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Effects of 100% Ethylene Oxide Test Gas on the Resistance of Ethylene Oxide Biological Indicators

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As of 31 Dec. 2014, the United States Environmental Protection Agency Clean Air Act will prohibit the sale and use of HCFC-based (hydrochlorofluorocarbon) products in the US, including Oxyfume ethylene oxide (EtO) sterilant blends such as Oxyfume 2000, which consists of 8.6% EtO and 91.4% HCFC-124. Biological indicators (BIs) manufacturers will, therefore, have to move to 100% EtO as the test gas for determining the resistance performance of EtO BIs by the end of 2014. In anticipation of this mandatory switch from Oxyfume 2000 to 100% EtO for BI testing, comparison studies were performed to determine if the switch from Oxyfume 2000 to 100% EtO would have any impact on BI resistance label claims.

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Biological indicators (BIs) are used during cycle development, validation, requalification, and routine monitoring of sterilisation processes. Published standards provide appropriate resistance performance characteristics (e.g., D-value) and thus govern the efforts of the manufacturers of biological indicators (1–3). Similarly, BI user guidance documents also reference appropriate selection and use of BIs, and the information provided in such documents will often guide end-user purchase specifications (4). In turn, information about appropriate resistance capabilities of the BIs are sometimes written into regulatory submissions when medical device or pharmaceutical manufacturers seek regulatory clearance for their products.

As of 31 Dec. 2014, the United States Environmental Protection Agency Clean Air Act will prohibit the sale and use of HCFC-based (hydrochlorofluorocarbon) products in the US, and that will include Oxyfume ethylene oxide (EtO) sterilant blends such as Oxyfume 2000, which consists of 8.6% EtO and 91.4% HCFC-124. (Oxyfume is a registered trademark owned by Honeywell International.) This requirement means that all BI manufacturers will have to move to 100% EtO as the test gas for determining the resistance performance of EtO BIs by the end of 2014. Currently, Oxyfume 2000 is often used by BI manufacturers for assessing BI EtO D-value label claims, so it is in the best interest of the BI community (manufacturers and end users) to assess the potential effects of this change.

There have been previous changes to the EtO gas used for BI testing. A previous version of an HCFC mixture, Oxyfume 2002, which consists of 10% EtO, 63% HCFC-124, and 27% HCFC-22, was eliminated by the Clean Air Act in December of 2009. At the time, many BI manufacturers were using Oxyfume 2002 as the source gas in their resistometers when performing BI EtO D-value resistance assessments. As the elimination of Oxyfume 2002 approached, comparative studies were performed to determine if the switch from Oxyfume 2002 to Oxyfume 2000 would have any impact on measured resistance performance. The results of these studies indicated that

Table I: Results from biological indicators manufactured by STERIS Corp, 3M, and Mesa Laboratories, tested for D-value at each facility. SCBI is self-contained biological indicator.

Product tested	Manufacturer label claims		Re-tested by facility:	D-value results (minutes)		Percent difference
	Population	D-value (minutes)		Oxyfume 2000	100% EtO	
Company A strip	1.2×10^6	3.2	A	3.2	2.5	-21.9%
			B	3.6	2.9	-19.4%
			C	2.7	2.7	0.0%
Company A disc	2.3×10^6	3.2	A	3.2	3.1	-3.1%
			B	3.3	2.9	-12.1%
			C	2.7	2.7	0.0%
Company B SCBI	2.7×10^6	3.7	A	3.6	2.6	-27.8%
			B	3.7	2.4	-35.1%
			C	3.2	2.2	-31.3%
Company B strip	2.2×10^6	4.1	A	3.6	2.9	-19.4%
			B	4.1	3.3	-19.5%
			C	2.9	2.2	-24.1%
Company C SCBI	2.7×10^6	3.6	A	3.7	3.2	-13.5%
			B	4.3	2.6	-39.5%
			C	3.5	2.6	-25.7%

Table II: Requirements for BI D-value when tested in EtO.

Reference	When tested at 600mg/L, 54 °C, 60% relative humidity
USP, Table I in Chapter <1035>	"Range of D-values for Selecting a Suitable Biological Indicator" Minimum 2.5 min, maximum 5.8 min.
ANSI/AAMI/ISO 11138-2:2006/(R)2010, paragraph 9.5	"…shall have a D value of not less than 2.5 min at 54 °C…"
EP 7.0, Section 5.1.2	"The D-value is not less than 2.5 min…"

USP = *United States Pharmacopeia*
ANSI = American National Standards Institute
AAMI = Association for the Advancement of Medical Instrumentation
ISO = International Organisation for Standardisation
EP = *European Pharmacopoeia*

the change in gas had no significant effect on the measured resistance of the BIs. As an example, MesaLabs EZTest lot G-162 displayed a D-value of 3.60 minutes when tested in Oxyfume 2002 or 3.58 minutes when tested in Oxyfume 2000. STERIS indicators showed similar results. STERIS VERIFY tested at 4.0 and 3.9 minutes, Spordex strips at 4.2 and 4.7 minutes, and Spordex discs at 3.4 and 3.5 minutes, when exposed in Oxyfume 2002 and Oxyfume 2000, respectively.

Comparability studies

In anticipation of this mandatory switch from Oxyfume 2000 to 100% EtO for BI testing, additional comparison studies were performed to determine if the switch from Oxyfume 2000 to 100% EtO would have any impact on BI resistance label claims. The test results for this change were markedly different than the change between mixed gasses. Despite programming both resistometers for identical exposure parameters (600 mg/L EtO, 54 °C, 60% relative humidity [RH]), Mesa BIs (both paper strip and self-contained versions) were showing a 26% to 39% reduction in measured D-value when tested in a resistometer using 100% EtO as the source gas. Having obtained these results, Mesa obtained strip and self-contained BIs (SCBIs) from other manufacturers, and when tested in Mesa resistometers, the same trend was observed. Specifically, the D-values when tested with 100% EtO were 29% to 51% lower than the results from the Oxyfume tests. (These data were the subject of a whitepaper [5] previously posted on the MesaLabs website).

Results and discussion

Based on these results and other preliminary tests performed by other BI manufacturers, this issue was brought up for discussion at the Association for Advancement of Medical Instrumentation, AAMI ST/WG 4, Biological indicators working group meeting in Alexandria, Virginia on 15 Oct. 2012. The result of that discussion was the decision to launch a collaborative effort amongst the three BI manufacturers that possessed the ability to perform both Oxyfume 2000 and 100% EtO exposures. Each manufacturer (STERIS Corporation, 3M, and Mesa Laboratories) agreed to exchange BIs to be tested by the other parties and share results. Exchanged BIs included spore disc, spore strip, and self-contained BI configurations.

The results in **Table I** are presented in a manner that protects the identity of the manufacturer. Of the 15 results, 13 showed a decrease in resistance when tested using 100% EtO as the

source gas and two instances showed no change in measured resistance. There were no observations of a higher measured resistance for BIs tested in 100% EtO. Specifically, the D-values in this round of testing are 0% to 39.5% lower when tested in 100% EtO as compared to the Oxyfume results, with an average reduction in measured D-value of 19.5%.

It is unknown what causes the lowered resistance measurement when 100% EtO is used as the resistometer source gas. The authors speculate that when using an Oxyfume blend gas, the HCFC competes with the EtO molecules for access to critical binding sites on the spores. Such competition would not exist when 100% EtO is the source gas for the resistometer cycles. With HCFC present in the exposure chamber and blocking EtO molecule access to the critical binding sites, the result is fewer alkylation reactions and thus a decreased lethal insult to the spore, despite both chambers providing 600 mg/L EtO, 54 °C, 60% RH conditions.

Recommended standards

The differences in measured D-value are cause for concern when considering the resistance recommendations that appear in current published standards. **Table II** shows the recommendations that appear in *United States Pharmacopeia (USP)*, International Organisation for Standardisation (ISO), and *European Pharmacopoeia (EP)*. When use of Oxyfume 2000 becomes prohibited and BI manufacturers switch to 100% EtO, the labeled D-value claims will likely show a pronounced downward shift consistent with the test results reported here, compared to historical values. The authors stress the fact that the BIs are not changing; the spores have not become less resistant to the sterilisation process. Rather, the "measuring stick" has changed with the changeover from Oxyfume 2000 to 100% EtO as the source gas used in resistometers. Because the measuring stick is changing, published standards will need to follow suit and adjust the verbiage in the relevant documents.

The data from this study indicate a decrease in measured value of up to 39.5% solely due to the use of 100% EtO as the supply gas. As such, the authors would recommend a change in published ranges to match. Whereas 2.5 minutes to 5.8 minutes were a suitable range of D-value for BIs tested in an HCFC blend gas, ISO and *EP* should consider lowering the "not less than 2.5-minutes" specification to "not less than 1.5-minutes" to accommodate the observed percent differences in the test data. *USP* provides a lower and upper range of resistance that is typical for EtO BIs. As such, the current *USP* citation of 2.5 to 5.8 minutes should be adjusted to perhaps 1.5 to 5.8 minutes (i.e., 2.5 – 40% = 1.5) for BIs that are tested using 100% EtO as the resistometer source gas.

Given that making changes to published standards can takes months, or even years to complete, the industry will likely experience a gap in time where available BIs (tested in 100% EtO) may not have a resistance label claim that meets the minimum values that currently appear in *USP, EP,* and American National Standards Institute (ANSI)/Association for the Advancement of Medical Instrumentation (AAMI)/ISO documents. Furthermore, end user purchase specifications and/or the information in the end user's regulatory submissions may also conflict with what is available from BI manufacturers, as the end user's stated values were based upon BIs that were resistance tested with an EtO/HCFC blend gas, rather than 100% EtO.

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