Ethylene Oxide

For use as a gaseous sterilant (PT2)

Document IIIA

Section 5

Effectiveness against target organisms and intended uses

February 2020

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Please refer to "Technical Notes for Guidance on Dossier Preparation including preparation and evaluation of study summaries under Directive 98/8 EC Concerning the Placing of Biocidal Products on the Market (Appendix 7.1 and 7.2)" for a list of the Standard Terms and Abbreviations used in this document.

Section A5		**Effectiveness against target organisms and intended uses**	
Subsection (Annex Point)			**Official use only**
5.1	**Function (IIA 5.1)**	Gaseous Sterilant	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA 5.2)**	Effective against bacteria, including bacterial spores, fungi and viruses.	x
5.2.1	**Organism(s) to be controlled (IIA 5.2)**	Bacteria (including spores), fungi and viruses	x
5.2.2	**Products, organisms or objects to be protected (IIA 5.2)**	Industrial sterilisation of single use medical devices, before these are made available on the market.	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA 5.3)**		
5.3.1	**Effects on target organisms (IIA 5.3)**	Ethylene oxide is used as a sterilant and kills all bacteria (including spores) and fungi. It also inactivates viruses. A number of review articles are available on the efficacy and mode of action of EtO. These include Richards *et al.* (1984), Page (1993) and Sintim-Damoa (1993), Russell (1998), Hoxey and Thomas (1999).	x
5.3.2	**Likely concentra-tions at which the A.S. will be used (IIA 5.3)**	400 to 1,000 mg/L under controlled conditions of temperature and humidity.	x
5.4	**Mode of action (including time delay) (IIA 5.4)**		

5.4.1 Mode of action	The mode of action of EtO is well understood. It is a highly reactive alkylating agent and reacts with proteins, amino acids and the nucleic acids, guanine (to give 7-(2'hydroxyethal) guanine). Reaction with the phosphated guanine component of DNA appears to result in bacterial cell to lose their ability to replicate (Michael & Stumbo, 1970). See also Marletta & Stumbo 1970, Winarno & Stumbo 1971 for further details on the mode of action of EtO.	
5.4.2 Time delay	This will be dependent on the temperature, humidity and concentration of ethylene oxide.	
5.5 Field of use envisaged (IIA 5.5)		
MG01: Disinfectants, general biocidal products	PT2 as a sterilant	
5.6 Use: industrial, professional, general public (IIA 5.6)	Industrial only	
5.7 Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA 5.7)		
5.7.1 Development of resistance	As EtO is used at concentrations required to achieve sterility there are no surviving populations to develop resistance. Many factors will affect the efficacy of EtO (see above) resulting in micro-organisms being more resistant under certain conditions e.g. low humidity. It should be noted however that this is not resistance to the EtO but a factor associated with the conditions under which the EtO is applied. Literature studies have identified no cases of true EtO resistance having been reported. Studies on resistance to EtO have identified the most resistant species to EtO (Dadd and Daly 1979). Fungal spores and vegetative bacteria exhibiting low resistance except after drying in organic material. Bacillus spores including *Bacillus subtilis var niger* (now *Bacillus atrophaeus* are reported to be amongst the most resistant species to EtO. *Bacillus atrophaeus* (formerly known as *Bacillus subtilis var. niger*) are used in biological indicators to validate EtO processes.	
5.7.2 Management strategies	Under specified use conditions ethylene oxide is a sterilant so there is no opportunity for micro-organisms to develop resistance as they are killed during the sterilisation process.	

5.8 Likely tonnage to be placed on the market per year (IIA 5.8)	See Confidential Appendix.	
Evaluation by Competent Authorities		
	Evaluation by Rapporteur Member State	
Date	02 November 2020	
Materials and Methods	Not relevant	
Results and discussion	Not relevant	
Conclusion	**Comment (5.2, 5.2.1)**: These are the target organisms claimed by the applicant. However, efficacy is only demonstrated against bacterial spores (*Bacillus atrophaeus*), in ISO 11135:2014 validation studies. *B. atrophaeus* is a recommended test organism for ethylene oxide sterilisation in accordance with ISO 11135:2014 due to a high resistance. However, it was concluded at WG-III-2020 that this is not sufficient for claiming efficacy also towards fungi and viruses. **Comment (5.3.1)**: At WG-III-2020, new information was required to demonstrate the efficacy of the active substance. Two ISO 11135:2014 validation studies were submitted by the applicant (please see Doc IIIB5.10/06 and /07 for details). The details on the information summarised in table A5.3.1-01 below has been suggested updated in line with Table A.12 in the CAR, to reflect the changes agreed in the RCOM table and at WG-III-2020. However, when it comes to the eCA's evaluation, please se Doc IIIB5. **Comment (5.3.2)**: The following application rate, i.e. the concentration of ethylene oxide in the sterilisation chamber, has been substantiated by the provided ISO 11135:2014 studies: 300 mg/L (based on approx. mean concentration from the two studies) Please note that other application rates may be used, as long as they are sufficiently validated according to ISO 11135:2014.	
Reliability	n/a	
Acceptability	Acceptable	
Remarks	The ISO standard ISO 11135:2014 (Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices), describes requirements that, if met, will provide an ethylene oxide sterilization process intended to sterilize medical devices, which has appropriate microbicidal activity. The eCA considers that ethylene oxide is a sufficiently effective biocidal agent for the intended biocidal use, i.e. for the sterilisation of single-use medical devices, when used in compliance with this ISO standard, and hence that the requirements for efficacy information on the active substance level is met.	

Table A5.3.1-01: Summary table of experimental data on the effectiveness of the active substance against target organisms at different fields of use envisaged, where applicable

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Sterilant	PT2	Gas mixture: 6 % ethylene oxide and 94 % carbon dioxide	*Bacillus atrophaeus ATCC 9372*	Validation of sterilisation acc. to ISO 11135:2014, in commercial sterilisation chamber on a medical device sterilisation facility. The "overkill approach" of ISO 11135:2014 was applied to validate the sterilisation efficacy: Three consecutive experiments (half-cycles) which must result in total inactivation of a microbial population of not less than 10^6.	Ethylene oxide concentration in sterilisation chamber (in each of the half-cycles): 291-318 mg/L 292-328 mg/L 300-332 mg/L Exposure time: 60 min Temperature: 55 ± 3 °C Humidity: 40-95 % Pressure: 2.0 ± 0.2 bar	Some modifications to the sterilisation process had to be made in order to meet the required sterilisation level. After these modifications, no microbial growth occurred in any of the half-cycles.	[redacted] (2018a)
Sterilant	PT2				Ethylene oxide concentration in sterilisation chamber (in each of the half-cycles): 269-295 mg/L 280-290 mg/L 280-300 mg/L Exposure time: 70 min Temperature: 60 ± 3 °C Humidity: 40-95 % Pressure: 2.0 ± 0.2 bar		[redacted] (2018b)
Sterilant	PT2	100% Ethylene oxide liquid	*Bacillus stearothermophilus* and *Bacillus subtilis*.	Evaluation of the influence on storage conditions of biological indicators on EtO sterilization	Biological indicators were exposed in the test chamber	The initial time taken to reduce the spore population of *B. subtilis* by 90% was 1.8 minutes. Storage at ambient, refrigerated and freezer conditions and as well as conditions of 20ºC and 20 and 44% relative humidity had no	R.R. Reich & L.L. Morien (1982)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
					Ethylene oxide concentration: 1,200 mg/L Temperature: 55 ± 1 ºC Relative humidity: 60 ± 10 %	significantly observable effect on ethylene oxide resistance.	
Sterilant	PT2	Ethylene Oxide	*Bacillus subtilis var. niger*	Spores hermetically sealed in various polymeric films were exposed to ethylene oxide at a concentration of 900 mg/L relative humidity of 40% and a temperature of 130ºF	EtO exposure occurred in a test chamber	In order of ethylene oxide resistance the study found that order polyethylene=poly vinyl chloride nylon< cellophane/polyethylene laminate< phenoxy < mylar polyethylene laminate. As the thickness of the polymeric film increased beyond a certain level the resistance to ethylene oxide increased. Spore strips that have been desiccated prior to ethylene oxide exposure demonstrated a high increase in ethylene oxide resistance. Introduction of moisture assists the sterilisation process as it helps heat the materials that are being processed. The load reaches higher temperatures more quickly and the ethylene oxide sterilises at a faster rate. Consequently it is recommended that pre-humidification remains a necessary part of ethylene oxide sterilization processes.	J.E. Doyle, A.W. McDaniel, K.L. West, J.E. Whitbourne, R.R. Ernst (1970)
Sterilant	PT2	Liquid Ethylene Oxide	*Bacillus subtilis* var. *niger*	Spores on glass beads were exposed to EtO in a test chamber	EtO exposure occurred in a test chamber. Dose rate of EtO was 440, 800 and 1500 mg/L	The concentration dependence of the ethylene oxide sterilisation reactions is the result of the physical phenomena of adsorption and diffusion and does not reflect a specific chemical reaction per se.	R.R. Ernst and J.J. Shull (1962)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Sterilant	PT2	Liquid Ethylene Oxide	Various	Various	Review Article	The review identifies that the activity of ethylene oxide depends on several factors. i)The activity of ethylene oxide is concentration and time dependent. The higher the concentration of ethylene oxide, the more rapid its sporicidal activity. Calculated values of 1/k (equivalent to the time in hours at 25ºC required to kill 90% [$t_{90\%}$] of *B.subtilis* spores dried on a cloth) were 7.2, 3.3, 1.6, 0.5 and 0.35 h for ethylene oxide concentrations of 22, 44, 88, 442 and 884 mg/L respectively. The values demonstrate that even at high concentrations, ethylene oxide has a slow rate of kill and that this needs to be taken into consideration when providing suitable conditions for sterilisation. ii)Activity is temperature dependent. Sporicidal activity of ethylene oxide is increased as temperature is raised. The relationship among temperature, concentration and time is complex. At concentrations of <880 mg/L and temperatures of 35ºC the temperature coefficient Q_{10} is 2.74 for each 10ºC rise in temperature. At each temperature, a critical concentration is reached, where an increase in concentration will no longer affect the rate of kill of bacterial spores. At higher concentrations and temperatures of about 32ºC the kinetics become zero order with respect to concentration and the Q_{10} value is around 1.9. iii)Activity is water vapour dependent. Of all the factors influencing ethylene oxide activity,	A.D. Russell (1990)

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
						moisture vapour is the most critical and the most variable. Conflicting results have been obtained, and this may be a reflection of the diverse test procedures used. The theory put forward to explain the conflicting data is that spores are characterised with respect to their immediate environment and relative moisture content as compared with the gross environment surrounding them. Water molecules carry ethylene oxide to reactive sites. In an environment with a relatively low moisture content relative to the active site then the dynamic exchange will be directed outward i.e. away from the spore. As the moisture content of the environment increases, the dynamic movement of water is towards the active site, the most ideal situation in practice. In situations where the relative humidity is above 40% the moisture content may have a diluting effect on ethylene oxide gas, reducing its availability to the spore. Reports suggest an optimum relative humidity of ca. 32%. iv) Activity depends on the type of organism. In the case of ethylene oxide, spores are often only 2-10 fold more resistant than are vegetative organisms. Spores of the thermophile *Bacillus stearthermophilus* and of certain other organisms may in fact be less resistant to ethylene oxide than some vegetative bacteria such as *S. aureus*, *Enterococcus faecalis* and *Deincoccus radiodurans*.	

Function	Field of use envisaged	Test substance	Test organism(s)	Test method	Test conditions	Test results: effects, mode of action, resistance	Reference*)
Sterilant	PT2	20 wt% Ethylene oxide and 80 wt% Carbon dioxide	*Bacillus subtilis* var. *globigii*	This study looked at the sterilisation of spore strips (to test different conditions) and medical devices (to validate identified efficient conditions) in commercial EO sterilisation bags of craft paper on one side and laminated polypropylene and polyester film on the other side.	The test with spore strips used a 40 L chamber with a mechanism for addition and removal of samples without affecting the EO concentration, humidity or temperature. The test with medical devices used a 800 L steriliser.	The study found that the minimum sterilising conditions were an EtO concentration of 400-500 mg/L a sterilising temperature of 55-60ºC with a sterilising time of 2 hours.	M. Furuhashi and T. Miyame (1982)

References:

[redacted] (2018a), Renewed functional assessment and renewed performance assessment according to EN 11135 of the program 2 bar / 55 °C / 120 min / 5 h of the ethylene oxide sterilizer. Unpublished.

[redacted] (2018b), Renewed functional assessment and renewed performance assessment according to EN 11135 of the program 2 bar / 60 °C / 140 min / 5 h of the ethylene oxide sterilizer. Unpublished.

R.R. Reich & L.L. Morien (1982), Influence of environmental storage relative humidity on biological indicator resistance, viability, and moisture content. Applied and Environmental Microbiology **43** (3) pp 609-614.

J.E. Doyle, A.W. McDaniel, K.L. West, J.E. Whitbourne, R.R. Ernst (1970), Ethylene oxide resistance of non-desiccated and desiccated spores of *Bacillus subtilis var. niger* hermetically sealed in various polymeric films. Applied Microbiology **20** (5) pp 793-797.

R.R. Ernst and J.J. Shull (1962), Ethylene oxide gaseous sterilization: I. Concentration and temperature effects. Applied Microbiology **10** pp 337-342.

A.D. Russell (1990), Bacterial Spores and Chemical Sporicidal Agents. Clinical Microbiology Reviews **3** (2) pp 99-199.

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Note: Full study summaries of all the above studies are included in Doc IIIB Section 5.

Mode of action References

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Hoxey, E.V. and Thomas, N. (1999) Gaseous Sterilisation. In Principles and Practice of Disinfection, preservation and Sterilization 3rd Edition (eds. Russell, A.D., Hugo, W.B. and Ayliffe, G.A.J.) 703-732. Blackwell scientific Ltd. Oxford.