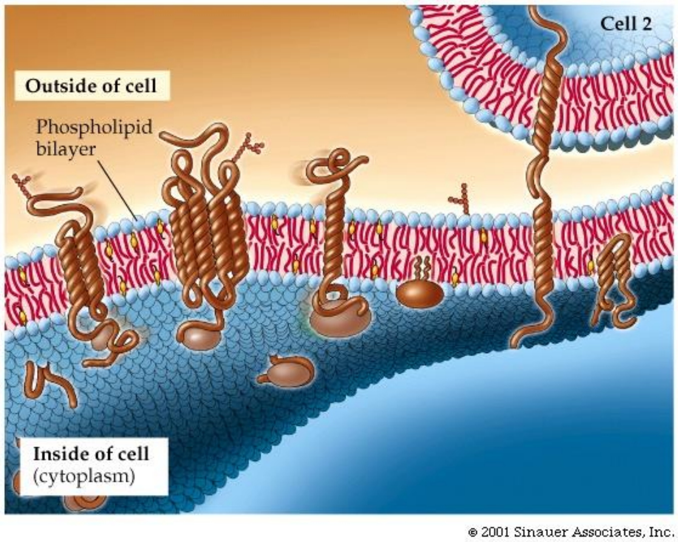
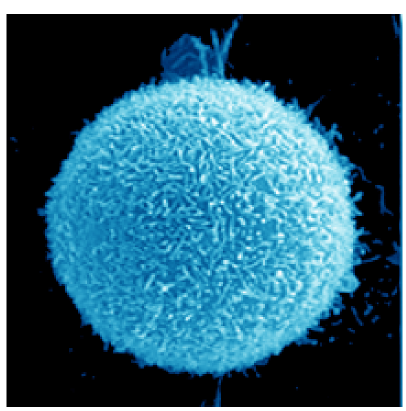
How does Ethylene Oxide sterilize?

The simple answer is that ethylene oxide rips apart cell membranes, causing microorganisms to die.



This is a diagram of a cell membrane showing the proteins on the surface of the cell and how they interact with other proteins.

Ethylene oxide is a colorless, flammable gas that reacts with water, organic and inorganic acids (such as amino acids, which make DNA), alcohols, phenols, and most of all amines. When it reacts with these different chemicals it undergoes a process called alkylation. Alkylation is a chemical reaction where a hydrogen atom is replaced with a hydroxyethyl radical. This process causes the amino acids that are found in proteins to change their shape. These proteins typically penetrate the cell membrane, forming a channel between the interior of the cell and the outside of the cell. When these proteins change their shape it causes a breach in the cell membrane, killing (lyzing) the microorganism.



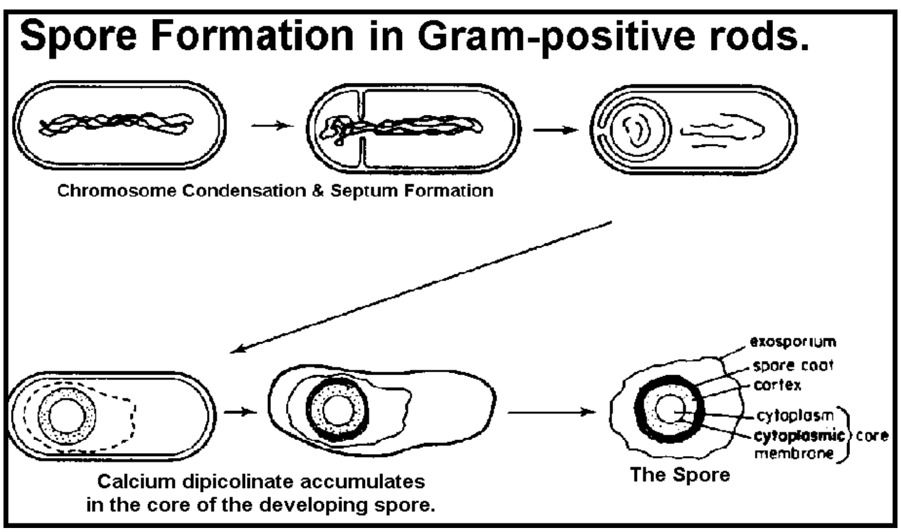
This is a photo taken with a microscope of the surface of a mast cell. The ridges are the surface proteins.

Bacterial spores: what are they and why does humidity matter?

Bacteria are microorganisms that can be pathogenic (disease causing) or saprophytic (not harmful). Some bacteria have an interesting defense mechanism called sporulation. This means that when the conditions are no longer optimal for growth they will be able to form a spore structure. Environmental changes that may trigger sporulation can include temperature changes, humidity changes, a lack of nutrients, or chemical changes. By forming a spore the bacteria can protect themselves from the harsh environment until the conditions improve.

The Spore Cycle

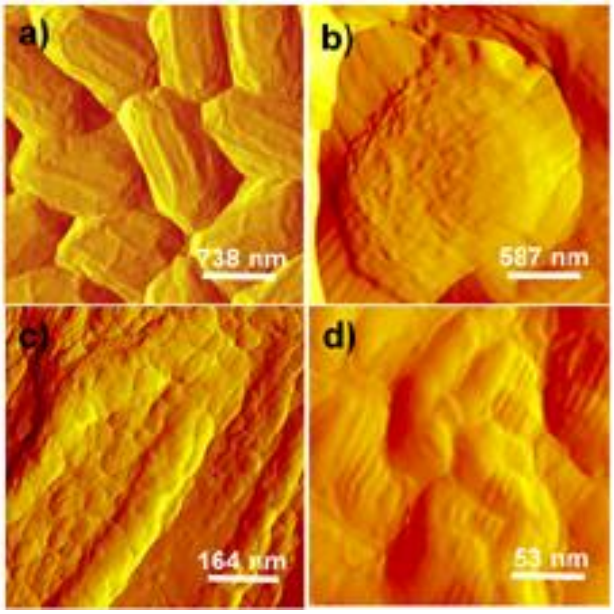
A bacterium becomes a spore in 4 steps. First the environment will become unsuitable, triggering the bacterium to replicate its DNA. Then the cell membrane begins to pinch in around the DNA copy, creating a protective wall around it. Then a peptidoglycan (very tough) wall forms between the two membranes and finally, a strong outer coating is made of proteins to surround the spore. These tough shells encase the spore, protecting it from changes in the environment like temperatures as high as 3,000 degrees C and as low as -269 degrees C. These bacterial spores are the most resilient life forms on Earth and some have been able to be revived after thousands of years. In the sterilization industry we use them as our biological indicators because they are so difficult to kill. If all the bacterial spores are killed, it is safe to assume all other microorganisms will be dead as well.



This is a diagram of a bacterium undergoing sporulation.

Humidity: How it affects spores

It has been known for some time that humidity has an effect on ethylene oxide sterilization. Studies have shown that with an optimal humidity can reduce kill time by half. Why this is true is unknown, however recent data offers some clues as to how humidity helps kill the spores in gas sterilization. Scientists have shown that spores will swell up in size in environments with a high relative humidity. This is important because scientists used to think that spores were dormant and did not interact with the environment. It is also important because it may be the link in understanding why humidity affects sterilization. It is now hypothesized that when the spores swell up in response to humidity, the pores on the surface of the cell expand, making it possible for the ethylene oxide to penetrate the spore and kill it.



This is a photo of a) a spore, b) a spore undergoing reactivation, c) a close up of the ridges of the spore, and d) a close up of (c) showing tiny 4-6 nm rods that are present along the spore ridges.

Citations:

Westphal et al. *Kinetics of size changes of individual Bacilius thuringiesnsis spores in response to changes in relative humidity*. PNAS March 18, 2003 (100)6: 3461-3466

Driks, A. *The dynamic spore*. PNAS March 18, 2003 (100)6: 3007-3009

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2004 Sales Week Presentation by Daryl Woodman

Dr. Mary Johnson, Spore Cycle Picture

Dr. Rong Wang, Spore Microscope Picture

Sintaeur Inc., Cell Membrane Picture

Dr. Ulrich Weisner, Mast Cell Picture