Determination of spore lethality at low Ethylene Oxide gas concentrations at atmospheric pressure.

PURPOSE:

This study was performed to establish the following:

a) To document that low levels of Ethylene Oxide in an atmospheric pressure environment can achieve a lethal state.
b) To establish whether there is any correlation between Ethylene Oxide concentration (at low concentrations) and cycle lethality (expressed as D-values).
c) To create a mathematical model or formula that could be used to predict the total log kill within a sterilization cycle using only two variables—gas concentration (mg/L) and temperature (°C).
d) To apply the mathematical model established above to an existing validated EOGas Series 3 process, to calculate a theoretical log reduction over the full exposure cycle.

D-Values are determined at different gas concentrations, spore reduction lethality can be calculated using the mathematical model, and the mathematical model can be applied to an established cycle.

BACKGROUND:

Unlike traditional pallet chambers, the Andersen Products, Inc. EOGas Series 3 process employs the use of a flexible sterilization bag or chamber at atmospheric pressure. The sterilization load is placed into the sterilization bag along with an Ethylene Oxide gas cartridge. The sterilization bag is hermetically sealed and placed into the sterilizer, the cartridge is activated through the wall of the bag, and the sterilizer door is closed. Once in the sterilizer, the sterilization load equilibrates with the sterilizer temperature and the Ethylene Oxide diffuses throughout the sterilization load (peaking at 1 to 2 hours). The gas then slowly diffuses through the permeable sterilization bag over the course of the 16-hour cycle. As a result, the concentration within the sterilization bag peaks rapidly and then slowly declines.

The sterilization bag:

The BIER vessel was not chosen as appropriate equipment in this study since it is difficult, if not impossible, to create the atmospheric pressure environment that is present in the EOGas sterilization bag—the injection of ETO creates pressure within the BIER vessel—a phenomenon that does not exist in the EOGas diffusion process (the flexible nature of the bag prevents the buildup of pressure that you would otherwise see in a rigid wall container or vessel). Since the purpose of a BIER vessel is to create a stable, reproducible exposure environment, we employed the use of a non-permeable sterilization bag—from which the Ethylene Oxide cannot escape; this allows us to create the static gas concentration that is necessary in establishing D-values at different gas concentrations.

Relative Humidity:

A level of humidity above 30% is typically used for effective sterilization. However studies have shown that it is the hydration of the spores that affects the rate at which sterilization is achieved[1] rather than the relative humidity during EO exposure. It has also been demonstrated that while relative humidity is a factor in sterilization efficiency, it has little effect on cycle lethality when the process range falls between 30% and 90%[2]. Within this range humidity can be considered a constant. For all D-values performed, we ensured that the exposure environment relative humidity fell within this range.

Temperature:

Temperature is a critical factor in defining the D-value. It is one of the defining process parameters for successful sterilization. Since the exposure phase within the EOGas process starts at room temperature and the sterilization load may take up to two hours to reach 50° C, it is important to consider the corrected D-value or the rate of lethality during this warm-up time. Conveniently, the Q10 factor can be employed to assist in calculating rates of lethality at sub-optimal temperatures.

The Q_{10} temperature coefficient:

The Q10 temperature coefficient is a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C. Q10 values have been demonstrated to range between 1.8 and 2.7 for sterilizing *Bacillus atrophaeus* spores, depending on the substrate[3]. For the sake of this study, we have adhered to the conservative approach and chosen a Q_{10} factor of 2.7. What this means is that for every 10°C drop in temperature (from 50°C) the D-value will be assumed to increase by a factor of 2.7. (For example, a D-value of 20 minutes at 50°C will correspond to a D-value of 146 minutes at 30°C).

Creating known D-values at different gas concentrations:

A series of D-values were generated at a process temperature of 50°C and gas concentrations of 150mg/L, 100mg/L, 75mg/L and 50mg/L[4]. When the gas concentration in mg/L is plotted against rate of kill (D-value), an inverse relationship between the two is evident—as gas concentration increases, the D-value decreases. Figure 1 demonstrates the linear nature of the D-value versus gas concentration ($r^2 = 0.98$). The linear nature of the graph allows us to use the slope as a known standard, from which any gas concentration between 50mg/L and 150mg/L can be correlated to a D-value—assuming the exposure temperature is at 50°C.

---

[1] Kereluk, R.A., Gammon, R.A., and Lloyd, R.S., "Microbiological Aspects of Ethylene Oxide Sterilization, III. Effects of Humidity and Water Activity on the Sporicidal Activity of Ethylene Oxide," *Appl. Micro*., Vol. 19, No. 1, Jan, 1970.

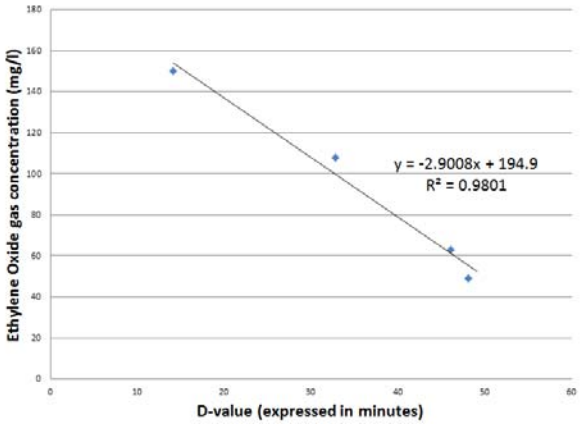
[2] Gregg A. Mosley, John R. Gillis, and James E. Whitbourne, "Calculating Equivalent Time for Use in Determining the Lethality of EtO Sterilization Processes" in Medical Device & Diagnostic Industry Magazine, February 2002

[3] Ernst RR. Ethylene oxide sterilization kinetics, *Biotechnol Bioeng Symp*. 1974;4;865-878

[4] Concentration of ETO in mg/L ± 5%

Determination of spore lethality at low Ethylene Oxide gas concentrations at atmospheric pressure.

Figure 1: D-Values determined at different ETO gas concentrations.



In order to establish reliable and repeatable D-value data, the sterilization variables must equilibrate prior to exposing the BI's—temperature, gas concentration and relative humidity. This was achieved through sealing a series of 10^4, 10^5 and 10^6 biological indicators into glass ampoules—ETO cannot penetrate sealed glass. By using a range of different starting spore populations, we increase the probability of finding a fraction negative time point. The glass ampoules were inverted and attached to the wall of an empty 36L rigid container—providing a static framework within the sterilization bag. The framework was inserted into the sterilization bag and an SEC Ethylene Oxide gas measurement sensor was attached to the other end of the bag allowing for direct ETO measurement. The sterilization bag was placed into a heated sterilizer at 50°C. The gas cartridge was activated and left for approximately two (2) to three (3) hours to allow the gas concentration and temperature to stabilize. Once a steady-state had been achieved, the inverted ampoule necks were snapped (by putting pressure on the ampoule neck against the container); thereby exposing the BI's to the stable sterilization environment. The sterilization bag was left in the sterilizer for a predetermined exposure times, then immediately removed and placed into a fume hood. The BI's were immediately transferred into media and incubated according to the manufacturer's instructions. The D-values were calculated using the Fraction-negative method (Stumbo Murphy Cochran procedure)—see table 1.

Table 1: D-values calculated at 150, 100, 75 and 50mg/l

ETO gas concentration mg/L	D-value (minutes)
150	14.15
100	32.82
75	46.08
50	48.15

The results clearly demonstrate a linear rate of kill, supporting published data that the rate of lethality is inversely proportional to gas concentration. By calculating the slope of the line, we can predict the D-value at any gas concentration between 50mg/L and 150mg/L and since we know the temperature for any given time-point, we can adjust the D-value using the Q_{10} factor.

Predicting log kill during the changing sterilization environment:
By dividing the total sterilization time (16 hours) into 192 equal five (5) minute intervals, and then measuring the temperature and sterilant gas concentration during each interval, we can calculate a theoretical log reduction within that specific time interval. Then, by calculating the sum of all 192 log reductions per time interval, we can calculate the total log reduction throughout the exposure cycle (*L*).

To calculate the theoretical total cycle spore reduction (*L*), we developed a mathematical equation using the Riemann sum shown in figure 2—this equation considers the temperature (°C) and gas concentration (mg/L) at each 5 minute interval. The first part of the equation simply states that the 16 hour exposure cycle was sub-divided into 192 equal time intervals, where gas concentration, temperature and therefore D-value are known (the D-values are based on an experimentally determined relationship to Ethylene oxide concentration—see Figure 1).

Figure 2: The lower bound analysis for log reduction of population

$$L = \sum_{i=1}^{192} \Delta L_i = \sum_{i=1}^{192} f(t_i)\Delta t = \sum_{i=1}^{192} \left(\frac{1}{\mathrm{Dval}(T(t_i), C(t_i))} \right) \Delta t$$

L = total log reduction over the 960 minute sterilization
i = index referring to each of 192 specific 5 minute intervals in the 960 minute sterilization
ΔL_i = the log reduction achieved in the ith interval
t_i = moment of time t in the ith interval
f = rate of log reduction per unit time, measured in log reductions per minute
Δt = 5 minutes, the duration of each interval
T = temperature
C = concentration of gas
Dval = D-value; the number of minutes required to achieve a one log reduction in the population

The second part of the equation states that the amount of log reduction occurring is computed as rate of kill (D-value defined as *f*) multiplied by time. The third part of the equation is an observation that the log reductions per minute are the reciprocal of minutes per log reduction. Since minutes per log reduction is the D-value, *f* becomes 1/Dval.

The use of the lower bound: A conservative approach
The temperature (*T*) and concentration (*C*) that are used to compute *f* are not measured across each entire interval. However, since these parameters and *f* are monotone across each interval, we can establish a lower bound for *f* (*ti*) for each interval by noting

$$f(t_i) \geq \min\{f(t_{start}), f(t_{end})\} = f_i$$

And then concluding,

Determination of spore lethality at low Ethylene Oxide gas concentrations at atmospheric pressure.

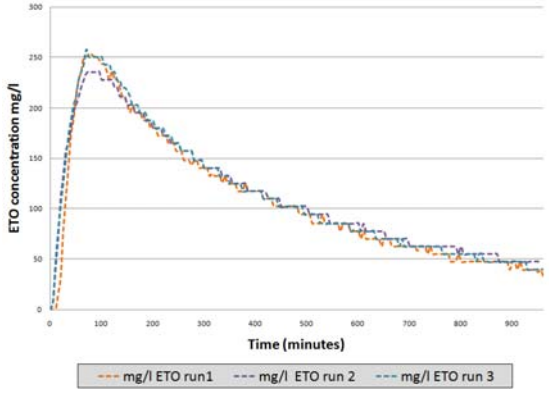
$$L = \sum_{i=1}^{192} f(t_i)\Delta t \quad \geq \quad \sum_{i=1}^{192} f_i \Delta t = L_{bound}$$

The measured (*T*) and (*C*) for the start and end of each interval is used to compute the corresponding Dval and *f*, and then *fi* for each interval, and then L_{bound} is computed from the above formula.

The computation of L_{bound} is asserted to be a lower bound of the true log reduction (L) due to the following conservative policies in the computation:
a) when the gas concentration exceeds the range defined in Figure 1—where we know the slope of the line is accurate, then we assumed the D-value did not continue to increase but defaulted to the maximum established value at (150mg/l), and
b) when the gas concentration dropped below 50mg/L, we conservatively used a kill rate of zero, and
c) we took the conservative approach when considering the Q_{10} value and chose a value of 2.7 to calculate log reduction at lower temperatures.

Applying the formula to an established cycle:
The above concept was applied to a series of three (3) consecutive exposure cycles performed using the Andersen Products, Inc., EOGas Series 3 process. Each sterilization load was kept constant[5]—a single sterilization bag containing 1080 sutures, an AN2018 ETO gas cartridge (containing 17.6g ± 5%), two Humidichips (providing relative humidity to the exposure cycle as the sterilization load is heated to 50°C) and an NIST traceable temperature and relative humidity datalogger. The three (3) consecutive profiles demonstrate a consistent gas concentration (figure 3), temperature (figure 4) and relative humidity (figure 5).

Figure 3: Three consecutive ETO concentration profiles (mg/L) over the 16 hour process



[5] Figures 3, 4 and 5 were generated from a previously validated process using sutures as the product load.

Figure 4: Three (3) consecutive temperature profiles over the 16 hour process

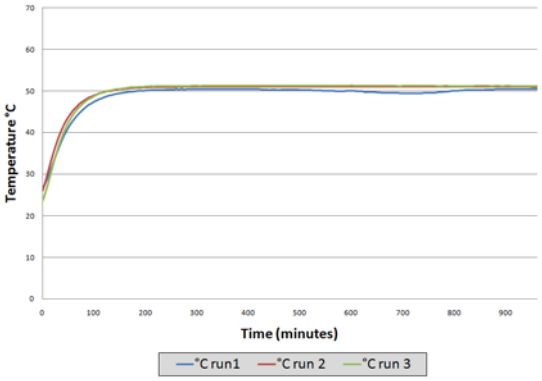


Figure 5: Three (3) consecutive relative humidity profiles over the 16 hour process

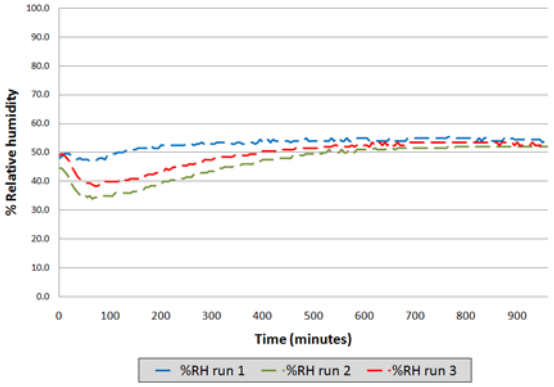


Table 2. Summary from three (3) consecutive sterilization processes (figures 1, 2 and 3)

	Average temperature (°C)	Average %RH	Average ETO concentration (mg/l)
Run 1	48.8	53.1	113
Run 2	49.9	46.5	108
Run 3	49.9	49.2	113

Results:
By applying this formula to our three (3) consecutive profiles, we can estimate the capability of the cycle by calculating the lower bound log reductions of all time intervals and then totaling to establish the total lower bound log reduction (L_{bound}). The total log reduction for runs 1, 2 and 3 are shown in table 3.

Table 3: The theoretical log reduction as calculated using the Riemann sum formula

Exposure cycle	Calculated total spore log reduction (*L*)
Run 1	33.6
Run 2	32.2
Run 3	36.1

CONCLUSION:
The following conclusions can be established from the study:
a) A sterilant level as low as 50mg/L at atmospheric pressure will achieve a lethal level, reducing the spore population by one (1) log (this is, by 90%) every 48 minutes—thereby achieving a sterility assurance level of 10^{-6} in less than ten (10) hours (48 minutes x 12 logs).

Determination of spore lethality at low Ethylene Oxide gas concentrations at atmospheric pressure.

b) A linear relationship between gas concentration and spore lethality (D-value) exists between 50mg/L and 150mg/L thereby allowing us to interpret known gas concentrations into rates of kill.

c) The D-Value vs. time linear relationship can be used to determine the model in Figure 1. The Spore Reduction Lethality model can use the varying temperature and gas concentration throughout the sterilization cycle to predict the total log kill of a 16-hour sterilization cycle in the EOGas Series 3 process.

d) When this model is applied to three (3) consecutive cycles, we can calculate a minimum theoretical log reduction (*L*) of 32.2—in excess of the industry required (*L*) of 12 (12 log reduction also known as the overkill cycle). This data provides supplemental evidence to demonstrate that the EOGas Series 3 sterilization cycle at 50°C using 17.6g ETO can achieve an SAL of $\geq 10^{-6}$.

Daryl L. Woodman, B.Sc. (Hons)
Andersen Scientific, Inc., July 07, 2011