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OBSERVATIONS REGARDING THE STERILIZING EFFECT OF ETHYLENE OXIDE/CARBON  
DIOXIDE MIXTURES ON BACTERIA AND BACTERIA SPORES

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## OBSERVATIONS REGARDING THE STERILIZING EFFECT OF ETHYLENE OXIDE/CARBON

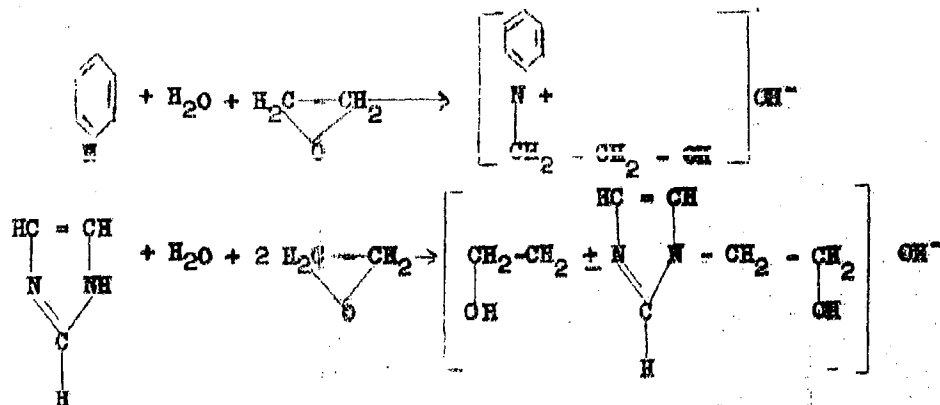
### DIOXIDE MIXTURES ON BACTERIA AND BACTERIA SPORES

[Following is the translation of a German-language report by K. Bucher, Hausmann Co. Laboratories, St. Gallen, Switzerland, in Reports to the Int. Cong. of Microb. Standardization, 1960, pages 397-401.]

The capacity for reacting of the 1, 2-epoxides -- unusual from the chemical aspect -- of which ethylene oxide represents the simplest homologue, has been exploited in the biological application fields also since the '30s. At first the strong biological effectiveness of ethylene oxide was used in anti-pest measures. The advantage of the possibility of a cold sterilization, particularly with thermolabile substances in the foodstuffs sector, was recognized next. In the pharmaceutical and medical field, where the problem of a real sterility naturally constitutes an essential condition, interest in cold sterilization methods awoke first with the appearance of the thermolabile artificial materials. Now, over the last ten years, a whole group of writings have been published that deal with the sterilizing effect of ethylene oxide. According to these studies, fungi, yeasts, and a whole group of bacteria types are killed with certainty by ethylene oxide (1, 2, 3, 4, 5, 6, 7). The sterilization has been described of, among other things, infusion instruments, catheters, cystoscopes, and other medical implements (6, 7, 8). Viruses, even, e.g. foot and mouth disease virus (9), are said to be inactivated by ethylene oxide. On the other hand however there have been voices raised according to which full sterility cannot be attained with ethylene oxide; or they question there being an effect on viruses (10). Thus Mayr and Kaemmerer (2) reported that dry gelatine could not be made sterile with only ethylene oxide in spite of the use of very high concentrations. The authors therefore recommend for this purpose mixtures of ethylene oxide + propylene oxide or ethylene oxide + methyl bromide. This situation shows clearly that published results cannot simply be taken over in every case, so long as completely similar test conditions do not exist. A broad and most interesting field of application is the cold sterilization of thermolabile pharmaceutical products. Thus according to Kaye and colleagues (11) penicillin in powder form can be sterilized by ethylene oxide without reduction of its activity, while streptomycin loses over a third of its activity in the same conditions. Diding (12) reported sterilizing results even in the case of aqueous penicillin solutions; but the layer of liquid to be sterilized must not amount to more than 1 cm.

One knows on the other hand that alterations of effect are to be expected owing to the strong liability of ethylene oxide to react with functional groups of many substances. Thus according to Windmueller and

colleagues (13) in many foodstuffs most of the vitamins of the B complex and also some of the amino acids, namely histidine, methionine, and lysine, which are essential for Man, are partly destroyed. The reaction proceeds as is shown in the following formulas.



These examples will serve to show that the sterilization of thermolabile pharmaceuticals involves a broad field that still requires much clarification.

Ethylene oxide has assumed by far the greatest importance among the compounds that are really suitable for cold sterilization.  $\beta$ -propiolactone has indeed been recommended as effective for similar purposes, but just recently Wisely (14) has drawn attention to certain carcinogenic qualities of this compound. Thus ethylene oxide may well rightly maintain the place it has won: it not being known to have side-effects of this sort.

For working in factory-type surroundings ethylene oxide is mostly used as a mix with carbon dioxide, either as T-gas, a mixture of 9 parts ethylene oxide + 1 part carbon dioxide, or as Cartox, a mixture of 1 part ethylene oxide + 9 parts carbon dioxide. The latter mix possesses the advantage of being as a mixture the only non-inflammable and non-explosive one. Cartox is preferred in our firm on the grounds of safety as well as of the simplicity of handling it. Since 1957 infusion instruments have been sterilized almost daily, and in this case on the basis of repeatedly conducted sterilization checks the procedure used may be judged to be reliable and sure. A qualifying reference must however be made to the fact that the components made out of artificial materials are poor in germs by their nature when properly manufactured and stored, and that the assembling of the infusion instruments takes place in hygienically unexceptionable and practically dust-free rooms, with the greatest cleanliness being maintained. In order to create the most difficult conditions for the checks, pieces of filter are placed in the check instruments, which have been exposed in the work-places for twelve hours. So as to be able to give

us a complete picture of the limits of the effectiveness of Cartox in the case of substances as well that are not of themselves as poor in germs as are many of the artificial materials, with their hardly humidifiable surfaces, the tests described below were carried out with bacteria and their spores respectively.

The preparation of the spores suspensions was carried out in accordance with a procedure that is modeled on a method described by Grundy and colleagues (6). Pieces of filter paper were used, about .3<sup>2</sup> cm in size, which had been dipped in spores suspensions that had been washed several times and which had been dried in a vacuum at 35°. For the tests with the vegetative bacteria forms, pieces of filter paper of the same size were also used, which were on the other hand dipped in infected prepared liquid culture mediums and afterwards kept on "Petri"-dishes at 20°. As our only concern was with the clarification of the total sterilizing effect without regard to any eventual quantitative conditions, the tests were transferred, as in vaccination, immediately after they had been Cartox-treated, onto bouillon-, or onto thioglyco- late-culture mediums which were incubated at 37° or 20° for 10 days. *Bac. subtilis* (Note: obtained from the Bacteriological Institute of Zuerich University.); soil bacteria (Note: obtained from the Bacteriological Institute of Zuerich University.); *bac. mesentericus* (Note: obtained from the Bacteriological Institute of the Cantonal Hospital St. Gallen.); *E. coli* (Note: obtained from the Bacteriological Institute of the Cantonal Hospital, St. Gallen.); *staphylococcus aureus* (Note: obtained from the Bacteriological Institute of the Cantonal Hospital, St. Gallen.); streptococci, of an undetermined variety (Note: obtained from the Bacteriological Institute of the Cantonal Hospital, St. Gallen.); and *lactobacillus fermenti*, were the sorts of germs used.

The infected pieces were placed:

- (a) in the droppers of the assembled infusion instruments, having the ends fitted with artificial material caps, which were closed with non-greased absorbent cotton;
- (b) in empty polyethylene bags, with sides .05 mm thick, and 6 x 4 cm in size;
- (c) in polyethylene bags of the same size, that contained 5 ml of sterile .9% sodium chloride solution.

All the polyethylene bags were sealed before the treatment with Cartox. The Cartox-treatment was conducted in a hermetable pressure chamber, which had been evacuated before the introduction of the gas up to 40 mm of the mercury column by means of a water jet pump. After Cartox-treatment had been carried out it was evacuated afresh; and filtered air was then allowed to flow in. Table 1 [see appendix] shows in the case of non-spore-forming bacteria the sterilizing effect of 1,000 mg ethylene oxide/l, corresponding to 4 atmospheres of Cartox, at 20°C after a 14 hour exposure period.

All the test series demonstrated that sterility is attained in the conditions mentioned.

The sterilizing of spore-formers (presented more difficulty) because the spores showed themselves to be obviously resistant to the Cartox-treatment. The results in question are shown grouped in Table 2.

It was shown that often no sterility was to be attained with a Cartox pressure of 4 atmospheres, even though the reaction temperature had been raised to 40°C or 50°C and the reaction period to 20 hours.

As a factor that should promise results, there remained a raising of the ethylene oxide concentration, i.e. a further raising of the Cartox pressure. However our attention was drawn by some information from our Cartox supplier to an additional possibility of an increase in the effect: the influence of an appropriate air-conditioning on that which is to be sterilized. According to this information, the degree to which they have been soaked is of essential importance in the killing of spores. A pre-air-conditioning with very high air-humidity several hours before sterilization is said to lead to good results.

The test of ours reproduced in Table 3 were therefore conducted with an air-humidity of practically 100% being maintained. This device seems in fact to have been successful.

In contrast to the earlier, insufficient, results, with a Cartox pressure of 4 atmospheres sterility could be attained in the tests without liquid after only 14 hours, regardless of the reaction temperature. In the case of the tests inserted in .9% sodium chloride solution on the other hand, the reaction temperature which was raised to 40°C seems to have been of some influence as regards the spores of bac. mesentericus and the soil bacteria. Although in the last conditions in every case sterility was achieved, we considered the area of safety with the sterilization of liquids somewhat too small. It seems to us that nothing less than a Cartox pressure of about 6 atmospheres is clear in its results, and of a sufficient degree of safety, for the amounts of liquid and the sorts of germs investigated; and this corresponds to a ethylene oxide concentration of about 1500 mg/l. In future in our opinion in the case of material containing spores, reaction temperatures of over 20°C and exposure times of at least 10 hours ought to be observed. At the same time, in order to be safe from surprises, it is an absolute prerequisite with preparations of the sort that there should be sterility checks over a long period.

To sum up, it can be established that:

The concentration of ethylene oxide is what is mainly decisive for the sterilizing effect. When this concentration is great enough, the reaction can be increased, by adequate humidification and raising of the

reaction temperature. For material containing spores, gas application times of over 10 hours must be selected. The sterilizing effect is as good in the case of thin-walled artificial material packaging as in that of open systems. With aqueous solutions sterility was likewise achieved in the test conditions.

As Cartox is not inflammable or explosive and as also the toxicity is relatively small, its working in factory-type pressurized systems may be judged to be simple and foolproof.

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FIGURE CAPTIONS

Table 1. Effect of ethylene oxide on non-spore-forming bacteria

Cartox pressure 4 atmospheres  
 Temperature 20 - 25°  
 Exposure period 14 hours

E. coli 0  
 Staphylococcus aureus 0  
 Streptococcus, undeter-  
 mined variety 0

0 = sterile.

X = growth.

Table 2. Effect of ethylene oxide on spore-forming bacteria

Cartox Pressure 4 atmospheres  
 Exposure period 20 hours

Reaction temperature 20° 40° 50°

Bac. subtilis ...  
 Bac. mesentericus...  
 Soil bacteria...

X = growth.

X<sub>40</sub> = 40% of tests were unsterile, 60% sterile.

Table 3. Effect of ethylene oxide on spores suspensions.

Pre-air-conditioning: 12 hours at  
 about 100% air-humidity

Cartox pressure 4 atmospheres 8 atmospheres

Reaction temperature 20°...

Exposure period 2 h ...

Spores of:

Bac. subtilis

A . . .

B . . .

C . . .

Bac. mesentericus

A . . .

B . . .

C . . .

Soil bacteria

A . . .

B . . .

C . . .

Lactobacillus

A . . .

- A: infected test in droppers, open system.
- B: infected test in sealed polyethylene bag.
- C: infected test in 5 ml sodium chloride solution in sealed polyethylene bag.
- 1. as representative of non-spore-building bacteria.

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