Ethylene Oxide

For use as a gaseous sterilant (PT2)

Document IIIA

Section 6

Toxicological and metabolic studies

December 2009

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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.

Acute Toxicity

6.1.1 Oral

Section A6.1.1/01 **Annex Point IIA 6.1.1**	**Acute toxicity – oral, rat**	
	1 Reference	**Official use only**
1.1 Reference	Smyth, H., Seaton, J and Fischer, L. (1941) The Single Dose Toxicity of Some Glycols and Derivatives J. Industrial Hygiene and Toxicology, 23, 259-268	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No guideline existed when the study was conducted	
2.2 GLP	No	
2.3 Deviations	None, not a guideline study	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2.2 Specification	Not reported	
3.1.2.3 Description	Not reported	
3.1.2.4 Purity	Not reported	X
3.1.2.5 Stability	Not reported	
3.2 Test Animals		
3.2.1 Species	Rat and guinea pig	
3.2.2 Strain	Rat: Wistar, guinea pig: Not reported	
3.2.3 Source	Not reported	
3.2.4 Sex	Rat: Male, guinea pig: Male and female	
3.2.5 Age/weight at study initiation	Rat: 90-120 g, guinea pig: 250-300 g	

3.2.6 Number of animals per group	In most cases, 10 animals per dose were used.	
3.2.7 Control animals	Not reported	
3.3 Administration/ Exposure		
3.3.1 Post exposure period	14 days	
3.3.2 Type	Oral (gavage)	
3.3.3 Concentration	Not reported	
3.3.4 Vehicle	Water	
3.3.5 Concentration in vehicle	1%	
3.3.6 Total volume applied	Not reported	
3.3.7 Controls	Not reported	
3.4 Examinations	Only mortality reported	
3.5 Method of determination of LD_{50}	Probit analysis described by Bliss[1]	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Clinical signs	Clinical signs and body weights were not reported	
4.2 Pathology	All ethers were stated to induce narcosis at dose levels close to LD50 or above. Gross pathology showed some degree of digestive tract irritation for all tested substances. The primary action of all substances is stated to be on kidneys, rarely proceeding as far as bloody urine and free blood beneath the capsule from the largest dosages. The liver was affected less, but bile was often orange or reddish.	
4.3 Other	None	
4.4 LD_{50}	Rat: 330 mg/kg (95% confidence limits 290-360 mg/kg) Guinea pig: 270 mg/kg (95% confidence limits 190-380 mg/kg). Deaths were reported to be delayed by about a week.	
	5 Applicant's Summary and conclusion	
5.1Materials and methods	Male rats and male and female guinea pigs were given single oral doses of ethylene oxide in water and mortality was recorded during the following 14 days.	

5.2 Results and discussion	The LD_{50} was 330 mg/kg (95% confidence limits 290-360 mg/kg) in male rats and 270 mg/kg (95% confidence limits 190-380 mg/kg) in guinea pigs.	
5.3 Conclusion	The acute oral toxicity of ethylene oxide was similar in the male rat and both sexes of guinea pig.	
5.3.1 Reliability	3	
5.3.2 Deficiencies	A guideline did not exist when this study was conducted. Compared with current requirements clinical signs of toxicity were not reported, but gross necropsy was conducted. The publication includes no details of the dose levels or group sizes used and there is little information on the time at which animals died. No information on test substance purity and batch is available.	x
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.4 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study. The summary of the applicant is acceptable with minor remarks added by eCa.	
Results and discussion		
Conclusion	The summary of the applicant is acceptable.	
Reliability	3 The summary of the applicant is acceptable with the following remark: clinical signs of toxicity and body weight changes were not reported, but gross necropsy was apparently conducted. No information on test substance purity and batch is available.	
Acceptability	Based on study limitations, it is not sufficient for assessment as a stand-alone, but can be accepted as a part of weight of evidence.	

Remarks	The applicant has not provided a waiver. However, according to Acute Toxicity [Ann IIA, VI. 6.1.] of BPD (98/8/EC), gases and volatile liquids should be administered by the inhalation route. Ethylene oxide is a gas, therefore inhalation exposure is considered the most relevant exposure route. As acute inhalation toxicity studies are available for ethylene oxide, performance of acute oral toxicity studies is considered to be not warranted.

6.1.2 Dermal

Section 6.1.2 **Annex Point 6.1.2**	**Acute toxicity - Dermal**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	An acute dermal toxicity study is not available. Ethylene oxide is a gas at room temperature and exposure is only by inhalation. Conducting a new study is not considered necessary and would not be a justified use of animals because there is no human exposure by that route.	
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2020	
Evaluation of applicant's justification	The justification of the applicant is acceptable. In accordance with the BPD (98/8/EC) TNsG on data requirements, dermal toxicity [Ann IIA, VI. 6.1.2.] must be reported in an active substance except for gases. Ethylene oxide is a gas, therefore inhalation exposure is considered the most relevant exposure route. According to acute Toxicity [Ann IIA, VI. 6.1.] of BPD (98/8/EC), gases and volatile liquids should be administered by the inhalation route. As acute inhalation toxicity studies are available for ethylene oxide, performance of acute dermal toxicity studies is considered not to be warranted.	
Conclusion	The justification of the applicant for nonperformance of an acute dermal toxicity study is acceptable. No acute dermal toxicity study needs to be conducted.	

Section 6.1.2 **Annex Point 6.1.2**	**Acute toxicity - Dermal**
Remarks	

6.1.3 Inhalation

Section A6.1.3/01 **Annex Point IIA6.1**	**Acute Toxicity**	
	1 Reference	**Official use only**
1.1 Reference	Jacobson, K.H., Hackley, E.B. and Feinsilver L. (1956) The Toxicity of Inhaled Ethylene Oxide and Propylene Oxide Vapors American Medical Association Archives of Industrial Health, 13, 237-244	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study was similar to OECD 403 Acute Inhalation Toxicity.	
2.2 GLP	No	
2.3 Deviations	Only one sex was used for each species and body weight was not reported.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2.2 Specification	Not reported: obtained from Matheson Company, East Rutherford, N.J.	
3.1.2.3 Description	Not reported	
3.1.2.4 Purity	Not reported	X
3.1.2.5 Stability	Not reported	
3.2 Test Animals		

3.2.1 Species	Rat, mouse and dog	
3.2.2 Strain	Rats: Not reported. Mice: Not reported. Dogs: Beagle	
3.2.3 Source	Not reported	
3.2.4 Sex	Rats and dogs: Male. Mice: Female	
3.2.5 Age/weight at study initiation	Not reported	
3.2.6 Number of animals per group	Rats and mice: 10. Dogs:3	
3.2.7 Control animals	Yes for pathology only. Rats and mice: 10. Dogs: 3	
3.3 Administration/ Exposure		
3.3.1 Postexposure period	14 days	
3.3.2Type	Inhalation	
3.3.3Concentration	See 3.3.8	
3.3.4Vehicle	None	
3.3.5 Concentration in vehicle	Not applicable	
3.3.6 Total volume applied	Not applicable	
3.3.7 Controls	Ambient air	
	Inhalation	
3.3.8 Concentrations	Nominal concentration Not reported	
	Analytical concentration Rats: 882, 1343, 1648, 1843, 1902 and 2208 ppm Mice: 533, 860, 882, 960, 1343 and 1365 ppm Dogs: 327, 710, 1393 and 2830 ppm	
3.3.9 Particle size	Not applicable	
3.3.10 Type or preparation of particles	Not applicable	
3.3.11Type of exposure	Not reported but probably whole body (exposure chambers were reported to have been used)	
3.3.12Vehicle	None	
3.3.13 Concentration in vehicle	Not applicable	

3.3.14 Duration of exposure	4 hours	
3.3.15 Controls	Ambient air	
3.4Examinations	Clinical signs of toxicity and gross pathology; blood counts (RBC, WBC, differential count, sedimentation rate, haemoglobin, haematocrit) for dogs (prior to exposure, so each dog served as its own control)	
3.5 Method of determination of LD_{50}	Bliss-Finney method for rats and mice[2] and Thompson-Weil method for dogs[3]	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Clinical signs	Rats: Frequent movement and preening, clear nasal discharge, lacrimation, diarrhoea, gasping (increasing in intensity during exposure) and, occasionally, salivation. Mice: Similar signs to rats except that diarrhoea was not observed Dogs: At 2800 ppm: lacrimation, clear nasal discharge, salivation, vomiting of a frothy colourless/yellow mucus, diarrhea and convulsions with laboured breathing. At 1400 ppm: similar signs except for diarrhea, convulsions and observable changes in respiration	
4.2 Pathology	Rats: Irritation in the upper respiratory passages, moderate congestion and petechial haemorrhages in the tracheal mucosa. The maximum pulmonary lesions consisted of a very minor degree of patchy scattered oedema sometimes involving the peribronchial zones. Mice: Details not reported Dogs: Moderate congestion in the lungs, dilatation of perivascular lymphatic spaces and perivascular oedema. No significant changes in RBC, WBC, differential count, sedimentation rate, haemoglobin or haematocrit were noted. Gross distension of the stomach was noted in all three species. Gross distension of the stomach was noted in all three species.	
4.3 Other	Mortality data are shown in Table 6.1.3/01-1.	
4.4 LD_{50}	The LD50 of ethylene oxide was 1460 (95% CI = 620-2550), 835 (95% CI = 623-1040 ppm) and 960 ppm (95% CI not specified) (equal to 2630, 1504 and 1730 mg/m3) in male rats, female mice and male dogs respectively. The other sex was not tested.	
	5 Applicant's Summary and conclusion	

5.1Materials and methods	Rats and mice (10 per group) and dogs (3 per group) were exposed by inhalation to varying concentrations of ethylene oxide for 4 hours. Clinical signs of toxicity and mortality were recorded for 14 days after exposure and dead and surviving animals were given a gross necropsy.	
5.2 Results and Discussion	Clinical signs of toxicity were frequent movement and preening, nasal discharge, lacrimation, diarrhoea, gasping and, occasionally, salivation, vomiting of a frothy colourless/yellow mucus and convulsions with laboured breathing. Deaths occurred. At necropsy macroscopic effects of exposure were irritation in the upper respiratory passages, moderate congestion and haemorrhages in the tracheal mucosa, scattered oedema in the lungs and peribronchial zones in rats and moderate congestion in the lungs, dilatation of perivascular lymphatic spaces and perivascular oedema in dogs. The LD_{50} of ethylene oxide was 1460, 835 and 960 ppm in rats, mice and dogs respectively.	
5.3 Conclusion	The acute inhalation toxicity of ethylene oxide was similar in rats, mice and dogs.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	Both sexes were not used in each species and body weight was not reported.	x
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.4 Purity* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and Discussion		
Conclusion		
Reliability	Reliability 2	
Acceptability	Acceptable. The study was also considered in the RAC opinion (2017) in their evaluation of classification for acute inhalation toxicity for ethylene oxide. The derived LC50 is comparable with values reported in other studies.	

Remarks	Information on test material not reported, no information on the test animal strain, only one sex per species was tested. No information on the concentrations at which clinical signs were observed, and individual data not available.

Table 6.1.3/01-1: Mortality in rats, mice and dogs exposed to ethylene oxide by inhalation for 4 hours

Species/ Concentration (ppm)	Mortality	% Mortality 14 days after exposure
Rats		
2208	3/10 1st hour, 9/10 1st day, 10/10 2nd day	100
1992	5/10 2nd hour, 9/10 1st day, 10/10 4th day	100
1843	6/10 1st day, 9/10 2nd day	90
1648	2/10 1st day, 3/10 2nd day, 4/10 4th day	40
1343	1/10 2nd day, 2/10 10th day	20
882	1/10 9th day, 2/10 14th day	20
Mice		
1365	2/10 on removal from chamber, 3/10 2nd hour, 10/10 1st day	100
1343	4/10 on removal from chamber, 7/10 1st day, 9/10 3 rd day, 10/10 4th day	100
960	1/10 1st hour, 5/10 1st day, 6/10 9th day, 7/10 12th day	70
882	2/10 1st day, 3/10 2nd day	30
860	3/10 2nd hour, 6/10 1st day	60
533	1/10 13th day	10
Dogs		
2830	1/3 3rd day, 3/3 5th day	100
1303	3/3 1st day	100
710	0/3 14th day	0
327	0/3 14th day	0

6.1.4 Skin and eye irritation

Section 6.1.4 **Annex Point 6.1.4**	**Skin irritation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements.* *The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	

Section 6.1.4 **Annex Point 6.1.4**	**Skin irritation**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	A dermal irritation study in laboratory animals is not available. Ethylene oxide is a gas at room temperature and exposure is normally only by inhalation. Ethylene oxide is known to be an irritant to skin in humans (Annex point 6.12.2) so conducting a new study is considered unecessary and would not be a justified use of animals.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2020	
Evaluation of applicant's justification	The applicant submitted a request for data waiving with regard to a dermal irritation study in laboratory animals, as ethylene oxide is a gas at room temperature and exposure is normally only by inhalation, and the substance is known to be irritaint to human skin. According to the BPD, a skin irritation study needs to be performed. However, handling of ethylene oxide gas is not easy, and performing such an experiment will be technical difficult. As ethylene oxide is flammable, explosive, carcinogenic, mutagenic and reproductive toxic, special precautions must be taken when handling the substance. There is information available from the CLH report on this endpoint on which RAC has concluded to classify ethylene oxide as corrosive to skin (Skin Corr. 1, H314). RAC based their conclusion on two rabbit studies, both with deviations, and publicly available human data. One of the rabbits studies (Hollingsworth et al, 1956) was submitted by the applicant for repeated dose toxicity. However, the studies/case had all some limitations and RAC decided that they did not allow for differentiation between the skin corrosion subcategories 1A/1B/1. The performance of a new skin irritation study is considered not warranted by the eCA.	
Conclusion	The justification of the applicant is acceptable. The performance of a skin irritation study is considered not warranted.	
Remarks		

Section A6.1.4/01 **Annex Point IIA 6.1.4**	**Acute toxicity - eye irritation**	

	1 Reference	**Official use only**
1.1 Reference	McDonald, T., Kasten, K., Hervey, R., Gregg, S., Borgmann, A. and Murchison, T. (1973) Acute Ocular Toxicity of Ethylene Oxide, Ethylene Glycol and Ethylene Chlorohydrin Bulletin of the Parental Drug Association, 27, 153-164	x
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No, guideline compliance is not claimed	
2.2 GLP	No	
2.3 Deviations	The study was designed to establish a NOAEL for eye irritation and neither the application regimen nor the scoring system were as described in OECD 405	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: Obtained from Eastman, Inc.	
3.1.3 Description	Not reported	
3.1.4 Purity	Not reported	x
3.1.5 Stability	Not reported	
3.2 Test animals		
3.2.1 Species	Rabbit	
3.2.2 Strain	New Zealand White	
3.2.3 Source	Not reported	
3.2.4 Sex	Male and female	
3.2.5 Age/ weight at study initiation	Approximately 2 kg	
3.2.6 Number of animals per group	6	
3.2.7 Control animals	Two groups of 6, one group treated with vehicle only and the other untreated	

3.3 Administration/ Exposure		
3.3.1 Preparation of test substance	Solution in balanced salt solution	
3.3.2 Amount of active substance instilled	Approximately 0.05 mL	
3.3.3 Exposure period	An application was made every 10 minutes for 6 hours, for a total of 36 applications	
3.3.4 Post exposure period	8 days after the last treatment	
3.4 Examinations		
3.4.1 Ophthalmoscopic examination	Cornea, iris, anterior chamber and lens were examined with the aid of a biomicroscope. These tissues, the retina and conjunctiva were also examined by histopathology.	
3.4.1.1 Scoring system	Conjunctival changes were graded according to the method of Draize and changes to the cornea, iris, anterior chamber and lens were graded by the method Baldwin.	
3.4.1.2 Examination time points	6 hours, 1, 2, 7 and 14 days after the start of treatment	
3.4.2 Other investigations	Three eyes from each test group were removed 6 hours and 14 days after the start of treatment for histopathology.	
3.5 Further remarks	At least four concentrations of ethylene oxide were tested, namely 0.1, 1.0, 5.0 and 20%	
	4 Results and Discussion	
4.1 Clinical signs	Lens and cornea showed signs of opacity. Changes in the conjunctiva were manifested as congestion, swelling and discharge. Flare and hyperemia were noted in the iris indicative of an irritant response in the anterior chamber. A purulent discharge was observed in the eyes of rabbits given the highest concentrations of ethylene oxide from day 7. The maximum non damaging concentrations of ethylene oxide varied from 0.1% for the conjunctiva to >20% for the lens and retina (Table 6.1.4/01-1).	
4.2 Average score		
4.2.1 Cornea	Not reported	
4.2.2 Iris	Not reported	
4.2.3 Conjunctiva	Not reported	
4.2.3.1 Redness	Not reported	
4.2.3.2 Chemosis	Not reported	
4.3 Reversibility	Changes in the iris and anterior chamber were reversible by around day 7. Once induced, corneal and lenticular damage was irreversible based	

	on biomicroscopic examination. At concentrations >1% conjunctivitis persisted to day 14.	
4.4 Other	None	
4.5 Overall Result	Ethylene oxide was an irritant to the eye of rabbits.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Varying concentrations of ethylene oxide in saline were instilled in the eye of rabbits. An application was made every 10 mins for 6 hours. The eyes were examined 1, 2, 7 and 14 days after the start of treatment using a biomicroscope and after 6 hours and 14 days by histopathology.	
5.2 Results and discussion	Treatment related changes were found in most ocular structures and the conjunctiva was the most sensitive part of the eye. Effects on the iris and anterior chamber were reversible by around day 7 but changes in the cornea, and lens were irreversible once induced. Conjunctivitis persisted to day 14 at concentrations > 1%	
5.3 Conclusion	At concentrations of 5.0 and 20%, ethylene oxide produced irreversible effects in rabbit eyes.	
5.3.1 Reliability	2	X
5.3.2 Deficiencies	The study was designed to establish a NOAEL for eye irritation and both the application regimen and the scoring system were not as described in OECD 405. However it did establish that ethylene oxide was an eye irritant.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	

Materials and Methods	*3.1.4 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.
Results and discussion	
Conclusion	
Reliability	Reliability 3
Acceptability	The study is not sufficient on its own to cover the endpoint, but it confirms the classification of ethylene oxide as causing serious damage to eyes.
Remarks	Not a guideline study but to establish a NOAEL for eye irritation. The application regimen nor the scoring system were as described in OECD 405. No information on purity. Study duration 14 days. Several concentrations were tested and in addition to clinical signs, histopathology was performed on the eyes. In addition, the BPD TNsG on data requirements states that: *where the active substance has shown to have potential corrosive properties, or is a severe skin irritant, eye irritation test shall not be necessary*. Therefore there is no data gap for eye irritation.

Table 6.1.4/01-1: No effect concentrations of ethylene oxide (%) for ocular damage after repeated instillation to the eye of rabbits for 6 hours

Tissue	Examination type		
	Macroscopic	Biomicroscopic	Microscopic
Conjunctiva	0.1%	-	0.1%
Cornea	-	1.0%	1.0%
Anterior chamber	-	5.0%	-
Iris/ciliary body	-	1.0%	1.0%
Lens	-	5.0%	>20%
Retina	-	-	>20%

6.1.5 Skin sensitisation

Section 6.1.5 **Annex Point 6.1.5**	**Skin sensitisation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A skin sensitisation study is not available. Ethylene oxide is a gas at room temperature and exposure is mainly by inhalation. Ethylene oxide is known to be a contact sensitiser in humans so conducting a new study is not considered necessary and would not be a justified use of animals.	x
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2020	
Evaluation of applicant's justification	The BPD indicates that an OECD skin sensitisation study needs to be performed. However, handling of ethylene oxide gas is not easy, and performing such an experiment will be technical difficult. As ethylene oxide is flammable, explosive, carcinogenic, mutagenic and reproductive toxic, special precautions must be taken when handling the substance. There is information available from the CLH report on this endpoint on which RAC has concluded that ethylene oxide should not classified as a skin sensitiser. The RAC based their conclusion on the overall weight in publicly available (human) data. The performance of a skin sensitisation study is considered not warranted by the eCA.	
Conclusion	The conclusion of the applicant that the performance of a new skin sensitisation study is not warranted, is supported by the eCA.	
Remarks		

6.2 Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study

Section A6.2/01 **Annex Point IIA 6.2**	**Metabolism studies in mammals**	
	1 Reference	**Official use only**
1.1 Reference	Filser, J. G. and Bolt, H. M. (1984) Inhalation pharmacokinetics based on gas uptake studies VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats Arch. Toxicol, 55, 219-223	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed.	
2.2 GLP	Not GLP	
2.3 Deviations	None, guideline compliance was not claimed	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Ethylene oxide: gas	
3.1.4 Purity	Ethylene oxide: 99.7%	
3.1.5 Stability	Not reported	
3.2 Test animals		
3.2.1 Species	Rat	
3.2.2 Strain	Sprague-Dawley	
3.2.3 Source	Ivanocas, Kissleg, Germany	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	150 - 280 g	

3.2.6 Number of animals per group	2 for inhalation exposure, 1 for intraperitoneal administration	
3.2.7 Control animals	2 rats per group, exposed to soda lime only in the dessicator chamber.	
3.3 Administration/ Exposure		
3.3.1 Administration	Whole-body inhalation exposure to ethylene oxide was performed in a desiccator jar chamber with soda lime to absorb carbon dioxide. Intraperitoneal administration of ethylene oxide was by injection as a gas.	
3.3.2 Dose level	Three exposure levels of approximately 100 to 1000 ppm by inhalation and 835 nL gas/g bw by intraperitoneal injection.	
3.4 Examinations		
3.4.1 Observations	None.	
3.4.2 Extraction and analysis	Concentration changes in the gas phase of the exposure system were determined at intervals during 0 to 5 hours by gas chromatography. Rate constants and pharmacokinetic parameters were calculated.	
	4 Results and Discussion	
4.1 Results of test	A first order elimination rate was observed after exposure to ethylene oxide by inhalation. After intraperitoneal injection the ethylene oxide concentration in exhaled air followed the Bateman exponential function. The kinetic parameters for distribution and metabolic elimination of ethylene oxide are shown in Table 6.2/01-1.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Rats were exposed to ethylene oxide either by inhalation (aproximately 100-1000 ppm) in a closed chamber or by intraperitoneal injection of the gas (835 nL/g bw). The concentration of ethylene oxide in the chamber was determined by gas chromatography at intervals during a 5 hour period after the start of the exposure or after intraperitoneal injection. Pharmacokinetic parameters were calculated.	
5.2 Results and discussion	There was a first order decline in the concentration of ethylene oxide from the atmosphere after exposure by inhalation. After intraperitoneal injection the concentration of ethylene oxide in exhaled air followed the Bateman exponential function.	
5.3 Conclusion	The uptake of ethylene oxide is not saturated at atmospheric concentrations of up to approximately 1000 ppm.	
5.3.1 Reliability	2	x
5.3.2 Deficiencies	None, the study was not conducted to any particular guideline	

	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	The summary of the applicant is acceptable.	
Results and discussion		
Conclusion	The summary of the applicant is acceptable.	
Reliability	3	
Acceptability	The study is non-GLP and non-guideline; the uptake of ethylene oxide was studied by measuring the concentrations of ethylene oxide in a closed chamber; no other parameters studied. Exposure levels are presented only as graphic representation. The study is not sufficient as a stand-alone, but can be used as a part of weight of evidence approach.	
Remarks		

Table 6.2/01-1: Pharmacokinetic parameters for distribution and metabolism of ethylene oxide

Compound	Parameter	Value
Ethylene oxide (oxirane)	$k_{12}V_1$	11100 $mL.h^{-1}$
	k_{21}	0.37 h^{-1}
	k_{eq}	30
	k_{st}[a]	1.52
	k_{el}	6.95 h^{-1}
	Cl_{tot}[a]	10600 $mL.h^{-1}$

[a] Calculated for V_1 approaching ∞

Cl_{lot}: total inhalatory clearance

k_{12}: influx rate constant

$k_{12}V_1$: influx process clearance

k_{eq}: equilibtirum constant (= $k_{12}V_1/k_{21}V_2$)

k_{st}: steady state constant

Section A6.2/02 **Annex Point IIA 6.2**	**Metabolism studies in mammals**	
	1 Reference	**Official use only**
1.1 Reference	Brown, C., Wong, B. and Fennell, T. (1996) *In vivo* and *in Vitro* Kinetics of Ethylene Oxide Metabolism in Rats and Mice Toxicology and Applied Pharmacology, 136, 8-19	

1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed.	
2.2 GLP	Not GLP	
2.3 Deviations	None, compliance with a guideline was not claimed.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Not reported	
3.1.4 Purity	>99.9%	
3.1.5 Stability	Not reported	
3.3 Test animals		
3.2.1 Species	Rat and mouse	
3.2.2 Strain	F-344 rats and B6C3F1 mice	
3.2.3 Source	Charles River Breeding Laboratories, Raleigh, North Carolina, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Rats: 10 – 12 weeks old. Male rats: 245 ± 10 g, female rats: 186 ± 7 g; Mice: 7 – 9 weeks old. Male mice: 25.8 ± 1.4 g, female mice: 20.8 ± 0.9 g	
3.2.6 Number of animals per group	5 per sex per species	
3.2.7 Control animals	None	
3.3 Administration/ Exposure		
3.3.1 Administration	Whole-body inhalation exposure to ethylene oxide for 4 hours in a 31 L exposure chamber. Ethylene oxide was mixed with filtered air (flow rate 10 L/min) to achieve the appropriate concentration. Each group had three exposures.	

3.3.2 Dose level	100 ppm or 300 ppm of ethylene oxide in air.	
3.4 Examinations		
3.4.1 Observations	One animal was removed from the exposure chamber at 3 and 4 hours into the exposure period and sacrificed, ethylene oxide concentrations in blood and tissues were determined to investigate whether steady state had been reached. The remaining animals were removed after 4 hours for sacrifice. For the elimination time course, rats and mice were killed between 2 and 20 minutes postexposure. After 3 hours animals were anaesthetised and blood was drawn within 2 minutes of removal from the exposure chamber. Following CO_2 anaesthesia, blood samples were collected by cardiac puncture. Muscle, brain and testis were removed and weighed. *In vitro* studies were conducted in which cytosolic and microsomal fractions prepared from male and female rat and mouse liver, kidney, testes (males only), lung and brain tissues were incubated with ethylene oxide. Incubation with ethylene oxide was conducted by addition of various volume of ethylene oxide (30-2000 µL) to give an initial solution concentration of 0.9-60 mM ethylene oxide. Activity was measured using ethylene oxide disappearance for up to 2 hours. Glutathione S-transferase kinetics was measured using a similar incubation but with added glutathione, and epoxide hydrolase activity kinetics were investigated by measurement of the hydrolysis product, ethylene glycol, as its dibenzoate ester. Metabolites were identified by NMR spectroscopy of incubations with [1,2-^{13}C]ethylene oxide . The kinetic constants, V_{max} and K_m, of enzymatically mediated ethylene oxide metabolism in different tissues were determined and comparisons were made by ANOVA using Student's *t* test. Differences in *in vivo* rate constants were compared using the same methods and steady state concentrations of ethylene oxide in tissues were compared using Welch's approximate *t* test.	
3.4.2 Extraction and analysis	*In vivo* ethylene oxide concentrations were determined in blood, brain, testes and muscle using headspace analysis. Determination of ethylene oxide concentration by headspace analysis was corrected for the loss of ethylene oxide reacting in the tissues post-mortem. Toxicokinetic parameters were calculated for blood and tissues.	
	4 Results and Discussion	
4.1 Results of test	*In vivo*: The mean actual exposure chamber concentrations were 99 ± 2 ppm and 327 ± 2 ppm. Ethylene oxide clearance from the blood of mice was more rapid than from the blood of rats. The $t_{1/2}$ for elimination in rats was 13.8 ± 3.0 min and 10.8 ± 2.4 min for males and females, respectively, at both concentrations. In mice the $t_{1/2}$ was 3.12 ± 0.2 min and 2.4 ± 0.2 min for males and females, respectively at 100 ppm. In mice only the $t_{1/2}$ at 300 ppm was higher, increasing to 5.4 ± 0.5 min in males and 5.6 ± 0.2 min in females. The rates of ethylene oxide disappearance from blood and other tissues were very similar and there were no statistically significant intra species differences between sexes in the elimination rate constant under either exposure condition (Table 6.2/02-1).	

	Tissue concentrations of ethylene oxide at 3 hours were very similar to those measured at 4 hours and analysis of ethylene oxide concentrations in blood, brain, muscle and testes indicated that the peak concentrations were equivalent in all tissues studied except for testes which were significantly lower (50% and 20% lower in the mouse and rat, respectively). The peak rat ethylene oxide concentrations were higher than those of mice at 100 ppm exposure and the peak tissue levels in mice were higher than those in rats at 300 ppm. *In vitro*: Three products were observed in mouse and rat liver cytosol after incubation with glutathione and [^{13}C]ethylene oxide: S-(2-hydroxyethyl)glutathione, ethylene glycol and 2-chloroethanol. The relative ethylene oxide metabolising activity was highest in liver, then in kidney and testes. Rat brain and rat and mouse lung showed only slight relative activity. The majority of the metabolic activity was localised in the cytosolic fraction in both the liver and the kidneys. The *in vitro* metabolism of ethylene oxide was catalyzed mainly by cytosolic glutathione S-transferase with the highest activity in the liver (Tables 6.2/02-2 and 6.2/02-3). The higher values in mice are consistent with the more rapid elimination in this species *in vivo* compared with rats.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	The kinetics of metabolism of ethylene oxide in rats and mice was investigated both *in vivo* and *in vitro*. Blood and tissue concentrations of ethylene oxide were measured following exposure to 100 or 300 ppm for 4 hours *in vivo*, and the activity of tissue cytosol and microsomes prepared from liver, kidneys, lung, brain and testes was determined *in vitro*. Metabolites were identified by NMR by using [1,2-^{13}C] ethylene oxide in the *in vitro* incubations.	
5.2 Results and discussion	Mice eliminated ethylene oxide more rapidly than rats following exposure to 100 ppm *in vivo* possibly because of a higher specific activity of cytosolic glutathione transferase activity. Mice showed a concentration dependent decrease in the rate of elimination of ethylene oxide, this apparent saturation of metabolism is mice is likely due to glutathione depletion and not enzymic saturation.	
5.3 Conclusion	Marked species differences were found in the rate of removal of ethylene oxide in rodents *in vivo* and these differences are consistent with the rate of metabolism *in* vitro. Metabolism of ethylene oxide was linear in rats in the dose range 100-300 ppm but was saturated in mice at the higher concentration.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	

	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is acceptable. Although the study is non-guideline and non-GLP, the experimental procedures and results are reported in sufficient details and can be used for evaluation purpose. The study is considered acceptable.	
Remarks		

Table 6.2/02-1: Peak tissue concentration of ethylene oxide in rats and mice

Group	Conc (ppm)	Tissue concentration (µg ethylene oxide/g tissue)			
		Blood	Muscle	Brain	Testes
Male mouse	100	0.29 (0.23 – 0.37)	0.30 (0.17 – 0.48)	0.36 (0.22 – 0.57)	0.15 (0.03 – 0.66)
	330	2.62 (1.87 - 368)	2.50 (1.84 – 3.38)	2.49 (1.83 – 3.39)	1.07 * (0.80 – 1.43)
Female mouse	100	0.41 ** (0.34 – 0.50)	0.35 (0.36 – 0.46)	0.36 (0.16 – 0.83)	-
	330	3.89 ** (2.90 – 5.22)	3.48 (2.59 – 4.69)	3.75 (2.92 – 4.82)	-
Male rat	100	0.57 *** (0.47 – 0.71)	0.60 (0.51 – 0.72)	0.57 (0.46 – 0.72)	0.10 * (0.06 – 0.15)
	330	2.38 (2.14 – 2.66)	2.16 (1.98 – 2.36)	2.22 (2.01 – 2.45)	0.57 * (0.49 – 0.67)
Female rat	100	0.72 *** (0.58 – 0.88)	0.66 (0.55 – 0.80)	0.56 (0.46 – 0.70)	-
	330	2.31 *** (1.98 – 2.70)	2.42 (2.03 – 2.90)	1.87 (1.56 – 2.23)	-

* = Statistically significant ($p < 0.0002$) difference compared to blood of same species and exposure.
** = Statistically significant ($p < 0.02$) difference compared to male mouse at same exposure.
*** = Statistically significant ($p < 0.003$) difference compared to same sex mouse blood at same exposure.

Table 6.2/02-2: Kinetic constants for the reaction of ethylene oxide with GSH in rodent tissue cytosol

Tissue	Species	Sex	V_{max} (nmol/mg protein/min)	K_M (mM)

Liver	Mouse	M	258 ±86.9 *	10.4 ± 2.3
		F	287 ± 49.0 *	11.0 ± 4.6
	Rat	M	52.7 ± 10.8	13.0 ± 1.4
		F	29.3 ± 4.9 **	8.2 ± 1.0 **
Kidney	Mouse	M	46.4 ± 17.0	7.1 ± 1.4
		F	46.0 ± 15.1	6.9 ± 1.8
	Rat	M	30.1 ± 3.0	10.8 ± 0.4
		F	34.3 ± 4.8	8.1 ± 3.3
Testis	Mouse	M	17.3 (n=1)	8.1 (n=1)
	Rat	M	20.3 ± 8.0	9.2 ± 0.6

* = Statistically significant ($p < 0.05$) difference compared to same sex rat liver V_{max}.
** = Statistically significant ($p < 0.05$) difference compared to male rat liver.

Table 6.2/02-3: Liver microsomal epoxide hydrolase activity (mean ± SD)

	Male mouse	**Male rat**
K_M (mg ethylene oxide/L)	9.0	9.0
V_{max} (nmol Eg/mg protein/min)	1.2 ± 0.8	1.8 ± 0.2
V_{max} (mg ethylene oxide hr^{-1} animal $^{-1}$)	0.14 ± 0.09	1.40 ± 0.16
V_{max} (mg ethylene oxide hr^{-1} kg bw^{-1})	5.23 ± 3.49	5.71 ± 0.63
V_{max}/K_M (hr^{-1})	0.6	0.6

Section A6.2/03 **Annex Point IIA 6.2**		**Metabolism studies in mammals**	
		1 Reference	**Official use only**
1.1	**Reference**	Brown, C., Asgharian, B., Turner, M. and Fennell T. (1998) Ethylene Oxide Dosimetry in the Mouse Toxicology and Applied Pharmacology, 148, 215-221	
1.2	**Data protection**	No	
1.2.1	**Data owner**	Data published	
1.2.2	**Criteria for data protection**	No data protection claimed	
		2 Guidelines and Quality Assurance	
2.1	**Guideline study**	Guideline compliance was not claimed.	
2.2	**GLP**	Not GLP	
2.3	**Deviations**	None	

	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Not reported	
3.1.4 Purity	Ethylene oxide: >99.9% Ethylene-d_4-oxide: >98%	
3.1.5 Stability	Not reported	
3.4 Test animals		
3.2.1 Species	Mouse	
3.2.2 Strain	B6C3F1	
3.2.3 Source	Charles River Breeding Laboratories, Raleigh, North Carolina, USA	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	Age: 7 - 9 weeks old Weight: 26 - 28 g	
3.2.6 Number of animals per group	13 animals	
3.2.7 Control animals	13 animals	
3.3 Administration/ Exposure		
3.3.1 Administration	Nose only inhalation exposure to ethylene oxide for up to 4 hours in a Cannon-style tower. Ethylene oxide was mixed with filtered air (flow rate 10 L/min) to achieve the appropriate concentration.	
3.3.2 Dose level	0, 50, 100, 200, 300 or 400 ppm ethylene oxide in air	
3.4 Examinations		
3.4.1 Observations	Respiratory physiology measurements were performed for each exposure group to determine effect on breathing parameters. One animal per exposure was placed in a plethysmograph tube for measurements of breathing frequency and tidal volume. Blood sampling was performed at 2, 3 or 4 hours into the exposure following anaesthesia with pentobarbital (90 mg/kg) via cardiac puncture. Liver, lung, kidney and testes were removed following blood sampling at the 4 hour time point for analysis of nonprotein sulfhydryl (GSH) content.	
3.4.2 Extraction and analysis	Glutathione concentrations in liver, lung, kidney and testes were determined by an automated clinical chemistry analyzer (COBAL) by measuring the absorbance at 412 nm, corresponding to GSH-5,5'-	

	dithiobis(2-nitrobenzoic acid) complex. Tissue non-protein sulfhydryl content was calculated from a standard curve of GSH prepared daily.	
	4 Results and Discussion	
4.1 Results of test	Time weighted average concentrations of ethylene oxide were 55±11, 104±6, 204±7, 301±5 and 400±11 ppm. No statistically significant differences were observed between control mice and any exposure group for breathing frequency and tidal volume. Blood ethylene oxide concentrations remained essentially constant throughout the sampling period (2 – 4 hours) for exposures of ≤200 ppm but increased throughout the exposure period at higher concentrations. The concentration of ethylene oxide in blood was linearly related to dose at concentrations up to and including 200 ppm but increased markedly at higher concentrations (Table 6.2/03-1). Glutathione levels in liver and lung showed concentration dependent depletion following exposure. Levels were significantly depressed compared with controls at exposure concentrations ≥100 ppm. In testes and kidneys, significant depletion only occurred at exposure concentrations of ≥300 ppm (Table 6.2-03/2).	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 13 mice were exposed to 0, 50, 100, 200, 300 or 400 ppm ethylene oxide by nose only inhalation for 4 hours. Blood was sampled from 4 rats per group 2, 3 and 4 hours after the start of exposure and breathing parameters were determined in the remaining animal. Mice were killed after 4 hours exposure and the liver, lungs, kidneys and testes were removed. The ethylene oxide concentration was determined in blood using headspace analysis and the glutathione content was determined in tissues using the Edman method.	
5.2 Results and discussion	Respiratory physiology parameters were not affected by exposure to ethylene oxide. Blood ethylene oxide concentrations remained essentially constant throughout the sampling period for exposures of ≤200 ppm but increased throughout the exposure period at higher concentrations. The concentration of ethylene oxide in blood was linearly related to dose at exposures up to and including 200 ppm but increased markedly at higher concentrations. GSH levels in liver and lung were significantly depressed compared with controls at exposure concentrations ≥100 ppm but in testes and kidneys, significant depletion only occurred at exposure concentrations of ≥300 ppm.	
5.3 Conclusion	Metabolism of ethylene oxide is only linear at concentrations of up to 200 ppm and metabolism shows signs of saturation at higher concentrations. Depletion of tissue glutathione appears to be responsible for the increase in blood concentration at ≥300 ppm.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	

	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is acceptable as a part of weight of evidence approach. Although the study is a non-guideline and non-GLP, the experimental procedures and results are reported with sufficient level of details to allow the assessment. Analytical concentrations of ethylene oxide were reported and were determined by a validated method.	
Remarks		

Table 6.2/03-1: Respiratory parameters and final blood concentrations after exposure by inhalation to ethylene oxide

Exposure concentration (ppm)	Breathing frequency (breaths/min)	Tidal volume (mL)	Ethylene oxide final blood concentration (µg/mL)
0	270 ± 33	0.23 ± 0.03	-
55	267 ± 32	0.20 ± 0.02	0.33 ± 0.05
104	244 ± 20	0.21 ± 0.02	0.64 ± 0.09
204	306 ± 19	0.18 ± 0.01	1.32 ± 0.25
301	212 ± 20	0.23 ± 0.03	5.07 ± 1.29
400	235 ±22	0.23 ± 0.02	10.83 ± 1.19

Table 6.2/03-2: Tissue glutathione levels at various ethylene oxide exposures

Exposure concentration (ppm)	Tissue glutathione concentration (mM)			
	Liver	Lung	Kidney	Testes
0	3.24 ± 0.40	1.04 ± 0.12	1.96 ± 0.16	2.40 ± 0.21
55	3.17 ± 0.26	0.87 ± 0.07	2.22 ± 0.12	2.61 ± 0.05
104	2.35 ± 0.42*	0.57 ± 0.07**	1.81 ± 0.12	2.24 ± 0.03
204	1.59 ± 0.36***	0.40 ± 0.03***	1.82 ± 0.09	2.51 ± 0.03
301	0.69 ± 0.10***	0.23 ± 0.03***	1.15 ± 0.28***	1.93 ± 0.05*
400	0.54 ± 0.02***	0.22 ± 0.02***	0.72 ± 0.04***	2.05 ± 0.04*

* = Significantly different from control value ($p < 0.05$).
** = Significantly different from control value ($p < 0.02$).
*** = Significantly different from control value ($p < 0.01$).

Section A6.2/04 **Annex Point IIA 6.2**	**Metabolism studies in mammals**	
	1 Reference	**Official use only**
1.1 Reference	Tardif, R., Goyal, R., Brodeur, J. and Gérin, M. (1987) Species Differences in the Urinary Disposition of Some Metabolites of Ethylene Oxide. Fundamental and Applied Toxicology, 9, 448-453	
1.2 Data protection	No	
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed.	
2.2 GLP	Not GLP	
2.3 Deviations	None	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Liquid gas	
3.1.4 Purity	99.7%	
3.1.5 Stability	Not reported	
3.5 Test animals		
3.2.1 Species	Mouse, rat and rabbit	
3.2.2 Strain	Mouse: Swiss CD-1 Rat: Sprague-Dawley Rabbit: Not reported	
3.2.3 Source	Mouse: Charles River Inc., St Constant, Québec, Canada Rat: Charles River Inc., St Constant, Québec, Canada Rabbit: Les Lapins Léonard, Mirabel, Québec, Canada	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	Mouse: 25 - 30 g Rat: 200 -225 g Rabbit: 1.5 - 2kg	

3.2.6 Number of animals per group	Mouse: 10 per group Rat: 5 per group Rabbit: 3 per group for intravenous administration and 4 per group for inhalation exposure	
3.2.7 Control animals	None	
3.3 Administration/ Exposure		
3.3.1 Administration	One intravenous injection of ethylene oxide (solution in distilled water at 0°C), or whole body inhalation exposure to ethylene oxide for 6 hours in a 35 L chamber with an air flow rate of 25 L/min.	
3.3.2 Dose level	Intravenous injection: 20 or 60 mg/kg ethylene oxide in distilled water; Inhalation exposure: 200 ppm ethylene oxide in air for 6 hours.	
3.4 Examinations		
3.4.1 Observations	Intravenous injection: Urine samples were collected at intervals of 0 – 6 hours and 6 – 24 hours. Inhalation exposure: 24 hour urine samples were collected following the exposure period.	
3.4.2 Extraction and analysis	2- Hydroxyethylmercapturic acid, and S-(2-hydroxyethyl)-L-cysteine were determined by HPLC. N-acetyl-S-carboxymethyl-L-cysteine and S-carboxymethyl-L-cysteine were determined together by the same method following deacetylation by acid hydrolysis. Ethylene glycol was determined by gas chromatography.	
	4 Results and Discussion	
4.1 Results of test	Mice excreted significant quantities of metabolites 2-hydroxyethylmercapturic acid, S-(2-hydroxyethyl)-L-cysteine, S-carboxymethyl-L-cysteine and ethylene glycol (8.3, 5.8, 1.9 and 3.3% of the 20 mg/kg dose, respectively) in 24 hours after the intravenous dose. This contrasts with excretion of only 2-hydroxyethylmercapturic acid (31%) and ethylene glycol (6%) in 24 hours by rats. Rabbits were found to only excrete ethylene glycol (2%). There were also species related differences in the rate of excretion of the metabolites, in rats larger amounts of 2-hydroxyethyl mercapturic acid were eliminated in the 6-24 hour period compared to the 0-6 hour collection whereas in mice approximately equal amounts were excreted during both periods (Table 6.2/04-1 and 6.2/04-2). There were no qualitative intraspecies differences in the metabolites excreted when an intravenous dose of 60 mg/kg was compared to inhalation exposure to 200 ppm ethylene oxide. There were, however, some quantitative changes.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Mice, rats and rabbits were exposed to ethylene oxide by intravenous administration (20 and 60 mg/kg bw) or by inhalation (200 ppm for 6 hours). Urine was collected during the 0-6 and 6-24 hour periods after treatment for intravenous administration and 0-24 hours period after inhalation exposure and analysed for 2-hydroxyethylmercapturic acid,	

	S-(2-hydroxyethyl)-L-cysteine, S-carboxymethyl-L-cysteine and ethylene glycol.	
5.2 Results and discussion	Qualitative and quantitative differences were found in the elimination of urinary metabolites of ethylene oxide in mice, rats and rabbits, and there were also species differences in the rate at which some metabolites were eliminated. The qualitative differences amongst the three species were found to be independent of the route of exposure or dose, but there were some differences in the amounts of metabolites eliminated in urine within each species.	
5.3 Conclusion	There are species differences in the metabolism of ethylene oxide which may have significance for the choice of animal model for human metabolism of ethylene oxide.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	The summary of the applicant is acceptable.	
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study addressed the identification of urinary metabolites of ethylene oxide in different species and, while it is not sufficient as a stand-alone ADME study, is acceptable as a part of weight of evidence. Although the study has a number of limitations (only one sex used, no information on the rabbit strain, no information on the validation of analytical methods for metabolite identification etc.), the experimental procedure and results are reported in sufficient details to be used for evaluation purpose.	
Remarks		

Table 6.2/04-1: Species differences in the metabolites of ethylene oxide after intravenous injection

Dose	Species	Time period (hours)	Urinary metabolites (% dose)			
			HMA	HC	CC	EG
Intravenous injection						
20 mg/kg	Mouse	0-6	3.3	1.9	0.7	0.5
		6-24	4.3	3.7	1.2	2.8
		0-24	8.3	5.8	1.9	3.3

	Rat	0-6	9.1	ND	ND	4.6
		6-24	21.7	ND	ND	1.3
		0-24	30.8	ND	ND	5.9
	Rabbit	0-6[a]	-	-	-	-
		6-24	ND	ND	ND	2.1
		0-24	ND	ND	ND	2.1
60 mg/kg	Mouse	0-6	3.5	3.1	2.2	1.2
		6-24	3.5	1.9	0.9	1.4
		0-24	7.0	5.0	3.2	2.6
	Rat (n=5)	0-6	5.7	ND	ND	5.25
		6-24	18.0	ND	ND	1.04
		0-24	23.7	ND	ND	6.3
	Rabbit (n=3)	0-6	-	-	-	-
		6-24	ND	ND	ND	2.8
		0-24	ND	ND	ND	2.8

HMA = 2-hydroxyethylmercapturic acid, HC = S-(2-hydroxyethyl)-L-cysteine, CC = S-carboxymethyl-L-cysteine, EG = ethylene glycol
ND = Not detected
[a] No urine voided

Table 6.2/04-2: Species differences in the metabolites of ethylene oxide after inhalation exposure

Dose	Species	Conc (ppm)	Urinary metabolites (µmol/100 g bwt)			
			HMA	HC	CC	EG
Inhalation exposure						
200 ppm	Mouse (n=10)	193 ppm	4.63 ± 0.84	2.62 ± 0.25	2.83 ± 0.81	0.77 ± 0.34
	Rat (n=5)	189 ppm	19.61 ± 5.27	ND	ND	1.84 ± 0.26
	Rabbit (n=4)	201 ppm	ND	ND	ND	2.56 ± 0.67

HMA = 2-hydroxyethylmercapturic acid
HC = S-(2-hydroxyethyl)-L-cysteine
CC = S-carboxymethyl-L-cysteine
EG = ethylene glycol
ND = Not detected

Section A6.2/05 **Annex Point IIA 6.2**	**Metabolism studies in mammals**	
	1 Reference	**Official use only**
1.1 Reference	Fennell, T. and Brown, C. (2001) A Physiologically Based Pharmacokinetic Model for Ethylene Oxide in	

	Mouse, Rat and Human Toxicology and Applied Pharmacology, 173, 161-175	
1.2 Data protection	No	
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	There is no guideline for this study type.	
2.2 GLP	Not GLP	
2.3 Deviations	Not applicable, there is no guideline for this study type.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Not reported	
3.1.4 Purity	Not reported	x
3.1.5 Stability	Not reported	
3.2 Test animals		
3.2.1 Species	Human, rat and mouse	
3.2.2 Strain	Rat: Not applicable, no samples used in this investigation Mouse: Blood and tissues were obtained from male $B6C3F_1$ mice Human: Cytosol was obtained from the liver of 3 male and 1 female donor and microsomes were obtained from the liver of 3 female and 2 male donors	
3.2.3 Source	Mouse blood and tissues: Charles River Laboratories, Raleigh, North Carolina, USA Human cytosol and microsomes: International Institute for the Advancement of Medicines, Scranton, Pennsylvania, USA	
3.2.4 Sex	Mouse: Male Human: Male and female	
3.2.5 Age/weight at study initiation	Not reported	
3.2.6 Number of animals per group	Not applicable	
3.2.7 Control animals	Not applicable	

3.3 Administration/ Exposure		
3.3.1 Administration and analysis	Liver microsomal and cytosolic fractions obtained from human donors were incubated with ethyelen oxide *in vitro* to determine glutathione transferase and epoxide hydrolase activity. All incubations were conducted at 37ºC and control incubations were conducted with heat inactivated cytosol or microsomes. Samples were analysed using the methods described in 6.2/02. Metabolic constants for the two major pathways of ethylene oxide (glutathione conjugation and hydrolysis) were calculated. Blood: and tissue:air partition coefficients were determined for mouse blood and mouse tissues using the vial equilibration technique.	
3.3.2 Dose level	Not applicable	
3.4 Model structure	A physiological model was constructed in Simusolv consisting of compartments for lung, liver, brain, testes, richly perfused organs, slowly perfused organs, fat, arterial blood and venous blood. Physiological parameters were obtained from a number of sources and metabolic parameters were either determined as part of this study or taken from published data.	
	4 Results and Discussion	
4.1 Results	Glutathione transferase activity with ethylene oxide was low in human liver cytosol. Reported values were approximately 25 times higher in mouse liver and 5 times higher in rat liver. In contrast epoxide hydrolase activity in human liver microsomes was similar to that reported in rat and mouse liver. A model was developed incorporating an uptake of ethylene oxide of 43-60% for the rat and 40% for the mouse which accurately predicted blood and tissue concentrations of ethylene oxide in rats and mice when compared with published data. For human exposure a pulmonary uptake of 78% was used and the resulting predictions of blood concentration at low exposure concentrations correlated well with published data from occupational exposure. Simulated blood concentrations for exposure to atmospheric concentrations of from 1 to 100 ppm were similar in the mouse, rat and human (Table 6.2/05-1) even though the amount of ethylene oxide metabolised is considerably greater in the mice and rats than in humans.	x
	5 Applicant‘s Summary and conclusion	
5.1 Materials and methods	A physiologically based pharmacokinetic model was developed using the results of *in vitro* experiments with human liver cytosol and microsomes and published data on the rates of metabolism of ethylene oxide in rodents.	
5.2 Results and discussion	The model accurately predicted available data on blood and tissue concentrations of ethylene oxide in rats, mice and humans, when the pulmonary uptake of ethylene oxide was set below 100%. Despite profound differences in the relative contribution of the different metabolic pathways, when differences in the uptake and metabolism are taken into account, simulated peak blood concentrations and areas under the curve are similar for rats, mice and humans at exposure concentrations of up to 200 ppm.	x

5.3 Conclusion	No significant species differences in systemic exposure are likely when rats, mice and humans are exposed to the same concentrations of ethylene oxide.	x
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	6 March 2020	
Materials and Methods	The summary of the applicant is acceptable with minor corrections made by the eCA: *3.1.4 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and discussion	The summary of the applicant is acceptable with some corrections made by the eCA: *4.1 Results should read:* To predict a significantly lower concentration in testes in comparison with other tissues, a diffusion-limited distribution to the testes was incorporated. *5.2 Conclusion should read:* Despite profound differences in the relative contribution of the different metabolic pathways, when differences in the uptake and metabolism are taken into account, simulated peak blood concentrations and areas under the curve are similar for rats, mice and humans at exposure concentrations of up to 100 ppm.	

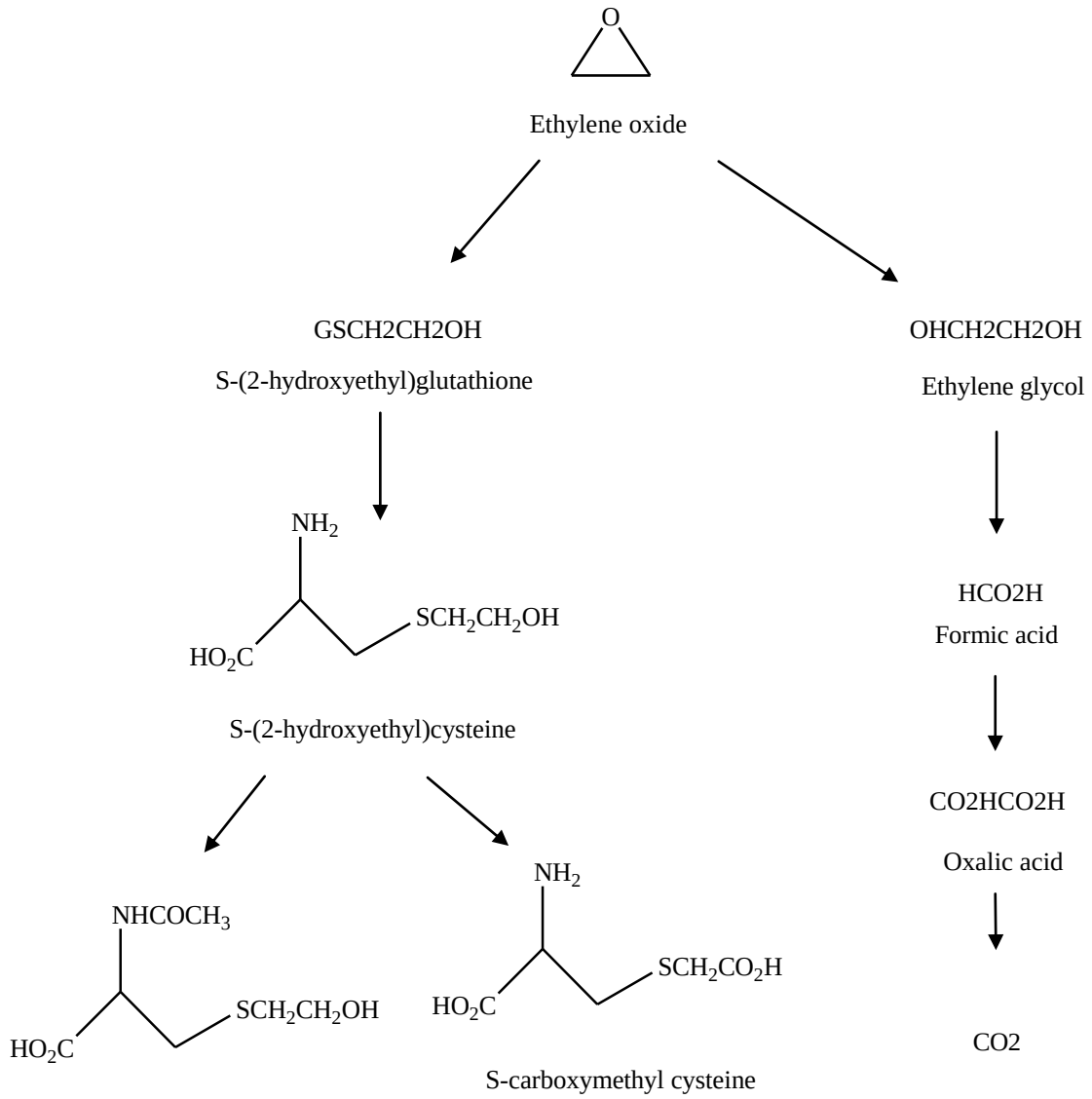
Conclusion	*5.3 Conclusion should read:* The pulmonary uptake seems to differ with a factor of 1.3 - 2 for the inhalation absorption of EtO, when comparing human vs rats and mice. When differences in the uptake and metabolism are taken into account in the PBPK modelling, simulated peak blood concentrations are similar for rats, mice and humans at exposure concentrations of up to 100 ppm. As for the simulated area under the curve (AUC) in blood the values were approximately 25% higher in human than in mice, and approximately 30% higher in humans compared with rat (when pulmonary uptake in the rat set to 43%), and approximately 30% higher in rat (when pulmonary uptake in the rat set to 60%) compared to mouse.
Reliability	2
Acceptability	PBPK models for ethylene oxide were developed to describe the exposure-tissue dose relationship in rodents and humans. The study report is considered to be comprehensive and acceptable for the assessment. The study is acceptable as a part of weight of evidence.
Remarks	

Table 6.2/05-1: Simulated maximum blood concentration and area under the blood concentration time curve following a 6 hour exposure to ethylene oxide

Conc (ppm)	Mouse[a]			Rat[b]			Rat[c]			Human[d]		
	Peak conc (mg/L)	AUC (mh.h/L)	Dose (mg/kg)	Peak conc (mg/L)	AUC (mh.h/L)	Dose (mg/kg)	Peak conc (mg/L)	AUC (mh.h/L)	Dose (mg/kg)	Peak conc (mg/L)	AUC (mh.h/L)	Dose (mg/kg)
1	0.007	0.044	0.33	0.007	0.043	0.09	0.010	0.059	0.13	0.009	0.056	0.039
3	0.022	0.13	1.0	0.022	0.13	0.28	0.029	0.18	0.39	0.027	0.11	0.12
10	0.074	0.44	3.3	0.072	0.43	0.93	0.098	0.59	1.3	0.094	0.57	0.39
33	0.246	1.48	10.7	0.240	1.43	3.0	0.330	1.97	4.3	0.311	1.88	1.3
50	0.376	2.25	16.4	0.369	2.20	4.6	0.509	3.03	6.5	0.475	2.87	1.97
100	0.767	4.58	32.9	0.763	4.53	9.3	1.059	6.28	13.0	0.965	5.38	3.95

[a]Pulmonary uptake in the mouse was set to 40%
[b]Pulmonary uptake in the rat was set to 43%
[c]Pulmonary uptake in the rat was set to 60%
[d]Pulmonary uptake in the human was set to 78%

Figure 6.2.1-1: Proposed metabolic pathway for ethylene oxide



6.3 Short term repeated dose toxicity

6.3.1 Repeated dose toxicity (oral)

Section 6.3 **Annex Point 6.3.1**	**Short term repeated dose toxicity (28 days)** **Repeated dose toxicity (oral)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only

Section 6.3 **Annex Point 6.3.1**	**Short term repeated dose toxicity (28 days)** **Repeated dose toxicity (oral)**	
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A 28 day oral toxicity study in the rat is not available. Ethylene oxide is a gas at room temperature, exposure is only by inhalation and a new study in a rodent species using oral administration is not considered necessary because there is no human exposure by that route.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2020	
Evaluation of applicant's justification	The justification of the applicant is acceptable, with minor adjustments of the text. "A 28 day oral toxicity study in the rat is not available. Ethylene oxide is a gas at room temperature, exposure is mainly by inhalation and a new study in a rodent species using oral administration is not considered necessary as exposure by inhalation is considered to be the relevant route."	
Conclusion	The justification of the applicant for non performance of an 28 day oral toxicity study in the rat is acceptable.	
Remarks		

6.3.2 Repeated dose toxicity (dermal)

Section 6.3 **Annex Point 6.3.2**	**Short term repeated dose toxicity (28 days)** **Repeated dose toxicity (dermal)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only

Section 6.3 **Annex Point 6.3.2**	**Short term repeated dose toxicity (28 days)** **Repeated dose toxicity (dermal)**	
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A 28 day dermal toxicity study in the rat is not available. Ethylene oxide is a gas at room temperature, exposure is only by inhalation and a new study in a rodent species using dermal administration is not considered necessary because there is no human exposure by that route.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20 January 2020	
Evaluation of applicant's justification	The justification of the applicant is acceptable, with minor adjustments of the text. "A 28 day dermal toxicity study in the rat is not available. As ethylene oxide is a gas at room temperature, dermal exposure to the liquid form is not anticipated. Dermal exposure to gaseous ethylene oxide can still occur, but the systemic exposure due to gaseous dermal exposure will be low when compared to respiratory exposure. Moreover, the available inhalation animal studies are usually done with a whole body exposure, so that dermal exposure is also covered by these studies. Therefore, a new study in a rodent species using dermal administration is not considered necessary."	
Conclusion	The justification of the applicant for non performance of an 28 day dermal toxicity study is acceptable.	
Remarks		

6.3.3 Repeated dose toxicity (inhalation)

Short term inhalation studies have been summarised in 6.4.3/01 and 6.4.3/02 as they were conducted together with subchronic toxicity studies. A 14 day dose range finding study has been conducted in mice and is summarised below.

Section A6.3.3/01 **Annex Point IIA 6.3.3**	**Repeated dose toxicity – inhalation, mouse**	
	1 Reference	**Official use only**
1.1 Reference	Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in $B6C3F_1$ Mice (Inhalation Studies) National Toxicology Program, Technical Report Series No 326, 1987	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No, this was a preliminary study	
2.2 GLP	No.	
2.3 Deviations	None, the study did not comply with any guideline	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	0018-1	
3.1.2 Specification		
3.1.2.1 Description	Not recorded	
3.1.2.2 Purity	>99%	
3.1.2.3Stability	No degradation observed over the course of the study	
3.2 Test animals		
3.2.1 Species	Mouse	
3.2.2 Strain	$B6C3F_1$	
3.2.3 Source	Charles River Breeding Laboratory, Portage, Michigan, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	7-9 weeks; weight: males 23.9-29.8 g, females 18.2-22.5 g	
3.2.6 Number of animals per group	5 per sex	

3.2.7 Control animals	5 per sex		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	14 days		
3.3.2 Frequency of exposure	6 hours per day, 5 days per week		
3.3.3 Post exposure period	None		
3.3.4 Oral	Not applicable		
3.3.5 Inhalation			
3.3.5.1 Concentrations	Nominal concentration	0, 50, 100, 200, 400 or 800 ppm	
	Analytical concentration	Not determined	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Whole body		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 5 days per week		
3.3.5.8 Controls	Ambient air		
3.3.6 Dermal	Not applicable		
3.3.7 Intrapertitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			
3.4.1.1 Clinical signs	Three times per day		
3.4.1.2 Mortality	As for clinical signs		
3.4.2 Body weight	Days 1 and 8 and at necropsy		
3.4.3 Food consumption	Not reported		

3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Not reported	
3.4.6 Haematology	Not reported	
3.4.7 Clinical chemistry	Not reported	
3.4.8 Urinalysis	Not reported	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	None	
3.5.2 Gross and histopathology	Tissues examined by histopathology: In surviving mice (6 animals): Skin, mandibular lymph nodes, salivary glands, larynx, trachea, lungs and bronchi, heart, thyroid gland, oesophagus, stomach, duodenum, colon, liver, gall bladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testis or ovaries/uterus, nasal cavity, brain, pituitary gland and eyes. In controls (2 animals): Eyes	
3.5.3 Other examinations	None	
3.5.4 Statistics	None	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Observations		
4.1.1 Clinical signs	Mice exposed to 800 ppm were hunched and listless.	
4.1.2 Mortality	All mice in the 800 ppm group died before the end of the exposure period. Other deaths were not clearly treatment related (Table 6.6.3/01-1).	
4.2 Body weight gain	There was no consistent effect of treatment (Table 6.6.3/01-1).	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Not reported	
4.5.2 Clinical chemistry	Not reported	
4.5.3 Urinalysis	Not reported	

4.6 Sacrifice and pathology		
4.6.1 Organ weights	Not reported	
4.6.2 Gross and histopathology	No effect of treatment on the survivors	
4.7 Other examinations	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 5 mice per sex were exposed to 0, 50, 100, 200, 400 or 800 ppm ethylene oxide by inhalation. The animals were observed three times daily and weighed on days 1 and 8 and before necropsy. Tissues were from surviving mice were examined by histopathology.	
5.2 Results and discussion	All mice exposed to 800 ppm ethylene oxide died but there was no treatment related mortality in the other groups. Body weight and histopathology were unaffected by treatment.	
5.3 Conclusion	There was only an effect of treatment at the highest exposure concentration.	
5.3.1 LO(A)EL	800 ppm	
5.3.2 NO(A)EL	400 ppm	
5.3.3 Other	None	
5.3.4 Reliability	2	x
5.3.5 Deficiencies	None , the study was not conducted to a guideline	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	Reliability 3	
Acceptability	It is a dose-range finding study and many parameters, required in the OECD test guideliens, were not assessed. The study is not sufficient for evaluation as a stand-alone, but can be considered as a part of weight of evidence.	
Remarks	Dose range finding study, with minimal parameters assessed.	

Table 6.3.3/01-1: Survival and body weight of mice exposed to ethylene oxide by inhalation for 14 days

	Survival	**Mean body weight (g)[1]**	

Concentration (ppm)		Initial	Final	Change	Final weight relative to controls (%)
Males					
0	5/5	29.0±0.8	30.2±0.8	+1.2±1.0	-
50	5/5	26.6±0.5	28.4±0.5	+1.8±0.6	94.0
100	5/5	25.0±1.1	27.6±1.4	+2.6±0.9	91.4
200	5/5	26.6±1.2	27.6±0.8	+1.0±0.5	91.4
400	5/5	26.8±0.4	29.4±0.5	+2.6±0.7	97.4
800	0/5[2]	27.2±0.9	-[3]	-[3]	-[3]
Females					
0	5/5	22.0±0.5	24.8±0.4	+2.8±0.5	-
50	5/5	20.6±1.4	21.8±1.4	+1.2±0.2	87.9
100	5/5	22.0±0.3	24.0±0.7	+2.0±0.6	96.8
200	3/5	19.2±1.0	21.0±1.7	+2.3±2.2	84.7
400	5/5	21.6±0.2	24.0±0.6	+2.4±0.6	96.8
800	0/5[2]	21.2±0.2	-[3]	-[3]	-[3]

[1] Mean ± standard error
[2] Days of death 7 and 11
[3] No data are reported due to 100% mortality in this group.

6.4 Subchronic toxicity

6.4.1 Subchronic oral toxicity test

Section 6.4.1 **Annex Point 6.4**	**Subchronic oral toxicity - rat and dog**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [x] **Limited exposure [x]**	**Technically not feasible [x]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A sub chronic oral toxicity study in the rat is not available. Ethylene oxide is a gas at room temperature, repeated exposure is only by inhalation and sub chronic toxicity studies conducted using that route are summarised in 6.4.3. Conducting a new study in a rodent species using oral administration is not considered necessary and would not be a justified use of animals. A sub chronic oral toxicity study is also unavailable in dogs. A study using oral administration would not be relevant for the reasons given above. Some data on toxicity to dogs following exposure by inhalation is summarised in 6.4.3/02 and although a NOAEL was not established the	x

Section 6.4.1 **Annex Point 6.4**	**Subchronic oral toxicity - rat and dog**	
	results show that there is no major difference in the toxicity of ethylene oxide between rats and dogs. A new dog study is considered unnecessary and exposure by inhalation would be technically difficult in this species.	
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	14 December 2017	
Evaluation of applicant's justification	The waiver for not performing sub-chronic oral studies is considered acceptable. The TNsG states that: *in cases where the potential inhalation exposure is significant, an inhalation study is required instead of the oral study*. Considering that ethylene oxide is a gas, inhalation is a most relevant exposure route. The performance of subchronic toxicity studies by oral route in rodent and non-rodent is not considered to be warranted.	
Conclusion	The justification of the applicant is acceptable. No subchronic toxicity study by oral route needs to be conducted.	
Remarks		

6.4.2 Subchronic dermal toxicity test

Section 6.4.2 **Annex Point 6.4.2**	**Subchronic dermal toxicity - rat**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	A sub chronic dermal toxicity study is not available. Ethylene oxide is a gas at room temperature, exposure is only by inhalation and sub chronic toxicity studies conducted using that route are summarised in 6.4.3. Conducting a new study in a rodent species using dermal administration is not considered necessary because there is no human exposure by that route and would not be a justified use of animals.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	

Section 6.4.2 **Annex Point 6.4.2**	**Subchronic dermal toxicity - rat**
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 January 2020
Evaluation of applicant's justification	The TNsG states that: a percutaneous study is required, where the potential dermal exposure is significant and route-to-route extrapolation is not possible. However, a percutaneous study may be necessary where it is justified that dermal route is more appropriate or specific effects of concern are different from the effects seen in the studies in other routes. As ethylene oxide is a gas at room temperature, dermal exposure to the liquid form is not anticipated. Dermal exposure to gaseous ethylene oxide can still occur, but the systemic exposure due to gaseous dermal exposure will not be significant when compared to respiratory exposure. Moreover, the available inhalation animal studies are usually done with a whole body exposure, so that dermal exposure is also covered by these studies.
Conclusion	A new study in a rodent and a non-rodent species using dermal administration is not considered necessary.
Remarks	

6.4.3 Subchronic inhalation toxicity test

Section A 6.4.3/01 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Hollingsworth, R., Rowe, V., Oyen, F., McCollister, D. and Spencer, H. (1956) Toxicity of Ethylene Oxide Determined in Experimental Animals American Medical Association Archives of Industrial Health, 13, 217-227	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No, guideline compliance was not claimed	
2.2 GLP	No	
2.3 Deviations	Not applicable, guideline compliance was not claimed	

	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification		
3.1.2.1 Description	Colourless gas	
3.1.2.2 Purity	97.0-98.6%	
3.1.2.3Stability	Not reported	
3.2 Test animals		
3.2.1 Species	Rat, mouse, guinea pig, rabbit and monkey	
3.2.2 Strain	Rat: Probably Wistar Mouse, guinea pig, rabbit and monkey: Not reported	
3.2.3 Source	Rat, guinea pig and rabbit: In house supply. Mice: Not reported. Monkeys: Imported	
3.2.4 Sex	Male and female (mice only female)	
3.2.5 Age/weight at study initiation	Not reported	
3.2.6 Number of animals per group	Rats: 5, 10 or 20/sex/group Mice: 5 or 10 females/group Rabbits: 1 or 2/sex/group Guinea pigs: 5 or 8/sex/group Monkeys: 1 or 2	
3.2.7 Control animals	Control animals were included but numbers were not always reported	
3.3 Administration / Exposure		
3.3.1 Duration of treatment	841 ppm: Up to 8 exposures 357 ppm: 7-123 exposures 204 ppm: 122-157 exposures 113 ppm: 122-157 exposures 49 ppm: 127-131 exposures	
3.3.2 Frequency of exposure	7 hours per day, 5 days per week	
3.3.3 Postexposure period	Normally one day after the final exposure though recovery periods were included in some experiments	
3.3.4 Oral	Not applicable	
3.3.5 Inhalation		

3.3.5.1 Concentrations	Nominal concentration	Not reported	
	Analytical concentration	841, 357, 204, 113 and 49 ppm (equal to 1.51, 0.64, 0.37, 0.20 and 0.09 mg/L)	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Not reported		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	7 hours per day, 5 days per week		
3.3.5.8 Controls	Yes but group size not clear		
3.3.6 Dermal	Not applicable		
3.3.7 Intrapertitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			
3.4.1.1 Clinical signs	Reported but frequency of observations not recorded		
3.4.1.2 Mortality	Yes		
3.4.2 Body weight	Reported but frequency of observations not recorded		
3.4.3 Food consumption	Not reported		
3.4.4 Water consumption	Not reported		
3.4.5 Ophthalmoscopic examination	Not reported		
3.4.6 Haematology	Yes, but list of parameters measured not reported and only positive findings included in the publication.		
3.4.7 Clinical chemistry	Yes, but list of parameters measured not reported and only positive findings included in the publication (mostly blood urea nitrogen)		
3.4.8 Urinalysis	Qualitative urine tests for blood, sugar, albumin and sediment were conducted on female rats, rabbits, guinea pigs, mice and monkeys exposed to 204 ppm 122-157 times.		
3.5 Sacrifice and pathology			

3.5.1 Organ weights	Yes but details not reported	
3.5.2 Gross and histopathology	Yes but details not reported The following information is indicated in the article regarding histopathological evaluation: 841 ppm: histopathological examination was conducted on 5 rats/sex and 5 guinea pigs/sex exposed 2 and 3 times, respectively, and killed 1 and 3 days after the last exposure. 357 ppm: histopathological examination was conducted on 8 guinea pigs/sex exposed to 357 ppm 123 times. 204 ppm: histopathological examination was conducted on 20 rats/sex, 8 guinea pigs/sex, 2 rabbits/sex and 2 female monkeys exposed 122-157 times. 49 ppm: histopathological examination was conducted on 20 rats/sex, 8 guinea pigs/sex, 2 rabbits/sex and 10 female mice exposed 127-131 times.	
3.5.3 Other examinations	Neurotoxicity was assessed using a range of pharmacological tests	
3.5.4 Statistics	Fisher *t*-test	
3.6 Further remarks	Only limited details of the experiments are included in the publication.	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	Rats: No clinical signs reported for animals exposed to 841 ppm but neurotoxic effects consisting of impaired sensory and motor function at the level of the lumbar and sacral regions, paralysis and atrophy of hind limb muscles were apparent at 357 ppm. These effects were reversible following 100-132 days recovery period. No clinical signs of toxicity were reported for rats exposed to lower concentrations of ethylene oxide. Mice: No clinical signs of toxicity reported Rabbits: No clinical signs of toxicity reported for rabbits exposed to 841 ppm. At 357 ppm sensory and motor function was impaired at the level of the lumbar and sacral regions, and paralysis and atrophy of hind limb muscles was recorded. These effects were reversible. Slight to marked paralysis of the rear legs towards the end of the exposure period was also reported in the 204 ppm group. There were no clinical signs of toxicity at lower concentrations. Guinea pigs: No clinical signs of toxicity reported Monkeys: A range of neurotoxic effects were reported at 357 and 204 ppm. At 357 ppm these consisted of impaired sensory and motor function at the level of the lumbar and sacral regions, paralysis and atrophy of hind limb muscles, knee jerk reflexes	

	poor or non existent, pain perception in hind quarters and genitalia very poor. No extensor reflex of the palms of the hind feet. Effects were reversible following 100-132 days recovery period. Effects at 204 ppm were less active knee jerk reflexes and abolition of withdrawal from superficial pain stimuli in hind feet, in skin on the back and in the legs; deep pain reflexes were unaffected. Partial paralysis and muscular atrophy were noted in the rear extremities. There were no adverse clinical signs in monkeys exposed to 113 ppm ethylene oxide.	
4.1.2 Mortality	Rats and mice: Deaths occurred amongst rats exposed to concentrations of ethylene oxide of 204 ppm and greater. Mortality at 204 and 357 ppm was thought to be mostly caused by secondary respiratory infection. Rabbits: Mortality occurred at ≥ 357 ppm Guinea pigs and monkeys: Deaths only occurred amongst animals exposed at the highest concentration of 841 ppm Rabbits: Mortality occurred at ≥ 357 ppm Guinea pigs and monkeys: Deaths only occurred amongst animals exposed at the highest concentration of 841 ppm	
4.2 Body weight gain	Rats: Reduction in body weight gain at 357, 204 and 113 ppm. No effect at 45 ppm. Mice and rabbits: No effect on body weight reported. Guinea pigs: Body weight gain reduced at 357 ppm in males, only a slight effect in females. Monkeys: Growth was depressed at 357 ppm.	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Rats: No adverse effect at ≤ 357 ppm Mice: Not reported Rabbits: No adverse effect at 357 or 204 ppm Guinea pigs: No adverse effect at 357 ppm Monkeys: No adverse effect at ≤ 357 ppm	
4.5.2 Clinical chemistry	Rats: No adverse effect at ≤ 357 ppm Mice: Not reported Rabbits: No adverse effect at 357 or 204 ppm Guinea pigs: No adverse effect at 357 ppm Monkeys: No adverse effect at ≤ 357 ppm	
4.5.3 Urinalysis	Qualitative urine tests on female rats, rabbits, guinea pigs, mice and monkeys exposed to 204 ppm 122-157 times for blood, sugar, albumin and sediment were all negative	
4.6 Sacrifice and pathology		

4.6.1 Organ weights	Rats: Marked increase in lung weight, moderate increase in kidney weight and slight increase in female liver weight at 204 ppm. Moderate increase in lung weights in both sexes was observed at 113 ppm. Mice: No effects reported Rabbits: No effects reported Guinea pigs: Slight increase in lung weight reported at 357 ppm Monkeys: No effects reported	
4.6.2 Gross and histopathology	Rats: At 841 ppm, respiratory tract irritation, slight haemorrhage and congestion of the lungs, light colouration of the liver, kidney enlargement and pale coloration and enlargement of the adrenals was found. Microscopically interstitial oedema, congestion and alveolar haemorrhage of the lungs, fatty degeneration of the liver, cloudy swelling of convoluted tubules in the kidneys and fat vacuoles in the adrenal cortex were observed. Evidence of liver regeneration was seen in animals killed 3 days after last exposure. Severe lung injury was also found at 357 ppm, and at 204 ppm the testes of males appeared small and there was microscopic evidence of slight tubular degeneration. Very slight cloudy swelling of a few convoluted tubules of male rat kidneys was also apparent and haemorrhage, congestion, emphysema and atelectasis of the lungs of females was observed both grossly and microscopically at 204 ppm. Mice: Respiratory tract irritation was found at 841 ppm. Severe lung injury was seen at 357 ppm which was probably secondary to primary lung irritation. Rabbits: Respiratory tract irritation at 841 ppm and slight generalised oedema and congestion of the lungs of males at 204 ppm. Guinea pigs: At 841 ppm respiratory tract irritation, slight haemorrhage and congestion of the lungs, light colouration of the liver, kidney enlargement and pale coloration and enlargement of the adrenals were detected. Microscopically interstitial oedema, congestion and alveolar haemorrhage of the lungs, fatty degeneration of the liver, cloudy swelling of convoluted tubules in the kidneys and fat vacuoles in the adrenal cortex were also found. There was some evidence for reversibility of these effects. Tubular degeneration in male testes and slight fatty infiltration in female adrenal cortex were found in guinea pigs exposed to 357 ppm. Monkeys: No adverse histopathology effects reported.	
4.7 Other examinations	None	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Groups of rats, mice, rabbits, guinea pigs and monkeys were exposed to 841, 357, 204, 113 or 49 ppm ethylene oxide for periods from 3 to 157 days. Mortality, body weight gain, signs of neurotoxicity, haematology and clinical chemistry were recorded for some groups and exposure times and a recovery period of 3 days was included for rats and guinea pigs exposed	

	to 841 ppm ethylene oxide. Most animals were given a macroscopic and microscopic examination at necropsy.	
5.2 Results and Discussion	Repeated exposure to 841 ppm caused deaths in all species tested. In rats adverse findings consisted of increased mortality, neurotoxicity, lung injury and a decrease in body weight gain at 357 ppm; mortality was also increased at 204 ppm and there were organ weight changes, histopathology of the testes, kidneys and lung and decreased body weight gain at this concentration level. The main cause of mortality at 357 ppm was considered to be secondary respiratory infection. Body weight gain was also decreased and lung weight increased at 113 ppm. Neurotoxic effects following exposure to 357 ppm were found to be reversible after 100-132 days recovery period. Severe lung injury and increased mortality was also found in mice exposed to 357 ppm but this was considered secondary to lung irritation. Blood urea nitrogen values and haematological values were within the normal range at ≤ 357 ppm. Mortality was also slightly increased at 204 ppm. In mice, mortality was slightly increased compared to controls at 204 ppm. Exposure to 851 ppm caused 100% mortality. Severe lung injury was observed at 357 ppm, which could have been secondary to the lung infection. No further details were reported. In rabbits, mortality was increased at 357 ppm and there were clinical signs of neurotoxicity but no associated histopathology at both 357 and 204 ppm. The only microscopic effect reported in these two groups was in the lungs of male rabbits exposed to 204 ppm. Effects on lungs, liver, kidneys and adrenals were noted in guinea pigs exposed to 841 ppm ethylene oxide and at 357 ppm there was a slight increase in lung weight, reduction in body weight gain and histopathology effects on the testes and adrenals. Signs of neurotoxicity were the principal adverse findings in monkey and these were apparent at 204 and 357 ppm ethylene oxide. Body weight gain was also reduced at 357 ppm.	x
5.3 Conclusion	Comparison of the effects reported in this study was complicated by the use of different exposure periods and group size and by the minimal details of the results. A range of macroscopic and microscopic histopathology findings were found, the lungs being affected in all species except monkey. Neurotoxic effects were also observed in rats, guinea pigs and monkeys but there was no associated pathology. Overall rats appeared to be the most sensitive of the species tested.	
5.3.1 LO(A)EL	Rats: 113 ppm. Mice 113 ppm. Rabbits: 204 ppm. Guinea pigs: 204 ppm. Monkeys: 204 ppm	x
5.3.2 NO(A)EL	Rats: 49 ppm. Mice: 49 ppm. Rabbits: 113 ppm. Guinea pigs: 113 ppm. Monkeys: 113 ppm	x

5.3.3 Other	None	
5.3.4 Reliability	3	
5.3.5 Deficiencies	The limited details of the results and the use of different group sizes and durations of exposure made intra species comparison of the effects at different dose levels and inter species comparisons difficult.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and Discussion	*5.2 Results and Discussion* should read: Based on the lack of information on mice exposed to 113 ppm the NOAEL for mice is set at 49 ppm and LOAEL at 204 ppm by the eCA. For guinea pigs, considering slightly increased lung weight in male mice exposed at 204 ppm, the NOAEL is set at 113 ppm. Based on the reported results, the following NOAELs are concluded by the eCA: Rats: 49 ppm. Mice: 49 ppm. Rabbits: 113 ppm. Guinea pigs: 113 ppm. Monkeys: 113 ppm. Based on the reported results, the following LOAELs are concluded by the eCA: Rats: 113 ppm. Mice 204 ppm. Rabbits: 204 ppm. Guinea pigs: 204 ppm. Monkeys: 204 ppm	
Conclusion		
Reliability	3	
Acceptability	The study is not sufficient as a stand-alone, but is acceptable as a part of weight of evidence.	
Remarks	Only limited information was available on the results for each species and no full review of data could be made. The limited details of the results and the use of different group sizes and durations of exposure made intra species comparison of the effects at different dose levels and inter species comparisons difficult.	

Section A 6.4.3/02 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only

1.1 Reference	Jacobson, H., Hackley, E. and Feinsilver, L. (1956) The Toxicity of Inhaled Ethylene Oxide and Propylene Oxide Vapors American Medical Association Archives of Industrial Health, 13, 237-244	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No, guideline compliance was not claimed	
2.2 GLP	No	
2.3 Deviations	Not applicable, guideline compliance was not claimed	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: obtained from Matheson Company, East Rutherford, N.J.	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	x
3.1.2.3Stability	Not reported	
3.2 Test animals		
3.2.1 Species	Rat, mouse and dog	
3.2.2 Strain	Rat: Not reported. Mouse: Not reported. Dog: Beagle	
3.2.3 Source	Not reported	
3.2.4 Sex	Rat and dog: Male. Mouse: Female	
3.2.5 Age/weight at study initiation	Not reported	
3.2.6 Number of animals per group	Rats: 20. Mice: 30. Dogs: 3 for both the 6 and 26 week exposures Satellite groups: Rats and mice: 15 for the 6 week study and 60 for the 6 month study. Five animals of each species were killed every other week until deaths reduced the number to < 5.	

3.2.7 Control animals	Equal number for rats and mice. 2 dogs for the 6 week exposure and 3 dogs for the 26 week exposure.		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	Up to 6 and 26 weeks		
3.3.2 Frequency of exposure	6 hours per day, 5 days per week		
3.3.3 Postexposure period	Recovery was investigated in some exposed animals.		
3.3.4 Oral	Not applicable		
3.3.5 Inhalation			
3.3.5.1 Concentrations	Nominal concentration	Six week exposure: Rats and mice: 400 ppm Dogs: 300 ppm Twenty six week exposure: 100 ppm	
	Analytical concentration	Six week exposure: Rats and mice: 406±42 ppm Dogs: 292±26 ppm Twenty six week exposure: Rats: 102±24 ppm Mice: 98±16 ppm Dogs: 100±13 ppm	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Not reported but probably whole body (exposure chambers were used)		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 5 days per week		
3.3.5.8 Controls	Room air		
3.3.6 Dermal	Not applicable		
3.3.7 Intraperitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			

3.4.1.1 Clinical signs	Yes, but frequency not reported	
3.4.1.2 Mortality	Yes, but frequency not reported	
3.4.2 Body weight	Yes, but frequency not reported	
3.4.3 Food consumption	Not reported	
3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Not reported	
3.4.6 Haematology	Yes, for dogs only but parameters were not reported	
3.4.7 Clinical chemistry	Yes, for dogs only Parameters: Calcium, urea and bilirubin	
3.4.8 Urinalysis	No	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	No	
3.5.2 Gross and histopathology	Yes, but tissues examined were not reported.	
3.5.3 Other examinations	None	
3.5.4 Statistics	Not reported	
3.6 Further remarks	None	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	Six weeks exposure: Rats: Reddish nasal discharge, diarrhoea, tendency toward the side position and laboured breathing. During the last week or two of exposure rats usually moved about on their front feet dragging their hindquarters. Surviving rats recovered over a period of several months after the end of exposure. Mice: None reported Dogs: Vomiting, occasional slight tremors and transient weakness in the hindlegs in two of the three animals. Twenty six weeks exposure: No clinical signs of toxicity in any species.	
4.1.2 Mortality	Six weeks exposure: Rats: Thirteen rats died during the treatment period compared with 0 control rats. Median survival time was 25.6 exposures. Mice: Twenty four mice exposed to ethylene oxide died compared with 3 controls. Median survival time was 21.6 exposures. Dogs: No deaths	

	Twenty six weeks exposure: Rats: Three exposed and control rats died. Mice: Eight mice died in the group exposed to ethylene oxide and 4 in the control group. Dogs: No deaths	
4.2 Body weight gain	Six weeks exposure: Rats: There was a progressive loss of body weight, averaging 100-240 g in rats initially weighing 400-450 g. Surviving rats regained weight over a period of several months after the end of exposure. Mice: Slightly increased loss in body weight compared with controls, probably not significant. Dogs: No significant change. Twenty six weeks exposure: Rats: Slight but probably not significant weight reduction compared with controls. Mice and Dogs: No effect	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Six weeks exposure: Dogs: Significant decrease in erythrocytes, haemoglobin and haematocrit in 2 dogs; in the third there was a decrease in haemoglobin and no change in erythrocytes or haematocrit. Twenty six weeks exposure: Dogs: Erythrocytes, haemoglobin and probably haematocrit were significantly reduced in one dog (RBC decrease ≥ 1,000,000, haemoglobin ≥ 2 g, haematocrit ≥8%) and there were slight decreases in these parameters in another dog.	
4.5.2 Clinical chemistry	Six weeks exposure: Dogs: No changes in blood calcium, blood urea and icteric indices. Twenty six weeks exposure:	

	Dogs: No changes in blood calcium, blood urea and icteric indices.	
4.5.3 Urinalysis	Not reported	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	Not reported	
4.6.2 Gross and histopathology	Six weeks exposure: Rats: Haemosiderosis in the spleen of some animals, usually late in the exposure period. Mice: no effects. Dogs: Congestion of the lungs and moderate focal alveolar collapse. There was an abundance of fat in the Mm. semimembranosus and semitendinosus and bundles of fibres were were squeezed and distorted out of shape by large fat globules. In other areas fat completely replaced the muscle fibres. Twenty six weeks exposure: Rats and mice: No treatment related effects. Dogs: Not examined.	
4.7 Other examinations	There were no significant changes in ECGs or rectal temperature.	x
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Groups of 20 rats and 30 mice were exposed to 0 or 400 ppm ethylene oxide 6 hours per day, 5 days per week for 6 weeks or to 0 or 100 ppm for 26 weeks. Clinical signs of toxicity and mortality were recorded and at the end of the treatment period the animals were necropsied and tissues examined by histopathology. Satellite groups of rats and mice were exposed to the same concentrations and killed in groups of 5 at fortnightly intervals for pathology. Groups of 3 dogs were also exposed to ethylene oxide concentrations of 300 ppm for 6 weeks or 100 ppm for 26 weeks. Two dogs were exposed to ambient air for 6 weeks and 3 for 26 weeks. In addition to the parameters investigated in the rodent experiments, blood was taken from the dogs for clinical chemistry and haematology.	
5.2 Results and Discussion	Exposure to 400 ppm ethylene oxide for 6 weeks was associated with increased mortality in both rats and mice. Rats lost body weight and displayed clinical signs of toxicity consisting of reddish nasal discharge and diarrhoea as well as symptoms of neurotoxicity. There was only a slight increase in body weight in mice and no signs of toxicity. Microscopic pathology revealed	

	haemosiderosis in rats and no changes in mice. There were no treatment related effects of exposure of rats to 100 ppm ethylene oxide for 26 weeks; the only effect in mice was a slight increase in mortality. Signs of neurotoxicity were also noted in dogs exposed to 300 ppm ethylene oxide for 6 weeks; vomiting was also recorded in these animals. Microscopic pathology showed that the lungs were congested and there was moderate focal alveolar collapse. Changes consistent with muscular atrophy were identified which explained the clinical signs of neurotoxicity. The only other finding was of normochromic anaemia in two of the dogs and possibly slight hypochromic anaemia in the third. Haematological changes associated with normochromic anaemia were also found in one and possibly two of the dogs exposed to 100 ppm ethylene oxide for 26 weeks but there were no clinical signs of neurotoxicity.	
5.3 Conclusion	Exposure to 400 ppm ethylene oxide increased mortality in rats and mice, and resulted in clinical signs of toxicity and neurotoxicity in rats. The only effect of exposure of rats and mice to 100 ppm for 26 weeks was a slight increased in mortality in mice. Neurotoxicity and anaemia resulted from exposure of dogs to 400 ppm for 6 weeks but anaemia was the only effect in dogs exposed to 100 ppm for 26 weeks.	
5.3.1 LO(A)EL	100 ppm for 26 weeks in mice and dogs.	
5.3.2 NO(A)EL	None, the study was not designed to establish a NOAEL.	
5.3.3 Other	None	
5.3.4 Reliability	3	
5.3.5 Deficiencies	Only limited details of the results are included in the publication.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.2 Purity* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	

Results and Discussion	*4.7 Other examinations* should read: Dogs, 6 and 26 weeks exposure: there were no significant changes in ECGs or rectal temperature.	
Conclusion	Based on the lack of adverse effects at 100 ppm during 26 weeks of exposure in rats, this concentration can be considered a NOAEL. For other species a NOAEL could not be set for either exposure duration, due to effects seen at the lowest dose level of 100 ppm.	
Reliability	3	
Acceptability	The study is not sufficient as a stand-alone, but can be accepted as a part of weight of evidence.	
Remarks	Due to low reliability of the study, the derived NOAEL in this study should not be used as part of an overall NOAEL in the rat.	

Section A 6.4.3/03 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Mori, K., Inoue, N., Fujishiro, K., Kikuchi, M. and Chiba, S. (1990) Biochemical Changes in Rat Erythrocytes Caused by Ethylene Oxide Exposure Fundamental and Applied Toxicology, 15, 441-447	
1.2 Data protection		
1.2.1 Data owner	Unknown	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance not claimed	
2.2 GLP	No	
2.3 Deviations	Not applicable, this was not a guideline study	
	3 Materials and Methods	
3.1 Test material		

3.1.1 Lot/Batch No	Not reported		
3.1.2 Specification	Obtained from Nissan Shoji Co., Tokyo, stated to be of reagent grade.		
3.1.2.1 Description	Not reported		
3.1.2.2 Purity	Not reported but ethylene oxide was obtained as a mixture with carbon dioxide containing 20% ethylene oxide		x
3.1.2.3Stability	Not reported		
3.2 Test animals			
3.2.1 Species	Rat		
3.2.2 Strain	Wistar		
3.2.3 Source	Seiwa Experimental Animals, Fukuoka, Japan		
3.2.4 Sex	Male		
3.2.5 Age/weight at study initiation	8 weeks		
3.2.6 Number of animals per group	28		
3.2.7 Control animals	28		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	6 hours per day, 3 days per week		
3.3.2 Frequency of exposure	2, 6 and 13 weeks		
3.3.3 Postexposure period	40 hours		
3.3.4 Oral	Not applicable		
3.3.5 Inhalation			
3.3.5.1 Concentrations	Nominal concentration	500 ppm. The carbon dioxide concentration was estimated to be 2300 ppm.	
	Analytical concentration	505±11.5 ppm	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Whole body		
3.3.5.5 Vehicle	None		

3.3.5.6 Concentration in vehicle	None	
3.3.5.7 Duration of exposure	6 hours per day, 3 days per week	
3.3.5.8 Controls	Ambient air	
3.3.6 Dermal	Not applicable	
3.3.7 Intrapertitoneal/ Intravenous/ Intratracheal instilation	Not applicable	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Reported but frequency not recorded	
3.4.1.2 Mortality	Reported but frequency not recorded	
3.4.2 Body weight	Reported but frequency not recorded	
3.4.3 Food consumption	Not reported	
3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Not reported	
3.4.6 Haematology	Yes Number of animals: 8 at 2 and 6 weeks and 12 at 13 weeks Parameters: Erythrocytes, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and reticulocytes. Heparinised blood was assayed for glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione peroxidase, glutathione reductase, ATP, 2,3-diphosphoglycerate and erythrocyte acetyl cholinesterase. Osmotic fragility and haemoglobin instability were also determined.	
3.4.7 Clinical chemistry	No	
3.4.8 Urinalysis	No	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	No	
3.5.2 Gross and histopathology	No	
3.5.3 Other examinations	No	
3.5.4 Statistics	Student's *t*-test	
3.6 Further remarks	None	

	4 Results	
4.1 Observations		
4.1.1 Clinical signs	All rats in the exposed group had an ataxic gait after 6-9 weeks of exposure	
4.1.2 Mortality	None reported	
4.2 Body weight gain	No effect of treatment	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Haemoglobin was decreased at 2 weeks and there was a further reduction by 6 weeks which was accompanied by a decrease in erythrocytes and increases in reticulocytes and mean cell volume. At 13 weeks erythrocyte counts had recovered slightly but haemoglobin and haematocrit were reduced and mean cell volume and reticulocytes were still increased (Table 6.4.3/03-1). Glutathione reductase activity in erythrocytes was reduced at all 3 time points and glutathione content was reduced slightly but significantly at 13 weeks (Table 6.4.3/03-2). The glutathione stability test gave a positive result. The only other significant change was in acetyl cholinesterase activity which was increased at 13 weeks. Haemoglobin stability was thought not to be affected at 13 weeks based on the negative results of the heat test and the isopropanol test (data not shown in the article).	
4.5.2 Clinical chemistry	Not reported	
4.5.3 Urinalysis	Not reported	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	Not reported	
4.6.2 Gross and histopathology	Not reported	
4.7 Other examinations	None	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Rats were exposed to 0 or 500 ppm ethylene oxide for 6 hours per day, 3 days per week for 2, 6 or 13 weeks. Clinical signs of toxicity were recorded and the effect on haematology and erythrocyte enzymes was investigated.	
5.2 Results and Discussion	Signs of toxicity consisting of ataxic gait were observed after 6-9 weeks. The haematology results showed macrocytic anaemia, normochromic anaemic and a high reticulocyte count. Measurements of reticulocyte enzymes showed a reduction in	

	glutathione reductase activity but other enzyme activities determined showed there was no significant effect on other enzymes in the hexose monophosphate cycle, no effect on the Embden-Meyerhof pathway or the Lapoport-Luebering cycle based on unchanged ATP or 2,3-diphosphoglycerate content . Membrane fragility and haemoglobin stability were also unaffected.	
5.3 Conclusion	The mechanism of ethylene oxide induced anaemia may be related to inhibition of glutathione reductase activity.	
5.3.1 LO(A)EL	500 ppm	
5.3.2 NO(A)EL	Not applicable	
5.3.3 Other	None	
5.3.4 Reliability	2	x
5.3.5 Deficiencies	None, compliance with regulatory guidelines was not claimed.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.2 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and Discussion		
Conclusion	Exposure to ethylene oxide at 500 ppm for a period up to 13 weeks caused macrocytic normochromic anaemia, which may be related to inhibition of glutathione reductase activity.	
Reliability	3	
Acceptability	The study is not sufficient as a stand-alone, based on limited parameters evaluated and limited exposure period (3 d/w), but can be accepted as a part of weight of evidence approach, specifically considering influence of ethylene oxide exposure on haematological parameters.	

Remarks	Only one concentration and 3 days/week tested, no gross necropsy and histopathology performed; only haematological parameters evaluated.	

Table 6.4.3/03-1: Haematology parameters after exposure to ethylene oxide

Parameter	Exposure period (weeks)					
	2		6		13	
	0 ppm	500 ppm	0 ppm	500 ppm	0 ppm	500 ppm
Erythrocytes ($x10^6/mm^3$)	8.83±0.48	8.44±0.81	9.14±0.27	7.16±0.54*	9.15±0.22	7.85±0.38*
Haemoglobin (g/dl)	17.15±0.50	16.23±0.66*	16.43±0.64	15.12±1.57*	16.28±0.48	14.67±0.59*
Haematocrit (%)	52.57±2.47	50.51±3.20	49.44±1.69	46.91±3.75	49.82±1.60	45.58±1.94*
Mean cell volume (μ^3)	59.45±1.63	60.25±2.63	54.08±1.00	65.50±2.02*	54.52±1.00	58.09±1.45*
Mean cell haemoglobin (μ μg)	19.45±1.02	19.35±1.36	19.16±0.25	19.83±0.90	18.39±0.30	18.72±0.54
Mean cell haemoglobin concentration (%)	32.65±1.34	32.18±1.14	33.23±0.27	32.23±1.49	32.68±0.53	32.18±0.56
Reticulocytes (%)	2.14±0.71	2.84±0.96	2.22±0.91	4.42±0.97*	1.82±1.24	3.09±1.26*

Table 6.4.3/03-2: Effect of 13 weeks exposure to ethylene oxide on erythrocyte enzymes

Enzyme activity (μmol NADPH/min/g haemoglobin)	Concentration (ppm)	
	0	500
Glucose-6-phosphate dehydrogenase	11.88±2.19 (8)	12.76±3.01 (8)
6-Phosphogluconate dehydrogenase	7.547±2.597 (8)	8.406±2.980 (8)
Glutathione reductase FAD(+) FAD(-)	 1.038±0.267 (12) 1.516±0.440 (12)	 0.400±0.140 (12)* 0.488±0.191 (12)*
Glutathione peroxidase	406.3±71.5 (8)	415.4±85.2

The number of animals in each group is given in brackets
* $p<0.05$

Section A 6.4.3/04 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Fujishiro, K., Mori, K. and Inoue, N. (1990) Chronic inhalation effects of ethylene oxide on porphyrin-heme metabolism Toxicology, 61, 1-11	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	

2.1 Guideline study	No, guideline compliance was not claimed		
2.2 GLP	No		
2.3 Deviations	Not applicable, not a guideline study		
	3 Materials and Methods		
3.1 Test material			
3.1.1 Lot/Batch No	Not reported		
3.1.2 Specification			
3.1.2.1 Description	Not reported		
3.1.2.2 Purity	Not reported but ethylene oxide was obtained as a mixture with carbon dioxide containing 20% ethylene oxide		x
3.1.2.3Stability	Not reported		
3.2 Test animals			
3.2.1 Species	Rat		
3.2.2 Strain	Wistar		
3.2.3 Source	Kyudo Co., Ltd., Japan		
3.2.4 Sex	Male		
3.2.5 Age/weight at study initiation	170 g 1 week prior to acclimatisation		
3.2.6 Number of animals per group	24		
3.2.7 Control animals	24		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	6 hours per day, 3 days per week		
3.3.2 Frequency of exposure	2, 6 or 13 weeks		
3.3.3 Postexposure period	40 hours		
3.3.4 <u>Oral</u>	Not applicable		
3.3.5 <u>Inhalation</u>			
3.3.5.1 Concentrations	Nominal concentration	500 ppm. The carbon dioxide concentration was estimated to be 2300 ppm.	

	Analytical concentration	500±10 ppm	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Whole body		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 3 days per week		
3.3.5.8 Controls	Ambient air		
3.3.6 Dermal	Not applicable		
3.3.7 Intraperitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			
3.4.1.1 Clinical signs	Not recorded		
3.4.1.2 Mortality	Not recorded		
3.4.2 Body weight	Determined at termination		
3.4.3 Food consumption	Not reported		
3.4.4 Water consumption	Not reported		
3.4.5 Ophthalmoscopic examination	Not reported		
3.4.6 Haematology	Yes, all animals at termination Parameters: Haemoglobin, haematocrit, erythrocyte count, mean cell volume, mean cell haemoglobin concentration and reticulocytes.		
3.4.7 Clinical chemistry	No		
3.4.8 Urinalysis	Yes, 24 hour urine samples were collected daily protected from light. Parameters: Coproporphyrin, δ-aminolevulinic acid and creatinine		
3.5 Sacrifice and pathology			
3.5.1 Organ weights	Liver		

3.5.2 Gross and histopathology	No	
3.5.3 Other examinations	Uroporphyrin, coproporphyrin and protoporphyrin and δ-aminolevulinic acid dehydratase were determined in liver homogenate, aminolevulinic acid synthase in liver 8000 x g pellet, cytochrome P-450, cytochrome b_5 and protohaem in liver microsomes and ferrochelatase in liver mitochondria. Protoporphyrin and δ-aminolevulinic acid dehydratase were also determined in erythrocytes.	
3.5.4 Statistics	Student's *t*-test	
3.6 Further remarks	None	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	Not reported	
4.1.2 Mortality	Not reported	
4.2 Body weight gain	No significant change	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Haemoglobin, haematocrit and erythrocyte count were significantly reduced but there were no differences in mean cell volume or mean cell haemoglobin concentration. Reticulocytes were increased (Table 6.4.3/04-1).	
4.5.2 Clinical chemistry	Not reported	
4.5.3 Urinalysis	Urinary coproporphyrin per mg creatinine showed a time dependent increase and was 5-6 fold greater than control after 13 weeks. Daily excretion of δ-aminolevulinic acid was also increased but not when corrected for creatinine concentration (Table 6.4.3/04-2).	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	No effect of treatment	
4.6.2 Gross and histopathology	Not reported	
4.7 Other examinations	Uroporphyrin and coproporphyrin in liver tended to increase (uroporphyrin by 37% after 13 weeks exposure), but there was no significant increase in protoporphyrin in liver or erythrocytes (Table 6.4.3/04-2). Hepatic cytochrome P-450 and and protohaem decreased significantly (Table 6.4.3/04-3); these	

	changes were already evident after 2 and 6 weeks of exposure. Hepatic microsomal total protein and cyrochrome b5 were not affected. Amongst the enzyme activities measured aminolevulinic acid synthase increased significantly, and ferrochelatase decreased by 25% after 13 weeks exposure but δ-aminolevulinic acid dehydratase was unaffected in the liver or erythrocytes (Table 6.4.3/04-4).	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Rats were exposed to 0 or 500 ppm ethylene oxide for 6 hours per day, 3 days per week for 2, 6 or 13 weeks. Haematology and urinalysis was conducted and the effect of exposure on liver porphyrins and enzymes involved in porphyrin-haem metabolism was also measured.	
5.2 Results and Discussion	Exposure to ethylene oxide caused a significant reduction in haemoglobin content and erythrocyte count, and a normocytic and normochromic anaemia was found. In the liver, cytochrome P-450 and protohaem were significantly reduced and the activity of δ-aminolevulinic acid and ferrochelatase increased. The concentration of porphyrins increased in liver, erythrocytes and urine; protoporphyrin was not increased in liver and erythrocytes, coproporphyrin increased in urine and liver and uroporphyrin also increased in liver.	
5.3 Conclusion	The results show that exposure to ethylene oxide causes alterations of haem-porphyrin metabolism in addition to anaemia.	
5.3.1 LO(A)EL	500 ppm (the only concentration tested)	
5.3.2 NO(A)EL	Not applicable, only one concentration was tested	
5.3.3 Other		
5.3.4 Reliability		x
5.3.5 Deficiencies		
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.2 Purity* should read: The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA	

	has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and Discussion		
Conclusion	Exposure to ethylene oxide at 500 ppm for 13 weeks significantly reduced haemoglobin, haematocrit and erythrocyte count and caused normocytic and normochromic anaemia. Exposure cased alterations in haem-porphyrin metabolism. Hepatic microsomal CYP-450 and prohaem decreased significantly, while the activity of hepatic ALA-synthase and ferrochelatase increased. In urea coproporhyrin was increased significantly.	
Reliability	3	
Acceptability	The study is not sufficient as a stand-alone, based on limited parameters evaluated and limited exposure period (3 d/w), but can be used as a part of weight of evidence, specifically considering influence of ethylene oxide exposure on haematological parameters.	
Remarks	Only one concentration and 3 days/week tested, no gross necropsy and histopathology performed; only haematological parameters evaluated.	

Table 6.4.3/04-1: Haematological effects associated with exposure to ethylene oxide for 13 weeks

Conc (ppm)	Haemoglobin (g/dl)	Haematocrit (%)	Erythrocytes (x 10^4 µL)	Mean cell volume (fL)	Mean cell haemoglobin concentration (%)	Reticulocytes (%)
0	15.6±0.7	45.4±2.8	869±38	52.2±2.3	34.4±0.8	10.9±3.4
500	14.0±0.7**	40.3±2.3**	763±36***	52.8±1.8	34.6±0.4	20.1±9.3*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 6.4.3/04-2: Effect of exposure to ethylene oxide for 13 weeks on porphyrins in liver, erythrocytes and urine

Conc (ppm)	Urine (µg/mg creatinine)		Liver (ng/g liver)			Erythrocytes (µg/dL erythrocytes)
	Coproporphyrin	δ-aminolevulinic acid	Uroporphyrin	Coproporphyrin	Protoporphyrin	Protoporphyrin
0	1.14±0.53	62.6±23.1	89.0±22.5	19.9±14.0	88.7±25.9	33.2±9.6
500	6.92±1.86**	114.1±38.0	122.1±27.2*	33.5±16.7	100.4±14.0	39.8±9.2

* $p < 0.05$, ** $p < 0.01$

Table 6.4.3/04-3: Effect of exposure to ethylene oxide for 13 weeks on liver parameters

Concentration (ppm)	Liver weight (g)	Microsomal protein (mg/g liver)	P-450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	Protohaem (nmol/mg protein)
0	9.5±0.5	27.0±4.8	0.61±0.08	0.21±0.04	1.10±0.12
500	9.1±1.1	26.4±3.9	0.48±0.09*	0.19±0.03	0.88±0.10*

* $p < 0.01$

Table 6.4.3/04-4: Effect of exposure to ethylene oxide for 13 weeks on enzymes of porphyrin haem metabolism

Concentration (ppm)	Aminolevulinic acid synthase (nmol ALA/h.g)	δ-Aminolevulinic acid dehydratase (µmol ALA/min per erythrocyte or mg protein)		Ferrochelatase (nmol haem/mg protein)
	Liver	Erythrocyte	Liver	Liver
0	20.5±1.5	2.62±0.54	140.8±18.1	1.20±0.18
500	27.3±3.4**	2.41±0.92	132.4±17.7	0.90±0.16*

* $p < 0.05$, ** $p < 0.01$

Section A 6.4.3/05 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Matsuoka, M., Igusu, H., Inoue, N., Hori, H. and Tanaka, I. (1990) Inhibition of creatine kinase activity by ethylene oxide British Journal of Industrial Medicine, 47, 44-47	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No guideline compliance claimed	
2.2 GLP	No	
2.3 Deviations	None, compliance with a guideline is not claimed	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: purchased from Showa Tansan Co., Ltd.	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported but ethylene oxide was obtained as a mixture with carbon dioxide containing 20% ethylene oxide	x

3.1.2.3Stability	Not reported		
3.2 Test animals			
3.2.1 Species	Rat		
3.2.2 Strain	Wistar		
3.2.3 Source	Not reported		
3.2.4 Sex	Male		
3.2.5 Age/weight at study initiation	160-180 g		
3.2.6 Number of animals per group	Probably up to 9		
3.2.7 Control animals	Probably up to 9		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	6 hours per day, 3 days per week		
3.3.2 Frequency of exposure	1 day, 4 and 12 weeks		
3.3.3 Postexposure period	40 hours		
3.3.4 <u>Oral</u>	Not applicable		
3.3.5 <u>Inhalation</u>			
3.3.5.1 Concentrations	Nominal concentration	500 ppm	
	Analytical concentration	Not reported	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Whole body		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 3 days per week		
3.3.5.8 Controls	Ambient air		
3.3.6 <u>Dermal</u>	Not applicable		

3.3.7 <u>Intrapertitoneal/ Intravenous/ Intratracheal instilation</u>	Not applicable	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Reported but details of frequency of observation not reported	
3.4.1.2 Mortality	Not reported	
3.4.2 Body weight	Reported but details of frequency not reported	
3.4.3 Food consumption	Not reported	
3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Not reported	
3.4.6 Haematology	No	
3.4.7 Clinical chemistry	Yes, probably all animals at termination Parameters: Total protein, albumin, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, cholinesterase, creatine kinase, amylase, glucose, total lipids, phospholipids, total cholesterol, high density lipoprotein cholesterol, triglycerides, fatty acids, blood urea nitrogen, creatinine, uric acid, thyroxine, tri-iodothyronine and thyroid stimulating hormone	
3.4.8 Urinalysis	No	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes, at least brain but results not reported	
3.5.2 Gross and histopathology	No	
3.5.3 Other examinations	The activity of creatine kinase, aspartate aminotransferase and lactate dehydrogenase were determined in brain, spinal cord, serum and a sample of gastrocnemius muscle. *In vitro* studies were also conducted in which the activity of creatine kinase, aspartate aminotransferase and lactate dehydrogenase were determined in rat brain homogenates exposed to ethylene oxide for 2 minutes in the presence and absence of dithiothreitol. Purified creatine kinase from rabbit muscle was exposed to ethylene oxide under similar conditions and sulphydryl content and creatinine kinase activity were determined.	
3.5.4 Statistics	Student's *t*-test	
3.6 Further remarks		

	4 Results	
4.1 Observations		
4.1.1 Clinical signs	The rats showed signs of ataxic gait from the sixth week of exposure.	
4.1.2 Mortality	Not reported	
4.2 Body weight gain	No effect on body weight gain	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Not reported	
4.5.2 Clinical chemistry	Serum creatine kinase was reduced by more than 40% after 12 weeks exposure to ethylene oxide. There was also a 20% reduction in serum triglycerides (Table 6.4.3/05-1).	
4.5.3 Urinalysis	Not reported	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	Not reported	
4.6.2 Gross and histopathology	Not reported	
4.7 Other examinations	Creatine kinase was inhibited in brain, spinal cord and muscle after 4 weeks exposure but neither aspartate aminotransferase activity nor lactate dehydrogenase activity was affected by treatment (Table 6.4.3/05-2). Creatine kinase was also inhibited by approximately 10% after a single 6 hour exposure to ethylene oxide. Creatine kinase activity in a brain homogenate was inhibited by 86.7% following exposure to ethylene oxide but there was no effect on the activity of either aspartate aminotransferase or lactate dehydrogenase. The degree of enzyme inhibition in brain homogenates and with the purified enzyme was similar in the presence or absence of dithiothreitol. There was also a decrease in catalytic activity when purified creatine kinase was exposed to ethylene oxide but the decrease in activity was less than the loss of sulphydryl groups and the addition of dithiothreitol had no effect on the loss of enzyme activity.	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Rats were exposed to 0 or 500 ppm ethylene oxide for 6 hours per day, 3 days per week for 1 day, 4 or 12 weeks. Signs of toxicity were noted and clinical chemistry parameters were measured after 12 weeks exposure. The activity of creatine	

	kinase, aspartate aminotransferase and lactate dehydrogenase were determined in brain, spinal cord and a sample of gastrocnemius muscle after 4 and 12 weeks exposure. *In vitro* studies were also conducted in which the effect of exposure to ethylene oxide on creatine kinase, aspartate aminotransferase and lactate dehydrogenase was investigated in rat brain homogenates and also on purified creatine kinase from rabbit muscle.	
5.2 Results and Discussion	Signs of neurotoxicity (ataxic gait) were noted from the sixth week of exposure but clinical chemistry effects after 12 weeks only consisted of a reduction in creatine kinase activity and triglycerides. Creatine kinase activity was also significantly reduced in the brain, spinal cord and muscle of rats exposed to ethylene oxide compared with controls but aspartate aminotransferase and lactate dehydrogenase were unaffected. After a single exposure there was a 10% reduction in creatine kinase activity. Exposure to ethylene oxide inhibited creatine kinase activity in brain homogenate *in vitro* and the activity of the purified enzyme from rabbit muscle was also inhibited. The addition of dithiothreitol had no protective effect so the decrease in activity could not be explained solely by the loss of sulphydryl groups.	
5.3 Conclusion	The neurotoxicity of ethylene oxide may result from inhibition of creatine kinase but this may not be the only mechanism of neuropathy because ethylene oxide may impair other enzymes or structures in the nervous system. Although creatine kinase has sulphydryl groups at its active site the loss of sulphydryl groups was greater than the loss of enzyme activity so some of the sulphydryl groups that were lost were not at the active site of the enzyme.	
5.3.1 LO(A)EL	500 ppm, the only concentration tested	
5.3.2 NO(A)EL	Not applicable, only one concentration was tested.	
5.3.3 Other	None	
5.3.4 Reliability	2	x
5.3.5 Deficiencies	None, it was not intended that the study comply with any particular guideline	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.2 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are	

	identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and Discussion		
Conclusion	The 12 weeks exposure to ethylene oxide at 500 ppm significantly inhibited creatine kinase activity in serum, brain, spinal cord and muscle, but had no effect on ASAT ad LDH activities. There was also a 20% reduction in serum triglycerides. Other biochemical parameters were not affected.	
Reliability	3	
Acceptability	The study is not sufficient as a stand-alone, based on limited parameters evaluated and limited exposure period (3 d/w), but can be used as a part of weight of evidence.	
Remarks	Only one concentration and 3 days/week tested, no gross necropsy and histopathology performed; only enzyme activities and clinical chemistry parameter parameters evaluated.	

Table 6.4.3/05-1: Activity of serum enzymes after rats were exposed to ethylene oxide for 12 weeks

Parameter	Concentration (ppm)	
	0	500
Creatine kinase (IU/mL)	4501±1234	2572±659**
Triglycerides (mg/dL)	47.4±7.4	37.5±7.1*

* $p < 0.05$; ** $p < 0.01$

Table 6.4.3/05-2: Effects of 4 or 12 weeks exposure to ethylene oxide on enzyme activities in selected tissues

Tissue	Concentration (ppm)	Period (weeks)	No of rats	Enzyme activity		
				Creatine kinase (IU/g)	Aspartate aminotransferase (Karmen unit/mg)	Lactate dehydrogenase (Wroblewski unit/mg)
Brain	0 500 0 500	4 4 12 12	4 6 6 6	315.9±10.8 232.0±4.5* 298.1±31.3 203.2±21.0*	72.1±1.6 71.7±2.5	136.5±6.7 136.5±4.6
Spinal cord	0 500	4 4	4 6	195.1±9.5 150.8±3.3*	40.8±2.3 40.0±1.5	63.4±1.4 63.6±2.8
Muscle	0 500	4 4	4 6	1854.8±175.5 1101.6±247.3*	61.8±5.7 59.4±8.2	202.6±22.3 219.5±32.9

* $p < 0.001$

Section A 6.4.3/06 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Snellings, W., Weil, C. and Maronpot R. (1984) A Subchronic Inhalation Study on the Toxicologic Potential of Ethylene Oxide in $B6C3F_1$ Mice Toxicology and Applied Pharmacology, 76, 510-518	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance not claimed but study was similar to OECD 413	
2.2 GLP	No	
2.3 Deviations	Only a limited number of tissues were examined by histopathology and the haematology and clinical chemistry measurements did not include all the parameters in OECD 413	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: purchased from Union Carbide Corporation, Seadrift, Texas	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	>99.9%	
3.1.2.3Stability	Purity was >99.9% throughout the study	
3.2 Test animals		
3.2.1 Species	Mouse	
3.2.2 Strain	$B6C3F_1$	
3.2.3 Source	Charles River Breeding Laboratory, Portage, Michigan, USA	
3.2.4 Sex	Male and female	

3.2.5 Age/weight at study initiation	Approx 9 weeks		
3.2.6 Number of animals per group	30 per sex		
3.2.7 Control animals	30 per sex		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	10 weeks for males, 11 weeks for females		
3.3.2 Frequency of exposure	6 hours per day, 5 days per week		
3.3.3 Postexposure period	None		
3.3.4 Oral	Not applicable		
3.3.5 Inhalation			
3.3.5.1 Concentrations	Nominal concentration	0, 10, 50, 100 or 250 ppm	
	Analytical concentration	Overall means (coefficient of variation) 10(6%) , 48(4%), 104(4%) or 236(4%) ppm	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Not reported		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 5 days per week		
3.3.5.8 Controls	Ambient air		
3.3.6 Dermal	Not applicable		
3.3.7 Intraperitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			
3.4.1.1 Clinical signs	Clinical signs of toxicity were recorded but frequency was not reported.		
3.4.1.2 Mortality	Mortality was recorded but frequency was not recorded		

3.4.2 Body weight	Body weights were recorded weekly.	
3.4.3 Food consumption	Not reported	
3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Not reported	
3.4.6 Haematology	Yes, 10 mice per sex per group at the end of the study Parameters: Erythrocyte count, packed cell volume, haemoglobin mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, white blood cell count and differential leukocyte count	
3.4.7 Clinical chemistry	Yes, 10 mice per sex per group at the end of the study Parameters: Glucose, blood urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase	
3.4.8 Urinalysis	Yes, 10 mice per sex per group at the end of the study Parameters: Bilirubin, glucose, ketones, nitrite, occult blood, pH, protein and urobilinogen	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes Liver, kidneys, adrenals, testes, thymus, spleen and brain	
3.5.2 Gross and histopathology	Yes, all dose groups Sciatic nerve, gastrocnemius muscle, brain and spinal cord from both sexes, testes from males and liver, sternal marrow and spleen from females	
3.5.3 Other examinations	A neuromuscular screening test was performed. Observations included locomotor activity, respiration patterns, corneal response, gait, tail and toe pinch reflex and righting reflex. Five mice were randomly selected for testing at an intermediate time point and at termination. The test was performed immediately after exposure.	
3.5.4 Statistics	Continuous variable data were analysed by Bartlett's test for homogeneity of variance, analysis of variance and Duncan's multiple range test. If Bartlett's test indicated heterogenous variance the F test was used. Student's *t* test was then used if the F value was not significant and Cochran *t* test was used if F was significant. Contingency data were analysed using Fisher's exact test and all other non-parametric data were compared using the multiple sum of ranks.	
3.6 Further remarks	None	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	No consistent clinical signs of toxicity	

4.1.2 Mortality	No treatment related effects on survival	
4.2 Body weight gain	No effect on body weight gain	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Erythrocyte count and haemoglobin were significantly reduced in males and erythrocyte count, packed cell volume and haemoglobin and mean cell haemoglobin were reduced in females at 250 ppm in both sexes. Other parameters were unaffected (Table 6.4.3/06-1).	
4.5.2 Clinical chemistry	No effect of treatment	
4.5.3 Urinalysis	No effect of treatment	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	In the 250 ppm group there was an increase in relative liver weight in females, a decrease in absolute testes weight in males and a decrease in both the absolute and relative spleen weight in both sexes. Absolute and relative spleen weight was also decreased in females exposed to 100 ppm (Table 6.4.3/06-2).	
4.6.2 Gross and histopathology	There were no treatment effects on histopathology.	
4.7 Other examinations	Statistically significant differences were noted in the 250, 100 and 50 ppm groups for abnormal posture during gait. Locomotor activity was also reduced in these groups. Abnormal reflex reactions were apparent in the 250 ppm group for righting, toe pinch and tail pinch and on one occasion in the 100 ppm group (Table 6.4.3/06-3).	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Groups of 30 male and female mice were exposed to 0, 10, 50, 100 or 250 ppm ethylene oxide 6 hours per day, 5 days per week for 10 weeks (males) or 11 weeks (females). Mortality, clinical signs of toxicity, including tests for neurotoxicity, and body weight were recorded. At the end of the exposure period samples of urine and blood were taken for clinical chemistry and haematology. Organs were weighed at necropsy and selected tissues were examined by histopathology.	
5.2 Results and Discussion	At 250 ppm there were minimal but statistically significant changes in red blood cell parameters consisting of reduced erythrocyte count and haemoglobin in both sexes and reduced packed cell volume and mean cell haemoglobin in females. Reduced locomotor activity and abnormal posture were observed at 250, 100 and 50 ppm groups and abnormal reflex reactions were apparent in the 250 ppm group and on one	

	occasion in the 100 ppm group. There were also changes in absolute and/or relative liver weights in females, testes in males and spleen in both sexes. Absolute and relative spleen weight was also decreased in females exposed to 100 ppm. There were no treatment related effects on histopathology.	
5.3 Conclusion	The lack of any microscopic findings suggest that the changes in organ weight at 250 ppm are not of toxicological significance and also provide no explanation for the neuromuscular effects in this group. The slight change in red blood cell parameters are indicative of a mild treatment related effect..	
5.3.1 LO(A)EL	50 ppm	
5.3.2 NO(A)EL	10 ppm	
5.3.3 Other	None	
5.3.4 Reliability	2	
5.3.5 Deficiencies	Only a limited number of tissues were examined by histopathology and the haematology and clinical chemistry measurements did not include all the parameters in OECD 413	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and Discussion		
Conclusion	Based on observed neurotoxicity effects at 50 ppm (reduced locomotor activity in females, hunched posture during gait in males) which became more prominent at higher concentrations, the concentration of 50 ppm is considered to be a LOAEL. The NOAEL is set at 10 ppm.	
Reliability	2	
Acceptability	The study is acceptable for assessment; however, it should be noted that not all parameters required by the modern guideline have been evaluated, and the study duration of 10/11 weeks was slightly lower than the required 13 weeks.	
Remarks		

Table 6.4.3/06-1: Haematology parameters for male and female mice exposed to ethylene oxide for 10 and 11 weeks respectively

Concentration (ppm)	Erythrocyte count ($x10^6/mm^3$)	Packed cell volume (%)	Haemoglobin (g/dl)	Mean cell volume (μm^3)	Mean cell haemoglobin (pg)	Mean cell haemoglobin concentration (%)
Males						

250	8.922±0.332 *	45.7±1.9	14.48±0.60*	52.6±0.8	16.1±0.3	31.6±0.7
100	8.534±1.908	44.2±8.7	14.02±2.58	54.1±4.3	16.7±1.9	31.8±1.3
50	9.174±0.316	47.0±2.4	14.81±0.56	52.7±1.0	16.1±0.3	31.4±1.0
10	9.335±0.432	47.4±1.9	15.04±0.58	52.4±1.2	16.0±0.0	31.8±0.9
0	9.346±0.530	47.8±2.8	15.16±0.75	52.7±1.1	16.1±0.3	31.9±0.7
Females						
250	8.694±0.451 **	44.7±4.0**	14.76±0.64**	52.7±0.7	17.00±0.8**	33.0±1.2
100	9.228±0.510	47.0±2.9	15.35±0.51	52.4±1.4	16.5±0.7	32.6±0.7
50	9.429±0.201	47.6±2.3	15.40±0.65	51.9±0.8	16.2±0.4	32.4±0.5
10	9.514±0.316	47.9±2.1	15.53±0.35	51.6±0.5	16.2±0.4	32.4±1.0
0	9.539±0.365	48.2±3.5	15.54±0.47	52.1±0.8	16.2±0.4	32.1±0.7

* p<0.05, ** p<0.01

Table 6.4.3/06-2: Organ weights for male and female mice exposed to ethylene oxide for 10 and 11 weeks respectively

Concentration (ppm)	Liver		Spleen		Testes
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)
Males					
250	1.428±0.225	5.180±0.549	0.052±0.010*	0.188±0.037*	0.106±0.008*
100	1.503±0.114	5.143±0.303	0.086±0.050	0.299±0.185	0.108±0.009*
50	1.492±0.213	5.168±0.298	0.066±0.014	0.230±0.036	0.108±0.004*
10	1.466±0.171	5.092±0.396	0.065±0.010	0.226±0.032	0.113±0.006
0	1.604±0.197	5.161±0.401	0.078±0.014	0.252±0.036	0.116±0.006
Females					
250	1.372±0.139	5.645±0.372*	0.060±0.009*	0.246±0.037*	-
100	1.179±0.150	5.026±0.418	0.068±0.011*	0.289±0.039*	-
50	1.310±0.156	5.239±0.440	0.080±0.013	0.321±0.042	-
10	1.270±0.134	5.164±0.299	0.085±0.013	0.348±0.052	-
0	1.306±0.149	5.269±0.428	0.083±0.010	0.335±0.033	-

* $p < 0.05$

Table 6.4.3/06-3: Percentage responders in neuromuscular test for male and female mice exposed to ethylene oxide for 10 and 11 weeks respectively

Time[b]	Sex	Concentration (ppm)				
		250	100	50	10	0
Reduced or no toe pinch reflex						
Intermediate	F	100**	40	40	40	0
Terminal	F	ND	ND	0	ND	0
Terminal	M	60	20	20	0	0
Reduced or no tail pinch reflex						
Intermediate	F	60	60	20	40	20
Terminal	F	60	40	20	ND	0
Terminal	M	100**	40	60	0	0
Abnormal righting reflex						
Intermediate	F	100*	100*	20	40	20
Terminal	F	80*	0	0	ND	0
Terminal	M	80*	60	20	0	0
Hunched posture during gait						
Intermediate	F	100*	100*	40	20	20
Terminal	F	100**	80*	80*	ND	0
Terminal	M	100**	100**	40	20	0
Reduced locomotor activity						
Intermediate	F	100	60	40	20	60
Terminal	F	100**	100**	60	ND	0
Terminal	M	100**	100**	80*	40	0

[a] n = 5, [b] Intermediate interval occurred during the 6th exposure week and terminal interval was just prior to sacrifice
* p <0.05, ** p <0.01

Section A 6.4.3/07 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F$_1$ Mice (Inhalation Studies) National Toxicology Program, Technical Report Series No 326, 1987	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	

	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance not claimed but study is similar to OECD 413	
2.2 GLP	No	
2.3 Deviations	Food consumption was not measured, clinical chemistry and haematology parameters were not determined and an ophthalmology examination was not included. A full range of tissues was not taken for histopathology.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	0018-1	
3.1.2 Specification	Not reported: supplied by Union Carbide, Inc., Torrance, CA	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	>99%	
3.1.2.3Stability	No degradation observed over the course of the study	
3.2 Test animals		
3.2.1 Species	Mouse	
3.2.2 Strain	$B6C3F_1$	
3.2.3 Source	Charles River Breeding Laboratories, Portage, Michigan, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	8-9 weeks	
3.2.6 Number of animals per group	10 per sex	
3.2.7 Control animals	10 per sex	
3.3 Administration / Exposure		
3.3.1 Duration of treatment	14 weeks	
3.3.2 Frequency of exposure	6 hours/day, 5 days/week	
3.3.3 Postexposure period	None	
3.3.4 Oral	Not applicable	
3.3.5 Inhalation		

3.3.5.1 Concentrations	Nominal concentration	0, 50, 100, 200, 400 or 600 ppm	
	Analytical concentration	Not reported	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Whole body		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure	6 hours per day, 5 days per week		
3.3.5.8 Controls	Ambient air		
3.3.6 Dermal	Not applicable		
3.3.7 Intrapertitoneal/ Intravenous/ Intratracheal instilation	Not applicable		
3.4 Examinations			
3.4.1 Observations			
3.4.1.1 Clinical signs	Twice daily		
3.4.1.2 Mortality	Twice daily		
3.4.2 Body weight	Before the start of exposure, weekly during exposure and at necropsy		
3.4.3 Food consumption	Not reported		
3.4.4 Water consumption	Not reported		
3.4.5 Ophthalmoscopic examination	Not reported		
3.4.6 Haematology	Not reported		
3.4.7 Clinical chemistry	Not reported		
3.4.8 Urinalysis	Not reported		
3.5 Sacrifice and pathology			
3.5.1 Organ weights	Yes, liver weight reported		
3.5.2 Gross and histopathology	Yes necropsy performed on all animals. Histology on controls and 2 highest dose groups. Tissues examined: Gross lesions and tissue masses, skin, liver,		

	ovaries/uterus, lungs, bronchi, heart, thymus, trachea, spleen, kidneys, adrenals, urinary bladder, sternebrae including marrow and nasal cavity	
3.5.3 Other examinations	None	
3.5.4 Statistics	Liver to body weight ratios were compared using Dunnett's test	
3.6 Further remarks	The study was a preliminary to the 2 year carcinogenicity study	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	All mice exposed to 600 ppm ethylene oxide had anorexia, dyspnea, decreased activity and were bloated and listless.	
4.1.2 Mortality	All mice in the 400 and 600 ppm groups died before the end of the treatment period.	
4.2 Body weight gain	No effect on body weight gain up to 200 ppm bodyweight effects at higher doses were not reported because there was 100% mortality.	
4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	Not reported	
4.5 Blood analysis		
4.5.1 Haematology	Not reported	
4.5.2 Clinical chemistry	Not reported	
4.5.3 Urinalysis	Not reported	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	The relative liver weight of female mice exposed to 50 ppm was significantly lower than controls (Table 6.4.3/07-1) but as there was no dose response this was considered not toxicologically significant.	
4.6.2 Gross and histopathology	Thymic lymphocyte necrosis and renal tubular necrosis were observed in both sexes at 600 ppm; lymphocytic necrosis of the spleen was apparent in males at 600 ppm. Renal tubular degeneration was also noted in both sexes at 100, 200 and 400 ppm and rhinitis of the nasal cavity was observed in both sexes at 200, 400 and 600 ppm. Loss of polarity of respiratory and olfactory epithelial cells, necrosis of epithelium, loss of cilia and trans migration of inflammatory cells with accumulation of purulent exudate in some mice were the most frequent alterations in the nasal portion of the respiratory tract. These dose related lesions were most pronounced in the dorsal turbinate areas (Table 6.4.3/07-2).	

4.7 Other examinations	None	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Groups of 10 mice of each sex were exposed to 0, 50, 100, 200, 400 or 600 ppm ethylene oxide 6 hours/day 5 days per week for 14 weeks. Animals were observed twice daily for mortality and signs of toxicity and body weights were recorded at weekly intervals. At the end of the exposure mice were given a gross necropsy and tissues were retained for histopathology.	
5.2 Results and Discussion	Clinical signs of toxicity consisting of anorexia, dyspnea, decreased activity bloating and listlessness were apparent at 600 ppm and all animals exposed to 400 and 600 ppm died before the end of the study. Histopathology revealed lymphocyte necrosis in the thymus and spleen (males only) at 600 ppm, renal tubular necrosis at 600 ppm with degeneration at 100, 200 and 400 ppm and rhinitis of the nasal cavity at 200, 400 and 600 ppm of both sexes.	
5.3 Conclusion	The NOAEL was 50 ppm.	
5.3.1 LO(A)EL	100 ppm	
5.3.2 NO(A)EL	50 ppm	
5.3.3 Other	None	
5.3.4 Reliability	2	
5.3.5 Deficiencies	Food consumption was not measured, clinical chemistry and haematology parameters were not determined and an ophthalmology examination was not included. A full range of tissues was not taken for histopathology.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and Discussion	The summary of the applicant is acceptable.	
Conclusion	The NOAEL is set at 50 ppm based on renal tubular degeneration at 100 ppm and above.	
Reliability	2	
Acceptability	The study is acceptable; however, it should be noted that not all parameters required by the modern guideline have been evaluated.	
Remarks	This NTP study, although not according to OECD guidelines, is a robust, peer-reviewed study.	

Table 6.4.3/07-1: Relative liver weights of mice exposed to ethylene oxide for 14 weeks

Concentration (ppm)	No of mice	Body weight (g)	Liver weight (mg)	Relative liver weight
Males				
0	10	30.5±3.63	1911±353	62.4±6.05
50	9	31.0±2.45	1794±271	57.8±6.28
100	9	32.4±2.24	1996±253	61.5±5.73
200	9	31.0±2.06	1858±256	59.8±5.14
Females				
0	10	27.1±1.79	1653±144	61.1±4.35
50	10	26.9±2.51	1508±220	56.0±5.66*
100	10	27.2±1.75	1614±121	59.4±4.16
200	10	26.4±1.51	1735±174	65.6±3.79

*** p<0.05**

Table 6.4.3/07-02: Incidence (no of observations/no examined) and severity[1] of mice exposed to ethylene oxide for 14 weeks

Tissue/lesion	Concentration (ppm)				
	0	100	200	400	600
Males					
Thymus Necrosis, lymphocytic Hypoplasia, lymphocytic	0/10 0/10	0/9 1/9 (0.1)	1/10 (0.2) 3/10 (0.4)	0/4 3/4 (2.8)	10/10 (3.8) 0/10
Spleen Necrosis, lymphocytic	0/10	0/1	0/9	0/10	5/10 (1.4)
Kidney Necrosis, tubular Degeneration, tubular Glomerulopathy, fibroid Congestion	0/10 0/10 0/10 0/10	1/10 (0.2) 5/10 (0.7) 0/10 0/10	1/10 (0.1) 6/10(0.7) 0/10 0/10	5/10 (1.4) 4/10 (1.5) 0/10 0/10	8/10 (2.3) 0/10 1/10 (0.4) 1/10 (0.2)
Nasal cavity Rhinitis	0/10	0/10	4/10 (0.4)	10/10 (2.8)	10/10 (3.3)
Female					
Thymus Necrosis, lymphocytic Hypoplasia, lymphocytic	0/10 0/10	0/10 0/10	0/10 1/10 (0.1)	0/5 5/5 (3.6)	6/10 (2.4) 4/10 (1.4)
Spleen Necrosis, lymphocytic	0/10	--	0/10	0/10	1/10 (0.3)
Kidney Necrosis, tubular Degeneration, tubular	0/10 2/10 (0.2)	0/10 0/10	0/10 8/10 (1.0)	5/10 (1.8) 6/10 (2.3)	5/10 (1.6) 4/10 (1.1)
Nasal cavity Rhinitis	0/9	0/9	8/10 (1.2)	9/9 (2.8)	10/10 (3.4)

[1] Severity ranked on a scale from 0 (normal) to 4 (most severe), mean value shown in brackets

Section A 6.4.3/08 **Annex Point IIA 6.4.3**	**Subchronic inhalation**	
	1 Reference	Official use only
1.1 Reference	Lynch, D., Lewis, T., Moorman, W., Burg, J., Lal, J., Setzer, J., Groth, D., Gulati, D., Zavos, P., Sabharwal, P., Ackerman, L., Cockrell, B. and Sprinz, H. (1984b) Effects on Monkeys and Rats of Long-term Inhalation Exposure to Ethylene Oxide: Major findings of the NIOSH study In: In hospital ethylene oxide sterilisation – Current issues in ethylene oxide toxicity and occupational exposure, Arlington, VA, Association for the Advancement of Medical Instrumentation, 7-10, AAMI Technology Assessment report No. 8-84 Setzer, J., Brightwell, W., Russo, J., Johnson, B., Lynch, D., Madden, G., Burg, J. and Sprinz, H.(1996) Neurophysiological and Neuropathological Evaluation of Primates Exposed to Ethylene Oxide and Propylene Oxide Toxicology and Industrial Health, 12, 667-682	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.3 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance not claimed.	
2.2 GLP	No	
2.3 Deviations	None, guideline compliance was not claimed	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	99.7%	
3.1.2.3Stability	Not reported	
3.2 Test animals		

3.2.1 Species	Monkey		
3.2.2 Strain	Cynomolgus		
3.2.3 Source	Not reported		
3.2.4 Sex	Male		
3.2.5 Age/weight at study initiation	Adult		
3.2.6 Number of animals per group	12		
3.2.7 Control animals	12 per group		
3.3 Administration / Exposure			
3.3.1 Duration of treatment	24 months		
3.3.2 Frequency of exposure	7 hours/day, 5 days/week for 2 years		
3.3.3 Postexposure period	8 years (for ophthalmoscopy)		
3.3.4 Oral	Not applicable		
3.3.5 Inhalation			
3.3.5.1 Concentrations	Nominal concentration	0, 50 and 100 ppm	
	Analytical concentration	Values not reported but chamber comcentrations were monitored and adjusted to maintain exposure at planned levels	
3.3.5.2 Particle size	Not applicable		
3.3.5.3 Type or preparation of particles	Not applicable		
3.3.5.4 Type of exposure	Inhalation		
3.3.5.5 Vehicle	None		
3.3.5.6 Concentration in vehicle	Not applicable		
3.3.5.7 Duration of exposure			x
3.3.5.8 Controls	Ambient air		
3.3.6 Dermal	Not applicable		

3.3.7 Intrapertitoneal/ Intravenous/ Intratracheal instilation	Not applicable	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Frequency not reported	
3.4.1.2 Mortality	Recorded but frequency of observations not reported	
3.4.2 Body weight	Recorded but frequency of observations not reported	
3.4.3 Food consumption	Not reported	
3.4.4 Water consumption	Not reported	
3.4.5 Ophthalmoscopic examination	Recorded but frequency of observations not reported	
3.4.6 Haematology	Recorded but frequency of observations not reported	
3.4.7 Clinical chemistry	Recorded but frequency of observations not reported	
3.4.8 Urinalysis	Recorded but frequency of observations not reported	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Not reported	
3.5.2 Gross and histopathology	Two animals per group were examined at the end of the exposure period; details of organs examined were not reported.	
3.5.3 Other examinations	Pulmonary function, neurophysiology, neuropathy, sister chromatid exchange and chromosomal aberrations in peripheral lymphocytes and sperm indices were examined.	
3.5.4 Statistics	Not reported	
3.6 Further remarks	None	
	4 Results	
4.1 Observations		
4.1.1 Clinical signs	Monkeys exposed to 100 ppm did not tolerate the exposures well but details of the signs of toxicity were not reported.	
4.1.2 Mortality	One monkey died in each of the ethylene oxide exposure groups. All animals in the control group survived.	
4.2 Body weight gain	There was a significant reduction in bodyweight gain ($p<0.05$) amongst monkeys exposed to 100 ppm ethylene oxide starting from the 25th week of exposure. There was no effect at 50 ppm.	

4.3 Food consumption and compound intake	Not reported	
4.4 Ophthalmoscopic examination	The incidence of cataracts during the last month of exposure was 0/11, 2/11 and 3/12 in animals exposed to 0, 50 or 100 ppm ethylene oxide respectively but the increased incidence was not statistically significant and was considered not to be related to exposure. The incidence of cataracts was 2/4, 2/3 and 4/4 respectively when assessed 10 years after the end of exposure. This was statistically significant in the 100 ppm group.	
4.5 Blood analysis		
4.5.1 Haematology	No treatment related effect on red or white blood cell counts.	
4.5.2 Clinical chemistry	No treatment related effect	
4.5.3 Urinalysis	No treatment related effect	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	Not reported	
4.6.2 Gross and histopathology	Neuropathological examination (2 monkeys per group) revealed demyelination in the very distal portion of the fasciculus gracilis in one monkey in the 50 and in the 100 ppm groups. Axonal end dystrophy of the nucleus gracilis of the medulla oblongata was observed in all three groups and there was no relationship to ethylene oxide exposure.	
4.7 Other examinations	No differences were detected in electroencephalograms or mean nerve velocities when groups of monkeys exposed to ethylene oxide were compared with controls but 2 of the 12 animals exposed to 100 ppm had decreased nerve conduction velocity measurements. Nerve conduction and velocity were re-assessed seven years after the termination of exposure but no treatment related effects were detected. Examination of sperm sampled at terminal necropsy showed treatment related reductions in sperm count and sperm motility and an increase in drive range. There was no effect on the incidence of abnormal sperm heads (Table 6.4.3/08-1). There were statistically significant increases in the incidence of sister chromatid exchange and chromosomal aberrations in lymphocytes of monkeys at both 50 and 100 ppm. Chromatid aberrations were also significantly greater in both groups exposed to ethylene oxide and combined chromatid plus chromosome aberrations were also increased (Table 6.4.3/08-2).	
	5 Applicant's Summary and Conclusion	
5.1 Materials and methods	Groups of 12 male cynomolgus monkeys were exposed to 0, 50 or 100 ppm ethylene oxide for 6-7 hours per day, 5 days per week for 104 weeks. Effects on body weight, ophthalmoscopy, haematology, clinical chemistry, pulmonary function, neurophysiology, sister chromatid exchange and chromosomal aberrations in peripheral lymphocytes and sperm indices were investigated. Two animals per group were killed at the end of	

	the exposure period and histopathology of the nervous system was evaluated. Some animals were followed for up to 10 years after exposure.	
5.2 Results and Discussion	Bodyweight gain was reduced in animals exposed to the highest concentration. Demyelination was found in the very distal portion of the fasciculus gracilis in one monkey in the 50 and in the 100 ppm groups. Decreased nerve conduction velocity was found in 2 of the 12 animals exposed to 100 ppm but when compared with the controls the difference in the group means was not statistically significant. No treatment related effects on nerve conduction or velocity were detected in a follow up investigation of the same monkeys 7 years after the end of the exposure period. Sperm count and motility were significantly reduced in animals from both treatment groups and there were also increases in chromosomal aberrations and sister chromatid exchange. The incidence of cataracts appeared to be elevated in a treatment related manner but the increase was not statistically significant at the end of the exposure period. Examination of 3 or 4 monkeys per group ten years after the end of exposure showed a statistically significant increase in cataracts.	
5.3 Conclusion	Exposure to ethylene oxide resulted in decreased sperm counts and mobility and chromosome damage. Although there was some neurophysiology and histopathology evidence of neurotoxicity the lack of a dose response suggests this may not be treatment related.	
5.3.1 LO(A)EL	50 ppm, the lowest concentration tested	
5.3.2 NO(A)EL	Not established	
5.3.3 Other	None	
5.3.4 Reliability	3	
5.3.5 Deficiencies	None, the study was not conducted to a particular guideline	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 february 2020	
Materials and Methods	*3.3.5.7 Duration of exposure* should read: 24 months	
Results and Discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable. The exposure level of 50 ppm is considered to be a LOAEL.	
Reliability	3	
Acceptability	The study has a number of limitations (not conducted according to a particular guideline, only male animals tested, limited parameters examined), but can be used as a part of weight of	

	evidence, in particular regarding effects on sperm quality and neurotoxic effects.	
Remarks		

Table 6.4.3/08-1: Sperm parameters in monkeys exposed to ethylene oxide for 24 months

Concentration (ppm)	Sperm motility (%)	Drive range (sec/0.2 mm)	Sperm concentration (x 10^8/mL)	Abnormal sperm heads (%)[1]
0	89.1±4.4	0.98±0.30	30.6±3.7	0.27±0.08
50	60.6±8.2*	3.6±1.2*	22.0±1.1*	0.23±0.13
100	59.0±29.9*	3.0±1.8*	18.2±9.1*	0.26±0.11

[1] Transformed using Freeman-Tukey modified Arcsine Transform
*** p<0.05**

Table 6.4.3/08-2: Sister chromatid exchange and chromosomal aberrations in peripheral lymphocytes of monkeys exposed to ethylene oxide for 24 months

Concentration (ppm)	SCE Frequency[1]	Abnormal cells[2]
0	5.42±1.04	0.58±0.56
50	10.19±1.71*	1.95±1.40
100	15.12±2.77*	3.69±2.67*

[1] Mean SCE/metaphase
[2] Cells with one or more chromatid and/or chromosome aberrations /100 metaphases
* p<0.05

6.5 Chronic toxicity

Combined chronic/carcinogenicity studies have been conducted and are summarised in section 6.7. Separate chronic toxicity studies have not been conducted.

6.6 Genotoxicity studies

6.6.1 *In vitro* gene mutation study in bacteria

Section A6.6.1/01 **Annex Point IIA 6.6.1**	**Genotoxicity in vitro – reverse mutation test**	
	1 Reference	**Official use only**
1.1 Reference	Pfeiffer, E. and Dunkelberg, H. (1980) Mutagenicity of Ethylene Oxide and Propylene Oxide and of the Glycols and Halohydrins Formed From Them During the Fumigation of Foodstuffs Food and Cosmetics Toxicology, 18, 115-118	

1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study was similar to OECD 471	
2.2 GLP	No	
2.3 Deviations	The study was not conducted in the presence of metabolic activation and cytotoxicity was not investigated.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: purchased from J. T. Baker Chemicals BV, Deventer, The Netherlands	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	99.7%	
3.1.2.3 Stability	Not reported	
3.2 Study Type		
3.2.1 Organism/cell type	*S. typhimurium* strains TA98, TA100, TA1535 and TA 1537	
3.2.2 Deficiencies / Proficiencies	None	
3.2.3 Metabolic activation system	None	
3.2.4 Positive control	β-propiolactone and benzo[a]pyrene-4,5-oxide	
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	20, 100 and 200 μmoles/plate, equivalent to 0.88, 4.4 and 8.8 mg/plate respectively	
3.3.2 Way of application	Solution in acetone	
3.3.3 Pre-incubation time	None	

3.3.4 Other modifications	The plate incorporation method of Ames, McCann and Yamasaki was modified using the procedure described by Pelon, Whiteman and Beasly (1977)[4]. The modification gave more reproducible results than the method of Ames et al.	
3.4 Examinations		
3.4.1 Number of cells evaluated	Not reported	
	4 Results and Discussion	
4.1 Genotoxicity		
4.1.1 without metabolic activation	There was a dose dependent increase in the number of revertant mutations in *S. typhimurium* strain TA100 and TA1535. There was no increase in the incidence of mutations in TA98 or TA1537. Results were reported graphically and are not available for tabulation.	
4.1.2 with metabolic activation	Not investigated	
4.2 Cytotoxicity	Evaluated but not reported for ethylene oxide	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Ethylene oxide in acetone was added to 10^8 bacteria in water. This was mixed with top agar containing sodium chloride and a biotin/histidine solution was poured into Petri dishes containing histidine free agar. The plates were incubated for 48 hours at 37ºC and the number of revertant colonies was counted. The experiment was repeated 6-10 times, individual experiments were performed independently and in duplicate.	
5.2 Results and discussion	There was a treatment related reproducible increase in the incidence of revertants in *S. typhimurium* strains TA 100 and TA1535 but no effect in strains TA98 and TA1537. Results were reported graphically and the results are not available for tabulation. Positve conrol substances gave the expected results.	
5.3 Conclusion	Ethylene oxide was mutagenic to bacteria under the conditions of the test.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	The study was not conducted in the presence of metabolic activation and cytotoxicity was not investigated. As a positive result was obtained these deficiencies are considered not significant.	

	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is not sufficient as a stand-alone, based on limited information on the tested substance and study design, but can be accepted as a part of weight of evidence approach.	
Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.	

6.6.2 *In vitro* cytogenicity study in mammalian cells

Section A6.6.2/01 **Annex Point IIA 6.6.2**	**Genotoxicity in vitro – chromosome aberration test**	
	1 Reference	**Official use only**
1.1 Reference	Zhong, B., Gu, Z., Whong, W-Z., Wallace, W and Ong, T. (1992) Comparative Study of Micronucleus Assay and Chromosomal Aberration Analysis in V79 Cells Exposed to Ethylene Oxide Teratogenesis, Carcinogenesis and Mutagenesis, 11, 227-233	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	No, but study is similar to OECD 473	
2.2 GLP	No	
2.3 Deviations	The test was not conducted in the presence of metabolic activation and cytoxicity was not investigated. No positive controls were included in the study.	

	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported; supplied by Liquid Carbonic Co., Chicago, Il.	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	100%	
3.1.2.3 Stability	Not reported	
3.2 Study Type		
3.2.1 Organism/cell type	Chinese hamster lung fibroblast cell line V79	
3.2.2 Deficiencies / Proficiencies	None	
3.2.3 Metabolic activation system	None	
3.2.4 Positive control	None	
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	0, 3500, 6900, 13800 and 27700 ppm for chromosomal aberrations and 0, 457, 1372, 4115 and 12344 ppm for micronucleus frequencies	
3.3.2 Way of application	Exposure to gas	
3.3.3 Pre-incubation time	30 minutes	
3.3.4 Other modifications	Micronuclei were determined in non dividing and dividing cells	
3.4 Examinations		
3.4.1 Number of cells evaluated	100 metaphases for chromosome aberrations, 2000 binucleated cells for micronuclei in non dividing cells and 1000 cells for micronuclei in dividing cells	
	4 Results and Discussion	
4.1 Genotoxicity		
4.1.1 without metabolic activation	Exposure to concentrations from 3500 to 27700 ppm resulted in an increase in the incidence of gaps, breaks, fragments and minutes (Table 6.6.2/01-1). A statistically significant increase in micronuclei was only found at the highest exposure concentration. Micronucleus frequencies were 1.5 to 3 fold higher in dividing cells but even so the increase was still only statistically significant at 12344 ppm. The proportion of binucleated cells decreased with increasing concentrations of ethylene oxide (Table 6.6.2/01-2).	

4.1.2 with metabolic activation	Not applicable, experiment was not conducted with metabolic activation	
4.2 Cytotoxicity	Not reported	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Approximately $3x10^6$ Chinese hamster lung V79 cells were exposed to ethylene oxide concentrations ranging from 0 to 27700 ppm for 30 minutes at 37ºC. The cultures were incubated in fresh medium for 24 hours after treatment and colcemid was added two hours before the end of the incubation. Cells were harvested, washed, fixed and stained with Giemsa and 100 metaphases were analysed for chromatid gaps and breaks, isochromatid gaps and breaks, fragments, deletions, minutes, acentric rings, dicentrics and endoreduplication. The incidence of micronucleus formation was determined in the same way except that cells were not treated with colcemid and after spreading on a slide the cells were stained with Diff-Quik. For the investigation of micronucleus formation in dividing cells cytochalasin B was added to the medium after exposure to ethylene oxide. The χ^2 test was used for statistical analysis.	
5.2 Results and discussion	Exposure of V79 cells to ethylene oxide caused a statistically significant and dose dependent increase in chromosomal aberrations at all concentrations tested. The incidence of micronuclei was only increased at 12344 ppm, the highest concentration tested.	
5.3 Conclusion	Ethylene oxide was clastogenic to Chinese hamster lung V79 cells *in vitro*.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	The test was not conducted in the presence of metabolic activation and cytoxicity was not investigated. No positive controls were included in the study. These deficiencies are not significant because a positive result was obtained in the study.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	10 January 2018	
Materials and Methods		
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is not sufficient as a stand-alone, based on limited information on the tested substance and study design, but can be accepted as a part of weight of evidence approach.	

Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.

Table 6.6.2/01-1: Chromosomal aberrations in Chinese Hamster V79 cells exposed to ethylene oxide

Concentration (ppm)	Aberrant cells (%)	Aberrations/100 metaphases[a]											
		Chromatid type			Chromosome type							Total	
		G	B	F and D	G	B	F and D	DC	AC	M	ER	With gaps	Without gaps
0	3	1	1	0	1	0	0	0	0	0	0	3	1
3500	17*	7	1	3	2	0	1	0	2	2	0	18*	9*
6900	22*	7	2	1	4	1	1	1	1	4	0	22*	11*
13800	31*	14	2	3	8	3	4	0	0	0	1	35*	13*
27700	35*	14	5	6	1	6	3	4	1	1	0	41*	26*

[a] G, gaps; B, breaks; F, fragments; D, deletions; DC, dicentric chromosome; AC, acentric ring; M, minutes; ER, endoreduplication
* $p<0.01$, χ^2 test compared with control

Table 6.6.2/01-2: Micronucleus frequencies in Chinese Hamster V79 cells exposed to ethylene oxide

Concentration (ppm)	Micronucleus frequency		Binucleated cells (%)
	Without cytochalasin B	With cytochalasin B	
0	2.50±1.29	6.0±0.00	91.3±1.5
457	2.75±0.96	9.6±2.83	82.3±3.2
1372	3.00±2.16	6.5±2.12	76.7±1.5
4115	4.75±2.75	8.5±2.12	55.7±3.1
12344	8.50±4.12**	12.0±1.41*	14.3±5.9

* $p< 0.05$, ** $p<0.01$ χ^2 test compared with control

6.6.3 *In vitro* gene mutation assay in mammalian cells

Section A6.6.3/01 **Annex Point IIA 6.6.2**	**Genotoxicity in vitro – gene mutation test**	
	1 Reference	**Official use only**
1.1 Reference	Tan, Eng-Lay, Cumming, R. and Hsie, A. (1981) Mutagenicity and Cytotoxicity of Ethylene Oxide in the CHO/HGPRT System Environmental Mutagenesis, 3, 683-686	
1.2 Data protection		
1.2.1 Data owner	Data published	

1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study is similar to OECD 476	
2.2 GLP	No	
2.3 Deviations	No significant deviations	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported; purchased from Fisher Scientific Co. (Itasca, Illinois, USA).	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	x
3.1.2.3 Stability	Not reported	
3.2 Study Type		
3.2.1 Organism/cell type	Chinese hamster ovary cells	
3.2.2 Deficiencies / Proficiencies	None	
3.2.3 Metabolic activation system	Post mitochondrial supernatant (S9) prepared from the livers of Sprague-Dawley rats treated with Arochlor 1254	
3.2.4 Positive control	Methyl methane sulphonate (in the absence of S9) and dimethylnitrosamine (in the presence of S9)	
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	Approximately 2 to 7.5 mM equivalent to 0.088 to 0.33 mg/mL	
3.3.2 Way of application	Solution in ice cold water	
3.3.3 Pre-incubation time	5 hours	
3.3.4 Other modifications	None	
3.4 Examinations		
3.4.1 Number of cells evaluated	200,000 cells for mutants, 200 cells for cytotoxicity	

	4 Results and Discussion	
4.1 Genotoxicity		
4.1.1 without metabolic activation	Increasing concentrations of ethylene oxide caused increases in mutation frequency. The results were shown graphically so tabulated data cannot be included in this summary but the mutation frequency was linearly related to the concentration of ethylene oxide. The positive control gave the expected result.	
4.1.2 with metabolic activation	The presence of metabolic activation had no effect on the mutagenicity of ethylene oxide. The positive control, dimethynitrosamine, increased mutation frequency as expected.	
4.2 Cytotoxicity	Increasing concentrations of ethylene oxide caused an increase in cytotoxicity. Relative survival was approximately 3% at a concentration of 10mM ethylene oxide. The presence of metabolic activation had virtually no effect on the mutagenicity of ethylene oxide.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	The mutagenicity of ethylene oxide was investigated in Chinese hamster ovary cells using hypoxanthine-guanine phosphoribosyl transferase to detect mutations. Approximately $1x10^6$ cells grown in monolayers in a modified Ham's F12 medium were incubated with 2 to 7.5 mM ethylene oxide for 5 hours at 37ºC. Incubations were also carried out in the presence of S9 prepared from the livers of rats treated with Arochlor 1254. Cells were rinsed with saline and incubated infresh medium overnight. Cytotoxicity was determined by counting colonies that arose from plating triplicate samples of 200 cells. Mutant cells were selected after 1 week subculture using five plates of 200,000 cells in hypoxanthine free medium containing 10µM 6-thioguanine. Mutation frequencies were corrected for cloning efficiency of the cells.	
5.2 Results and discussion	There were dose dependent increases in both mutation frequency and cytotoxicity in the presence and absence of metabolic activation and the mutation frequency was linearly related to the concentration of ethylene oxide. The positive control gave the expected result. Relative survival was approximately 3% at a concentration of 10mM ethylene oxide.	
5.3 Conclusion	Under the conditions of the test ethylene oxide caused mutations at the hypoxanthine-guanine phosphoribosyl transferase gene in Chinese hamster ovary cells. The mutation frequency was unaffected by the presence of metabolic activation.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	As ethylene oxide gave a positive result in this test any deviations are considered not significant.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	

Materials and Methods	*3.1.2.2 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.
Results and discussion	The summary of the applicant is acceptable.
Conclusion	The summary of the applicant is acceptable.
Reliability	2
Acceptability	The study is not sufficient as a stand-alone, based on limited information on the tested substance and study design, but can be accepted as a part of weight of evidence approach.
Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.

Section A6.6.3/02 **Annex Point IIA 6.6.3**	**Genotoxicity in vitro – gene mutation test**	
	1 Reference	**Official use only**
1.1 Reference	Hatch, G., Conklin, P., Christensen, C., Anderson, T., Langenbach, R. and Nesnow, S. (1986) Mutation and Enhanced Virus Transformation of Cultured Hamster Cells by Exposure to Gaseous Ethylene Oxide	
1.2 Data protection		
1.2.1 Data owner	Unknown	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance not stated but study design was similar to OECD 476	
2.2 GLP	No	
2.3 Deviations	A metabolic activation system was not used and the cells were exposed to only 4 concentrations of ethylene oxide	

	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported: purchased from Matheson Gas Co., East Rutherford, New Jersey, USA	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	10% in nitrogen	
3.1.2.3 Stability	Not reported	
3.2 Study Type		
3.2.1 Organism/cell type	V79 Chinese hamster lung cells (V79) and Syrian hamster embryo cells (SHE)	
3.2.2 Deficiencies / Proficiencies	None	
3.2.3 Metabolic activation system	None	
3.2.4 Positive control	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and benzo(a)pyrene	
3.3 Administration / Exposure; Application of test substance		
3.3.1 Concentrations	0, 625, 1250, 5000 and 7500 ppm for treatment of V79 cells and 0, 625, 1250 and 5000 ppm for treatment of SHE cells	
3.3.2 Way of application	Exposure to vapour	
3.3.3 Pre-incubation time	Cells were exposed for 2 hours	
3.3.4 Other modifications	Details of the procedure used are included in 5.1 of this summary	
3.4 Examinations		
3.4.1 Number of cells evaluated	V79 cells: 200 per dish for plating efficiency, $2x10^5$ for ouabain mutants and $5x10^6$ cells for 6-thioguanine resistant mutants SHE cells: 700 per dish for both survival and SA7 virus transformation	
	4 Results and Discussion	
4.1 Genotoxicity		

4.1.1 without metabolic activation	V79 cells: There was a concentration response relationship for both markers in two independent experiments (Table 6.6.3/02-1). The positive control, MNNG, also gave a positive response for both markers. SHE cells: There was a concentration related increase in virus transformation when cells were exposed to ethylene oxide for 2 hours but transformation was not increased when cells were exposed for 20 hours prior to treatment with the virus. Benzo(a)pyrene, the positive control, gave the expected response (Table 6.6.3/02-2).	
4.1.2 with metabolic activation	Experiment not conducted	
4.2 Cytotoxicity	V79 cells and SHE cells: There was significant toxicity at 2500 and 5000 ppm	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	V79 cells in a supplemented Williams medium E were exposed to 0-7500 ppm ethylene oxide for 2 hours. Two dishes each containing 4-6x10^6 cells were exposed at each concentration and the cells were then reseeded to determine enhancement of transformation. SHE cells were exposed in a similar way to 0-5000 ppm ethylene oxide for 2 or 20 hours. V79 cells were cultured for a further 24 hours after exposure and then seeded into Petri dishes to determined plating efficiency and frequency of ouabain resistant mutants. The same cells were used to determine the frequency of 6-thioguanine resistant mutants and plating efficiency. SHE cells were inoculated with SA7 virus after treatment. Survival was determined after further periods of plating and sensitivity to virus transformation was determined by staining with crystal violet.	
5.2 Results and discussion	After 2 hour exposure to ethylene oxide there were treatment related increases in the incidence of both ouabain and 6-thioguanine resistant V79 cells and increased sensitivity to virus transformation in SHE cells. There was no increase in sensitivity to virus transformation in SHE cells that were exposed for 20 hours. The lack of an effect on SHE cells after 20 hours exposure may have been due to a decline in ethylene oxide concentration during the exposure.	
5.3 Conclusion	Ethylene oxide caused both mutation and enhanced virus transformation.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	A metabolic activation system was not used and the cells were exposed to only 4 concentrations of ethylene oxide. These deficiencies are considered not significant because a positive result was obtained in the test.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	

Materials and Methods	
Results and discussion	The summary of the applicant is acceptable.
Conclusion	The summary of the applicant is acceptable.
Reliability	2
Acceptability	The study is well described, and can be accepted as a part of weight of evidence approach.
Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.

Table 6.6.3/02-1: Incidence of ouabain and 6-thioguanine resistant mutants after exposure of V79 cells to ethylene oxide for 2 hours

Concentration (ppm)	Ouabain resistance				6-Thioguanine resistance			
	Plating efficiency[a] (%)		Mutants/10^6 survivors		Plating efficiency[b] (%)		Mutants/10^6 survivors	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
7500	6	0	128	0	30	0	283	0
5000	14	0.2	41	0	48	39	52	478
1250	31	14	6	29	58	40	19	96
625	53	ND	6	ND	41	ND	9	ND
0	47	23	1	4	47	50	3	15
MNNG[c] 0.5 µg.mL	24	15	103	82	38	44	401	607

[a] Determined from plates receiving 200 cells 24 hours after exposure
[b] Determined from plates receiving 200 cells 5 days after exposure
[c] Cells treated for 4 hours in buffer

Table 6.6.3/02-1: Enhancement of virus transformation after exposure of SHE cells to ethylene oxide for 2 hours

Concentration (ppm)	% Survival[a]			Number of SA7 foci[b]			Enhancement ratio[c]		
	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
5000	0	0	0	0	0	0	0	0	0
2500	11	62	19	47	115	30	9.9**	4.6**	7.2**
1250	80	82	76	99	77	31	2.9**	2.4**	1.9*
625	112	87	58	52	73	33	1.1	2.1**	2.6**
0	100	100	100	43	40	22	1.0	1.0	1.0
Benzo(a)pyrene[d] 0.5 µg/mL	93	91	87	94	100	54	1.8**	2.8**	2.8**

* $p<0.05$, ** $p<0.01$
[a] Cloning efficiencies (% of controls) were Exp 1 6.1%, Exp 2 9.9% and Exp 3 7.1%
[b] Number from $2x10^6$ cells
[c] Determined by dividing the transformation frequency of treated cells by the value for control cells
[d] Cells treated for 20 hours (Exp 1) or 2 hours (Exp 2 and 3)

6.6.4 If positive in 6.6.1, 6.6.2 or 6.6.3, then an *in vivo* genotoxicity study will be required (bone marrow assay for chromosomal damage or a micronucleus test)

Section A6.6.4/01 **Annex Point IIA 6.6.4**	**Genotoxicity in vivo – micronucleus test**	
	1 Reference	**Official use only**
1.1 Reference	Farooqi, Z., Törnquist, M., Ehrenburg, L. and Natarjan, A. (1993) Genotoxic Effects of Ethylene Oxide and Propylene Oxide in Mouse Bone Marrow Cells Mutation Research, 288, 223-228	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study is similar to OECD 474 and OECD 475	
2.2 GLP	Not GLP	
2.3 Deviations	Insufficient number of animals in each group, no evidence that maximum tolerated dose was used, no information on toxicity of the selected dose to bone marrow cells, ie the ratio of polychromatic to normochromatic cells was not reported. Only mean data are reported with no standard deviations and only one time point was used.	
	3 Materials and Methods	
3.1 Test material		
3.1.1Lot/Batch number	Not reported	
3.1.2 Specification	Not reported;purchased from Fluka	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	x
3.1.2.3 Stability	Not reported	
3.1.2.4 Maximum tolerable dose	Not reported	
3.2 Test Animals		
3.2.1 Species	Mouse	
3.2.2 Strain	Swiss albino	

3.2.3 Source	Not reported	
3.2.4 Sex	Female	
3.2.5 Age/weight at study initiation	9-10 weeks	
3.2.6 Number of animals per group	4	
3.2.7 Control animals	4 per group	
3.3 Administration/ Exposure		
3.3.1 Number of applications	One	
3.3.2 Interval between applications	Not applicable	
3.3.3 Post exposure period	24 hours after dosing	
	Intraperitoneal injection	
3.3.10 Vehicle	Phosphate buffered saline	
3.3.11 Concentration in vehicle	Not reported	
3.3.12 Total volume applied	Not reported	
3.3.13 Dose applied	0, 30, 60, 120 or 150 mg/kg bw	
3.3.14 Substance used as Positive Control	None	
3.3.15 Controls	Not reported whether the controls received vehicle only or were not dosed	
3.4 Examinations		
3.4.1 Clinical signs	Not reported	
3.4.2 Tissue	Bone marrow cells	
3.5 Further remarks	Chromosomal aberrations and sister chromatid exchange were also investigated in this study.	
	4 Results and Discussion	
4.1 Clinical signs	Not reported	
4.2 Haematology / Tissue examination	Not reported	

4.3 Genotoxicity	There was an increase in micronuclei in the bone marrow of mice treated with 120 and 150 mg/kg bw (Table 6.6.4/01-1). There was also a dose dependent increase in chromosomal aberrations and sister chromatid exchanges but these were only reported graphically. Chromosome aberrations consisted of chromatid breaks, 65%; exchanges, <0.5%; isochromatid breaks, 23% and gaps, 12%. Linear regression lines were fitted to the increases in both chromosomal aberrations and sister chromatid exchanges. Chromosomal aberrations/cell were = 0.08(±0.06) + 0.267(±0.03) Dose (mmol/kg); r = 0.982, n = 5, p = 0.002 Sister chromatid exchange/cell were = 0.50(±0.07) + 0.94(±0.03) Dose (mmol/kg), r = 0.99	
4.4 Other	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 4 Swiss albino mice received a single intraperitoneal dose of 0, 30, 60, 120 or 150 mg/kg bw ethylene oxide in phosphate buffered saline. Animals were killed 24 hours later and bone marrow was removed from the femurs. Bone marrow smears were prepared and stained with May-Grünwald solution prior to analysis for micronuclei. For analysis of chromosome aberrations, colchicine was administered 22 hours after treatment to arrest dividing cells in metaphase and the animals were killed 2 hours later. Cells were obtained from the bone marrow, subjected to hypotonic shock, fixed and stained with Giemsa. For analysis of sister chromatid exchange a bromo deoxyuridine tablet was implanted subcutaneously immediately before mice were exposed to ethylene oxide. The mice were killed 28 hours after exposure and bone marrow cells were obtained and stained using the same procedure as for chromosome aberrations. Sister chromatid differentiation was obtained using the fluorescence plus technique developed by Perry and Wolf[5].	
5.2 Results and discussion	There was an increase in micronuclei in mice exposed to 120 and 150 mg/kg bw ethylene oxide but no effect at lower doses. There was also a dose dependent increase in chromosomal aberrations and sister chromatid exchange.	
5.3 Conclusion	Ethylene oxide was clastogenic *in vivo* under the conditions of the test.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	There are a number of deficiencies in this study when compared with the corresponding OECD guidelines (see 2.3). However, there were dose dependent changes in the incidence of micronuclei, chromosomal aberrations and sister chromatid exchanges so the result is considered valid despite the deficiencies in the method and lack of reporting detail.	

	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods	*3.1.2.2 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and discussion		
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is not sufficient as a stand-alone, based on limited information on the tested substance and study design, but can be accepted as a part of weight of evidence approach.	
Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.	

Table 6.6.4/01-1: Incidence of micronuclei in polychromatic erythrocytes following exposure to ethylene oxide

Dose (mg/kg bw)	Polychromatic erythrocytes		Micronuclei per 1000 polychromatic erythrocytes
	With 1 micronucleus	With 2 micronuclei	
0	1	0	0.5
30	3	1	5
60	14	0	14
120	20	1	22
150	40	1	42

6.6.5 If negative in 6.6.4 but positive in some of *in vitro* tests then undertake a second *in vivo* study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow

Section 6.6.5/01 **Annex Point 6.6.5**	**DNA damage in tissue other than bone marrow**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	A study is not available and is considered unnecessary. Ethylene oxide was mutagenic in bacteria and mammalian cells *in vitro*. It was clastogenic in mammalian cells *in vitro*, caused an increase in micronuclei in an *in vivo* test in mice and was also positive in the dominant lethal test in mice. Ethylene oxide clearly has the potential to cause DNA damage in other tissues and there is no need to conduct for example a liver UDS study. Even if it gave a negative result it would not affect the overall assessment of the genotoxicity of ethylene oxide.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	10 January 2018	
Evaluation of applicant's justification	The BPD states: *If negative in 6.6.4 but positive in some of in vitro tests then undertake a second in vivo study to examine whether mutagenicity or evidence of DNA damage can be* demonstrated in tissue other than bone marrow. [Ann IIA, VI. 6.6.5.] The *in vivo* test in 6.6.4 was positive, therefore no further testing inder 6.6.5 is necessary.	
Conclusion	No furhter *in vivo* testing in somatic cells necessary.	
Remarks		

6.6.6 If positive in 6.6.4 then a test to assess possible germ cell effects may be required

Section A6.6.6/01 **Annex Point IIA 6.6.6**	**Genotoxicity in vivo – Rodent dominant lethal test**	
	1 Reference	**Official use only**
1.1 Reference	Generoso, W., Cain, K., Cornett, C., Cacheiro, N. and Hughes, L (1990) Concentration-response curves for ethylene oxide-induced heritable translocations and dominant lethal mutations. Environmental and Molecular Mutagenesis, 16, 126-131	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Not a guideline study but the design was similar to OECD 478	
2.2 GLP	No	
2.3 Deviations	A positive control was not used. Treated males were mated towards the end of the exposure period rather than at intervals during the exposure period.	
	3 Materials and Methods	
3.1 Test material		
3.1.1Lot/Batch number	Not reported	
3.1.2 Specification		
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	99.7%	
3.1.2.3 Stability	Not reported	
3.1.2.4 Maximum tolerable dose	Not reported	
3.2 Test Animals		
3.2.1 Species	Mice	
3.2.2 Strain	C3H x 101, T-stock, SEC x 101 and (SEC x C57BL)F_1	
3.2.3 Source	Not reported	

3.2.4 Sex	C3H x 101, males; T-stock, (SEC x 101)F_1 and (SEC x C57BL)F_1 females	
3.2.5 Age/weight at study initiation	Approximately 12 weeks	
3.2.6 Number of animals per group	24 males, 48 T-stock females and 48 (SEC x 101)F_1 females for the dominant lethal study 48 males, 24 T-stock females and 24 (SEC x C57BL)F_1 females for the heritable translocation study	
3.2.7 Control animals	24 males, 48 T-stock females and 48 (SEC x 101)F_1 females for the dominant lethal study 24 (SEC x C57BL)F_1 females and two groups of 24 males and 24 T-stock females for the heritable translocation study	
3.3 Administration/ Exposure		
3.3.1 Number of applications	47 to male mice	
3.3.2 Interval between applications	None	
3.3.3 Post exposure period	1 day	
3.3.4 Concentrations	0, 165, 204, 250 or 300 ppm	
3.3.5 Way of application	Exposure to vapour on weekdays for 6 weeks and then daily beginning the 7th week for 2.5 weeks. Exposure was for 6 hours per day.	
3.3.6 Substance used as Positive Control	None	
3.3.7 Controls	Treatment received by control mice was not documented.	
3.4 Examinations		
3.4.1 Clinical signs	Not reported	
3.4.2 Examination of uterine content	Implants per pregnant female, number of live embryos, dead implants and number of females with one or more dead implants were reported	
3.5 Further remarks	For the heritable translocation study the sequential method was used to identify sterile and semisterile translocation carriers. Ten semisteriles, the majority of steriles and males of questionable sterility were analysed cytogenetically. Dominant lethal data were evaluated according to the positive criteria proposed by the International Commission for Protection Against Environmental Mutagens and Carcinogens Final Report[6] which puts primary emphasis on changes in the average number of living embryos.	

	4 Results and Discussion	
4.1 Clinical signs	Not reported	
4.2 Haematology / Tissue examination	Not applicable	
4.3 Genotoxicity	There was a marginal level of dominant lethal effects at 204 ppm as indicated by a small but significant reduction in the average number of living embryos and an increase in the number of females with one or more dead implantations in one of the two stocks of females. There was no effect at 165 ppm. Effects at higher doses were concentration dependent but not linear with dose and only at 300 ppm was there a statistically significant reduction in total implants (Table 6.6.6/01-1). The incidences of heritable translocations were increased at all exposure concentrations (Table 6.6.1/01-2). All of the 10 semisterile males that were analysed cytogenetically were confirmed as carriers of translocations whereas all the 11 controls were found to be cytogenetically normal (Table 6.6.6/01-3).	
4.4 Other	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Male mice were subjected to repeated exposures to ethylene oxide concentrations of 0, 165, 204, 250 or 300 ppm during an 8.5 week period. The males were mated with two different strains of female mice and the offspring were investigated for dominant lethal mutations and heritable translocations.	
5.2 Results and discussion	There was a dose response for both dominant lethal mutations and heritable translocations but the response curves were concave up. Heritable translocations were found at all concentrations of ethylene oxide but dominant lethal mutations were only statistically significant at exposure concentrations ≥204 ppm.	
5.3 Conclusion	Exposure of male mice to ethylene oxide resulted in transmission of cytogenetic effects to the offspring.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	A positive control was not used. Treated males were mated towards the end of the exposure period rather than at intervals during the exposure period. As a positive result was obtained in this study the deviations are considered not to have invalidated the study.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	10 January 2018	
Materials and Methods	The summary of the applicant is acceptable.	
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	

Reliability	2
Acceptability	The study is not sufficient as a stand-alone, based on limited information on the tested substance and study design, but can be accepted as a part of weight of evidence approach.
Remarks	Reliability of 3 could also be considered. However, the study provides useful information and should not be discarded; the deviations are minor in light of the positive result. Therefore reliability 2 was assigned.

Table 6.6.6/01-1: Uterine findings and incidence of dominant lethal mutations in mice exposed to ethylene oxide

Concentration (ppm)	Female strain	Mated females	Pregnant females	Implants/ pregnant females	Living embryos/ pregnant females[a]	Dead implants (%)	Females with ≥1 dead implants[b]	Dominant lethals (%)[c]
165	T-stock	40	35	8.3	6.3	24	27	6
	(SECx101)F_1	31	21	8.2	7.6	7	11*	8
Control	T-stock	45	38	8.1	6.7	18	27	-
	(SECx101)F_1	31	25	8.6	8.3	3	6	-
204	T-stock	44	39	8.3	6.1	27	36**	14
	(SECx101)F_1	38	31	8.4	7.5*	12	21**	13
Control	T-stock	39	38	8.4	7.1	17	25	-
	(SECx101)F_1	40	36	9.1	8.6	6	14	-
250	T-stock	40	35	7.9	5.4**	32	32**	23
	(SECx101)F_1	29	28	8.0	6.0**	25	24**	24
Control	T-stock	39	37	8.4	7.0	16	24	-
	(SECx101)F_1	29	24	8.4	7.9	6	9	-
300	T-stock	36	27	5.2**	2.7**	48	25**	60
	(SECx101)F_1	31	21	7.2	4.2**	42	18**	45
Control	T-stock	39	34	8.3	6.7	19	9	-
	(SECx101)F_1	34	27	8.0	7.7	4	6	-

[a] Comparison between treatment and control groups is by one-sided Mann-Whitney nonparametric analysis
[b] Comparison between treatment and control groups is by one-sided chi-square test in a 2x2 contingency table
[c] % Dominant lethals = [1-living embryos per treated pregnant female/ living embryos per treated control female] x 100
* $p<0.05$, ** $p<0.01$

Table 6.6.6/01-2: Ethylene oxide induction of heritable translocations

Stock of females	Conc (ppm)	Progeny tested	No of semi steriles	Observed no. of steriles	Corrected no. of steriles[a]	Frequency of translocation carriers (%)[b]
T-stock	0	1451	1[c]	7	0	0.07(0.0036 to 0.45)
	165	610	9	8	5	2.30(1.3 to 3.9)
	204	399	24	6	4	7.02(4.8 to 10.1)
	250	354	26	17	15	11.58(8.5 to 15.5)
	300	100	27	6	6	33.00(24.1 to 43.2)

(SEC x C57BL)F1	0	2068	1[c]	11	0	0.05(0.0025 to 0.31)
	165	1143	20	18	12	2.80(2.0 to 4.0)
	204	1021	45	13	7	5.09(3.9 to 6.7)
	250	812	56	37	32	10.84(8.8 to 13.2)
	300	427	80	31	29	25.53(21.5 to 30.0)
Combined	0	2068	1[c]	11	0	0.05(0.0025 to 0.31)
	165	1143	20	18	12	2.80(2.0 to 4.0)
	204	1021	45	13	7	5.09(3.9 to 6.7)
	250	812	56	37	32	10.84(8.8 to 13.2)
	300	427	80	31	29	25.53(21.5 to 30.0)

[a] Corrected for frequency of steriles in the respective control group
[b] Values in parenthesis are 95% confidence limits
[c] All 25 meiocytes scored cytogenetically had multivalent chromosomes. The rearrangement is considered to be a new occurrence in the germ cell line of one of the parents since all 21 of the proband's male full sibs tested normal.

Table 6.6.6/01-3: Multivalent association amongst semisterile males[a]

Male no.[b]	Conc (ppm)	2II	RIV	CIV	CIII+I	19II+I+I	Others
1125	204	13	0	8	3	1	0
1755	204	0	20	2	1	2	0
939	204	10	7	3	2	3	0
2944	165	2	17	6	0	0	0
4908	250	9	3	3	0	10	0
5847	250	1	19	4	0	1	0
5980	250	0	15	5	5	0	0
5492	250	0	17	4	3	1	0
7338	300	8	5	3	1	7	1
7583	300	4	1	11	6	3	0

[a] Chromosomal configurations of 25 cells are presented as bivalents (2II), ring of 4 (RIV), chains of 4 (CIV), chain of 3 plus 1 univalent (CIII+I) and 19 bivalents plus 2 univalents (19II+I+I)
[b] picked at random amongst among semisterile progeny of exposed males

6.6.7 If the results are negative for the three tests 6.6.1, 6.6.2 and 6.6.3, then further testing is normally only required if metabolites of concern are formed in mammals, and in Chapter 1.4 further guidance is given on the non-submission of data. (See also the Technical Guidance Document for the Risk Assessment New and Existing Chemicals)

Not applicable, ethylene oxide was not negative in 6.6.1, 6.6.2 or 6.6.3.

6.7 Carcinogenicity study

Section A6.7/01 **Annex Point IIA 6.7**	**Carcinogenicity – lifetime feeding study, rat**	
	1 Reference	**Official use only**
1.1 Reference	Lynch, D., Lewis, T., Moorman, W., Burg, J., Groth, D., Khan, A., Ackerman, L. and Cockrell, B. (1984a) Carcinogenic and Toxicologic Effects of Inhaled Ethylene Oxide and Propylene Oxide in F344 Rats Toxicology and Applied Pharmacology, 76, 69-94 Lynch, D., Lewis, T., Moorman, W., Burg, J., Lal, J., Setzer, J., Groth, D., Gulati, D., Zavos, P., Sabharwal, P., Ackerman, L., Cockrell, B. and Sprinz, H. (1984b) Effects on Monkeys and Rats of Long-term Inhalation Exposure to Ethylene Oxide: Major findings of the NIOSH study In: In hospital ethylene oxide sterilisation – Current issues in ethylene oxide toxicity and occupational exposure, Arlington, VA, Association for the Advancement of Medical Instrumentation, 7-10 (AAMI Technology Assessment report No. 8-84	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but study was similar to OECD 453	
2.2 GLP	Not GLP	
2.3 Deviations	None	
	3 Materials and Methods	
3.1Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification	Not reported: obtained from Union Carbide Corp., Chicago, Illinois, USA	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	99.7%	
3.1.2.3 Stability	Not reported	
3.2 Test Animals		
3.2.1 Species	Rat	

3.2.2 Strain	Fischer 344/HAPBR	
3.2.3 Source	Harlan Industries, Indianapolis, IN, USA	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	Approx 110 g (estimated from graphical presentation), 6 weeks	
3.2.6 Number of animals per group	80	
3.2.6.1 at interim sacrifice	No interim sacrifice	
3.2.6.2 at terminal sacrifice	80	
3.2.7 Control animals	2 groups of 40	
3.3 Administration/ Exposure		
3.3.1 Duration of treatment	2 years	x
3.3.2 Interim sacrifice(s)	No interim sacrifice	
3.3.3 Final sacrifice	2 years (486 exposure days with an average exposure duration of 6.9 h/day	
3.3.4 Frequency of exposure	7 hours per day, 5 days per week	
3.3.5 Post exposure period	None	
3.3.6 Type	Inhalation	
3.3.7 Concentration	0, 50 or 100 ppm	
3.3.8 Vehicle	None	
3.3.9 Concentration in vehicle	Not applicable	
3.3.10 Total volume applied	Not applicable	
3.3.11 Controls	Exposed to room air only	
3.4 Examinations		
3.4.1 Body weight	Weekly for weeks 1-10, biweekly for weeks 11-30, monthly for weeks 31-95 and weekly for weeks 96-105	
3.4.2 Food consumption	Not reported	
3.4.3 Water consumption	Not reported	
3.4.4 Clinical signs	Twice daily	

3.4.5 Macroscopic investigations	Not reported	
3.4.6 Ophthalmoscopic examination	Not reported	
3.4.7 Haematology	Haematology indices including haematocrit, haemoglobin, red and white blood cell counts, clotting time and differential blood cell counts at 104 weeks. Differential slides were evaluated on all rats killed in a moribund condition during the last 6 months of the study as an aid in detecting mononuclear cell leukemia.	
3.4.8 Clinical Chemistry	Alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, blood urea nitrogen, creatinine, sorbitol dehydrogenase and albumin/globulin ratio at termination (15 rats/group).	
3.4.9 Urinalysis	Acetone, albumin, glucose, blood, casts, crystals, white blood cells, red blood cells, bacteria and epithelial cells at termination (15 rats/group).	
3.4.10 Pathology	All animals that died or were killed were given a gross necropsy.	
3.4.10.1 Organ Weights	Lungs, liver, kidneys, adrenals, spleen, testes and brain	
3.4.11 Histopathology	34 tissues (not specified) plus all gross lesions. Although the study design did not indicate which 34 organs were assessed for histopathology, the tables in the study specify non-neoplastic lesions in 17 different organs, and neoplastic lesions in 30 different organs.	
3.4.12 Other examinations	None reported	
3.5 Statistics	Group bodyweights were compared using multivariate analysis of covariance and, if significant, the Duncan multiple range test. Survival was compared using the actuarial method and the Lee-Desu statistic. Organ weights, haematology and clinical chemistry parameters were compared using the Kruskal-Wallis test followed by multiple comparisons if significant. The Mantel-Haenszel method, the Peto method, and the χ^2 test were used to compare tumour incidences.	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Body weight	Body weight gain was significantly reduced at 100 ppm from week 9 and at 50 ppm from week 14 until the end of the study.	
4.2 Food consumption	Not reported	
4.3 Water consumption	Not reported	
4.4 Clinical signs	Signs of murine pulmonary infections appeared in all groups after approximately 8 months and rats were treated with tetracycline in drinking water for 3 weeks. The animals were treated again for 2 weeks after 16 months exposure and for 3 weeks after 20 months exposure. Exposure to ethylene oxide continued during the 8 and 20 month tetracycline treatment periods but was suspended during the 16 month treatment. There was a statistically significant reduction in survival in both	

	exposure groups. Median survival time was 720, 690 and 653 days for rats exposed to 0, 50 and 100 ppm, respectively.	
4.5 Macroscopic investigations	Not reported	
4.6 Ophthalmoscopic examination	Not reported	
4.7 Haematology	Statistically significant differences between the percentages of neutrophils and lymphocytes between control and exposed groups but as there were no statistically significant differences in total white blood cell counts these differences were considered not to be biologically significant. The WBC count was statistically increased in rats with leukemia at 50 ppm ($p = 0.02$), but not in the 100 ppm group ($p = 0.09$).	
4.8 Clinical Chemistry	Statistically significant increase in aspartate transaminase in both the 50 and 100 ppm groups.	
4.9 Urinalysis	No effect of treatment. No data analyses were performed on the rat urinalysis measurements; however, the frequency distributions appear uniform across groups.	x
4.10 Pathology	Hepatosplenomegaly at 50 and 100 ppm.	
4.11 Organ Weights	Treatment related increases in absolute and relative lung weight but these may have been due to the infection. Absolute kidney and brain weights were decreased but this may have been due to the reduction in body weight. Testes weights were highly variable due to the high incidence of interstitial cell tumours seen in this strain of rat. Absolute and relative adrenal weights were also reduced in treated rats (Table 6.7/01-1).	
4.12 Histopathology	At both 50 and 100 ppm there was a higher incidence of inflammatory lesions of the lungs, nasal cavity, trachea and internal ear and there were also effects on the spleen. Changes in lungs were consistent with chronic respiratory disease. Non neoplastic lesions were also noted in the adrenals and eyes at both 50 and 100 ppm and in skeletal muscle at 100 ppm (Table 6.7/01-2). The skeletal muscle myopathy, consisting of multifocal areas of atrophy and degeneration of skeletal muscle fibers, was statistically significantly increased at 100 ppm. These changes were not accompanied by any changes in the nerves which were detectable by light microscopy. Number of rats with multifocal mineralization of the posterior layers of the choroid/sclera portion of the eye was also increased in exposed rats. Other non neoplastic changes were typical of aged rats of this strain and the incidences were not treatment related. There was a statistically significant increase in mononuclear cell leukaemia in rats that received 50 ppm but the incidence was non significant at 100 ppm. The lack of statistical significance at the higher exposure may have been due to reduced survival. There was also a statistically significant increase in the incidence of peritoneal mesotheliomas at 100 ppm and mixed cell gliomas in the brain at 50 and 100 ppm. Two rats in the 50 ppm group and 4 in the 100 pm group had increased numbers of glial cells which may have represented incipient gliomas. No other tumours were considered to have resulted from treatment (Table 6.7/01-2).	x
4.13 Other examinations	None	

4.14 Time to tumours	Not reported	
4.15 Other	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 80 male Fischer rats were exposed to 0, 50 or 100 ppm ethylene oxide by inhalation 7 hours/day, 5 days/week for 2 years. Body weights and clinical signs of toxicity were recorded during the treatment period; haematology, clinical chemistry and urinalysis parameters were determined at the end of the treatment period. Animals were given a gross necropsy at termination, selected organs were weighed and tissues were examined by histopathology.	
5.2 Results and Discussion	Survival was reduced in both treatment groups and there was also a reduction in body weight gain. A reduction in aspartate transaminase was the only treatment related effect on clinical chemistry and there were no treatment related effects on haematology or urinalysis. Absolute kidney and brain weights and absolute and relative adrenal weights were reduced at 50 and 100 ppm. There was an increase in the incidence of mononuclear cell leukaemia at 50 ppm and brain glioma and peritoneal mesothelioma at 100 ppm. Non-neoplastic lesions were recorded in the adrenals (cortical nodular hyperplasia, multifocal cortical vacuolation and multifocal cortical hyperplasia), nasal cavity (suppurative rhinitis) and spleen (focal fibrosis and extramedullary haematopoiesis). Effects were also reported in the lungs but these were probably the results of an infection and were considered not related to treatment.	
5.3 Conclusion	Ethylene oxide was carcinogenic in the rat.	
5.3.1 Reliability	2	x
5.3.2 Deficiencies	Only male rats were used and only two dose levels. Food consumption was not reported, haematology and clinical chemistry parameters were only determined at termination.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods	*3.3.1 Duration of treatment* should read: 2 years (486 exposure days with an average exposure duration of 6.9 h/day)	
Results and discussion	*4.9 Urinalysis* should read: No data analyses were performed on the rat urinalysis measurements; however, the frequency distributions appear uniform across groups. *4.12 Histopathology*: The lack of statistical significance at the higher exposure may have been due to reduced survival (19% vs 49% in controls).	

Conclusion	The conclusion of the applicant is acceptable. Ethylene oxide was carcinogenic in rats. The lowest tested concentration of 50 ppm is considered to be a LOAEL for general toxicity and carcinogenicity.
Reliability	Reliability 3
Acceptability	The study is acceptable with the following remarks: only one sex and two dose levels were tested; no data are reported on food consumption; a disease outbreak occurred at 8 and 16 months; no interim kills.
Remarks	

Table 6.7/01-1: Absolute and relative organ weights of male rats following a 2 year exposure to ethylene oxide

Exposure (ppm)		Lungs	Liver	Kidneys	Adrenals	Testes	Brain
0	Abs	2.97±0.62	14.80±4.22	3.59±0.65	0.08±0.02	5.03±2.47	2.19±0.12
	Rel[1]	8.96±2.24	44.26±11.92	10.79±2.47	0.25±0.08	15.17±7.63	6.58±0.78
50	Abs	4.23±2.74	12.88±2.98	3.14±0.37*	0.10±0.02*	5.51±10.75	2.10±0.11*
	Rel[1]	15.31±11.32*	45.45±13.12	10.98±2.04*	0.34±0.12*	17.82±31.41	7.34±0.95*
100	Abs	3.18±0.51	13.44±2.26	3.14±0.36*	0.10±0.02*	3.89±2.27	2.04±0.13*
	Rel[1]	10.50±3.11	43.50±8.41	10.15±1.57	0.31±0.06*	12.40±6.55	6.67±1.30

[1]Organ/body weight x 1000, * $p \leq 0.05$

Table 6.7/01-2: Incidence of treatment related histopathology effects in rats following a 2 year exposure to ethylene oxide

Organ	0 ppm	50 ppm	100 ppm
Non neoplastic lesions			
Adrenal glands			
Cortical nodular hyperplasia	0/78	2/77	14/78*
Multifocal cortical vacuolation	0/78	25/77*	42/78*
Multifocal cortical hyperplasia	0/78	16/77*	36/78*
Lungs			
Acute bronchopneumonia	11/79	21/79*	30/80*
Chronic pneumonia, focal	6/79	28/79*	42/80*
Oedema	0/79	14/79*	10/80*
Nasal cavity			
Suppurative rhinitis	12/76	63/75*	62/75*
Spleen			
Focal fibrosis	6/77	9/79	23/76*
Extramedullary haematopoiesis	34/77	53/79*	46/76*
Eyes			
Cataract, unilateral	2/77	3/79	9/78
Skeleta muscle			
Multiocal myopathy	7/77	10/78	43/70
Neoplastic lesions			
Brain			
Glioma	0/76	2/77	5/79*
Body cavity			
Peritoneal mesothelioma	3/78	9/79	21/79*
Spleen			
Mononuclear cell leukemia	24/77	38/79*	30/76

*** p<0.05**

Section A6.7/02 **Annex Point IIA 6.7**	**Carcinogenicity – lifetime feeding study, rat**	
	1 Reference	**Official use only**
1.1 Reference	Snellings, W., Weil, C. and Maronpot, R (1984) A Two-Year Inhalation Study of the Carcinogenic Potential of Ethylene Oxide in Fischer 344 Rats Toxicology and Applied Pharmacology, 75, 105-117 Garman, R., Snellings, W. and Maronpot, R. (1985) Brain Tumours in F344 Rats Associated with Chronic Inhalation Exposure to Ethylene Oxide Neurotoxicology, 6(1), 117-138 Garman, R. and Snellings, W.M. (1986) Frequency, Size and Location of Brain Tumours in F-344 rats	

	Chronically Exposed to Ethylene Oxide Food and Chemical Toxicology, 24, 145-153	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study is similar to OECD 451	
2.2 GLP	Not GLP	
2.3 Deviations	None	
	3 Materials and Methods	
3.1Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification	Not reported: obtained from Union Carbide Corp., Seadrift, Texas, USA	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	>99.9%	
3.1.2.3 Stability	>99.9% pure throughout the study	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Fischer 344	
3.2.3 Source	Microbiological Associates, Walkersville, Maryland, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Approximately 6 weeks	
3.2.6 Number of animals per group	120 per sex	
3.2.6.1 at interim sacrifice	10 rats per sex after 6 and 12 months and 20 rats per sex after 18 months	
3.2.6.2 at terminal sacrifice	90 per sex	
3.2.7 Control animals	2 groups of 120 rats per sex	
3.3 Administration/ Exposure		

3.3.1 Duration of treatment	Females 24 months; males 25 months	
3.3.2 Interim sacrifice(s)	6, 12 and 18 months	
3.3.3 Final sacrifice	Females 24 months; males 25 months	
3.3.4 Frequency of exposure	6 hours per day, 5 days per week	
3.3.5 Post exposure period	None	
3.3.6 Type	Inhalation	
3.3.7 Concentration	0, 10, 33 and 100 ppm	
3.3.8 Vehicle	None	
3.3.9 Concentration in vehicle	Not applicable	
3.3.10 Total volume applied	Not applicable	
3.3.11 Controls	Room air only	
3.4 Examinations		
3.4.1 Body weight	Routinely determined during study but frequency not reported.	
3.4.2 Food consumption	Not reported	
3.4.3 Water consumption	Not reported	
3.4.4 Clinical signs	Routinely determined during study but frequency not reported.	
3.4.5 Macroscopic investigations	Rats were palpated at intervals for abnormal tissue masses.	
3.4.6 Ophthalmoscopic examination	Not reported	
3.4.7 Haematology	No details of investigations included in the publication but some effects were found.	
3.4.8 Clinical Chemistry	Not reported	
3.4.9 Urinalysis	Not reported	
3.4.10 Pathology	Gross examination was performed on all rats	
3.4.10.1 Organ Weights	No details of all organs weighed were included in the publications but it included brain and spleen.	
3.4.11 Histopathology	Approximately 50 tissues examined after 6 and 24 months exposure from the control and 100 ppm groups. The same tissues were examined from rats that died or were killed during the treatment period. Fifteen tissues were examined at the 12 and 18 month sacrifice. Only tissues with gross lesions were examined at any interim kill for the	

	10 and 33 ppm groups and approximately 20 tissues were examined from rats in these groups at the terminal sacrifice.	
3.4.12 Other examinations	None	
3.5 Statistics	Mortality and tumour incidence data were compared using Fischer's exact test for two tailed test probabilities. Time adjusted trend analysis was also used to compensate for differential mortality.	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Body weight	There was a statistically significant decrease in body weight gain in both sexes at 100 ppm from the end of the first month of exposure and body weight gain was also reduced in female rats in the 33 ppm groups from week 10. Results were only presented graphically and could not be tabulated.	
4.2 Food consumption	Not reported	
4.3 Water consumption	Not reported	
4.4 Clinical signs	An outbreak of viral sialodacryoadenitis was confirmed in the 15 month of the study. There was a reduction in body weight and exposure to ethylene oxide was stopped for 2 weeks during which time body weight and most clinical signs returned to normal. There was a treatment related increase in mortality at 100 ppm from the 22^{nd} and 23^{rd} months of the study. There was no statistically significant difference in the cumulative percentage mortality at 10 or 33 ppm but there was a numerical increase in both sexes in the 33 ppm group from the 21^{st} month. Results were only presented graphically and could not be tabulated.	
4.5 Macroscopic investigations	Not reported	
4.6 Ophthalmoscopic examination	Not reported	
4.7 Haematology	Peripheral blood smears showed that there were increased numbers of rats with malignant mononuclear cells in the exposed groups.	
4.8 Clinical Chemistry	Not reported	
4.9 Urinalysis	Not reported	
4.10 Pathology	Gross pathology not reported	
4.11 Organ Weights	Relative spleen weights were increased in rats with mononuclear cell leukaemia and brain weights were higher than the control range in two animals that had brain tumours.	
4.12 Histopathology	There was no statistically significant increase in tumour incidence amongst rats killed at 6, 12 or 18 months. After 24 months exposure there was a treatment related increase in mononuclear cell leukaemia, peritoneal mesotheliomas, brain tumours and subcutis fibroma (Table 6.7/02-1 and 6.7/02-2). Mononuclear cell leukaemia was increased in all three exposure groups	

	but this was only statistically significant in females exposed to 100 ppm Mononuclear cells were principally present in spleen and liver. Mortality adjusted trend analysis showed a significant positive trend in both sexes (females $p<0.005$, males $p<0.05$), and significant findings were also obtained when results from rats that died or were killed in a moribund condition were also included in the analysis. Peritoneal mesotheliomas were increased in males at 100 and 33 ppm without statistical significance, but significant findings were obtained when results from rats that died or were killed in a moribund condition were included in the analysis. Trend analysis, when adjusted for mortality, indicated a relationship between exposure and the induction of this tumour ($p<0.005$). Brain neoplasms were also numerically increased particularly in males in the 100 and 33 ppm groups. A time adjusted trend test, including all the animals that died, showed significant probabilities indicating a positive trend for both sexes (males $p<0.01$, females $p<0.05$). Information on brain pathology was reported in the later publications by Garman, Snellings and Maronpot (1985) and Garman and Snellings (1986). The brain tumours consisted of granular cell tumours, gliomas and malignant reticuloses/microgliomatoses/ reticulum cell sarcomas and a revised analysis of tumour incidence based on tumours found at the 18 and 24 month sacrifice and in rats that died or were terminated during the treatment period is shown in Table 6.7/02-3. The incidence of pituitary adenomas was similar in all groups but trend analysis indicated that this tumour developed earlier in females exposed to 100 ppm than in males. The only other tumour which was significantly increased was fibroma of the subcutis in male rats. When the incidence of this tumour in rats that died or were killed in a moribund condition was included in the analysis the ratio of the number of rats with the tumour to the number at risk was significantly increased for the 100 ppm group (Table 6.7/02-4). This tumor was considered an incidental tumor and was statistically evaluated accordingly. Exposure to ethylene oxide increased the frequency of rats with multiple neoplasms. The frequency of male rats with multiple primary tumours was significantly greater at 100 ppm and the frequency of female rats was significantly greater at all three exposure levels (Table 6.7/02-5). The incidence of other tumours shown in Tables 6.7/02-1 and 6.7/02-2 could not be related to treatment.	
4.13 Other examinations	None	
4.14 Time to tumours	Not reported, but at the 6-, 12-, and 18-month intervals no significant differences were noted for the incidence of primary neoplasms among groups. Most of mononuclear cell leukemia and brain tumors occurred in months 24-25 of exposure.	
4.15 Other	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 120 rats of each sex were exposed to concentrations of 0, 10, 33 or 100 ppm ethylene oxide by inhalation 6 hours per day, 5 days per week for up to approximately 2 years. Ten rats per sex were sacrificed after 6 and 12 months exposure and 20 rats per sex were sacrificed after 18 months exposure. Animals which died or were terminated during the study were given a post mortem at termination and tissues were retained for histopathology. Some haematology parameters, for example blood	

	smears, were also investigated and some organ weights were also recorded but experimental details were not included in the publication.	
5.2 Results and Discussion	There was no treatment related increase in tumour incidence amongst rats killed 6, 12 or 18 months after the start of exposure. After 24 months exposure there was a treatment related increase in mononuclear cell leukaemia, peritoneal mesotheliomas, brain tumours and subcutis fibroma. Mononuclear cell leukaemia was increased in all three exposure groups and was only statistically significant in females exposed to 100 ppm but there was a significant positive trend in both sexes. Peritoneal mesotheliomas were increased in males at 100 and 33 ppm without statistical significance but again trend analysis indicated a relationship between exposure and tumour incidence. Brain tumours were increased, also not statistically significantly, particularly in males in the 100 and 33 ppm groups, and a trend test showed a positive trend for both sexes. Fibroma of the subcutis was significantly increased in male rats in the 100 ppm group. The incidence of pituitary adenomas was similar in all groups but trend analysis indicated that this tumour developed earlier in females exposed to 100 ppm than in males. Exposure to ethylene oxide increased the frequency of rats with multiple neoplasms. The frequency of male rats with multiple primary tumours was significantly greater at 100 ppm and the frequency of female rats was significantly greater at all three exposure levels.	
5.3 Conclusion	Ethylene oxide was carcinogenic in the rat.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	No deficiencies as a carcinogenicity study.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	27 February 2020	
Materials and Methods		
Results and discussion		
Conclusion	The summary of the applicant is acceptable. Based on the increased prevalence of mononuclear cell leukemia and a statistically significant increase in the number of rats with multiple tumors at all dose levels the lowest tested concentration of 10 ppm is considered to be a LOAEL for carcinogenicity.	
Reliability	2	
Acceptability	The study is acceptable, with the following remarks: not all experimental details are reported; a disease outbreak occurred at 15 months; however, these shortcoming are not considered to have an influence on the study conclusions.	
Remarks		

Table 6.7/02-1: Primary tumours with a greater than 5% incidence in male rats killed after 24 months exposure[a]

Tumour type	Exposure group (ppm)				
	100	33	10	0	0
No of animals examined	30	39	51	48	49
Primary neoplasms % (No of rats with tumours)					
Adrenal pheochromocytoma	37(11)	26(10)	20(10)	23(11)	31(15)
Brain tumours	10(3)	3(1)	0(0)	2(1)	0(0)
Pancreas islet cell adenoma[1]	17(5)	3(1)	4(2)	4(2)	10(5)
Peritoneal mesothelioma	13(4)	10(4)	4(2)	2(1)	2(1)
Pituitary adenoma	41(12)	33(13)	29(15)	33(16)	27(13)
Skin basal cell tumour	7(2)	0(0)	0(0)	0(0)	0(0)
Spleen mononuclear cell leukemia	30(9)	31(12)	18(9)	10(5)	16(8)
Subcutis fibroma	36*(11)	3(1)	17(9)	2(1)	4(2)
Testes interstitial cell tumour[2]	97(29)	95(37)	94(48)	100(48)	94(46)
Thyroid follicular adenoma	13(4)	3(1)	4(2)	2(1)	4(2)
Thyroid parafollicular adenoma	13(4)	3(1)	4(2)	10(5)	8(4)
Thyroid parafollicular carcinoma	0(0)	0(0)	8(4)	0(0)	4(2)

[a] Number of rats with tumours (in brackets) have been calculated by the Notifier to aid comparison with tumour incidence in other studies
[1] Examined in the 33 and 10 ppm groups only if gross lesions present
[2] Includes unilateral and bilateral tumours
[*] 0.01>p>0.001

Table 6.7/02-2: Primary tumours with a greater than 5% incidence in female rats killed after 24 months exposure[a]

Tumour type	Exposure group (ppm)				
	100	33	10	0	0
No of animals examined	26	48	54	60	56
Primary neoplasms % (No of rats with tumours)					
Adrenal pheochromocytoma[1]	4(1)	4(2)	6(3)	3(2)	2(1)
Brain tumours	8(2)	4(2)	0(0)	0(0)	2(1)
Mammary gland					
Adenoma[2]	8(2)	4(2)	0(0)	2(1)	0(0)
Adenocarcinoma[2]	8(2)	4(2)	4(2)	0(0)	4(2)
Pituitary adenoma	42(11)	50(24)	43(23)	47(28)	46(26)
Spleen mononuclear cell leukemia	58*(15)	29(14)	20(11)	8(5)	11(6)
Thyroid parafollicular adenoma	0(0)	8(4)	7(4)	12(7)	4(2)
Uterine polyp	28(7)	8(4)	24(13)	20(12)	15(8)

[a] Number of rats with tumours (in brackets) have been calculated by the Notifier to aid comparison with tumour incidence in other studies
[1] Includes unilateral and bilateral tumours
[2] Examined in the 33 and 10 ppm groups only if gross lesions present
[*] 0.01>p>0.001

Table 6.7/02-3: Adjusted ratios[1] of primary brain tumour frequencies for rats exposed to ethylene oxide for 2 years

Sex	Concentration of ethylene oxide			
	100	33	10	0
Males	7/87**	5/85*	1/92	1/181
Females	4/80	3/92	1/94	1/188

[1] Adjusted ratio = No of rats with tumour/No alive when first tumour was observed in any group
* p<0.05, ** p=0.01

Table 6.7/02-4: Selected ratios of the number of rats with tumours to the number at risk[1] for rats exposed to ethylene oxide for 2 years

Tumour type	Sex	Exposure group		
		100	0	0
Adrenal pheochromocytoma[2]	M	22/107 (21%)	17/106 (16%)	19/105 (18%)
Mammary gland adenoma	F	3/68 (4%)	1/85 (1%)	0/82 (0%)
Mammary gland carcinoma	F	2/43 (5%)	0/44 (0%)	0/47 (0%)
Pancreas islet cell adenoma	M	10/59 (17%)	2/64 (3%)	8/65 (12%)
Pituitary adenoma	M	27/93 (29%)	28/95 (29%)	22/96 (23%)
Skin basal cell tumour	M	2/56 (4%)	0/64 (0%)	0/68 (0%)
Pituitary adenoma	F	32/106 (30%)	38/109 (35%)	38/106 (36%)
Subcutis fibroma	M	15/58* (26%)	1/68 (1%)	3/69 (4%)
Testes interstitial cell tumour[2]	M	75/91 (82%)	86/94 (91%)	86/94 (91%)
Thyroid follicular adenoma	M	6/52 (11%)	1/63 (2%)	2/66 (3%)
Thyroid parafollicular adenoma	M	5/91 (5%)	5/96 (5%)	7/94 (7%)
Uterine polyp	F	18/107 (17%)	17/109 (16%)	11/109 (10%)

[1] Number at risk is the number alive when the first tumour was detected
[2] Includes unilateral and bilateral tumours
* p<0.01

Table 6.7/02-5: Number of primary benign and malignant neoplasms[1] among rats killed after 24 months exposure

Parameter	Exposure group (ppm)					
	100	**33**	**10**	**0**	**0**	**Combined control groups**
Males						
No of animals examined	30	39	51	48	49	97
Mean number of neoplasms/neoplasm bearing rat[2]	4.1	3.3	3.0	3.2	3.3	3.3
Percentage of rats with						
1 or more neoplasms[2]	100	100	100	100	98	99
2 or more neoplasms	100	97	94	98	98	98
3 or more neoplasms	83	72	61	75	76	75
4 or more neoplasms	63*	41	35	35	31	33
5 or more neoplasms	47**	18	10	10	12	11
6 or more neoplasms	17	5	2	2	8	5
7 or more neoplasms	7	3	0	0	2	1
Percentage of rats with						
1 or more malignancies[3]	40	51	33	27	33	30
2 or more malignancies	17*	5	4	2	2	2
Females						
No of animals examined	26	48	54	60	56	116
Mean number of neoplasms/neoplasm bearing rat[2]	2.2	1.7	1.8	1.3	1.3	1.3
Percentage of rats with						
1 or more neoplasms[2]	92	81	81	80	79	79
2 or more neoplasms	62**	42*	44*	20	20	20
3 or more neoplasms	42**	17	13	3	5	4
4 or more neoplasms	12	0	6	0	0	0
5 or more neoplasms	4	0	0	0	0	0
Percentage of rats with						
1 or 2 malignancies[3]	58**	35**	31	10	21	16
2 or more malignancies	19**	6	6	0	0	0

[1] The presence of the same neoplasm in bilateral organs was counted as two with the exception of mononuclear cell leukemia, peritoneal mesothelioma and multiple liver tumours of the same type which were each tabulated as one per rat. For certain tissues only gross lesions were examined for the 10 and 33 ppm exposure groups.
[2] Includes benign and malignant
[3] Liver neoplastic nodules were not considered as malignant
[*] $p<0.05$ when compared to combined controls, [**] $p<0.05$ when compared to separate and combined controls

Section A6.7/03 **Annex Point IIA 6.7**	**Carcinogenicity – lifetime feeding study, mouse**	
	1 Reference	**Official use only**
1.1 Reference	Toxicology and Carcinogenesis Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F$_1$ Mice (Inhalation Studies) National Toxicology Program, Technical Report Series No 326, 1987	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but the study was similar to OECD 451	
2.2 GLP	Not GLP	
2.3 Deviations		
	3 Materials and Methods	
3.1Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification		
3.1.2.1 Description	Gas with ether like odour	
3.1.2.2 Purity	>99%	
3.1.2.3 Stability	Not reported	
3.2 Test Animals		
3.2.1 Species	Mouse	
3.2.2 Strain	B6C3F$_1$	
3.2.3 Source	Frederick Cancer Research Centre, Frederick, MD, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	8 Weeks	
3.2.6 Number of animals per group	50 per sex	
3.2.6.1 at interim sacrifice	0	

3.2.6.2 at terminal sacrifice	50 per sex	
3.2.7 Control animals	50 per sex	
3.3 Administration/ Exposure		
3.3.1 Duration of treatment	102 weeks	
3.3.2 Interim sacrifice(s)	No interim sacrifice	
3.3.3 Final sacrifice	102 weeks	
3.3.4 Frequency of exposure	6 hours per day, 5 days per weeks	
3.3.5 Post exposure period	None	
3.3.6 Type	Inhalation	
3.3.7 Concentration	0, 50 or 100 ppm (nominal); 0, 49.7 ± 3.6 and 99.3 ± 7.8 ppm (analytical)	
3.3.8 Vehicle	Not applicable	
3.3.9 Concentration in vehicle	Not applicable	
3.3.10 Total volume applied	Not applicable	
3.3.11 Controls	Room air only	
3.4 Examinations		
3.4.1 Body weight	Weekly for the first 13 weeks and then monthly	
3.4.2 Food consumption	Not reported	
3.4.3 Water consumption	Not reported	
3.4.4 Clinical signs	Mice were observed twice daily and given a clinical examination once per week	
3.4.5 Macroscopic investigations	Necropsy was performed on all animals.	
3.4.6 Ophthalmoscopic examination	Not reported	
3.4.7 Haematology	Not reported	
3.4.8 Clinical Chemistry	Not reported	
3.4.9 Urinalysis	Not reported	
3.4.10 Pathology	Grossly visible lesions were recorded at necropsy	
3.4.10.1 Organ Weights	Not reported	

3.4.11 Histopathology	Gross lesions and tissue masses, mandibular lymph nodes, mammary gland, skin, salivary glands, sternebrae, thyroid gland, parathyroids, small intestine (3 sections), colon, liver, prostate/testis or ovaries/uterus, gallbladder, lungs, bronchi, heart, esophagus, stomach, brain (3 sections), thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, pituitary gland, nasal cavity and nasal turbinates	
3.4.12 Other examinations	None	
3.5 Statistics	Probability of survival was estimated by the product-limit procedure of Kaplan-Meier. Dose related effects on survival was analysed by the method of Cox and Tarone. Tumour incidence data were analysed using life table analysis, incidental tumour analysis and unadjusted analyses.	
3.6 Further remarks	None	
	4 Results and Discussion	
4.1 Body weight	Mean body weights were unaffected.	
4.2 Food consumption	Not reported	
4.3 Water consumption	Not reported	
4.4 Clinical signs	No treatment related signs of toxicity and no significant differences in survival between any groups of either sex (Table 6.7/03-1).	
4.5 Macroscopic investigations	Not reported	
4.6 Ophthalmoscopic examination	Not reported	
4.7 Haematology	Not reported	
4.8 Clinical Chemistry	Not reported	
4.9 Urinalysis	Not reported	
4.10 Pathology	Gross pathology not reported	
4.11 Organ Weights	Not reported	
4.12 Histopathology	Significant or noteworthy changes in the incidence of mice with neoplastic or non neoplastic lesions were apparent in the lung, Harderian gland, haematopoietic system, uterus and mammary gland (Table 6.7/03-2). There was a significant positive trend in the incidence of alveolar/bronchiolar carcinomas in the lungs of both sexes of mice. The combined incidences of benign and malignant tumours also showed a positive trend and were significantly greater than controls in the 100 ppm group. At 100 ppm there were also significant increases in the incidences of adenomas and carcinomas in females and carcinomas only in males. In the Harderian gland papillary cystadenocarcinomas occurred with significant positive trends in both sexes and the incidences were significantly greater than in controls. One papillary cystadenocarcinoma	

	was observed at the higher exposure in both sexes. The incidence of malignant lymphomas in female mice exposed to 100 ppm was higher than in controls and there was a positive trend. Adenocarcinomas of the uterus occurred in female mice with a positive trend and the incidence was marginally increased at the higher exposure. Adenocarcinomas and combined adenocarcinomas and adenosquamous carcinomas were increased in the mammary gland of low dose female mice.	
4.13 Other examinations	Not reported	
4.14 Time to tumours	Not reported	
4.15 Other	Not reported	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 50 mice per sex were exposed to 0, 50 or 100 ppm ethylene oxide 6 hours per day, 5 days per week for 102 weeks. Mice were observed twice daily and given a weekly clinical examination. Body weight was measured at intervals. At the end of the exposure period animals were necropsied and tissues were examined by histopathology.	
5.2 Results and Discussion	In both male and female mice there was a dose related increase in the incidence of benign or malignant neoplasms in the lung and benign neoplasms in the Harderian gland. Ethylene oxide also caused an increase in malignant tumours of the uterus, mammary gland and lymphomas in female mice.	
5.3 Conclusion	There was clear evidence of a carcinogenic effect of ethylene oxide in $B6C3F_1$ mice.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods		
Results and discussion		
Conclusion	The summary of the applicant is acceptable with minor corrections made by the eCa. The concentration level of 50 ppm is considered to be a LOAEL for carcinogenicity. The eCa considers the study to have reliability 2, as it is a well performed NTP study, and the deviations such as lack of assessments for interim kill, food and water consumption, and differential blood count are considered not to affect the carcinogenicity evaluation	
Reliability	2	

Acceptability	The study is acceptable.
Remarks	

Table 6.7/03-1: Survival of mice exposed to ethylene oxide for two years

Sex	Exposure concentration (ppm)		
	0	**50**	**100**
Males	28/50	31/50	34/50
Females	25/50	24/50	31/50

Table 6.7/03-2: Incidence of neoplastic lesions in mice exposed to ethylene oxide for two years

Tumour type	Sex	Exposure concentration		
		0	**50**	**100**
Lung				
Alveolar/bronchiolar adenoma	M	5/50	11/50	11/50
Alveolar/bronchiolar carcinoma		6/50	10/50	16/50* (p=0.014)
Alveolar/bronchiolar adenoma or carcinoma		11/50	19/50	26/50 (p=0.002)
Alveolar/bronchiolar adenoma	F	2/49	4/48	17/49 (p<0.001)
Alveolar/bronchiolar carcinoma		0/49	1/48	7/49* (p=0.006)
Alveolar/bronchiolar adenoma or carcinoma		2/49	5/48	22/49 (p<0.001)
Harderian gland				
Papillary cystadenoma	M	1/43	9/44* (p=0.008)	8/42* (p=0.013)
Papillary cystadenoma	F	1/46	6/46 (p=0.055)	8/47* (p=0.016)
Haematopoietic system				
Malignant lymphomas	F	9/49	6/48	22/49** (p=0.004)
Uterus				
Adenocarcinoma	F	0/49	1/47	5/49
Adenoma		0/49	1/47	0/49
Adenoma or carcinoma		0/49	2/47	5/49
Mammary gland				

Adenocarcinoma	F	1/49	6/48	4/49
Adenosquamous carcinoma		0/49	2/48	2/49
Adenocarcinoma or adenosquamous carcinoma		1/49	8/48	6/49

* p<0.05, ** p<0.01

6.8 Reproductive toxicity

6.8.1 Teratogenicity test

Section A6.8.1/01 **Annex Point IIA 6.8.1**	**Teratogenicity study -– rat**	
	1 Reference	**Official use only**
1.1 Reference	Snellings, W. M., Maronpot, R. R., Zelenak, J. P. and Laffoon, C. P. (1982) Teratology Study in Fischer 344 Rats Exposed to Ethylene Oxide by Inhalation Toxicology and Applied Pharmacology, 64, 476-481	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed but study design was similar to OECD 414	x
2.2 GLP	Not GLP	
2.3 Deviations	Dosing was from day 6 to 15 inclusive. Body weight was not determined during gestation.	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification	Not reported	x
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	>99.9%	
3.1.2.3 Stability	Not reported; purchased from Union Carbide Corp.	
3.2 Test Animals		

3.2.1 Species	Rat	
3.2.2 Strain	Fischer 344	
3.2.3 Source	Microbiological Associates, Walkersville, Maryland, USA	
3.2.4 Sex	Female	
3.2.5 Age/weight at study initiation	14 weeks prior to mating	
3.2.6 Number of animals per group	22 mated females per treatment or negative control group, 11 mated females per positive control group	
3.2.7 Control animals	Two negative and two positive (aspirin, oral) control groups of 11 mated females each	
3.3 Administration/ Exposure		
3.3.1 Duration of exposure	Exposure commenced on Day 6 of gestation until Day 15 and was for 6 hours per day. On Gestation Day 20, females of all groups were anesthetized and the foetuses delivered by caesarean section	
3.3.3 Type	Inhalation	
3.3.4 Concentration	0, 10, 33 or 100 ppm (nominal concentration)	
3.3.5 Vehicle	Not applicable	
3.3.6 Concentration in vehicle	Not applicable	
3.3.7 Total volume applied	Not applicable	
3.3.8 Controls	Negative controls: room air only; positive controls: one group received 500 mg/kg of aspirin by gavage on Day 9 of gestation and one group received 625 mg/kg of aspirin by gavage on Day 10 (aspirin suspended in 0.2% CMC).	
3.4 Examinations		
3.4.1 Body weight	Weights of foetuses were recorded.	
3.4.2 Food consumption	Not reported	
3.4.3 Clinical signs	Not reported	
3.4.4 Examination of uterine content	Examination for number and position of implantation sites, viable foetuses, dead foetuses, early resorption sites and late resorption sites. Numbers of corpora lutea were recorded in each ovary of each female.	
3.4.5 Examination of foetuses		

3.4.5.1General	Gross examination for developmental defects and external abnormalities. Body weight, sex and crown to rump length were recorded.	
3.4.5.2 Skeleton	Half of each litter was fixed in 10% neutral buffered formalin for skeletal analysis. All foetuses from the 100 ppm and control groups underwent skeletal examination.	
3.4.5.3 Soft tissue	Half of each litter was fixed in Bouin's fixative for examination of the soft tissues. All foetuses from the 100 ppm and control groups underwent visceral examination.	
3.5 Further remarks	The coefficient of variation of the chamber concentrations was 0.8, 2.8 and 1.2% for exposures of 100, 33 and 10 pppm respectively.	
	4 Results and Discussion	
4.1 Maternal toxic Effects	There were no treatment related clinical signs of toxicity in females.	
4.2 Teratogenic / embryo-toxic effects	There were no treatment related effects on parameters associated with preimplantation loss, or embryo and foetal resoption. There was a significant effect on depression of body weights for male and female foetuses in the 100 ppm treatment group ($p < 0.05$), but no significant differences in crown to rump length and no gross abnormalities were recorded in either sex (Table 6.8.1/01-1). Skeletal abnormalities were observed in the 100 ppm treatment group and in the air controls. These were classified as variations in the ossification of sternebrae (split or poorly ossified) and distal thoracic vertebral centra (bilobed). The percentage of litters and the percentage of foetuses with distal thoracic vertebral centra variations in the 100 ppm group were elevated compared to the controls but were not statistically significant. There were no statistically significant differences between the 100 ppm and control groups for the presence of visceral alterations such as renal dilation (Table 6.8.1/01-2). In the positive control aspirin treated rats, there were statistically significant differences for many parameters examined, including body weight and length, and number of foetuses with skeletal abnormalities.	
4.3 Other effects	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 22 rats (11 rats for each positive control group) were exposed to 0, 10, 33 and 100 ppm ethylene oxide by inhalation for 6 hours per day from day 6 to day 15 of gestation. A positive control of aspirin was used, with gavage administration at 500 mg/kg and 625 mg/kg using positive control groups of 11 rats each. On day 20 of gestation the foetuses were removed and examined. The females were necropsied and their uteri examined. The foetal examinations included skeletal and visceral examination.	
5.2 Results and discussion	No clinical signs of toxicity were recorded in parental females. There was a significant effect on depression of body weights for male and female foetuses in the 100 ppm treatment group ($p < 0.05$), but no significant differences in crown to rump length and no gross abnormalities were recorded in either sex. Skeletal abnormalities were observed in the 100 ppm treatment group and in the air controls,	

	classified as variations in the ossification of sternebrae and distal thoracic vertebral centra. These differences were not statistically significant.	
5.3 Conclusion	Ethylene oxide is considered not to be a teratogen by inhalation in the rat at doses up to 100 ppm, the highest concentration tested, but was foetotoxic.	
5.3.1 LO(A)EL maternal toxic effects	Not established	
5.3.2 NO(A)EL maternal toxic effects	100 ppm ethylene oxide, the highest dose tested. Limited number of parameters examined. Exposure to 100 ppm ethylene oxide resulted in a statistically significant depression of fetal body weight, but no changes in crown-rump length. No statistically significant increases in skeletal or visceral variations were seen; vertebral variations were only slightly (nonsignificantly) elevated: 11% of the foetuses (in 42% of litters) showed these variations at the high dose, whereas in two control groups the incidences were 5-7% (in 18-19% of the litters). Renal pelvic dilatation occurred in 29% of the pups (in 78% of the litters) at the high dose vs 20-28% of the pups (in 59-81% of the litters) in two control groups. The author explained the latter finding by individual variations in the development of the renal papilla. Since no information on maternal weight gain or other maternal effects was given, it is unclear if these the observed effects on fetal body weights and vertebral variations were specific developmental effects or related to maternal toxicity.	
5.3.3 LO(A)EL embryotoxic / teratogenic effects	100 ppm ethylene oxide	
5.3.4 NO(A)EL embryotoxic / teratogenic effects	33 ppm ethylene oxide	
5.3.5 Reliability	2	
5.3.6 Deficiencies	Dosing was from day 6 to 15 inclusive. Maternal body weight was not determined during gestation.	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Guidelines and Quality Assurance	2.1 Guideline study should read: Guideline compliance was not claimed but study design was similar to OECD 414 (1981)	
Materials and Methods	The summary of the applicant is acceptable with minor clarifications made by the eCa. 3.1.2 Specification should read: Purchased from Union Carbide Corp.	
Results and discussion		
Conclusion		

Reliability	2
Acceptability	The study is not sufficient as a stand-alone, based on limited maternal parameters evaluated, but can be accepted as a part of weight of evidence approach.
Remarks	

Table 6.8.1/01-1: Caesarean data for pregnant rats exposed to ethylene oxide by inhalation

Parameter	**Exposure group (ppm)**				
	100	**33**	**10**	**Control I (air) 0**	**Control II (air) 0**
Females pregnant	19	22	20	21	17
Litters totally resorbed	0	1	0	0	0
Viable foetuses/dam	8 ± 3	8 ± 4	9 ± 3	8 ± 3	9 ± 3
Pre-implantation loss/dam (%)	18 ± 20	26 ± 29	15 ± 18	17 ± 22	19 ± 22
Early resorption sites/dam	1.0 ± 1.2	1.0 ± 1.7	0.4 ± 0.7	0.9 ± 1.6	0.6 ± 1.0
Late resorption sites/dam	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2	0.1 ± 0.2
Total resorption sites/dam	1.0 ± 1.4	1.1 ± 1.7	0.4 ± 0.7	1.0 ± 1.6	0.6 ± 1.0

Table 6.8.1/01-2: Summary of foetal data after maternal exposure to ethylene oxide

Parameter	Exposure group (ppm)				
	100	**33**	**10**	**Control I (air) 0**	**Control II (air) 0**
Weight male foetuses (g)	3.1* ± 0.2	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.4	3.3 ± 0.2
Weight female foetuses (g)	2.9* ± 0.1	3.1 ± 0.3	3.0 ± 0.3	3.1 ± 0.3	3.0 ± 0.2
Crown to rump length (male) (mm)	36 ± 1	36 ± 2	37 ± 1	37 ± 1	36 ± 1
Crown to rump length (female) (mm)	35 ± 1	35 ± 2	36 ± 1	35 ± 2	35 ± 1
Foetuses with one or more gross abnormalities (%)	0	0	0	0	0
Incidence of foetal alterations					
No. of foetuses/No. of litters examined: external examination	154/19	-	-	175/21	149/17
No. of foetuses/No. of litters examined: skeletal examination	75/19	-	-	87/21	74/17
No. of foetuses/No. of litters examined: visceral examination	79/18[1]	-	-	88/21	75/17
% affected, foetuses (litters) – external alterations	0 (0)	-	-	0 (0)	0 (0)
% affected, foetuses (litters) – skeletal alterations: variation ossification sternebrae	4 (11)	-	-	7 (29)	1 (6)
% affected, foetuses (litters) – skeletal alterations: variation ossification vertebrae	11 (42)	-	-	5 (19)	7 (18)
% affected, foetuses (litters) – visceral alterations: renal pelvic dilation	29 (78)	-	-	28 (81)	20 (59)
% foetuses/litter affected, Q_2 (QD): variation ossification sternebrae	0 (0)	-	-	0 (10)	0 (0)
% foetuses/litter affected, Q_2 (QD): variation ossification vertebrae	0 (12)	-	-	0 (0)	0 (0)
% foetuses/litter affected, Q_2 (QD): renal pelvic dilation	20 (15)	-	-	33 (13)	17 (20)

* = $p > 0.05$ when compared to Controls I and II

[1] One dam had only one foetus which was skeletally examined

Section 6.8.1/02 **Annex Point 6.8.1**	**Teratogenicity study - rabbit**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [x] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	A study was included in the submission to the US EPA in which rabbits were exposed by inhalation to 0 and 150 ppm ethylene oxide for 7 hours per day during gestation days 7-19 or 1-19[5]. There was no evidence of maternal or developmental toxicity. Further details of this study are not available so a study summary cannot be compiled. As ethylene oxide has already been shown to produce developmental effects in the rat at lower concentrations than used in the rabbit study, a new study would not provide data that would affect the risk assessment. It would be an unnecessary use of animals and therefore should not be required.	x
Undertaking of intended data submission [ ]	*No undertaking provided; submission of data/information is not considered necessary*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	January 2020	
Evaluation of applicant's justification	Teratogenicity study in rabbits is not provided by the applicant. In the study by Hackett (1982) which has been published by Hardin (1983), exposure of rabbits to ethylene oxide during gestation (GD 1-19 and 7-19) did not result in adverse developmental effects, while adverse effects were reported in the same study in rats exposed in the same manner. This suggests that rabbits are less sensitive to developmental toxicity of ethylene oxide than rats. The publication of Hardin et al. (1983) was recovered from the public domain and assessed by the eCA.	
Conclusion	In any case, a performance of an additional developmental toxicity study with rats would not provide data which would influence the risk assessment as EtO is a genotoxic carcinogen. The performance of a developmental toxicity study with rabbits is not considered to be justified in view of animal welfare.	
Remarks		

6.8.2 Two generations reproduction study

Section A6.8.2/01	**Multigeneration reproduction toxicity study - rat**	

Annex Point IIA 6.8.2		
	1 Reference	**Official use only**
1.1 Reference	Snellings, W. M., Zelenak, J. P. and Weil, C. S. (1982) Effects on Reproduction in Fischer 344 Rats Exposed to Ethylene Oxide by Inhalation for One Generation Toxicology and Applied Pharmacology, 63, 382-388	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	Guideline compliance was not claimed. The study was similar to OECD 415.	
2.2 GLP	Not GLP	
2.3 Deviations	A single generation was investigated. Body weight was measured at 2 week intervals and food consumption was not measured. Litters were reduced to 10 pups per group irrespective of sex on day 4. Organs were not weighed at necropsy and not examined by histopathology and sperm parameters were not investigated. Developmental parameters were not investigated in the offspring. Purity unknown and no information on test substance batch.	
	3 Materials and Methods	
3.1Test material		
3.1.1 Lot/Batch number	Not reported	
3.1.2 Specification	Not reported; purchased from Union Carbide Corp	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	x
3.1.2.3 Stability	Not reported	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Fischer 344	
3.2.3 Source	Microbiological Associates, Walkersville, Maryland, USA	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	3 to 4 weeks	

3.2.6 Number of animals per group	30 per sex	
3.2.7 Mating	One male was placed with one female; the male was replaced after one week if no vaginal plug had been observed.	
3.2.8 Duration of mating	Two weeks unless a vaginal plug was observed during the mating period.	
3.2.9 Deviations from standard protocol	None	
3.2.10 Control animals	Two control groups were used and exposed only to room air.	
3.3 Administration/ Exposure		
3.3.1 Animal assignment to dosage groups	Random assignment to one of five groups.	
3.3.2 Duration of exposure before mating	12 weeks	
3.3.3 Duration of exposure in general P, F1, F2 males, females	Parents exposed for 6 hours per day, 5 days per week for 12 weeks prior to cohabitation, then 6 hours per day, 7 days per week during cohabitation. After 2 weeks of mating, only the females were exposed for 6 hours per day, 7 days per week. Females in which a vaginal plug was observed were exposed until day 19 of gestation. Five days after parturition, the dams were separated from their pups for approximately 6.75 hours per day and were exposed for 6 hours per day, 7 days per week until day 21 postpartum.	
3.3.4 Type	Inhalation	
3.3.5 Concentration	0, 10, 33 or 100 ppm	
3.3.6 Vehicle	Not applicable	
3.3.7 Concentration in vehicle	Not applicable	
3.3.8 Total volume applied	Not applicable	
3.3.9 Controls	Room air only	
3.4 Examinations		
3.4.1 Clinical signs	Not reported	
3.4.2 Body weight	Every two weeks until the cohabitation period. Each litter was weighed as a unit on days 4 and 14 postpartum. On day 21 each pup was weighed individually.	
3.4.3 Food/water consumption	Not reported	
3.4.4 Oestrus cycle	Not reported	

3.4.5 Sperm parameters	Not reported	
3.4.6 Offspring	Ratio of number of pups born to number of implantation sites, number of litters, number of pups born dead or alive and survival indices were reported.	
3.4.7 Organ weights P and F1	Not reported	
3.4.8 Histopathology P and F1	The uteri of the F0 generation were fixed and stained to determine the number of implantation sites and pregnancy status.	
3.4.9 Histopathology F1 not selected for mating, F2	Not applicable, this was only a one generation study	
3.5 Further remarks	Mean chamber concentrations were 96, 32 and 10 ppm compared with the target concentrations of 100, 33 and 10 ppm.	
	4 Results and Discussion	
4.1 Effects		
4.1.1 Parent males	No treatment related effects on body weight during 12 weeks of exposure and no mortality.	
4.1.2 Parent females	No treatment related effects on body weight during 12 weeks of exposure. No dams died during gestation or lactation periods.	
4.1.3 F1 males	No treatment related effect on survival. See F1 females below.	x
4.1.4 F1 females	No statistically significant effects on survival rate using the ratio of pups alive on day 4 postpartum to the number of pups born alive on day 0 per female, or using the ratio of pups alive on day 14 or 21 postpartum to the number of pups alive on day 4 per pregnant female. No statistically significant effects on body weights in the 100 ppm exposure group when the body weights were determined at day 4, 14 or 21 postpartum (tables 6.8.2/01-1 and 6.8.2/01-2).	x
4.1.5 Reproductive indices	The fertility indices (% of females pregnant and % of males proven fertile) indicate lower fertility for the 100 ppm exposure group than either control group but the difference was not statistically significant. There were significantly more females in the 100 ppm group with a gestation period >22 days than in controls (4 females 23 days, 2 females 25/26 days, 1 female undeterminable), but the length of the gestation periods were not significantly different.	
4.1.6 Offspring data	The number of pups born per litter and the number of implantation sites per pregnant female were significantly lower in the 100 ppm group than in either control group. No statistically significant effects on survival rate using the ratio of pups alive on day 4 postpartum to the number of pups born alive on day 0 per female, or using the ratio of pups alive on day 14 or 21 postpartum to the number of pups alive on day 4 per pregnant female. No statistically significant effects on body weights in the 100 ppm exposure group when the body weights were determined at day 4, 14 or 21 postpartum (tables 6.8.2/01-1 and 6.8.2/01-2).	

	The ratio of the number of foetuses born to the number of implantation sites per female was also statistically significantly lower in the 100 ppm group than for either control group (Table 6.8.2/01-3).	
4.2 Other	None	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 30 rats per sex were exposed to 0, 10, 33 or 100 ppm ethylene oxide for 6 hours per day for 12 weeks before mating, during a 2 week mating period, then during gestation and lactation. Body weight was measured at intervals and gestation period was recorded. At the end of lactation the uteri from the females were examined for implantations. The numbers of pups born dead and alive and pup weights were noted and survival indices calculated.	
5.2 Results and discussion	After 12 weeks of exposure there were no significant differences in body weight gain in the F0 generation compared to control animals. Treatment related effects were limited to animals exposed to 100 ppm, there were fewer pups per litter, fewer implantation sites and a smaller proportion or pups were born relative to the number of implantation sites. The length of gestation for the 100 ppm treated rats was slightly longer than for the control groups, There was no effect on survival or growth rate in the pups during lactation.	x
5.3 Conclusion	Treatment related reproductive effects occurred in the 100 ppm treatment group, with no statistically significant effects reported in the 10 and 33 ppm groups.	
5.3.1 LO(A)EL maternal toxic effects	Not established	
5.3.2 NO(A)EL maternal toxic effects	100 ppm, the highest dose tested	
5.3.3 LO(A)EL reproduction	100 ppm, the highest dose tested	
5.3.4 NO(A)EL reproduction	33 ppm	
5.3.5 LO(A)EL offspring	Not established	
5.3.6 NO(A)EL offspring	100 ppm, the highest dose tested	
5.3.7 Reliability	2	
5.3.8 Deficiencies	A single generation was investigated. Body weight was measured at 2 week intervals and food consumption was not measured. Litters were reduced to 10 pups per group irrespective of sex on day 4. Organs were not weighed at necropsy and not examined by histopathology and sperm parameters were not investigated. Developmental parameters were not investigated in the offspring.	

	Evaluation by Competent Authorities
	Evaluation by Rapporteur Member State
Date	3 March 2020
Materials and Methods	The summary of the applicant is acceptable with minor clarifications made by the eCA: *3.1.2.2 Purity*: The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.
Results and discussion	4.1.3. The sentence should read “Not applicable”. The study was a one-generation study, thus no survival of F1 generation was examined. 4.1.4. The sentence should read “Not applicable”. The study was a one-generation study, thus no survival of F1 generation was examined.
Conclusion	The summary of the applicant is acceptable with the following comments made by the eCA: 5.2. The eCA does not agree with the applicant that the gestation length was still within historical control values, as no further information on the historical controls was made available (species, lab). Moreover the study describes that normal reported gestation length is 21-23 days, hence only 3 were longer than 23 days, which according to the eCA is therefore not within “normal / historical range”. Ethylene oxide is considered to produce reproductive effects (gestation length, number of pups and implantation sites) in the 100 ppm treatment group, with no statistically significant effects reported in the 10 and 33 ppm groups. Limited number of parameters were evaluated in the study; no information is available on organ weights and histopathology of reproductive organs, oestrous cycle and sperm parameters in parental animals; only one generation was included.
Reliability	2
Acceptability	The study is not sufficient as a stand-alone, based on limited parental parameters evaluated, but can be accepted as a part of weight of evidence approach.
Remarks	

Table 6.8.2/01-1: Reproductive parameters for rats exposed to ethylene oxide

Parameter	Exposure group (ppm)				
	100	**33**	**10**	**Control I 0**	**Control II 0**
No. of pregnant females	17/27 (63%)	25/28 (89%)	25/30 (83%)	24/29 (83%)	19/28 (68%)
No. of males proven fertile	15/22 (68%)	20/23 (87%)	19/23 (83%)	17/21 (81%)	12/20 (60%)
No. of litters totally resorbed	2	0	0	0	0
No. of pups at Day 0 postpartum	64	212	237	222	174
No. of pups born dead	0	1	3	0	0
0 to 4 days survival index, Q_2 (Q)[1]	100 (8)	100 (0)	100 (0)	100 (0)	100 (0)
4 to 14 days survival index, Q_2 (Q) [1]	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
14 to 21 days survival index, Q_2 (Q) [1]	100 (8)	100 (0)	100 (0)	100 (0)	100 (0)

[1] = Q_2 is the median. (Q) is the semi-interquartile range. Number of pups alive at later interval/number of pups alive at earlier interval/pregnant female (x100)

Table 6.8.2/01-2: Neonatal body weights (g)

Postpartum day	Exposure group (ppm)				
	100	**33**	**10**	**Control I 0**	**Control II 0**
4	8.5* ± 0.8	7.7 ± 0.7	7.8 ± 0.9	8.0 ± 0.8	7.6 ± 0.8
14	14.8 ± 4.4	16.1 ± 1.5	16.7 ± 1.5	16.8 ± 1.4	16.8 ± 2.1
21 (male)	23.6 ± 5.3	22.6* ± 2.6	23.7 ± 2.2	24.4 ± 2.9	25.8 ± 3.9
21 (female)	21.8 ± 4.3	22.6 ± 2.6	24.0 ± 3.2	24.1 ± 3.1	24.8 ± 3.1

* = $p < 0.05$ in comparison to either control group

Table 6.8.2/01-3: Median number of stained implantation sites per pregnant rat and median number of foetuses born per number of implantation sites

Quartile	Exposure group (ppm)				
	100	**33**	**10**	**Control I 0**	**Control II 0**
Median number of stained implantation sites per pregnant rat					
Q_2 (median)	6.0*,**	11.0	11.0	11.0	10.0
(Q_1-Q_3)	(3.5 – 9.5)	(9.0 – 11.0)	(10.0 – 11.0)	(9.25 – 11.0)	(9.0 – 11.0)
Median number of foetuses born per number of implantation sites (%)					
Q_2 (median)	57***	90	92	92	100
(Q_1-Q_3)	(40 – 76)	(81 – 96)	(84 – 100)	(90 – 100)	(89 – 100)

* = $p < 0.001$ in comparison to Control I. ** = $0.01 > p > 0.001$ in comparison to Control II.

6.9 Neurotoxicity studies

No individual study summaries on neurotoxicity studies in animals have been submitted by the applicant.

6.10 Mechanistic studies

Section A6.10/01 **Annex Point IIA 6.10**		**Mechanistic studies**	
		1 Reference	**Official use only**
1.1	**Reference**	Segerbäck, D. (1990) Reaction Products in Hemoglobin and DNA after *in vitro* Treatment with Ethylene Oxide and N-(2-hydroxyethyl)-N-nitrosourea Carcinogenesis, 11, 307-312	
1.2	**Data protection**	No	
1.2.1	**Data owner**	Unknown	
1.2.2	**Criteria for data protection**	No data protection claimed	
		2 Guidelines and Quality Assurance	
2.1	**Guideline study**	There is no guideline for this study type.	
2.2	**GLP**	Not GLP	
2.3	**Deviations**	Not applicable, there is no guideline for this study type.	
		3 Materials and Methods	
3.1	**Test material**		
3.1.1	Lot/Batch No	Not reported	
3.1.2	Specification	Not reported; purchased from The Radiochemical Centre, Amersham, UK	
3.1.3	Description	Not reported	
3.1.4	Purity	^{14}C labelled ethylene oxide: 95.2%, specific activity: 1200 GBq/mol	
3.1.5	Stability	Not reported	
3.3	**Test animals**		
3.2.1	Species	Human, rat and mouse	
3.2.2	Strain	Rat: Fischer Mouse: CBA	

3.2.3 Source	Not reported	
3.2.4 Sex	Not reported	
3.2.5 Age/weight at study initiation	Not reported	
3.2.6 Number of animals per group	Not reported	
3.2.7 Control animals	Not reported	
3.3 Administration/ Exposure		
3.3.1 Administration	Human, rat and mouse erythrocytes and lysed human erythrocytes were incubated with ^{14}C ethylene oxide for 30 minutes at 37°C. Calf thymus DNA was also incubated with ^{14}C ethylene oxide under the same conditions, for three hours.	
3.3.2 Dose level	16 kBq/mL for incubation with erythrocytes (equivalent to 0.59 µg/mL) and 50 kBq/mL for incubation with DNA (equivalent to 1.83 µg/mL)	
3.4 Examinations		
3.4.1 Observations	Not applicable	
3.4.2 Extraction and analysis	Globin was obtained from the erythrocytes or lysed erythrocytes and hydrolysed under acidic conditions. Synthetic standards were added and the amino acids were analysed by ion exchange chromatography followed by thin layer chromatography. Amounts of the modified amino acids were determined by liquid scintillation counting of the ion exchange chromatography fractions. DNA samples were subjected to acid hydrolysis and the hydrolysates analysed on Aminex A-5 resin after the addition of synthetic standards. UV absorption was monitored and the amount of DNA was calculated from the absorption of the guanine and adenine peaks. Fractions were analysed for radioactivity to quantify the amounts of modified bases present.	
	4 Results and Discussion	
4.1 Results of test	The main reaction products obtained from haemoglobin were 2-hydroxyethyl derivatives of the SH group in cysteine (HOEtCys), N-terminal group in valine (HOEtVal),the hydroxyl group in serine (HOEtSer) and the two imidazole nitrogens in histidine (N^K-HOEtHis and N^T-HOEtHis). The reactivities of the nitrogens in valine and histidine were approximately the same in all three species but the reactivity of the cysteine in mouse and rat haemoglobin was 12 and 170 higher times higher, respectively, than in human haemoglobin (Table 6.10/01-1). There were no differences in the rate constants in experiments with intact or lysed erythrocytes. The main product formed by incubation of ethylene oxide with DNA was N-7-(2-hydroxyethyl)guanine. O^6-(2-hydroxyethyl)guanine and N-3-(2-hydroxyethyl) adenine was formed to a lesser extent (0.5% and 4.4% respectively of the alkylation of guanine-N-7). Two other peaks	

	suspected to be N-7-(2-hydroxyethyl)adenine and N-1-(2-hydroxyethyl)adenine were also present as well as an unknown adduct.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Human, rat and mouse erythrocytes, lysed human erythrocytes and calf thymus DNA were incubated with ^{14}C ethylene oxide *in vitro*. Globin was isolated from erythrocytes and lysed erythrocytes. Globin or DNA was hydrolysed to obtain free amino acids or bases which were analysed by ion exchange chromatography. Synthetic standards were added prior to chromatography to identify modified amino acids and bases which were quantified by liquid scintillation counting.	
5.2 Results and discussion	The main adducts formed with haemoglobin were S-(2-hydroxyethyl)cysteine, N-(2-hydroxyethyl) valine, O-(2-hydroxyethyl)serine and both N^K- and N^T-(2-hydroxyethyl) histidine. The reactivities with valine and histidine were approximately the same in all three species but the reactivity of the cysteine in mouse and rat haemoglobin was 12 and 170 higher times higher respectively than in human haemoglobin. There were no differences in the rate constants in experiments with intact or lysed erythrocytes. The main product formed by incubation of ethylene oxide with DNA was N-7-(2-hydroxyethyl)guanine. Five other minor adducts were also present, two were definitely identified and two others were tentatively identified.	
5.3 Conclusion	Ethylene oxide readily forms adducts with both haemoglobin and DNA *in vitro*. There are some quantitative differences in the adducts formed in rat, mouse and human erythrocytes.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods		
Results and discussion		
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is not performed according to a particular guideline; however, it provides enough information for the assessment with regard to interaction of ethylene oxide with DNA. Therefore it is acceptable as a part of weight of evidence.	
Remarks		

Table 6.10/01-1: Second order rate constants for the *in vitro* alkylation of cysteine-*S*, valine-N^2 and histidine-N^K and –N^T in humans, mouse and rat haemoglobin by ethylene oxide at 37°C and pH 7.4

Species	k_Y (1-(g Hb)$^{-1}$.h^{-1}) x 10^4			
	Cysteine-*S*	Valine-N^2	Histidine-N^K	Histidine-N^T
Human[a]	0.06 ± 0.01	0.45 ± 0.06	0.38 ± 0.05	0.37 ± 0.07
Mouse[b]	0.70 ± 0.22	0.32 ± 0.10	0.37 ± 0.14	0.21 ± 0.05
Rat[c]	10	0.46	0.62	0.27

[a] n=6 in two independent experiments, [b] n=4 in two independent experiments, [c] One determination

Section A6.10/02 **Annex Point IIA 6.2**	**Metabolism studies in mammals**	
	1 Reference	**Official use only**
1.1 Reference	Wu, K-Y., Ranasinghe, A., Upton, P.B., Walker, V. E. and Swenberg, J. A. (1999) Molecular Dosimetry of Endogenous and Ethylene Oxide-Induced N7-(2-Hydroxyethyl) Guanine Formation in Tissues of Rodents Carcinogenesis, 20, 1787-1792	
1.2 Data protection	No	
1.2.1 Data owner	Data published	
1.2.2 Criteria for data protection	No data protection claimed	
	2 Guidelines and Quality Assurance	
2.1 Guideline study	There is no guideline for this type of study	
2.2 GLP	Not GLP	
2.3 Deviations	Not applicable	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch No	Not reported	
3.1.2 Specification	Not reported	
3.1.3 Description	Not reported	
3.1.4 Purity	Ethylene oxide: 99%	
3.1.5 Stability	Not reported	
3.4 Test animals		

3.2.1 Species	Rats and mice	
3.2.2 Strain	F-344 rats and B6C3F1 mice	
3.2.3 Source	Not reported	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	9 weeks old	
3.2.6 Number of animals per group	10 animals per group	
3.2.7 Control animals	10 animals per group, air exposure only	
3.3 Administration/ Exposure		
3.3.1 Administration	Whole-body inhalation exposure to ethylene oxide for 6 hours per day, 5 days per week for 4 weeks in a stainless steel and glass inhalation chamber.	
3.3.2 Dose level	0, 3, 10, 33 or 100 ppm ethylene oxide in filtered air	
3.4 Examinations		
3.4.1 Observations	After the last exposure, the animals were immediately sacrificed by exsanguination and the liver, spleen, brain and lung were removed.	
3.4.2 Extraction and analysis	DNA was extracted from the whole lung, spleen and brain and from up to 2 g of liver using an automated phenolic extraction procedure. DNA content was determined by UV and guanine was determined by HPLC analysis after acid hydrolysis. N7-(2-hydroxyethyl)guanine (7-HEG) was released from the DNA by thermal hydrolysis, derivatised and quantified using gas chromatography coupled with high-resolution mass spectrometry (GC-HRMS). The one way Student's test was used to evaluate differences in 7-HEG content between control and ethylene oxide exposed animals. Linear regression was used to examine dose response relationships.	
	4 Results and Discussion	
4.1 Results of test	Analysis of control animals showed that endogenous 7-HEG varied from 0.2 ± 0.1 to 0.3 ±0.2 pmol/µmol guanine in tissues of rats and mice. Tissue and species specific dose-response relationships were found for 7-HEG in ethylene oxide exposed animals. The dose-response relationship was linear for mouse liver, brain and spleen for exposures in the range 3 to 100 ppm. The mouse lung response was slightly sub-linear between 33 and 100 ppm of ethylene oxide. In rat liver and spleen the relationships were linear between 3 and 100 ppm, with slightly sublinear responses for brain and lung between 33 and 100 ppm. The number of 7-HEG adducts present in rats exposed to 3 ppm ethylene oxide was 5.3 – 12.5 times higher than in the control. The number of 7-HEG adducts present in mice exposed to 3 ppm ethylene oxide was only 1.3 to 2.5 times higher than in the control (Table	

	6.2/05-1). Statistically higher amounts of 7-HEG accumulated in tissues of rats than in mice exposed to the same concentrations of ethylene oxide.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Groups of 10 rats and mice were exposed to 0, 3, 10, 33 or 100 ppm ethylene oxide for 6 hours per day, 5 days per week for 4 weeks. The amount of DNA, guanine and N7-(2-hydroxyethyl) guanine was determined in liver, spleen, lung and brain at the end of the exposure period.	
5.2 Results and discussion	There were tissue and species specific dose responses for the formation of N7-(2-hydroxyethyl) guanine in both rats and mice. The dose-response relationship was linear for mouse liver, brain and spleen for exposures in the range 3 to 100 ppm. The mouse lung response was slightly sub-linear between 33 and 100 ppm of ethylene oxide. In rat liver and spleen the relationships were linear between 3 and 100 ppm, with slightly sublinear responses for brain and lung between 33 and 100 ppm.	
5.3 Conclusion	Ethylene oxide reacted directly with DNA to form N7-(2-hydroxyethyl) guanine. Adducts were formed in all tissues investigated which included target tissues for carcinogenesis (spleen and brain of rats and lungs of mice) and non-target tissues (liver and lung of rats and liver, spleen and brain of mice). The dose responses for 7-HEG adduct formation at low exposures to ethylene oxide were greater in rats than in mice.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods	The summary of the applicant is acceptable.	
Results and discussion	The summary of the applicant is acceptable.	
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is acceptable as a part of weight of evidence.	
Remarks		

Table 6.2/05-1: 7-HEG (pmol/µmol of guanine ± SD) in tissues of animals exposed to ethylene oxide for 4 weeks

Dose (ppm)	Liver	Spleen	Brain	Lung
F-344 rats				
0	0.3 ± 0.2 (n=12)	0.2 ± 0.1 (n=16)	0.2 ± 0.1 (n=9)	0.2 ± 0.1 (n=8)
3	1.6 ± 0.6 (n=4)[a]	2.5 ± 0.6 (n=4)[a]	1.8 ± 0.3 (n=4)[a]	1.6 ± 0.4 (n=4)[a]
10	3.3 ± 0.6 (n=4)[a]	4.0 ± 0.7 (n=4)[a]	5.1 ± 0.8 (n=4)[a]	4.3 ± 1.0 (n=4)[a]
33	8.8 ± 0.3 (n=4)[a]	8.8 ± 3.9 (n=5)[a]	10.7 ± 1.5 (n=2)[a]	11.2 ± 3.6 (n=5)[a]
100	34.1 ± 3.3 (n=4)[a]	30.8 ± 5.9 (n=4)[a]	53.2 ± 10.9 (n=5)[a,b]	56.0 ± 7.4 (n=2)[a,b]
B6C3F1 mice				
0	0.3 ± 0.2 (n=9)	0.2 ± 0.1 (n=8)	0.3 ± 0.1 (n=8)	0.3 ± 0.2 (n=9)
3	0.4 ± 0.1 (n=4)[a]	0.5 ± 0.1 (n=4)[a]	0.6 ± 0.2 (n=6)[a]	0.5 ± 0.1 (n=4)[a]
10	1.0 ± 0.1 (n=4)[a]	1.4 ± 0.4 (n=4)[a]	2.5 ± 0.6 (n=7)[a]	1.8 ± 0.2 (n=4)[a]
33	3.8 ± 0.2 (n=4)[a]	5.6 ± 0.8 (n=4)[a]	9.2 ± 1.3 (n=5)[a]	6.2 ± 0.4 (n=4)[a]
100	12.2 ± 2.0 (n=4)[a]	16.6 ± 5.0 (n=4)[a]	25.5 ± 5.9 (n=4)[a,c]	29.6 ± 7.1 (n=4)[a,c]

a 7-HEG in exposed tissue was significantly greater than the control ($p < 0.05$).
b 7-HEG in brain and lung were significantly greater than those in liver and spleen ($p < 0.005$).
c 7-HEG in brain and lung were significantly greater than those in liver and spleen ($p < 0.05$).

6.11 Studies on other routs of administration (parenteral routs)

No information provided.

6.12 Medical data in anonymous form

6.12.1 Medical surveillance data on manufacturing plant personnel if available

No information provided.

6.12.2 Direct observation, e.g. clinical cases, poisoning incidents if available

No information provided.

6.12.3 Health records, both from industry and any other available sources

No information provided.

6.12.4 Epidemiological studies on the general population, if available

Section A6.12.4/01 **Annex Point IIA6.12.4**	**Epidemiological Study** *Cohort study*	
	1 Reference	**Official use only**
1.1 Reference	Steenland, K., Stayner, L., Greife, A., Halperin, W., Hayes, R., Hornung, R. and Nowlin, S. (1991) Mortality Among Workers Exposed to Ethylene Oxide New England Journal of Medicine, 324, 1402-1407 Steenland, K., Stayner, L. and Deddens, J. (2004) Mortality Analysis in a Cohort of 18235 Ethylene Oxide Exposed Workers: Follow up Extended from 1987 to 1998 Occupational and Environmental Medicine, 61, 2-7	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Companies with letter of access		
1.2.3 Criteria for data protection	No data protection claimed	
	Guidelines and Quality Assurance	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not applicable, occupational exposure	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	See comment above	x
3.1.2.3 Stability	Not reported	
3.2 Type of study	Cohort study	
3.3 Method of data collection	Record review: US National Death Index which provided cause of death and via the US Social Security Administration and Internal Revenue Service. 98.5% were traced successfully.	
3.4 Test Persons / Study Population		
3.4.1 Selection criteria	Workers at chemical factories where ethylene oxide was produced or converted into other substances or where it was used as a sterilant. Only workers with at least 3 months exposure to ethylene oxide from the 1940s to the 1980s were included. Workers from 14 plants were selected because they had at least 400 person years at risk before 1978.	

3.4.2 Number of test persons per group/cohort size	Cohort size was 18235, 1222 were steriliser operators	
3.4.3 Sex	45% male and 55% female	
3.4.4 Age	Not reported but as cumulative exposure averaged 26.9 years a wide range of ages would have been involved. The data were adjusted for the date of birth (within 5 years). Death rates in the exposed cohorts were stratified according to age, race, sex and calendar year	
3.4.5 Diseases	Not reported	
3.4.6 Smoking status	Not reported, but presumably both	
3.5 Controls		
3.5.1 Type of control	National population of the United States	
3.5.2 Number of test persons per group/cohort size	Not reported but would be much greater than the size of the cohort for the exposed group	
3.5.3 Sex	Male and female	
3.5.4 Age	Not reported. Not reported, but the death rates in the control cohort were stratified according to age, race, sex and calendar year	
3.5.5 Diseases	Not reported	
3.5.6 Smoking status	Not reported, but presumably both	
3.6 Administration/ Exposure		
3.6.1 Exposure Route	Not reported but presumably mainly by inhalation.	
3.6.2 Exposure Situation	Workplace, range of activities involved in the manufacture and use of ethylene oxide. The subjects averaged 4.9 years of exposure.	
3.6.3 Exposure concentration(s)	Steriliser operators: 4.3 ppm calculated based on 627 personal 8-hour samples obtained at the 13 out of 14 study plants between 1976 through 1985 Other workers: 2.0 ppm calculated based on 1888 personal samples Exposures were also modelled according to published [1,2] procedures to estimate historical levels. Many companies installed improved engineering controls in 1978 and exposures were thought to have been higher before that year.	
3.6.4 Method(s) to determine exposure	Personal monitoring using charcoal tubes. Data were obtained for exposure after 1977.	
3.6.5 Postexposure period	Average follow up period was 16.1 years	
3.7 Examinations		
3.7.1 Type of disease	Cancer, including hematopoietic, brain-nervous, digestive and respiratory systems, urinary organ and breast cancers	

3.7.2 Parameters	Standard mortality ratios (SMRs) were calculated for 99 causes of death using the NIOSH life table program. Internal exposure-response analyses were conducted using Cox regression analysis for haemopoietic and breast cancer.	
3.8 Further remarks	None	
	4 Results and Discussion	
4.1 Exposure		
4.1.1.1 Number of measurements	627 for steriliser operators and 1888 for other workers exposed to ethylene oxide	
4.1.1.2 Average concentrations	8-Hour time weighted averages (TWA) were: Steriliser operators: 4.3 ppm Other workers: 2.0 ppm Many companies installed improved engineering controls in 1978 and exposures were thought to have been higher before that year. Prior to 1984 the US occupational exposure limit was 50 ppm.	
4.1.1.3 Standard deviation	Not reported	
4.1.1.4 Date(s) of measurement(s)	Data were obtained for exposure after 1977.	
4.1.2 Other	None	
4.2 Number of cases for each disease / parameter under consideration	Results are shown for mortality from all causes and for heart disease and various cancers in Table 6.12.4/01-1.	
4.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)	There was little evidence for excess mortality for the cohort as a whole and no individual cancer site showed a significant excess at the 0.05 level with the exception of bone cancer for which there were only 6 deaths. Neither all haemopoietic cancers nor non-Hodgkin's lymphoma showed any increase. However, internal exposure response analyses found positive trends for haemopoietic cancers which were limited to males with a 15 year lag. The trend was driven by lymphoid tumours (non-Hodgkin's lymphoma, myeloma and lymphocytic leukaemia) which also have a positive trend with cumulative exposure for males with a 15 year lag. Haemopoietic cancer trends were somewhat weaker in the later analysis than in the earlier study and analysis restricted to the post 1987 data did not show any significant positive trends (exposure levels dropped sharply in the early 1980s). There was also an excess incidence of breast cancer in the highest cumulative exposure quartile using a 20 year lag.	
4.4 Other Observations	Cumulative exposure averaged 26.9 ±65.7 years (median 5.6 years). Exposure duration for males (37.8±87.6, median 7.6 years) was higher than that for females (18.2±38.2, median 4.6 years).	
	5 Applicant's Summary and conclusion	

5.1 Materials and methods	Mortality and cause of death data for a cohort of 18235 workers exposed to ethylene oxide (of whom 1222 were steriliser operators) was compared with the general US population. Only workers with at least 3 months exposure to ethylene oxide from the 1940s to the 1980s were included. Workers from 14 plants were selected because they had at least 400 person years at risk before 1978 and the cause of death was obtained from the US National Death Index. Life table analyses were conducted using the NIOSH life table program to calculate standard mortality ratios. Internal exposure-response analyses were conducted using Cox regression analysis for haemopoietic and breast cancer. Data were obtained for exposure after 1977 using personal monitors and exposures in previous years were estimated by modelling.	
5.2 Results and Discussion	98.5% of the cohort was traced successfully and there were 2852 deaths (data from the follow-up until 1998). Cumulative exposures averaged 26.9±65.7 years and monitoring data showed the 8-hour TWA exposure to be 4.3 ppm for steriliser operators and 2.0 ppm for other workers. No cancer site showed a significant excess at the 0.05 level with the exception of bone cancer based on small numbers of tumours. Internal exposure-response analyses found positive trends for haemopoietic cancers in males with a 15 year lag and breast cancer in females with a 20 year lag. Analyses of haemopoietic cancers in the post 1987 period did not show any significant positive trends.	
5.3 Conclusion	There was no overall evidence of excess cancer mortality in the cohort. However, exposure-response analyses showed an association between increased exposure and some types of haemopoietic cancer in males and breast cancer in females when 15 and 20 year lag periods, respectively, were taken into consideration. Exposure levels dropped considerably in the early 1980s and exposure during the period associated with the increase in haematopoietic and breast tumours would have been greater than the measured values reported in this study. Analyses of haemopoietic cancers in the period after 1987 did not show any evidence of a positive trend.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	None	
5.4 Other	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	

Materials and Methods	The summary of the applicant is acceptable with minor additions made by the eCa. *3.1.2.2 Purity*: The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study. 3.6.2 the following information should be added: .“The subjects averaged 4.9 years of exposure to the gas“. Many companies installed improved engineering controls in 1978 and exposures were thought to have been higher before that year.
Results and Discussion	
Conclusion	The conclusion of the applicant is acceptable with minor additions made by the eCa.
Reliability	2
Acceptability	The study is acceptable.
Remarks	

[1]Greife, A., Hornung, R., Stayner, L. and Steenland, K. Development of a model for use in estimating exposure to ethylene oxide in a retrospective cohort mortality study. Scandinavian Journal of Work and Environmental Health, 14, 29-30, 1988.

[2]Hornung, R., Greife, A., Stayner, L., Steenland, K., Herrick, R., Elliot, L., Ringenburg, V. and Morawetz, J. Statistical model for prediction of retrospective exposure to ethylene oxide in an occupational mortality study. American Journal of Industrial Medicine, 25, 825-836, 1994

Table 6.12.4/01-1: Mortality in the ethylene oxide cohort (n=18325*)

Cause (ICD-9 code)	Observed deaths	SMR (95% CI)	Male SMR (95% CI)	Female SMR (95% CI)
All causes	2852	0.90 (0.88-0.93)	0.94 (0.89-0.99)	0.86 (0.81-0.91)
Coronary heart disease (410-414)	669	0.92 (0.86-0.98)	1.04 (0.85-1.04)	0.87 (0.78-0.99)
All cancers (140-208)	860	0.98 (0.92-1.03)	0.94 (0.95-1.16)	0.92 (0.84-1.01)
Stomach (151)	25	1.07 (0.74-1.49)	0.87 (0.44-1.52)	1.34 (0.71-2.29)
Pancreas (157)	38	0.92 (0.69-1.21)	1.03 (0.64-1.61)	0.82 (0.45-1.30)
Lung (162)	258	1.05 (0.95-1.17)	1.05 (0.89-1.23)	1.05 (0.86-1.27)
Prostate (185)	37	1.29 (0.91-1.78)	1.29 (0.91-1.78)	Not applicable
Kidney (189.0-189.2)	21	1.19 (0.80-1.72)	1.51 (0.85-2.49)	0.78 (0.28-1.28)
Brain (191-192)	14	0.59 (0.36-0.91)	0.52 (0.19-1.13)	0.65 (0.25-7.37)
Bone (170)	6	2.82 (1.23-5.56)	3.51 (0.96-8.98)	2.04 (0.25-7.37)
Breast cancer (174)	103	0.99 (0.84-1.17)	2.04 (0.05-11.37)	0.99 (0.81-1.20)
All haematopoietic (200-208)	79	1.00 (0.79-1.24)	1.09 (0.79-1.47)	0.91 (0.84-1.25)
Non-Hodgkin's lymphoma (200, 202)	31	1.00 (0.72-1.35)	1.29 (0.78-2.01)	0.73 (0.38-1.29)
Hodgkin's disease	6	1.24 (0.53-2.43)	1.83 (0.59-4.27)	0.47 (0.05-11.87)
Myeloma (203)	13	0.92 (0.54-0.87)	0.61 (0.17-1.56)	1.19 (0.54-2.26)
Leukaemia (204-208)	29	0.99 (0.71-1.36)	0.97 (0.52-1.63)	1.020.57-1.68)

*These mortalities include the entire cohort. Subsequent exposure response analyses are based on a reduced cohort in which one small plant without adequate exposure data (4% of the cohort) was excluded

Section A6.12.4/02 **Annex Point IIA6.12.4**	**Epidemiological Study** *Cohort study*	
	1 Reference	**Official use only**
1.1 Reference	Steenland, K., Whelan, E., Deddens, J., Stayner, L. and Ward, E.(2003) Ethylene Oxide and Breast Cancer Incidence in a Cohort Study of 7576 Women (United States). Cancer Cause and Control, 14, 531-539	

1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Companies with letter of access	None	
1.2.3 Criteria for data protection	No data protection claimed	
	Guidelines and Quality Assurance	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not applicable, occupational exposure to ethylene oxide	
3.1.2 Specification	Not reported	
3.1.2.1 Description	Not reported	
3.1.2.2 Purity	Not reported	x
3.1.2.3 Stability	Not reported	
3.2 Type of study	Cohort study based on women in the cohort described in the NIOSH study summarised in 6.12.4/01.	
3.3 Method of data collection	Interviews, death certificates, cancer registries and medical records. Interviews were obtained for 68% of the cohort.	
3.4 Test Persons / Study Population		
3.4.1 Selection criteria	Cohort study involving workers at chemical factories where ethylene oxide was produced or converted into other substances or where it was used as a sterilant. Only workers with at least 3 months exposure to ethylene oxide from the 1940s to the 1980s were included. Workers from 14 plants were selected because they had at least 400 person years at risk before 1978. Cancer incidence information was sought by interviews of 7576 women (76% of those in the original cohort) or their next of kin who had worked for at least one year.	
3.4.2 Number of test persons per group/cohort size	7576	
3.4.3 Sex	Females	
3.4.4 Age	Not reported, but the analyses were stratified by age (5 year categories), calendar time and race/ethnicity. Mean year of birth for included cases was 1932 (SD = 11.3).	
3.4.5 Diseases	Not reported	

3.4.6 Smoking status	Not reported, but presumably both	
3.5 Controls	Yes	
3.5.1 Type of control	National population of the USA	
3.5.2 Number of test persons per group/cohort size	Not reported but would be much greater than the size of the cohort for the exposed group	
3.5.3 Sex	Female	
3.5.4 Age	Not reported	
3.5.5 Diseases	Not reported	
3.5.6 Smoking status	Not reported	
3.6 Administration/ Exposure		
3.6.1 Exposure Route	Not reported, presumably mainly inhalation	
3.6.2 Exposure Situation	Workplace, range of activities involved in the manufacture and use of ethylene oxide. Median cumulative exposure was 14.0 ppm-years for cases included in the study, mean exposure duration was 13.0 years (SD = 9.2).	
3.6.3 Exposure concentration(s)	Steriliser operators: 4.3 ppm Other workers: 2.0 ppm Exposures were also modelled according to published procedures[17,18] to estimate historical levels. Many companies installed improved engineering controls in 1978 and exposure was thought to be higher before that year.	
3.6.4 Method(s) to determine exposure	Personal monitoring using charcoal tubes. Data were obtained for exposure after 1977.	
3.6.5 Postexposure period	Follow up for breast cancer incidence was continued until the end of December 1998.	
3.7 Examinations		
3.7.1 Type of disease	Invasive female breast cancer (ICD 9th revision code 174) and in situ breast cancer (ICD 9th revision code 233.0)	
3.7.2 Parameters	Life table analyses of the cohort were done using the NIOSH Life-Table Analysis system using referent rates from SEER (Surveillance, Epidemiology and End results). Internal exposure-response analyses were conducted using Cox regression with interviews for the entire cohort and for the sub cohort.	
3.8 Further remarks		
	4 Results and Discussion	
4.1 Exposure		

4.1.1.1 Number of measurements	627 for steriliser operators and 1888 for other workers exposed to ethylene oxide	
4.1.1.2 Average concentrations	8-Hour time weighted average were: Steriliser operators: 4.3 ppm Other workers: 2.0 ppm Many companies installed improved engineering controls in 1978 and exposures were thought to have been higher before that year. Prior to1984 the US occupational exposure limit was 50 ppm.	
4.1.1.3 Standard deviation	Not reported	
4.1.1.4 Date(s) of measurement(s)	Data were obtained for exposure after 1977.	
4.1.2 Other	None	
4.2 Number of cases for each disease / parameter under consideration	5139 interviews were obtained corresponding to 68% of the cohort.	
4.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)	Completed interviews were obtained for 5139 (68%) of the 7576 women in the cohort. The principal reason for no interview was failure to locate the respondent. There were 319 incidents of breast cancer identified in the cohort to the end of 1998 and 39% had died by the end of 1998 (124/319). The data did not indicate any overall excess of breast cancer incidence among the cohort as a whole compared to the US population (Table 6.12.4/02-1) but cancer incidence was probably under ascertained because of the inability to locate some cohort members. Respondents in the upper quartile of cumulative exposure with a 15-year lag period had a 27% increase in breast cancer incidence compared with the non exposed population and this was increased to 34% after excluding *in situ* cases.	
4.4 Other Observations	For the entire cohort the average duration of exposure was 10.7±9.2 years and 1327 of the cohort (18%) had died.	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	Breast cancer incidence was studied in a cohort of of 7576 women employed for at least one year and exposed to ethylene oxide for an average of 10.7 years. Breast cancer incidence was ascertained via interviews, death certificates, cancer registries and medical records. Interviews were obtained with 68% of the cohort. The standardised incidence ratio was calculated using the NIOSH Life-Table Analysis system with referent rates from SEER (Surveillance, Epidemiology and End results). Internal exposure-response analyses were conducted using Cox regression for the entire cohort and for the sub cohort with interviews.	
5.2 Results and Discussion	There was no increase in breast cancer in the cohort as a whole compared with the US population but respondents in the upper quartile of cumulative exposure with a 15 year lag period had a 27% increase in breast cancer incidence compared with the non exposed population.	

5.3 Conclusion	The results suggest that ethylene oxide exposure is associated with an increased incidence of breast cancer but there are some uncertainties in the findings due to inconsistencies in the exposure response data and possible biases due to patterns of non response and cancer ascertainment.	
5.3.1 Reliability	2	
5.3.3 Deficiencies	None	
5.4 Other	None	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods	The summary of the applicant is acceptable with minor additions made by the eCA: *3.1.2.2 Purity:* The purity is not reported in the published article. As discussed in the CAR, section A.1.2. Composition of the substance (reference specifications), the production of ethylene oxide consistently yields the active substance in high purity (generally above 99 %). It is not expected that today's production process is significantly different from the production process at the time when this article was written. The principles of the ethylene oxide production has remained unchanged since the 1930s. In the current production of ethylene oxide, some impurities are identified, but none detected above significant level (all below 0.01 %). Furthermore, based on the identity of these impurities and the hazardous profile of the active substance itself, the eCA has no reason to believe that the impurities will have any impact on the findings of this study.	
Results and Discussion	The summary of the applicant is acceptable with minor additions made by the eCa.	
Conclusion	The summary of the applicant is acceptable with minor additions made by the eCa.	
Reliability	2	
Acceptability	The study is acceptable.	
Remarks		

Table 6.12.4/02-1: Rate ratios for breast cancer incidence by cumulative exposure to ethylene oxide (ppm-days) for the entire cohort

	0 (lagged out)	<647	647-2026	2026-4919	4919-14620	>14620	Combined exposed	Test for trend for cumulative exposure or log cumulative exposure [b]
15-Year lag								
External referent[a]	0.88 (0.67-1.04)	0.77 (0.56-1.03)	0.77 (0.56-1.03)	0.94 (0.69-1.25)	0.83 (0.61-1.11)	1.27 (0.94-1.69)	0.89 (0.78-1.01)	Linear, $p = 0.002$, log $p = 0.05$
Observed case[c]	81	45	46	46	45	48	230	
		<855	855-2596	2596-6343	6343-16447	>16447		
No lag								
External referent[a]	Not applicable	0.74 (0.57-0.97)	0.81 (0.62-1.04)	0.92 (0.70-1.18)	0.91 (0.70-1.17)	1.02 (0.79-1.30)	0.87 (0.77-0.97)	Linear, $p = 0.16$ log $p = 0.08$
Observed case[c]	Not applicable	60	62	63	62	64	311	

[a] External referent is US population, SEER cancer incidence rates, 1970-1998, indirectly stratified for age (5 year categories), ethnicity (white/non white) and calendar time (5 year categories)
[b] Test for trend (internal referent) calculated by Poisson distribution adjusted for age (5 year categories), calendar time (5 year categories) and ethnicity (white/non white)
[c] 311 of 319 cases were included, 8 cases were diagnosed before 1970 when SEER rates became available

Section A6.12.4/03 **Annex Point IIA6.12.4**	**Epidemiological Study** *Meta analysis*	
	1 Reference	**Official use only**
1.1 Reference	Teta, M., Sielken, R. L., and Valdez-Flores, C.(1999) Ethylene Oxide Cancer Risk Assessment Based on Epidemiological Data: Application of Revised Regulatory Guidelines Risk Analysis: 19, 1135-1155	
1.2 Data protection		
1.2.1 Data owner	Data published	
1.2.2 Companies with letter of access	None	
1.2.3 Criteria for data protection	No data protection claimed	
	Guidelines and Quality Assurance	
	3 Materials and Methods	
3.1 Test material		
3.1.1 Lot/Batch number	Not applicable, this was a meta analysis of data from 10 other studies.	
3.1.2 Specification	Not applicable, this was a meta analysis of data from 10 other studies.	
3.1.2.1 Description	Not applicable, this was a meta analysis of data from 10 other studies.	
3.1.2.2 Purity	Not applicable, this was a meta analysis of data from 10 other studies.	
3.1.2.3 Stability	Not applicable, this was a meta analysis of data from 10 other studies.	
3.2 Type of study	Meta analysis	
3.3 Method of data collection	Data taken from 10 cohort studies	
3.4 Test Persons / Study Population	Non-entry field	
3.4.1 Selection criteria	Ethylene oxide epidemiological studies available as of 1993 were identified and examined as a part of a meta-analysis. One study focusing on breast cancer in females from 1995 was excluded	
3.4.2 Number of test persons per group/cohort size	A total of approximately 33000 workers	
3.4.3 Sex	Male and female	

3.4.4 Age	Not reported	
3.4.5 Diseases	Not reported	
3.4.6 Smoking status	Not reported, but presumably both	
3.5 Controls	Yes	
3.5.1 Type of control	Varied according to the study	
3.5.2 Number of test persons per group/cohort size	Not reported	
3.5.3 Sex	Male and female	
3.5.4 Age	Not reported	
3.5.5 Diseases	Not reported	
3.5.6 Smoking status	Not reported	
3.6 Administration/ Exposure		
3.6.1 Exposure Route	Not reported	
3.6.2 Exposure Situation	Workplace, range of activities involved in the manufacture and use of ethylene oxide	
3.6.3 Exposure concentration(s)	Only three of the studies included quantitative exposure estimates. Levels would have varied.	
3.6.4 Method(s) to determine exposure	Direct measurement and modelling	
3.6.5 Postexposure period	See Table 6.12.4/-3-1	
3.7 Examinations		
3.7.1 Type of disease	All cancers, pancreatic, brain, stomach cancer, leukaemia, non-Hodgkin's lymphoma	
3.7.2 Parameters	Meta analysis according to revised EPA guidelines.	
3.8 Further remarks		
	4 Results and Discussion	
4.1 Exposure		
4.1.1.1 Number of measurements	Not reported	
4.1.1.2 Average concentrations	Not reported but would have varied during the period under consideration. In the early years of ethylene oxide production (1940s) levels averaged around 14 ppm and about 5-10 ppm in the 1950s but peak ppm values are known to have been much higher than the	

	estimated 8-hour time weighted average. From the late 1960s to the mid 1980s levels in sterilant operations were reported to be from 20-75 ppm and only in 1984 was the exposure limit in the USA reduced from 50 ppm to 1 ppm time weighted average.	
4.1.1.3 Standard deviation	Not applicable	
4.1.1.4 Date(s) of measurement(s)	See response in 4.1.1.2	
4.1.2 Other	None	
4.2 Number of cases for each disease / parameter under consideration	A total of 876 cancers in the 10 studies (vs. 928 expected)	
4.3 SMR (Standard mortality ratio), RR (relative risk), OR (Odds ratio)	For all cancers the SMR standardised for age, sex and calendar year was 0.94 (95% confidence interval 0.85-1.05). For pancreatic, brain, and stomach cancer, leukaemia and non-Hodgkins lymphoma the SMR ranged from 0.94-1.34 but these were not statistically different from 1.0 (Table 6.12.4/03-2). The meta-SMR for non-Hodgkin lymphoma was moderately increased with borderline statistical significance (95% CI = 0.96-1.89), but there were no positive trends with duration, intensity or latency.	
4.4 Other Observations	There were no statistically significant positive trends with duration, intensity or latency with the exception of brain tumours; however, the trend with latency was based on only four studies which provided brain cancer data by time since first exposure. The meta-SMR for brain cancer (0.96) was not elevated based on seven studies that reported results for this cause. There were more leukaemia cases than expected in the longest latency category (14 compared with 7.9 expected).	
	5 Applicant's Summary and conclusion	
5.1 Materials and methods	A meta-analysis was conducted using data from 10 cohorts of workers exposed to ethylene oxide. The incidence of all cancers and cancer of the pancreas, brain, stomach, leukaemia and non-Hodkin's lymphoma was comparable with the expected values.	
5.2 Results and Discussion	The analysis involved nearly 33,000 workers in which there were 876 cancers. There was no statistically significant increase in the incidence of all cancers or cancer of the pancreas, brain, stomach, leukaemia or non-Hodgkin's lymphoma in the exposed group. There were no statistically significant positive trends with duration, intensity or latency for any cancer type, with the exception of brain cancer; however, the trend with latency was based on only four studies which provided brain cancer data by time since first exposure. The meta-SMR for brain cancer (0.96) was not elevated based on seven studies that reported results for this cause. However, there were more leukaemia cases than expected in the longest latency category (14 observed vs. 7.9 expected). "	

5.3 Conclusion	Exposure to ethylene oxide did not result in an increase in the risk of cancer.	
5.3.1 Reliability	2	
5.3.3 Deficiencies	None	
5.4 Other	None	
	Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State	
Date	3 March 2020	
Materials and Methods		
Results and Discussion		
Conclusion	The summary of the applicant is acceptable.	
Reliability	2	
Acceptability	The study is acceptable.	
Remarks		

Table 6.12.4/03-1: Details of cohort studies used in meta-analysis

Author	Country	Workers	Cancers	Average duration (years)	Average observation (years)
Hogstedt	Sweden	175	20	3-30	?
Hogstedt	Sweden	355	13	9-13	?
Hagmar	Sweden	2170	40	?	11.6
Kiesselbach	Germany	2658	68	9.6	15.5
Morgan[a]/Divine	USA	767	19	>20	?
Greenburg[a]/Teta	USA	1896	110	5.4	27.2
Steenland	USA	18254	343	4.9	16.1
Bisanti	Italy	1971	43	ca. 7	ca. 9
Gardner	UK	2876	85	?	?
Olsen	USA	1361	75	5.7	24.5

[a] Excluded from meta-analysis

Table 6.12.4/03-2: SMRs and trends in ethylene oxide meta-analysis

Endpoint	Observed/ Expected	Meta-SMR[a]	95% Confidence interval	Duration	Intensity	Latency
All cancer	876/928	0.94	0.85-1.05[b]	-	-	-
Pancreas	37/39	0.95	0.69-1.31	No	No	No
Brain	25/26	0.96	0.49-1.91[b]	No	No	Yes[c]
Stomach	59/48	1.23	0.71-2.13[b]	No	No	No
Leukaemia	35/32	1.08	0.61-1.93[b]	No	No	No
w/o Hogstedt study	30/32	0.95	0.64-1.35	-	-	-
Non Hodgkins lymphoma	33/25	1.34	0.96-1.89	No	No	No

[a] SMR = Standardised mortality ratio
[b] Adjusted for heterogeneity
[c] $p<0.05$, based on 4 studies with latency data for brain