrug and toxic compound extrusion (MATE) family. The involvement of multidrug efflux pumps in bacterial resistance to various compounds including QACs, phenolics, and intercalating agents has been widely reported, particularly in Staphylococcus aureus.

Another mechanism that can contribute to the reduction in the concentration of a toxic compound is degradation. Degradation has been well described for metallic salts with an enzymatic reduction and for aldehydes with the involvement of aldehydes dehydrogenase. The degradation of phenols, such as triclosan, by environmental strains has been reported, but there is little evidence that such degradation takes place in clinical isolates. In addition, some bacteria express enzymes such as catalases, superoxide dismutase, and alkyl hydroxyperoxidases to prevent and repair free radical-inducing damage caused by oxidizing agents.

Bacteria in biofilms have been shown to be more resistant to antimicrobials than their planktonic counterparts. Resistance results from a multicomponent mechanism involving phenotypic adaptation following attachment to surfaces, impairment of biocide penetration, and enzymatic inactivation, and the induction of multidrug resistance operons and efflux pumps.

Future Use

The last 50 years have witnessed an important increase in the number of biocides and their usage in the healthcare environment. When used correctly (ie, compliance with disinfection/antiseptic regimens), biocides have an important role to plan in controlling infection. There is still some uncertainty as to the extent of their use in the healthcare environment. Should they be reserved for the disinfection of critical and semi-critical items/areas only, or should they be used also on noncritical devices/surfaces? Should the use of biocide-embedded products (eg, plastics, fabrics) be encouraged or banned? There is no doubt that the use of chemical biocides creates selective pressure, however it is yet unclear in practice whether such pressure favors the emergence of bacterial resistance.

Biocides are essential in prevention and controlling infections in the healthcare environment and the benefits from their usage currently outweigh possible disadvantages. Disinfection of noncritical surfaces and items, and the usage of biocide-containing products, need to be reviewed, although the incorporation of biocides into medical devices to prevent bacterial infection is promising, if controlled and assessed appropriately.

The full text of this article is available from Nicole Kenny nkenny@virox.com

Reversing Natural Selection

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place, so there is plenty of scope for interference between the two.

Whether Dr. Kishony's discovery has any clinical implications remains to be seen. As he observes, the experiments were performed on bacteria that live in the laboratory under highly controlled conditions. Nevertheless, this study opens a novel way of looking at the problem of resistance. Using one drug to neutralize resistance to another looks worthy of further research.

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