Ethylene Oxide

For use as a gaseous sterilant (PT2)

Document IIIA

Section 4

Analytical methods for detection and identification

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.

4.1 Analytical methods for the determination of pure active substances and, where appropriate, for relevant degradation products, isomers and impurities of active substances and their additives (e.g. stabilisers)

4.1.1 Methods for the determination of pure active substance in the active substance as manufactured

Please see confidential annex.

4.1.2 Methods for the determination of significant and/or relevant impurities and additives (eg. stabilisers) in the active substance as manufactured

Please see confidential annex.

4.2 Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, and where relevant in/on the following:

4.2.1 Residues in soil

Section A4.2.1 **Annex Point IIA 4.2.1**	**Residues in soil**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Based on the proposed use of ethylene oxide as a gaseous sterilising agent, environmental exposure is expected to be extremely minimal. During the sterilisation process gaseous ethylene oxide is introduced into a sealed, stainless steel chamber containing the products for sterilisation. On completion of the process, ethylene oxide from the chamber is exhausted to the atmosphere via a catalytic converter which converts the gas to carbon dioxide and water, typically with efficiency greater than 99.9%. The sterilisation chamber is then repeatedly flushed with nitrogen or air to remove the remaining ethylene oxide from the chamber and the sterilised products are subjected to high rates of air exchange to remove any residual ethylene oxide from the product and packaging. The exhaust from this phase is also treated via the catalytic converter. In a relatively small number of sterilisation plants acid scrubbers containing a solution of water and 5% sulphuric acid are used as an alternative to the catalytic conversion system and ethylene oxide from the sterilisation chamber is dissolved in the acid solution, which converts ethylene oxide to ethylene glycol with efficiency typically in the range of 99.5 to 99.9%. Neutralised solution from the acid scrubbers is transported to waste water treatment facilities as toxic waste water, where it undergoes specialist handling and treatment, thus minimising any potential environmental exposure. Direct exposure to soil and the natural terrestrial environment will therefore not occur following use of ethylene oxide as proposed and any indirect exposure is expected to be negligible.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** February 2020	

Evaluation of applicant's justification	The following sentence should specify the application to soil compartments: "Based on the proposed use of ethylene oxide as a gaseous sterilising agent, environmental exposure, **e.g. to soil compartments,** is expected to be extremely minimal".
Conclusion	The justification from the applicant is considered acceptable. Submission of specific data is not required. The exposure to soil is considered negligible by the ecotoxicological experts.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

4.2.2 Residues in water (including drinking water and surface water)

Section A4.2.2 **Annex Point IIA 4.2.21**	**Residues in water (including drinking water and surface water)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Ethylene oxide is intended for use as a gaseous sterilising agent; no application is envisaged for use in water. During the sterilisation process gaseous ethylene oxide is introduced into a sealed, stainless steel chamber which contains the products for sterilisation. On completion of the process, ethylene oxide from the chamber is exhausted to the atmosphere via a catalytic converter which converts the gas to carbon dioxide and water, typically with efficiency greater than 99.9%. The sterilisation chamber is then repeatedly flushed with nitrogen or air to remove the remaining ethylene oxide from the chamber and the sterilised products are subjected to high rates of air exchange to remove any residual ethylene oxide from the product and packaging. The exhaust from this phase is also treated via the catalytic converter. In a relatively small number of sterilisation plants acid scrubbers containing a solution of water and 5% sulphuric acid are used as an alternative to the catalytic conversion system and ethylene oxide from the sterilisation chamber is dissolved in the acid solution, which converts ethylene oxide to ethylene glycol with efficiency typically in the range of 99.5 to 99.9%. Neutralised solution from the acid scrubbers is transported to waste water treatment facilities as toxic waste water, where it undergoes specialist handling and treatment, thus minimising any potential environmental contamination. Direct exposure to the natural aquatic environment and potentially to drinking water supplies will therefore not occur following use of ethylene oxide as proposed and any indirect	

	exposure is expected to be negligible.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	February 2020	
Evaluation of applicant's justification	The application's justification is deemed to be acceptable.	
Conclusion	Applicant's justification is acceptable. Submission of specific data is not required. The exposure to soil is considered negligible by the ecotoxicological experts.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

4.2.3 Residues in air

Official monitoring methods for measuring ethylene oxide concentrations in air have historically been available from the United States Department of Labor, Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH). These methods were developed using hydrobromic acid coated carbon adsorption tubes to convert ethylene oxide to stable 2-bromoethanol. This eliminates air sampling tube retention issues with EtO and loss of trapped EtO due to stability issues. The methods used older analytical GC column techniques and relied on derivitisation prior to GC-ECD detection. The petroleum charcoal used as the basis of these methods was discontinued and therefore an updated method was developed by OSHA. This most recent OSHA method (with validation data where available) is presented below along with other available methods which are considered to be appropriate for this dossier.

Section A4.2.3 **Annex Point IIA 4.2.3/01**	**Residues in air**	
	1 Reference	**Official use only**
1.1 Reference	Shah, Y. (2007) Ethylene oxide OSHA Method 1010	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Samples are collected with 7-cm × 4-mm i.d. × 6-mm o.d. glass sampling tubes packed with two sections of HBr coated carbon beads (HBr-CB). The front section contains 100 mg and the back section contains 50 mg of HBr-CB. Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate. After sampling the tubes are capped and sent to the laboratory for analysis. If shipment is delayed store the tubes under refrigeration. Record sample air volumes (litres), sampling time (minutes), and sampling rate (L/min) for each sample, along with any potential interference.	x
3.1.2 Cleanup	Remove the plastic end caps from the sample tube and carefully transfer the front section of HBr-CB along with the front glass wool plug to a 2-mL vial. Transfer the centre glass wool plug and back-up section of HBr-CB to a separate 2-mL vial. Discard the glass tube. Add 1.0 mL of methanol to each vial and immediately seal the vials with PTFE-lined caps. Shake the vials on a shaker for 30 min. Shaking is recommended to obtain complete sample extraction.	
3.2 Detection	Non-entry field	
3.2.1 Separation method	A gas chromatograph column capable of separating 2-bromoethanol from the extraction solvent is required. A GC is used with a DB-5 capillary column, 60 m x 0.32 mm id, df = 1.0 µm.	

	GC conditions: column: initial 100 °C, hold 9 min, program at 5 °C/min to 150 °C, hold 1 min injector: 200 °C detector: 250 °C run time: 20 min column gas flow: 2.1 mL/min (hydrogen) injection size: 1.0 µL (20:1 split)
3.2.2 Detector	Electron capture (ECD)
3.2.3 Standard(s)	Standards of 2- bromoethanol in methanol are required for this method. By diluting this stock solution, concentrations close to the target level can be achieved. A 2x target level stock standard (2ppm) can be prepared by injecting 7.0µl of 2-bromoethanol into a 1.0 mL volumetric flask containing 0.5 mL methanol and then diluting up to the mark with methanol. A working standard can then be prepared by dilution of this stock standard.
3.2.4 Interfering substance(s)	There are no known interferences to the sampling process. Any compound that produces an ECD response and has a similar retention time as 2-bromoethanol is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte
3.3 Linearity	Non-entry field
3.3.1Calibration range	Calibration was over the range 5.4 to 43.2 µg EtO per sampling tube. This range corresponds to 0.25 to 2 times the target concentration.
3.3.2 Number of measurements	Not reported
3.3.3 Linearity	An external standard calibration method is used. A calibration curve can be constructed by plotting response of standard injections versus micrograms of analyte per sample. Response of the GC-ECD was shown to be linear over the range tested.
3.4 Specifity: interfering substances	No significant interferences to this analysis have been observed. Methanol, ethanol, n-propanol, 2-chloroethanol, ethylene glycol, and n-butanol, all of which form esters with HFBI, and are chromatographed under the existing conditions, are not interferences. In the event that an interference is observed, selection of alternative GC conditions will be necessary. Confirmation of the derivative by GC/MS is a highly useful means of compound identification.
3.5 Recovery rates at different levels	The recovery of EtO from samples used in a 20-day storage test remained above 98.6% when the samples were stored at 23°C. The mean extraction efficiency for desorption of EtO from dry HBr-CB into methanol over the range of the reliable quantification limit (RQL) to 2 times the target concentration (0.21 to 42.3 µg per sample) was 97.9%. The extraction efficiency

	was not affected by the presence of water.	
3.5.1 Relative standard deviation	Not reported	
3.6 Limit of determination	The detection limit of the overall procedure is 63 ng/sample (2.92 ppb or 5.25 µg/m^3). This is the amount of EtO spiked on the sampler that will give a detector response that is significantly different from the response of a sampler blank. The reliable quantitation limit is 210 ng/sample (9.72 ppb or 17.5 µg/m^3). This is the amount of EtO spiked on the sampler that will give a detector response that is considered the lower limit for precise quantitative measurement.	x
3.7 Precision	The precision of the overall procedure at the 95% confidence level for the ambient temperature 20-day storage test (at the target concentration) is ±10.74%. This includes an additional 5% for sampling pump variability.	
3.7.1 Repeatability	Six samples were collected from a controlled test atmosphere and submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after 9 days of storage at 4 °C. No individual sample result deviated from its theoretical value by more than the reported precision value. Reproducibility Data for ETO theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) 21.78 | 21.24 | 97.5 | -2.5 21.83 | 20.87 | 95.6 | -4.4 22.60 | 23.27 | 102.9 | 2.9 21.87 | 22.20 | 101.5 | 1.5 21.50 | 22.60 | 105.1 | 5.1 21.88 | 22.19 | 101.4 | 1.4	
3.7.2 Independent laboratory validation	Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch of OSHA.	
	4 Applicant's Summary and conclusion	
4.1 Materials and methods	Samples are collected with 7-cm × 4-mm i.d. × 6-mm o.d. glass sampling tubes packed with two sections of HBr coated carbon beads (HBr-CB). The front section contains 100 mg and the back section contains 50 mg of HBr-CB. Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate. After sampling the tubes are capped and sent to the laboratory for analysis. If shipment is delayed store the tubes under refrigeration. Record sample air volumes (litres), sampling time (minutes), and sampling rate (L/min) for each sample, along with any potential interference. Remove the plastic end caps from the sample tube and carefully transfer the front section of HBr-CB along with the front glass	

	wool plug to a 2-mL vial. Transfer the centre glass wool plug and back-up section of HBr-CB to a separate 2-mL vial. Discard the glass tube. Add 1.0 mL of methanol to each vial and immediately seal the vials with PTFE-lined caps. Shake the vials on a shaker for 30 min. Shaking is recommended to obtain complete sample extraction. Results are expressed in ppm or mg/m^3 EtO.
4.2 Conclusion	The method described in this procedure has been updated from earlier methods to use modern GC capillary column chromatography, safe solvents and does not require derivitisation. The method has been shown in to provide a reliable, convenient, and accurate means of measuring EtO exposures. This method has been tested under a broad range of conditions in the laboratory. This method can be used to accurately measure short term exposures in the workplace as well as all day measurements. The practical lower limit of the method (210 ng/sample (9.72 ppb or 17.5 µg/m^3)) is below the proposed minimum exposure levels.
4.2.1 Reliability	1
4.2.2 Deficiencies	None

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	Acceptable, but please consider the remarks mentioned below.
Results and discussion	Acceptable
Conclusion	The methods for the determination of residue in air is deemed to be acceptable if the remarks below are taken into consideration. Submission of specific data is not required.
Reliability	1
Acceptability	Acceptable
Remarks	Point 3.1.1: The first sentence should read "Samples are collected with 7-cm × 4-mm i.d. × 6-mm o.d. glass sampling tubes packed with two sections of HBr coated carbon beads (HBr-CB) separated with glass wool plugs. Point 3.6: Please note that the detection limit of the analytical procedure from was calculated to be 5.58 pg.
	Comments from ...
Date	
Materials and Methods	

Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3 **Annex Point IIA 4.2.3/02**	**Residues in air**	
	1 Reference	**Official use only**
1.1 Reference	3M Company (1997) Determination of Ethylene oxide in air using 3M 3550/3551 Ethylene oxide monitors	
1.2 Data protection		
1.2.1 Data owner	3M	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Sampling is performed with an Ethylene oxide monitor (3M Brand 3550/3551). Sampling rate is controlled by molecular diffusion. Collection layer has about 160 mg of treated activated carbon in a Teflon matrix. The personal 3M monitor is worn by the operator and no pumping device is required. The monitor is a badge containing a chemically treated charcoal disk which converts adsorbed ethylene oxide to 2-bromoethanol. It is presumed the charcoal is impregnated with hydrogen bromide, but the exact coating procedure is proprietary information. Ethylene oxide is collected by diffusion, thus no sampling pumps are needed, but a minimum air velocity must exist during sampling to ensure that badge starvation does not occur.	
3.1.2 Cleanup	Using a pipette or a syringe add 1.5 mL of the desorption solution (10% dichloromethane in methanol) to each monitor through the centre port (the port is immediately resealed). After 30 minutes, with occasional gentle agitation, the eluent is transferred into a graduated 2 mL vial and a 1 µL aliquot is automatically injected	

	into the gas chromatograph.
3.2 Detection	
3.2.1 Separation method	Gas Chromatograph - equipped with a J&W DB225 capillary column (15 m x 0.25 mm i.d., 0.25 µm df).
3.2.2 Detector	Electron capture detector (ECD).
3.2.3 Standard(s)	A solution of 4 mL Ethylene oxide gas in 10 mL of deionised water is prepared and spiked onto 3M brand Ethylene oxide monitors. The amounts spiked are 5 µl (3.59 µg), 15 µL (10.77 µg), 20 µL (14.36 µg), 40µL (28.72µg) and 70µL (50.26µg). A standard curve can also be prepared from dilutions of 2 bromo-ethanol in 10% methylene chloride and calculating the equivalent amount of EtO.
3.2.4 Interfering substance(s)	Acetone and isopropanol can both be brominated by the chemistry on the 3550/3551 monitors. Use care to ensure that each column has adequate resolution to effectively separate the brominated acetone and isopropanol derivatives.
3.3 Linearity	Non-entry field
3.3.1Calibration range	Using the above spiked standards, the calibration range is nominally 2.4 to 33.5 µg/mL.
3.3.2 Number of measurements	Not reported
3.3.3 Linearity	A best line fit is calculated by the regression technique to correlate peak height with the amount spiked on the monitors. The ECD detector should give a linear response over the calibrated range.
3.4 Specifity: interfering substances	Acetone and isopropanol can both be brominated by the chemistry on the 3550/3551 monitors. Use care to ensure that each column has adequate resolution to effectively separate the brominated acetone and isopropanol derivatives.
3.5 Recovery rates at different levels	Time weighted average concentrations can be calculated by knowing the length of sampling period, the contaminant weight determined by gas chromatography, the recovery coefficient and the calculation constant either A or B. Constant A is used to calculate the concentration when expressed in units of milligrams per cubic metre (mg/m^3). Constant B is used when expressed in units of parts per million (ppm). The calculation constants A and B are calculated by: A= 1000/ Sampling Rate (cc/min) B= 1000x 24.45 /Sampling Rate x molecular weight The time-weighted-average concentration in milligrams per cubic meter (mg/m^3) of ethylene oxide in the environment sampled can be calculated from the following expression:

	C (mg/m^3) = W x A/ r x t The time-weighted-average concentration in parts per million (ppm) of the contaminant can be calculated from the following expression: C (ppm) = W x B/ r x t Where: W = equivalent EtO weight recovered (micrograms) r = recovery coefficient t = length of sampling period (minutes) When the standard curve is prepared by spiking EtO gas onto monitors, the recovery is automatically taken into account and r is set to 1.00. If 2-bromoethanol is used for the standard curve, it is necessary for the laboratory to determine and use a recovery correction. Experiments in the laboratory have shown a recovery of 85% (r = 0.85). These expressions calculate EtO concentration as 25ºC and a pressure of 760 mm Hg. Every 6 degrees above or below 25ºC requires a one percent correction to the calculated time- weighted-average concentration.
3.5.1 Relative standard deviation	Not reported
3.6 Limit of determination	The limit of quantitation in the laboratory has been determined to be 1 µg. This allows detection of 0.75 ppm with a 15 min sample or 0.02 ppm with an 8 hr sample.
3.7 Precision	Not reported
3.7.1 Repeatability	Not reported
3.7.2 Independent laboratory validation	Evaluated method. This method has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch of OSHA (OSHA method 49). Satisfactory performance was shown using 3M monitors.
	4 Applicant's Summary and conclusion
4.1 Materials and methods	Ethylene oxide vapours are adsorbed on chemically treated charcoal where they are converted to 2-bromoethanol, desorbed with 10% methylene chloride in methanol and quantitated using a gas chromatograph equipped with an electron capture detector.
4.2 Conclusion	This method can be used to analyse ethylene oxide concentrations in the air down to a concentration of 1 µg per sample. This allows detection of 0.75 ppm with a 15 min sample or 0.02 ppm with an 8 hr sample.
4.2.1 Reliability	2
4.2.2 Deficiencies	This method is a guideline on use of the 3M brand ethylene oxide monitor rather than an independent laboratory method.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	Acceptable, but please take the remark below into consideration.
Results and discussion	Acceptable
Conclusion	The methods for the determination of residue in air is deemed to be acceptable given that the remark below is taken into consideration. Submission of specific data is not required.
Reliability	2
Acceptability	Acceptable
Remarks	
	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

4.2.4 Residues in body fluids and tissues

Section A4.2.4 **Annex Point IIA 4.2.4/01**	**Residue in body fluids and tissues [Blood]**	
	1 Reference	**Official use only**
1.1 Reference	Brown, C.D., Wong, B.A., Fennell, T.R. (1996) *In Vivo* and *in Vitro* Kinetics of ethylene oxide Metabolism in rats and Mice. Toxicology and Applied Pharmacology **136** pp 8-19	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Blood samples were incubated in gently rotating heating block at a temperature of 37ºC for 30 minutes, to equilibrate blood / tissue and vapour phases. Following incubation, head space samples (1.0 mL) were analysed by injection onto the GC system. Blood or tissue EtO concentrations calculated using headspace results appropriate blood/tissue : air partition coefficient, volume of blood/tissue and volume of sampling vials.	
3.1.2 Cleanup	None required	
3.2 Detection	Non-entry field	
3.2.1 Separation method	EtO in blood or tissues was determined by gas chromatography (GC). Separation was achieved with a 10 ft 10% SP1000 packed column.	
3.2.2 Detector	Flame ionisation detection (FID).	
3.2.3 Standard(s)	EtO	
3.2.4 Interfering substance(s)	Not reported	

3.3 Linearity	Standard curve development was conducted with gas bags containing defined concentrations of EtO.	
3.3.1Calibration range	Not reported, appropriate for concentrations measured.	
3.3.2 Number of measurements	Not reported	
3.3.3 Linearity	Not reported	
3.4 Specifity: interfering substances	Not reported	
3.5 Recovery rates at different levels	Not reported	
3.5.1 Relative standard deviation	Not reported	
3.6 Limit of determination	Not reported. Lowest results obtained were 0.23 ppm in blood and 0.10 ppm in tissue.	x
3.7 Precision	Non-entry field	
3.7.1 Repeatability	Not applicable	
3.7.2 Independent laboratory validation	Not applicable	
	4 Applicant's Summary and conclusion	
4.1 Materials and methods	Samples were incubated in gently rotating heating block at a temperature of 37ºC for 30 minutes, to equilibrate blood / tissue and vapour phases. Following incubation, head space samples (1.0 mL) were analysed by injection onto the GC system. Blood or tissue EtO concentrations calculated using headspace results appropriate blood/tissue : air partition coefficient, volume of blood/tissue and volume of sampling vials.	
4.2 Conclusion	Analysis of ethylene oxide concentration in blood and tissues using gas chromatography with flame ionisation detection was able to detect EtO concentrations at levels ranging from of 0.23 to 2.70 µg/g (ppm) in blood and from 0.10 to 4.82µg/g (ppm) in tissue.	x
4.2.1 Reliability	2	
4.2.2 Deficiencies	The method has not been independently verified	

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	Acceptable
Results and discussion	Acceptable
Conclusion	The methods for the determination of residue in body fluids and tissues is deemed to be acceptable given that the remark below is taken into consideration. Submission of specific data is not required.
Reliability	3 This study report focusses mainly on the kinetics in mouse and rats, and not so much on the analytical method. This weakens the reliability
Acceptability	Acceptable
Remarks	Point 3.6: Please note that the values given are mean values. The applicability of this study should be restricted to tissue only as the LOQ or lowest mean result obtained in blood is considerably higher than the common limit for monitoring the content in blood.
	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4 **Annex Point IIA 4.2.4/02**	**Residue in body fluids and tissues [Blood]**	
	1 Reference	**Official use only**
1.1 Reference	Fennell, T.R., Snyder, R.W., Parkinson, C., Murphy, J., James, R.A. (2004) The Effect of Ethylene exposure on Ethylene Oxide in Blood and on Hepatic Cytochrome P450 in Fischer Rats. Toxicological Sciences **81** pp7-13	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Blood samples were removed from ice and incubated in the headspace autosampler oven at a temperature of 37ºC. Head space samples (1.0 mL) were analysed by injection onto the GC system.	x
3.1.2 Cleanup	None required	
3.2 Detection	Non-entry field	
3.2.1 Separation method	EtO in blood was determined by gas chromatography (GC with a headspace auto sampler). Separation was achieved with a 30 m x 0.53 mm i.d., 1µm df DB wax capillary column. Helium was used as the carrier gas at a flow rate of 5 mL/min	
3.2.2 Detector	Flame ionisation detection (FID).	
3.2.3 Standard(s)	EtO	
3.2.4 Interfering substance(s)	Not reported	
3.3 Linearity	Standard curve development was conducted with gas bags containing defined concentrations of EtO. Blood samples spiked with EtO were used to develop appropriate analytical conditions and a standard curve. Standards were prepared with vials containing blood into which defined volumes of EtO:air mixtures	

	were added
3.3.1Calibration range	Not reported
3.3.2 Number of measurements	Not reported
3.3.3 Linearity	Not reported
3.4 Specifity: interfering substances	The paper hypothesises that CO_2 may interfere when low resolution GC-MS is used for the analysis of EtO
3.5 Recovery rates at different levels	Ethylene oxide concentrations of between 0.13µg/ml and 0.06 µg/ml were recovered from blood samples. Upon exposure to 1000 ppm ethylene, blood EtO concentration rapidly reached a peak of 0.1µg/ml and remained constant during the exposure. Following exposure to 600 ppm ethylene EtO reached 0.13 µg/ml and then declined to 0.06 µg/ml and exposure to 300 ppm ethylene resulted in a peak blood concentration of 0.08 µg/ml which declined to 0.06 µg/ml.
3.5.1 Relative standard deviation	Not reported
3.6 Limit of determination	Not reported. Lowest results obtained were 0.06 ppm in blood.
3.7 Precision	Non-entry field
3.7.1 Repeatability	Not applicable
3.7.2 Independent laboratory validation	Not applicable
	4 Applicant's Summary and conclusion
4.1 Materials and methods	Ethylene oxide in blood was quantitated by gas chromatography with flame ionisation detection. Separation was achieved with a 30 m x 0.53 mm i.d., 1µm df DB wax column. Helium was used as the carrier gas at a flow rate of 5 mL/min. Head space samples (1.0 mL) were analysed with an injector temperature of 150ºC, a detector temperature of 300ºC and an initial oven temperature of 35ºC. Samples were removed from ice and incubated in the headspace autosampler oven at a temperature of 37ºC. Ethylene oxide eluted at approximately 2.6 min. After 3.5 min a temperature gradient of 70-100ºC was used to recondition the column for the next injection. Samples were compared to a standard curve that was developed by adding known quantities of EtO to blood samples.
4.2 Conclusion	Analysis of ethylene oxide concentration in blood using gas chromatography with flame ionisation detection was able to detect EtO concentrations at levels as low as 0.06 µg/mL (ppm).
4.2.1 Reliability	2
4.2.2 Deficiencies	The method has not been independently verified

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	Acceptable
Results and discussion	Acceptable
Conclusion	The methods for the determination of residue in body fluids and tissues is deemed to be acceptable given that the remark below is taken into consideration. Submission of specific data is not required.
Reliability	3 This study report focusses mainly on the kinetics in mouse and rats, and not so much on the analytical method. This weakens the reliability. The method should have been independently verified to make the reliability better
Acceptability	Acceptable
Remarks	Point 3.1.1: The first sentence should read " Blood samples were removed from ice and incubated in the headspace autosampler oven at a temperature of 37ºC for 10 minutes.
	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4 **Annex Point IIA 4.2.4/03**	**Residue in body fluids and tissues [Blood]**	
	1 Reference	**Official use only**
1.1 Reference	Brugnone, F., Perbellini, L., Faccini, G.B., Pasini, F., Bartolucci G.B. and DeRosa, E. (1986) Ethylene Oxide Exposure – Biological Monitoring by Analysis of Alveolar Air and Blood. Int Arch Occup Environ Health (1986) **58**: pp 105-112	
1.2 Data protection	Non-entry field	

1.2.1 Data owner	Not applicable
1.2.3 Criteria for data protection	No data protection claimed
	2 Guidelines and Quality Assurance
2.1 Guideline study	No
2.2 GLP	No
2.3 Deviations	Not applicable
	3 Materials and Methods
3.1 Preliminary treatment	Non-entry field
3.1.1 Enrichment	Blood samples (3 mL) were transferred to at a 12 mL glass vial fitted with screw cap and Teflon membrane septum. Vials were placed in a heated area at a temperature of 37ºC for 2 to 3 hours prior to GC headspace analysis. Head space samples (1.0 mL) were analysed by injection onto the GC system.
3.1.2 Cleanup	None required
3.2 Detection	Non-entry field
3.2.1 Separation method	EtO in blood was determined by gas chromatography (GC headspace analysis). Separation was achieved with a 2.4 m x 2 mm i.d., packed steel GC column using Carbowax 1500 0.2% on Carbopack C 80-100 mesh. Nitrogen was used as the carrier gas at a flow rate of 12 mL/min.
3.2.2 Detector	Flame ionisation detection (FID).
3.2.3 Standard(s)	EtO
3.2.4 Interfering substance(s)	Not reported
3.3 Linearity	Not reported
3.3.1Calibration range	Not reported
3.3.2 Number of measurements	Not reported
3.3.3 Linearity	Not reported
3.4 Specifity: interfering substances	Not reported
3.5 Recovery rates at different levels	Not reported
3.5.1 Relative standard deviation	Not reported

3.6 Limit of determination	Reported as 0.05 µg/L EtO in blood	x
3.7 Precision	Non-entry field	
3.7.1 Repeatability	Not reported	
3.7.2 Independent laboratory validation	Not applicable	
	4 Applicant's Summary and conclusion	
4.1 Materials and methods	Blood samples (3 mL) were transferred to at a 12 mL glass vial fitted with screw cap and Teflon membrane septum. Vials were placed in a heated area at a temperature of 37ºC for 2 to 3 hours prior to GC headspace analysis. Head space samples (1.0 mL) were analysed by injection onto the GC system. EtO in blood was determined by gas chromatography (GC headspace analysis). Separation was achieved with a 2.4 m x 2 mm i.d., packed steel GC column using Carbowax 1500 0.2% on Carbopack C 80-100 mesh. Nitrogen was used as the carrier gas at a flow rate of 12 mL/min.	
4.2 Conclusion	Analysis of ethylene oxide concentration in blood using gas chromatography with flame ionisation detection was able to detect EtO concentrations in the measured range of 4.0 to 103.7 µg/mL.	
4.2.1 Reliability	3	
4.2.2 Deficiencies	No description of calibration or accuracy. The method has not been independently verified. The technique of GC-headspace analysis is confirmed as being appropriate for analysis of EtO in blood.	

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	Acceptable
Results and discussion	Acceptable
Conclusion	The methods for the determination of residue in body fluids and tissues is deemed to be acceptable given that the remark below is taken into consideration. Submission of specific data is not required.

Reliability	3 No description of calibration or accuracy and no independently verification result in a weaker reliability for the method.
Acceptability	Acceptable
Remarks	Please note that the LOQ for the method should be corrected to 4 µg/L which is the lowest measured concentration of EtO in blood in the study. This measurement seems more reliable.
	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

4.3 Analytical methods including recovery rates and the limits of determination for the active substance, and for residues thereof, in/on food or feedstuffs and other products where relevant

Section A4.3 **Annex Point IIA 4.3**	**Residues in/on food or feedstuffs and other products where relevant[**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Ethylene oxide is intended for use as a gaseous sterilising agent; no application is envisaged for use in food or feedstufs. Direct exposure to food or feedstuffs will not occur following the use of ethylene oxide as proposed and any indirect exposure is expected to be negligible.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2020.
Evaluation of applicant's justification	Ethylene oxide will not be in contact with food and feeding stuff with the proposed usage. The wavier by the applicant is therefore deemed to be acceptable.
Conclusion	Acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Medical devices that have been sterilised with ethylene oxide (EtO) may retain small amounts of EtO residuals, either as EtO or as potential reaction products such as ethylene chlorhydrin (ECH) or ethylene glycol (EG). To allow for monitoring of such residuals, various published literature methods are available and have been summarised below as residues in or on 'other products'.

Section A4.3 **Annex Point IIA 4.3/01**		**Residues in/on food or feedstuffs and <u>other products</u> where relevant** **[Residuals on medical devices]**	
		1 Reference	**Official use only**
1.1	**Reference**	T. Harper, L. Cushinotto, N. Blaszko, J. Arinaga, F. Davis, C. Cummins, M. DiCicco (2008) Round-robin evaluation of a solid phase microextraction-gas chromatographic method for reliable determination of trace level ethylene oxide in sterilized medical devices. Biomedical Chromatography **22** pp136-148	
1.2	**Data protection**		
1.2.1	Data owner	Not applicable	
1.2.3	Criteria for data protection	No data protection claimed	
		2 Guidelines and Quality Assurance	
2.1	**Guideline study**	No	
2.2	**GLP**	No	

2.3 Deviations	Not applicable
	3 Materials and Methods
3.1 Preliminary treatment	
3.1.1 Enrichment	Solid-phase microextraction (SPME) is an equilibrium sampling method. The amount of analyte absorbed/adsorbed by the fibre coating at equilibrium is proportional to analyte concentration in a sample. A polymer coated fused silica fibre has the ability to extract volatile and non-volatile organic analytes from aqueous and gaseous media and concentrate them onto the fibre. The analytes are then thermally desorbed from the SPME fibre onto an analytical separations unit such as a GC equipped with a capillary column. Three independent laboratories all used a 75µm coating Carboxen®/PDMS (CAR-PDMS) SPME fibre (Supelco, Inc). The conditions used in this round robin study were 30 min optimal simulated use extraction time with refrigeration (3ºC) for greatest EtO peak area sensitivity, no stirring, at neutral pH and with no added salt. Aqueous EtO standards were poured into 10 mL headspace vials and crimp capped. Vials were mostly filled with ~10% headspace remaining, leaving room for the SPME needle. The PTFE-lined septa of the EtO standard vials were pierced with the SPME septum piercing needle ensuring that the needle remained in the headspace of the vial and that the fibre did not come into contact with the EtO standard solution. Once the SPME holder was in place the fibre was exposed and submerged into the solutions. The SPME assembly was then placed in the refrigerator for 30 min. After 30 minutes the SPME fibre was retracted and injected into the GC for quantitative analysis. The septum of the GC inlet was pierced with the septum piercing needle and the EtO was thermally desorbed from the SPME by depressing the plunger exposing the fibre to the heated inlet for exactly 10 seconds. After this the SPME fibre was retracted and the assembly removed from the GC inlet. For the application of this technique to medical devices, aqueous extractions are conducted and then determined using the above approach.
3.1.2 Cleanup	None required
3.2 Detection	Non-entry field
3.2.1 Separation method	The three independent laboratories involved in this study all used different GC systems, parameters GC capillary column manufacturers and capillary column stationary phases in assessing this method. All three laboratories used capillary GC capillary columns instead of packed columns due to their higher efficiencies.

<table>
<tr><td>3.2.2 Detector</td><td>Flame ionisation detector (FID)</td></tr>
<tr><td>3.2.3 Standard(s)</td><td>Standards were prepared by initially flowing gaseous ethylene oxide into a flask chilled with dry ice. Once liquid EtO was collected in the bottom of the flask, 5-7 drops were weighed into a flask[1] containing 50 mL ultra pure water. This was then diluted to the 100 mL mark. Dilutions from the stock solution were made to prepare EtO standards in the concentrations of 0.01, 0.05, 0.10, 1.00 and 5.00 ppm EtO.</td></tr>
<tr><td>3.2.4 Interfering substance(s)</td><td>Not reported</td></tr>
<tr><td>3.3 Linearity</td><td></td></tr>
<tr><td>3.3.1Calibration range</td><td>0.01 to 5.00 ppm</td></tr>
<tr><td>3.3.2 Number of measurements</td><td>The various EtO standards were extracted three times with three separate SPME fibres for a total of nine simulated use extractions per EtO standard concentration at each laboratory.</td></tr>
<tr><td>3.3.3 Linearity</td><td>The linearity of the SPME-GC method was determined by constructing calibration curves for each of the three independent laboratories. Each point on the calibration curves represents the mean obtained from the nine replicate simulated use extractions or each of the 0.01, 0.05, 0.10, 1.0 and 5.0 ppm standards. The linear correlations that were calculated were excellent over 2.7 orders of magnitude. (0.01-5.0). R values of ≥0.99997 were calculated indicating that minimally 99.994 % of the variability is explained by the independent variables.

Although it was not extended further in this study, the linear range for this method is expected to be much greater. The advantages of using the SPME-GC method for analysing EtO residues in sterilised medical devices are gained at trace-level analysis and therefore the wide range reported would be well suited to the methods purpose.</td></tr>
<tr><td>3.4 Specifity: interfering substances</td><td>Not reported</td></tr>
<tr><td>3.5 Recovery rates at different levels</td><td>The interlaboratory accuracy levels for the various standard concentrations were:
<table><tr><td>Concentration (ppm)</td><td>Mean recovery level (95% confidence interval)</td></tr><tr><td>0.01</td><td>30.7 (24.1-37.3)</td></tr><tr><td>0.05</td><td>113.8 (104.5-123.1)</td></tr><tr><td>0.10</td><td>102.4 (98.8-106.0)</td></tr><tr><td>1.00</td><td>99.3 (96.7-101.9)</td></tr><tr><td>5.00</td><td>110.1 (108.1-112.1)</td></tr></table></td></tr>
</table>

<table>
<tr><td></td><td></td></tr>
<tr><td>3.5.1 Relative standard deviation</td><td>Not reported</td></tr>
<tr><td>3.6 Limit of determination</td><td>The inter laboratory Limit of determination was 0.0150 ppm.

The intra/inter-laboratory LOD and LOQ of the SPME method are given below.

<table>
<tr><td></td><td>LOD (ppm)</td><td>LOQ (ppm)</td><td>Coefficient of variation</td></tr>
<tr><td>Laboratory 1</td><td>0.0033</td><td>0.011</td><td>15.84</td></tr>
<tr><td>Laboratory 2</td><td>0.0030</td><td>0.010</td><td>9.53</td></tr>
<tr><td>Laboratory 3</td><td>0.0153</td><td>0.051</td><td>2.44</td></tr>
<tr><td>Inter-laboratory</td><td>0.0150</td><td>0.050</td><td>20.56</td></tr>
</table>
</td></tr>
<tr><td>3.7 Precision</td><td>Non-entry field</td></tr>
<tr><td>3.7.1 Repeatability</td><td>Intra-laboratory reproducibility was effectively demonstrated in the 0.01-5.00 ppm EtO concentration range. The inter-laboratory reproducibility was demonstrated in the 0.05-5.00 ppm EtO concentration range due to laboratory 3 failing to demonstrate repeatability at the 0.01 ppm level</td></tr>
<tr><td>3.7.2 Independent laboratory validation</td><td>This study compared the results after 3 independent laboratories had used the SPME-GC method to test standards of EtO.

The method was then successfully applied to analyse EtO residues in several sterilised/aerated medical device samples of various polymeric compositions.</td></tr>
<tr><td></td><td><h2>4 Applicant's Summary and conclusion</h2></td></tr>
<tr><td>4.1 Materials and methods</td><td>Three independent laboratories all used a 75µm coating Carboxen®/PDMS (CAR-PDMS) SPME fibre (Supelco, Inc).

The conditions used in this round robin study were 30 min optimal simulated use extraction time with refrigeration (3ºC) for greatest EtO peak area sensitivity, no stirring, at neutral pH and with no</td></tr>
</table>

	added salt. Aqueous EtO standards were poured into 10 mL headspace vials and crimp capped. Vials were mostly filled with ~10% headspace remaining, leaving room for the SPME needle. The PTFE-lined septa of the EtO standard vials were pierced with the SPME septum piercing needle ensuring that the needle remained in the headspace of the vial and that the fibre did not come into contact with the EtO standard solution. Once the SPME holder was in place the fibre was exposed and submerged into the solutions. The SPME assembly was then placed in the refrigerator for 30 min. After 30 minutes the SPME fibre was retracted and injected into the GC for quantitative analysis. The septum of the GC inlet was pierced with the septum piercing needle and the EtO was thermally desorbed from the SPME by depressing the plunger exposing the fibre to the heated inlet for exactly 10 seconds. After this the SPME fibre was retracted and the assembly removed from the GC inlet. For the application of this technique to medical devices, aqueous extractions are conducted and then determined using the above approach.
4.2 Conclusion	The use of SPME technique allows EtO residuals of 0.05 ppm to be determined on medical devices. Using aqueous extraction will provide information on potential in-use exposure of patients rather than a total contained residual amount.
4.2.1 Reliability	2
4.2.2 Deficiencies	Method is presented as a potentially useful technique but without actual in-use findings or extraction conditions.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	-
Results and discussion	-
Conclusion	-
Reliability	-
Acceptability	-
Remarks	Residues in medical devices are not a part of this evaluation, as this is considered to be covered by other legislations. Thusly, the method for detection in medical equipment are included for information purposes only. The method has not been evaluated by the eCA.

	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.3 **Annex Point IIA 4.3/02**	**Residues in/on food or feedstuffs and <u>other products</u> where relevant** **[Residuals on medical devices]**	
	1 Reference	**Official use only**
1.1 Reference	M. DiCicco, B. Lang, T.I. Harper (2009) Optimization of a novel headspace-solid-phase microextraction-gas chromatographic method by means of a Doehlert uniform shell design for the analysis of trace level ethylene oxide residuals in sterilized medical devices. Biomedical Chromatography **23** pp647-657	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	A novel headspace solid phase microextraction (SPME)-gas chromatographic method developed for analysing trace levels of ethylene oxide in sterilised medical devices was used. Prior to use all of the SPME fibres were conditioned according to the suppliers instructions by inserting them into the GC inlet. A series of preliminary experiments were carried out in order to determine the optimal experimental conditions for EtO Thee final optimum conditions at which the GC peak area response was at a maximum while not affecting the EtO chromatographic peak quality were 0.333 min HS-SPME desorption time, 3.0ºC extraction temperature, 174.0ºC GC inlet temperature and 3.0 min extraction time. The PTFE lined septa of the standard vials were pierced with the SPME fibre sheath and once the SPME holder was in position the fibre was exposed to the gaseous EtO standards. Once the extraction of EtO was complete, the SPME fibre was drawn back into the fibre holder and immediately injected into the GC inlet for quantitative analysis. Following desorption time, the SPME fibre was withdrawn back into the fibre holder for reuse.	
3.1.2 Cleanup	None required	

<table>
<tr><td>3.2 Detection</td><td>Non-entry field</td></tr>
<tr><td>3.2.1 Separation method</td><td>An Agilent Technologies GC 6890N gas chromatograph using a Restek Rtx-502.2 capillary column, 60 m x 0.32 mm id and 1.8 µm df. The GC inlet temperature was 174ºC and the head pressure was 31.6 psi using He carrier gas.</td></tr>
<tr><td>3.2.2 Detector</td><td>Flame ionisation detector (FID)</td></tr>
<tr><td>3.2.3 Standard(s)</td><td>Gaseous standards were prepared in the following manner: A sealed 20 mL HS vial attached to a bubbling apparatus was flushed with 99.9% EtO from a lecture bottle for 15 minutes. Using gas tight syringes (0.1-10.0 µL and 0.25-25.0 µL) volumes of EtO stock were extracted to sealed individual 20 mL HS vials (crimp capped using PTFE lined septa) that had been previously purged with dry nitrogen. Temperature and atmospheric pressure within the laboratory were recorded during the preparation of EtO stock/standards and the mass of the various standards was then calculated using a simple ideal gas law expression.</td></tr>
<tr><td>3.2.4 Interfering substance(s)</td><td>Not reported</td></tr>
<tr><td>3.3 Linearity</td><td>Non-entry field</td></tr>
<tr><td>3.3.1Calibration range</td><td>A calibration curve was constructed using several EtO standards ranging from 1 to 100 ng.</td></tr>
<tr><td>3.3.2 Number of measurements</td><td>Six replicate extractions performed with a single SPME fibre were performed. Similarly the low, medium and high standards were tested 18 times (3 different SPME fibres, 6 replicates per fibre).</td></tr>
<tr><td>3.3.3 Linearity</td><td>The method demonstrates excellent linear range for two orders of magnitude with an R value of 0.9999.
Linear regression statistics figures of merit from calibration curve.

<table>
<tr><td>Slope</td><td>1.9342</td></tr>
<tr><td>y—intercept</td><td>1.6010</td></tr>
<tr><td>Linear correlation coefficient (R)</td><td>0.999</td></tr>
<tr><td>Range (ng)</td><td>1.0-100.0</td></tr>
<tr><td>Number of data points</td><td>4</td></tr>
<tr><td>p of slope</td><td>1.0600×10^{-4}</td></tr>
<tr><td>p of y-intercept</td><td>0.2844</td></tr>
<tr><td>Standard error about the regression line</td><td>1.5581</td></tr>
</table>
The novel optimised HS-SPME-GC method was applied to analysing trace residue levels on several sterilised/aerated cardiovascular stents. The trace levels were found to be within the</td></tr>
</table>

<table>
<tr><td></td><td>linear range of the HS-SPME-GC method. An additional calibration curve was constructed using EtO standards that were subjected to the same heating as the cardiovascular stents as this would enable assist in gauging potential matrix interference. The resulting slope (1.9756) was fairly comparable to the slope calculated from standards indicating no apparent matrix effects as a consequence of heating/volatilising.</td></tr>
<tr><td>3.4 Specifity: interfering substances</td><td>Not reported</td></tr>
<tr><td>3.5 Recovery rates at different levels</td><td>The absolute recovery results from EtO spiked cardiovascular stents are provided. The absolute recovery for the 100 mg EtO spike was good and near target, although the recoveries were higher compared with the respective nominal mass for the 10.0 and 1.0 ng EtO spike levels.
<table>
<tr><td>Stent mass (g)[a]</td><td>EtO Standard/ spike mass (ng)</td><td>Recovery level (ppm)</td><td>Absolute recovery and CV (%)[1]</td></tr>
<tr><td>0.0410</td><td>1.0</td><td>0.024</td><td>121.48 (3.89)</td></tr>
<tr><td>0.0454</td><td>10.0</td><td>0.22</td><td>114.18 (8.99)</td></tr>
<tr><td>0.0458</td><td>100.0</td><td>2.2</td><td>92.52 (1.47)</td></tr>
</table>
1 - Mean values on n = 3 determinations</td></tr>
<tr><td>3.5.1 Relative standard deviation</td><td>1.47 to 8.99%</td></tr>
<tr><td>3.6 Limit of determination</td><td>The reported lower limit of quantification (LLOQ) was 1.0 ng in 20 mL. This equates to 50 parts per trillion. Attesting to the high sensitivity of the HS-SPME-GC method, the limit of detection was roughly 20 pg.</td></tr>
<tr><td>3.7 Precision</td><td>Non-entry field</td></tr>
<tr><td>3.7.1 Repeatability</td><td>The repeatability values were good with CV values of ~ 5% or lower. The values are summarised below.
<table>
<tr><td>EtO mass (ng)</td><td>Accuracy (%)</td><td>Repeatability (CV; %)</td><td>Reproducibility (CV; %)</td></tr>
<tr><td>1.0</td><td>97.73</td><td>5.03</td><td>20.98</td></tr>
<tr><td>10.0</td><td>105.36</td><td>2.86</td><td>7.65</td></tr>
<tr><td>50.0</td><td>98.16</td><td>4.54</td><td>-</td></tr>
<tr><td>100.0</td><td>100.40</td><td>5.58</td><td>14.21</td></tr>
</table>
The reproducibility CV of SPME fibres was reasonable at the medium and high concentrations but was somewhat diminished at</td></tr>
</table>

	the low limit of quantification.
3.7.2 Independent laboratory validation	Not performed on this work.
	4 Applicant's Summary and conclusion
4.1 Materials and methods	This study reports the development of a novel highly sensitive Headspace solid phase microextraction-gas chromatographic method developed for analysing trace levels of EtO residues in sterilised medical devices. Experiments with HS-SPME desorption time, extraction temperature, GC inlet temperature and extraction time were performed in order to optimise the experimental conditions for EtO analysis. The final optimal experimental conditions were 0.333 min HS-SPME desorption time, 3.0ºC extraction temperature, 174ºC GC inlet temperature and 3.0 min extraction time. Standards of EtO were sealed in 20 mL HS vials prior to testing. SPME fibres were exposed to EtO and then injected into a GC to thermally desorb the EtO for quantitative analysis. The method has demonstrated excellent linear range for two orders of magnitude (1.0-100 ng) and the repeatability values were good.
4.2 Conclusion	The evaluation of this HS-SPME-GC method and its application to analysing trace levels of EtO residues on sterilised/aerated cardiovascular stents yielded largely good results. The benefit of this method lies in the ability to quantify trace levels of EtO on devices that may not have been detectable by conventional sampling. It also removes the need to pool devices to attain the required sensitivity for analysis. Using aqueous extraction will provide information on potential in-use exposure of patients rather than a total contained residual amount.
4.2.1 Reliability	2
4.2.2 Deficiencies	This method is currently limited for use determining trace levels of EtO on small medical devices.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	-
Results and discussion	-
Conclusion	-
Reliability	-
Acceptability	-

Remarks	Residues in medical devices are not a part of this evaluation, as this is considered to be covered by other legislations. Thusly, the method for detection in medical equipment are included for information purposes only. The method has not been evaluated by the eCA.
	Comments from ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.3 **Annex Point IIA 4.3/03**	**Residues in/on food or feedstuffs and <u>other products</u> where relevant** **[Residuals on medical devices]**	
	1 Reference	**Official use only**
1.1 Reference	European Committee for Standardization (2008) Biological evaluation of medical devices – Part 7: Ethylene oxide sterilization residuals (NBN EN ISO 10993-7:2008)	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No: Standard method	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Two scenarios are considered in the ISO document: 1) **simulated-use extraction** extraction to demonstrate compliance with the requirements of this part of ISO 10993, by evaluating	

	residue levels available to the patient or user from devices during the routine use of a device with water extraction to simulate product use. 2) **exhaustive extraction** extraction until the amount of EtO or ECH in a subsequent extraction is less than 10 % of that detected in the first extraction, or until there is no analytically significant increase in the cumulative residue levels detected. The ISO document reviews and summarises various analytical methods for EtO residuals (including ECH and EG). Time between removal of samples from a controlled aeration area and the beginning of extraction should be held to a minimum. Samples shall be shipped on dry ice on overnight delivery. Test samples may also be taken directly from the product load at the desired aeration interval and immediately placed in a headspace vial which is then shipped to the analytical laboratory. Samples may be extracted and the extraction fluid shipped to the laboratory for analysis. If the extraction fluid is water then the fluid is kept at ice-cold temperatures. The volume of fluid used to extract residues from devices or representative sections of them should be sufficient to maximise extraction efficiency while maintaining detection sensitivity. Samples shall be extracted for a time equivalent to or exceeding the maximum time for single use, and at temperatures that provide the most realistic simulated challenge. Simulated-use extraction: Water is commonly used for the recovery of residual EtO. Devices that are wholly or partially in contact with the body should be extracted at 37ºC whereas those devices having no immediate body contact should be extracted at 25ºC. If the assay is not performed immediately then the extract should be decanted into a PTFE septum capped vial. The headspace of the vial should be less than 10% of the total volume. Exhaustive extraction: Exhaustive extraction represents an acceptable alternative to simulated-use extraction. It produces results greater or equal to the one that the patient may receive. Such extraction also precludes measurement of dose as a function of time. A variety of extraction fluids have been used for exhaustive recovery of residual EtO. Thermal desorption followed by headspace analysis is an example of a procedure that does not use extraction fluid. Headspace analysis may not be feasible or preferred for intact testing of large or complex devices. For solvent extraction procedures, selection of a suitable extraction fluid depends on the material of the device and its components.
3.1.2 Cleanup	Optional, usually not required.
3.2 Detection	Non-entry field

3.2.1 Separation method	Gas chromatograph determination. Any method that can be shown to be analytically sound and meets the performance criteria described in ISO 10993 can be used. Capillary column techniques can detect EtO, ECH and EG in one sequence.
3.2.2 Detector	Commonly FID or ECD. GC-MS can be used to confirm identity.
3.2.3 Standard(s)	Two alternatives can be used for the preparation of standards. 1) Use of prepared standards from commercial sources 2) Preparation of standards either volumetrically, by diluting known volumes of EtO gas or gravimetrically, by diluting a known mass of EtO liquid. ECH and EG standards can be similarly prepared.
3.2.4 Interfering substance(s)	Not discussed.
3.3 Linearity	Non-entry field
3.3.1 Calibration range	Accuracy should ideally be assessed using a minimum of nine determinations over a minimum of three concentration levels covering a specified range (i.e. three replicates each at three different concentrations). The range should cover the target concentrations of concern.
3.3.2 Number of measurements	Not discussed.
3.3.3 Linearity	Regression analysis of the standard concentration versus peak area or peak height should be performed using a minimum of five concentrations. The linearity of the correlation data should be determined along with the reproducibility of the slope and the intercept. The minimum correlation coefficient for the standard curve should be 0.95
3.4 Specifity: interfering substances	Not discussed.
3.5 Recovery rates at different levels	Not discussed
3.5.1 Relative standard deviation	Not discussed
3.6 Limit of determination	The method detection limit is the smallest amount that can be detected with reasonable confidence. Determination of the signal-to-noise ratio is performed by comparing measured signals of known low concentrations with blank samples and establishing the minimum concentration that can be reliably detected. A signal-to-noise ratio of 3:1 is generally accepted.
3.7 Precision	Non-entry field
3.7.1 Repeatability	Repeatability can be assessed using a minimum of nine determinations covering the specified range of standards used (i.e. three replicates each at three different concentrations. Repeatability can be calculated as the relative standard deviation of the peak area. The % relative standard deviation should not

<table>
<tr><td></td><td>exceed 5% for the range of standards used.</td></tr>
<tr><td>3.7.2 Independent laboratory validation</td><td>Interlaboratory evaluations are considered within the ISO document.

EtO methods

An interlaboratory evaluation was conducted at 13 laboratories using several EtO methods on a series of samples with analytical values distributed from about 40 ppm to about 350 ppm. The estimated total coefficient of variation of the methods is given in Table K.1.

Table K.1 — Comparison of intra- and interlaboratory variations
<table><tr><th>EO method</th><th>Intralaboratory</th><th>Interlaboratory</th></tr><tr><td>Headspace method</td><td>3,7 %</td><td>21,3 %</td></tr><tr><td>Acetone method</td><td>4,1 %</td><td>16,3 %</td></tr><tr><td>DMF method</td><td>2,9 %</td><td>8,3 %</td></tr><tr><td>Aqueous method</td><td>2,7 %</td><td>17,0 %</td></tr></table>
ECH methods

An interlaboratory evaluation was conducted for ECH. The estimated total coefficient of variation of the methods was as follows:

• Intralaboratory 7.46 %

• Interlaboratory 10.99 %

These data were obtained for ECH concentrations of about 3.0 ppm to 100 ppm.</td></tr>
<tr><td></td><td><h2>4 Applicant's Summary and conclusion</h2></td></tr>
<tr><td>4.1 Materials and methods</td><td>This document was produced by the European Committee for Standardization guide to the evaluation of the biological evaluation of medical devices: Ethylene oxide sterilization residuals. The document specifies the allowable limits for residual ethylene oxide in individual EtO –sterilised medical devices, procedures for the measurement of EtO and methods for determining compliance so that devices may be released. Guidance on sampling, extraction, gas chromatographic procedures and the application of the standards is provided. Similar discussions are presented for the reaction products ECH and EG.</td></tr>
<tr><td>4.2 Conclusion</td><td>This document provides guidance on the analytical methods for the biological evaluation of medical devices that have been sterilised with ethylene oxide depending on the devices intended use and the level and duration of exposure that a patient could expect. The potential for residuals of reaction products ECH and EG is addressed with analysis techniques.</td></tr>
<tr><td>4.2.1 Reliability</td><td>2 (ISO guideline document)</td></tr>
</table>

4.2.2 Deficiencies	This document is a guide to the assessment of EtO derived residual exposure rather than actual specific methods.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	February 2020
Materials and Methods	-
Results and discussion	-
Conclusion	-
Reliability	-
Acceptability	-
Remarks	Residues in medical devices are not a part of this evaluation, as this is considered to be covered by other legislations. Thusly, the method for detection in medical equipment are included for information purposes only. The method has not been evaluated by the eCA
	Comments from ...
Date	
Materials and Methods	
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Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.3 **Annex Point IIA 4.3/04**	**Residues in/on food or feedstuffs and <u>other products</u> where relevant** **[Residuals on medical devices]**	
	1 Reference	**Official use only**
1.1 Reference	E. De Rudder, E. De Graeve, R. Van Severen, P. Braeckman (1986) Quantification of ethylene chlorohydrin and ethylene glycol as potential reaction products in gas-sterilized medical-grade plastics. Journal of Clinical and Hospital Pharmacy **11** pp125-130	
1.2 Data protection	Non-entry field	
1.2.1 Data owner	Not applicable	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No	
2.2 GLP	No	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Preliminary treatment	Non-entry field	
3.1.1 Enrichment	Accurately weighed medical plastic polymer (nominally 700 mg) is extracted using 13% v/v ethyl acetate, 66% v/v of twice distilled water and 21% v/v of methanol, spiked with 10 ppm propylene glycol as an ISTD. Gas-tight tubes are used for the extraction with 2 mL of the above solvent mixture, stood for 2 hours at 80°C. Aliquots (10 µL) are taken of the cooled solvent for injection into the GC system.	
3.1.2 Cleanup	Not required.	
3.2 Detection	Non-entry field.	
3.2.1 Separation method	GLC-analyses were carried out on an HP 5750 G gas chromatograph. Isothermal elution of ethylene chlorohydrin (ECH) was performed at 100ºC, after this the temperature was raised to 120 ºC. The injector temperature was set at 200ºC and the detector temperature at 180ºC. Helium was used as a carrier gas. A packed GC column (3 m x 2.0 mm id) with 5% Carbowax was used for separation. A modern equivalent capillary GC column would be acceptable.	
3.2.2 Detector	Flame ionisation detection (FID)	
3.2.3 Standard(s)	Standard solutions containing 2-15 ppm of ECH and 4-20 ppm of	

<table>
<tr><td></td><td>ethylene glycol (EG) were used as the extraction liquid in order to construct a curve..</td></tr>
<tr><td>3.2.4 Interfering substance(s)</td><td>Not reported</td></tr>
<tr><td>3.3 Linearity</td><td>Non-entry field.</td></tr>
<tr><td>3.3.1Calibration range</td><td>Ethylene chlorohydrin: 2-15 ppm
Ethylene glycol: 4-20 ppm</td></tr>
<tr><td>3.3.2 Number of measurements</td><td>Not reported</td></tr>
<tr><td>3.3.3 Linearity</td><td>By plotting the peak area ratios of ethylene chlorohydrin/propylene glycol and ethylene glycol/propylene glycol versus the ECH and EG concentrations, calibration curves were obtained. Details are provided in the tables below.

Slopes of the calibration curve for the determination of ECH in different polymers (concentration range 2-15 ppm)
<table>
<tr><td>Polymer</td><td>Slope</td><td>Y-intercept</td><td>Correlation coefficient</td></tr>
<tr><td>-</td><td>0.0566</td><td>0.0221</td><td>0.9993</td></tr>
<tr><td>Polyvinylchloride (PVC)</td><td>0.0567</td><td>-0.0051</td><td>0.9996</td></tr>
<tr><td>Silicon Rubber (SR)</td><td>0.0606</td><td>0.0060</td><td>0.9991</td></tr>
<tr><td>Polyethylene (PE)</td><td>0.0582</td><td>0.0161</td><td>0.9997</td></tr>
<tr><td>Polyproylene (PP)</td><td>0.0578</td><td>0.0178</td><td>0.9999</td></tr>
</table>

Slopes of the calibration curve for the determination of EG in different polymers (concentration range 4-20 ppm)
<table>
<tr><td>Polymer</td><td>Slope</td><td>Y-intercept</td><td>Correlation coefficient</td></tr>
<tr><td>-</td><td>0.0224</td><td>-0.0075</td><td>0.9994</td></tr>
<tr><td>PVC</td><td>0.0237</td><td>0.0621</td><td>0.9990</td></tr>
<tr><td>SR</td><td>0.0240</td><td>0.0321</td><td>0.9989</td></tr>
<tr><td>PE</td><td>0.0229</td><td>0.0097</td><td>0.9987</td></tr>
<tr><td>PP</td><td>0.0220</td><td>-0.0093</td><td>0.9993</td></tr>
</table></td></tr>
</table>

<table>
<tr><td>3.4 Specifity: interfering substances</td><td>Ethylene oxide interferes with the detection of ethylene glycol at levels of above 2ppm.</td></tr>
<tr><td>3.5 Recovery rates at different levels</td><td>For the quantification of the extraction efficiency, the peak area ratios of a standard solution containing 10 ppm of both ECH and EG with and without blank polymer were compared. Results are shown in the table below.

Quantification of the extraction efficiency (n=5) for the analysis of ethylene chlorohydrin and ethylene glycol from plastics

<table>
<tr><td rowspan="2">Polymer</td><td>ECH 10ppm</td><td rowspan="2">Without polymer</td><td>EG 10ppm</td></tr>
<tr><td>With polymer (%)</td><td>With polymer (%)</td></tr>
<tr><td>PVC</td><td>96</td><td>100</td><td>98</td></tr>
<tr><td>SR</td><td>104</td><td>100</td><td>102</td></tr>
<tr><td>PP</td><td>100</td><td>100</td><td>103</td></tr>
<tr><td>PE</td><td>94</td><td>100</td><td>100</td></tr>
</table>
</td></tr>
<tr><td>3.5.1 Relative standard deviation</td><td>The intra-analytical variance checked for this standard solution is 1.1 % for ECH and 3.2% for EG (n=5). The inter analytical variance for the determination of ECH and EG in polymers is about 2% (n=5).</td></tr>
<tr><td>3.6 Limit of determination</td><td>The lower limits of detection are 1 ppm of ethylene chlorohydrin and 2 ppm of ethylene glycol.</td></tr>
<tr><td>3.7 Precision</td><td>Non-entry field</td></tr>
<tr><td>3.7.1 Repeatability</td><td>Not reported</td></tr>
<tr><td>3.7.2 Independent laboratory validation</td><td>Not performed</td></tr>
<tr><td></td><td><h2>4 Applicant's Summary and conclusion</h2></td></tr>
<tr><td>4.1 Materials and methods</td><td>This study describes a method used for determination of ethylene chlorohydrin and ethylene glycol residues following sterilisation of plastic medical devices with ethylene oxide. This paper describes a gas chromatographic method for the simultaneous quantification of ECH and EG. The optimum extraction liquid was prepared by mixing 13% v/v of ethyl acetate, 66% v/v of twice distilled water and 21% v/v of methanol and was spiked with 10 ppm propylene glycol (ISTD). This mixture was used for extraction of components in /on plastics carried out in glass tubes. For the elaboration of a calibration curve, known concentrations were added to the extraction solution By plotting the peak area ratios of ethylene chlorohydrins/propylene glycol and ethylene glycol/propylene glycol individual curves were obtained for each</td></tr>
</table>

	of the polymers used in the test. Samples were analysed by treating 700 mg of polymer with 2 mL of spiked extraction liquid and standing tubes for 2 hours at 80ºC before determination by gas chromatography with FID. The behaviour of ethylene oxide under the conditions of the test were investigated by spiking standard solutions with 1000 ppm of EtO. Half of the solutions were heated at 80ºC for 2h while the other half were kept at room temperature. GLC analysis of the samples showed no difference in the ECH concentration but large amounts of the EG were found in the heated sample due to hydrolysis of EtO. Results imply that quantification of EG is only possible if EtO levels are below 2 ppm.
4.2 Conclusion	This gas-liquid chromatographic method allows simultaneous analysis of ethylene chlorohydrin and ethylene glycol in gas-sterilised medical grade plastics.
4.2.1 Reliability	2
4.2.2 Deficiencies	As EtO concentration interferes with the analysis of EG aeration time necessary for EtO concentration to drop below 2 ppm may be required before assessment of ECH and EG concentrations.

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
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