

SHORT REPORT

# Lack of sporicidal activity in diallyl-disulphide-oxide and a nanotechnology-product for sterilizing medical and dental instruments

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## Abstract

The objective of this study was to evaluate sporicidal activity in products commercially available for sterilisation of medical and dental instruments. In simulated use and under worst-case scenario conditions, *Bacillus atrophaeus* spores were suspended in diallyl disulphide oxide (DDO), a nanotechnology product (NANO) or three super oxidized water (SOW) solutions. After 6 hours exposure, the spore suspensions were filtered, washed and incubated.

The DDO and NANO formulations evaluated lack sporicidal activity and are inadequate to sterilise instruments. The three SOW solutions evaluated are sporicidal but their activities are inhibited by protein. Their respective sterilisation contact times remain to be determined.

In conclusion, the results identify formulations that pose a risk to patients, and unveil failures in the regulatory evaluation of products. The absence of science-based regulatory standards results in the commercialisation of unsafe products without the required sporicidal activity. To strengthen national and local health systems, the international infection prevention community must critically review the regulatory expectations for sporicidal products and disseminate information on the basics of antimicrobial effectiveness testing.

**Keywords:** Sterilisation; Surgical instruments; Dental instruments

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## Introduction

Healthcare-associated infections (HAIs) take a high toll in human lives and affect hundreds of millions of patients worldwide.<sup>1</sup> The Latin American Study of Adverse Events (IBEAS) revealed that HAIs are a major health problem in the hospitals of Argentina, Colombia, Costa Rica, Mexico and Peru.<sup>2</sup>

Ineffective instrument reprocessing ranks high among the diverse challenges that adversely affect patient safety. Recently, hard to clean instruments have been involved in multi-drug resistant bacteria outbreaks in healthcare facilities in the United States (US).<sup>3,4</sup> HAIs traceable to inefficient instrument reprocessing have occurred also in diverse medical tourism destinations.<sup>5-7</sup> Countries with a science-based regulatory framework can take timely action to protect patients. On 17 March 2015, the US Food and Drug Administration (FDA) issued reviewed guidance for the formulation and scientific validation of reprocessing instructions for reusable medical devices.<sup>8</sup>

Sterilisation is the safest way to process critical and semi-critical instruments between patients. Instruments must be thoroughly cleaned and then sterilised in a fully validated system. In the second decade of the 21<sup>st</sup> century, however, many instruments, particularly heat-sensitive instruments are immersed in liquid chemical sterilants/high-level disinfectants (S/HLD). Most industrialised nations regulate the expected anti-microbial activity of S/HLD but standards may be notably different among these countries. In the US, the FDA requires all instrument S/HLD candidates to demonstrate sporicidal activity. Then, these sporicidal products are challenged with mycobacteria to define the time required to act as HLD.<sup>9</sup> In the European Community (EC) the CEN/TC 216 technical committee produces current and future disinfectant testing standards.<sup>10</sup> Standard EN 14885-2006 Indicates test methods to be used to substantiate claims for products intended for Instrument disinfection, including mycobacterial/tuberculocidal (EN 14348, EN 14563), bactericidal (EN 13727, EN 14561) and fungicidal (EN 13624, EN 14562) activity tests<sup>11</sup> but the terms “sterility, sterile, Sterilisation, sterilant” fall outside the scope of CEN/TC 216.

Most countries in Latin America still lack science-based standards. This leads to the commercialisation of unsafe products without the required sporicidal activity. Travelling patients and health care workers deployed in response to international emergencies must pay attention to the reliability of locally available S/HLD products.

Among a wide diversity of formulations, diallyl disulphide oxide (DDO), nanotechnology-based (NANO) products and diverse super-oxidized water (SOW) solutions are emerging in developing and underdeveloped nations as germicides with purported efficacy as “instrument sterilant” in their label claims. A serious threat to patient safety is that these products’ labels present limited information from the manufacturers on their active ingredients and in most cases, the recommended exposure times to achieve sterilisation have no precedent in the peer-reviewed literature.

To strengthen national and local health systems, the international infection prevention community must critically review the regulatory expectations for sporicidal products and disseminate information on the basics of antimicrobial effectiveness testing. Sporicidal potency tests demonstrate the potential usefulness of a chemical product as a S/HLD for medical and dental instruments.<sup>9,12</sup> A liquid chemical sterilant must demonstrate no failures in the Association of Official Analytical Communities (AOAC) sporicidal official method 966.04<sup>13</sup> and accept no survivors in simulated-use and in-use tests with a challenge inoculum of 6 logs of spores.<sup>9</sup> A list of S/HLD products is available at [www.fda.gov/medicaldevices/deviceregulationandguidance/reprocessingofsingle-usedevices/ucm133514.htm](http://www.fda.gov/medicaldevices/deviceregulationandguidance/reprocessingofsingle-usedevices/ucm133514.htm). The FDA’s sporicidal list doesn’t include a DDO formulation or a NANO product, and Sterilox<sup>®</sup> is the only SOW product cleared for interstate trade in the US as a HLD only.

In the absence of a science-based regulatory process, independent testing conducted at higher education institutions helps health care professionals make informed choices when selecting S/HLD. The suspension method described provides a screening

tool to assess sporicidal activity.<sup>14-16</sup> A simulated use test under worst case conditions is one of many evaluations required by regulatory agencies in the US and the EC. The aim of this investigation was to evaluate sporicidal activity in products commercially available for sterilisation of medical and dental instruments.

## Materials and methods

### Liquid chemical “sterilants”

Five chemical products registered and sold as sporicidal solutions for instrument sterilisation were evaluated before the expiration dates printed on their labels. The sealed product samples were purchased from medical supply outlets. Information on tests used for regulatory purposes was not available. The products evaluated and presented in Table I, where: DDO = Accua Aséptic Hp (Grupo Medifarma S.A. de C.V., Morelos, México), NANO = Eviter (Gresmex S.A. de C.V. México, D.F.), SOW 1 = Microdacyn<sub>60</sub> (More Pharma Corporation, México, D.F.), SOW 2 = Estericide Qx (Esteripharma S.A. de C.V. México, D.F.), and SOW 3 = OxOral (Esteripharma S.A. de C.V. México, D.F.).

Immediately before evaluation, products requiring dilution were prepared following the indications on their respective labels using distilled-and-deionised sterile water. Sporicidal activity was evaluated against *Bacillus atrophaeus* ATCC-9372 spores (SPS Medical, Rush, NY) as described previously.<sup>14-16</sup> The sporicidal potency test was conducted in triplicate. Briefly, following strict aseptic technique, 10<sup>6</sup> spores were suspended in 50 mL of the chemical product under evaluation and maintained at room temperature (~22°C) for 6 hours. For worst case scenario conditions the exposure time was 6 hours in the presence of 1% bovine serum albumin (Sigma Chemical Co. St. Lois, MO) as organic challenge or with an inorganic challenge in a mixture containing 1% of each of calcium chloride, sodium bicarbonate and magnesium sulphate (J.T. Baker®, Avantor Performance Materials. Center Valley, PA.). After exposure, the suspension was placed in a filtration funnel (Millipore, Bedford, MA), and the chemical product was removed by suction through a 0.22 µm polyvinylidene filter membrane (Durapore®, Millipore). The spores retained on the membrane were rinsed with 100 mL of 1% sodium bisulfite (NaHSO<sub>3</sub>) and 200 mL of sterile distilled water

to remove any traces of the product under evaluation. To culture the surviving spores, the filters were placed face-down onto tryptic soy agar (Difco; BD, Franklin Lakes, NJ) and incubated for 5 days at 37°C. A FDA cleared 7.5% H<sub>2</sub>O<sub>2</sub> formulation (Sporox II®; Sultan Healthcare, Englewood, NJ) and 2% glutaraldehyde (Tiodex® Laboratorios Químicos Arvi, S.A. San José, Costa Rica) served as controls for sporicidal activity. Sterile distilled water was used as a negative control. Survival and growth of *B. atrophaeus* was confirmed by colony morphology and light microscopy on stained smears.

## Results

Under best scenario conditions, the SOW solutions showed sporicidal activity after six hour contact time (Table II). Neither DDO nor the nanotechnology product showed sporicidal activity and were no longer tested.

Subject to worst case scenario conditions for 6 hours, the sporicidal activity in SOW solutions 1, 2 and 3 was nullified in the presence of 1% albumin, while hard water did not inhibit the sporicidal activity in these products.

No *B. atrophaeus* growth was observed after exposure of spores to the 7.5% H<sub>2</sub>O<sub>2</sub> formulation (FDA cleared for sterilisation in six hours) or 2% glutaraldehyde, which achieves sterilisation in ten hours. These sporicidal activities were not inhibited by protein or hard water.

## Discussion

The results of this sporicidal-potency test show that both the diallyl disulphide oxide formulation and the nanotechnology product evaluated lack sporicidal activity and are inadequate to sterilise medical and dental instruments. The results indicate also that the presence of protein inhibits the sporicidal activity in the SOW solutions tested, confirming reports that the activity of SOW is reduced by organic contamination.<sup>17</sup> Sterilox® is the only SOW FDA-cleared as a HLD to be freshly generated on site and used on rigorously pre-cleaned instruments.<sup>18</sup>

**Table I. Chemical products evaluated\***

Product	Active ingredient	Recommended contact time	Label claim
DDO	0.008g/100mL diallyl disulphide oxide	15 min	S
NANO	NA	20 min	S
SOW 1	<80 ppm Cl	15 min	S
SOW 2	0.004% active Cl	15 min	S
SOW 3	NA	15 min	S

\*Registered and sold as a sterilants for immersion of instruments.

DDO = diallyl disulphide oxide

NANO = Nanotechnology product.

SOW = Superoxidized water.

NA = Not available.

S = Sterilant

**Table II. Sporicidal activity at 6 hours in emerging germicidal formulations.**

	Best case scenario	1% albumin	Hard water
DDO	+	a	a
NANO	+	a	a
SOW 1	-	+	-
SOW 2	-	+	-
SOW 3	-	+	-
7.5% H <sub>2</sub> O <sub>2</sub>	-	-	-
2.8% glutaraldehyde*	-	-	-
H <sub>2</sub> O	+	+	+

DDO = diallyl disulphide oxide

NANO = Nanotechnology product.

SOW = Superoxidized water.

\* Glutaraldehydes  $\geq 2\%$  are FDA-cleared as sterilants with contact times  $\geq 10$  h

a = Not done, because product failed best scenario conditions.

+ = Growth (failure).

- = No growth.

Essential oils, such as eugenol from clove (*Syzygium aromaticum*), have antiseptic activity. Diallyl disulphide oxide is a component of garlic oil and may have antiseptic activity. No information is available on the composition of the nanotechnology-based product.

The qualitative test system employed provides a screening tool to give a single indication of presence of, or lack of, sporicidal activity. Based on FDA criteria, if growth occurs, the product failed for the intended purpose of sterilising medical and dental instruments. A microbial count of survivors is therefore irrelevant. A simulated use test under worst scenario conditions is one of many evaluations required by regulatory agencies, where products are tested also with the spores dried onto the surfaces of instruments and in the presence of organic soiling.

In this investigation, all products were evaluated under optimal conditions that favoured their performance. Sodium-bisulfite is used to inactivate glutaraldehyde.<sup>19</sup> In this investigation, sodium-bisulfite was used also to neutralize hydrogen peroxide and SOWs, because it can efficiently remove chlorine and hypochlorite salts and readily reacts with dissolved oxygen. For the DDO and NANO formulations, the comparison of the inactivation efficacy of sodium-bisulfite against that of sodium-thiosulfate was unnecessary because these products lacked sporicidal activity.

The sporicidal potency challenge applied in this study does not define the immersion time required to achieve sterilisation. As examples, the 2% glutaraldehyde used as positive control showed sporicidal activity resulting in no-growth after 6 hours exposure. However, glutaraldehydes  $\geq 2\%$  are FDA-cleared as sterilants with contact times  $\geq 10$  hours, while the 7.5% H<sub>2</sub>O<sub>2</sub> product is cleared as a sterilant in six hours at 20°C or as a HLD in 30 minutes at 20°C. No SOW has been FDA cleared as a sterilant. Sterilox<sup>®</sup> passes the AOAC Sporicidal Activity Test in 24 hrs at 25°C but is cleared as a HLD only, with an immersion time of 10 minutes at 25°C.

Not all SOW formulations are created equal. Nosocomial contamination of SOW with mycobacteria has been reported.<sup>20</sup> Therefore, the stability and shelf life of each SOW must be validated. Also, the

recommended immersion time for each SOW remains to be determined using the AOAC sporicidal official method 966.04.

The results of this investigation identify commercially available formulations that pose a risk to patients, and unveil failures in the regulatory evaluation of products intended for immersion of medical instruments. Products without specification of active agents and submission of scientifically clear data on efficacy testing and related conditions must never ever be used for processing of medical devices.

The international infection control community must be aware that many nations need help to develop and enforce scientifically sound standards for the selection and use of disinfectants. Introduction of a simplified and reproducible protocol for testing sporicidal activity will help in the identification of reliable products and the exclusion of unsafe formulations even in resource-limited settings. A sustained international effort, led by infection control officers, policy makers, researchers, educators, and consultants, is required to strengthen national and local health systems, and avoid the marketing of ineffective products that may endanger human lives.

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