REGULATION (EU) NO 528/2012 CONCERNING THE MAKING AVAILABLE ON THE MARKET AND USE OF BIOCIDAL PRODUCTS

***Assessment of active substances***

**COMPETENT AUTHORITY REPORT**

# Ethylene oxide

# Product type 2

**Disinfectants and algaecides not intended for direct application to humans or animals**

**EC Number:** 200-849-9

**CAS Number:** 75-21-8

**Applicant:** EtO BPD Consortium

**Contact details of evaluating CA:** Norwegian Environment Agency, Oslo, Norway

**Version number: 4** **Date:** 21.04.2021

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# STATEMENT OF SUBJECT MATTER AND PURPOSE OF THE CAR

This assessment report has been established as a result of the assessment of the active substance ethylene oxide in product-type 2 (disinfectants and algaecides not intended for direct application to humans or animals), carried out in the context of Regulation (EU) No 528/2012 (BPR) with a view to the possible approval of this substance.

On 1 December 2009 the Norwegian competent authorities received a dossier from the applicant. The Evaluating Competent Authority (eCA) accepted the dossier as complete for the purpose of the assessment on 6 April 2010, based on the provisions of Directive (EC) No 98/8 (BPD).

On 5 March 2020, the Evaluating Competent Authority submitted to ECHA a copy of the assessment report containing the conclusions of the assessment, hereafter referred to as the competent authority report (CAR).

Subject to Article 90 of the BPR, the eCA's evaluation has been carried out in accordance with the provisions of the BPR, but on the basis of the information provided in the dossier submitted under the BPD.

Before submitting the CAR to ECHA, the applicant was given the opportunity to provide written comments in line with Article 8(1) of the BPR.

According to the biocides Review Program Regulation/Biocides working procedure:

- If the CMR-based exclusion criteria are met, the RAC opinion on CLH needs to be available at the time of submitting the CAR.

- If the substitution criteria are met because of CMR properties, it is highly preferable and therefore strongly recommended that the RAC opinion on harmonised classification and labelling is available at the time of submitting the CAR. In any case if the substitution criteria are met, a CLH dossier needs to be submitted by the time of submitting the CAR.

- Regarding substances not considered to meet the exclusion or substitution criteria, if changes are proposed to an already existing harmonised classification and labelling, or no harmonised classification and labelling is available for the active substance, it is strongly recommended that a CLH dossier is submitted by the time of submitting the CAR.

In the case of ethylene oxide, a harmonised classification according to Regulation (EC) No 1272/2008 already existed at the time of submitting the CAR to ECHA (with the most recent amendments being adopted in the 14$^{th}$ ATP). No changes to the harmonised classification are proposed by the eCA.

In order to review the CAR and the comments received on it, consultations of technical experts from all Member States (peer review) were organised by ECHA. Revisions agreed upon were presented at the Biocidal Products Committee and its Working Groups meetings and the competent authority report (CAR) was amended accordingly.

The aim of the assessment report is to support the opinion of the Biocidal Products Committee and a decision on the approval of ethylene oxide for product-type 2 and, should it be approved, to facilitate the authorisation of individual biocidal products. In the assessment of applications for product authorisation, the provisions of the BPR shall be applied, in particular the provisions of Chapter IV, as well as the common principles laid

down in Annex VI.

For the implementation of the common principles of Annex VI, the content and conclusions of the assessment report, which is available from the web-site of ECHA shall be taken into account.

However, where conclusions of this assessment report are based on data protected under the provisions of the BPR, such conclusions may not be used to the benefit of another applicant, unless access to these data for that purpose has been granted to that applicant.

# BPC OPINION

Please see the BPC opinion on the application for approval of the active substance ethylene oxide in product type 2 (ECHA/BPC/272/2020), adopted on 3 December 2020.

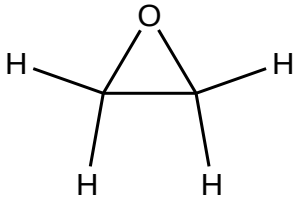
# ASSESSMENT REPORT

# SUMMARY

# 1. PRESENTATION OF THE ACTIVE SUBSTANCE

## 1.1 IDENTITY OF THE ACTIVE SUBSTANCE

Table 1.A.1 Main constituents

| Main constituent(s) | |
|---|---|
| **ISO name** | Ethylene oxide |
| **IUPAC or EC name** | Oxirane / Ethylene oxide |
| **EC number** | 200-849-9 |
| **CAS number** | 75-21-8 |
| **Index number in Annex VI of CLP** | 603-023-00-X |
| **Minimum purity / content** | 99.9% |
| **Structural formula** |  |



No relevant or significant impurities or additives are present in the active substance. Please see confidential annex for further information.

## 1.2 INTENDED USES AND EFFECTIVENESS

Table 1.A.2 Use of the active substance

| **Product type** | **2** |
|---|---|
| **Intended use pattern(s)** | Industrial sterilisation of single use medical devices, which cannot be sterilised by other means, before these are made available on the market. |
| **Users** | Industrial users |

Table 1.A.3 Effectiveness of the active substance

| **Function** | Sterilising agent for the sterilisation of single-use medical devices before they are made available on the market |
|---|---|
| **Organisms to be controlled** | Bacteria (including spores), fungi and viruses (These are the target organisms intended by the applicant. However, please note that efficacy of the active substance is only demonstrated for bacteria.) |

| | |
|---|---|
| **Limitation of efficacy including resistance** | Many factors will affect the efficacy of ethylene oxide, resulting in micro-organisms being more resistant under certain conditions, e.g. low humidity. However, no reports of true ethylene oxide resistance are known to the eCA. It is expected that the development of resistance towards a substance with such a basic mode of action towards several vital molecules within the cell is unlikely. |
| **Mode of action** | Ethylene oxide is a highly reactive alkylating agent and reacts with proteins, amino acids and the nucleic acids. |

# 2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE ACCORDING TO THE CLP CRITERIA

A revised harmonised classification is available for ethylene oxide, according to Regulation (EC) No 1272/2008 (CLP) as amended by Commission Delegated Regulation (EU) 2020/217 of 4 October 2019 (see table 2.1). The classification as amended by Commission Delegated Regulation (EU) 2020/217, i.e. the 14th ATP to CLP, shall apply from 9 September 2021, but may be used before this date. The eCA does not propose any changes to the revised harmonised classification.

## 2.1 HARMONISED CLASSIFICATION AND LABELLING FOR THE ACTIVE SUBSTANCE ACCORDING TO REGULATION (EC) NO 1272/2008

Table 2.A.4 Harmonised classification and labelling of the active substance

| Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors, ATE values | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hazard Class and Category Code(s) | Hazard statement(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| '603-023-00-X | ethylene oxide; oxirane | 200-849-9 | 75-21-8 | Flam. Gas 1<br>Press. Gas<br><br>Carc. 1B<br><br>Muta. 1B<br><br>Repr. 1B<br><br><br><br>Acute Tox. 3<br>Acute Tox. 3<br><br>STOT SE 3<br><br>STOT SE 3<br><br><br>STOT RE 1 | H220 Extremely flammable gas<br><br>H350 May cause cancer<br>H340 May cause genetic defects<br><br>H360Fd May damage fertility. Suspected of damaging the unborn child.<br>H331 Toxic if inhaled<br>H301 Toxic if swallowed<br>H335 May cause respiratory irritation<br>H336 May cause drowsiness or dizziness<br>H372 Causes damage to organs (nervous system)<br>H314 Causes severe skin burns and eye damage | GHS02<br>GHS08<br>GHS06<br>GHS05<br>Dgr | H220<br><br>H350<br><br>H340<br><br>H360Fd<br><br><br><br>H331<br>H301<br><br>H335<br><br>H336<br><br><br>H372 | | inhalation: ATE = 700ppm (gases) oral: ATE = 100 mg/kg bw' | U* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Skin Corr. 1<br><br>Eye Dam. 1 | H318 Causes serious eye damage | | (nervous system) H314 | | | |

* Note U (of CLP as amended by Commission Delegated Regulation (EU) 2016/918 of 19 May 2016): When put on the market gases have to be classified as 'Gases under pressure', in one of the groups compressed gas, liquefied gas, refrigerated liquefied gas or dissolved gas. The group depends on the physical state in which the gas is packaged and therefore has to be assigned case by case. The following codes are assigned: Press. Gas (Comp.), Press. Gas (Liq.), Press. Gas (Ref. Liq.), Press. Gas (Diss.). Aerosols shall not be classified as gases under pressure (See Annex I, Part 2, Section 2.3.2.1, Note 2).

## 2.2 PROPOSED CLASSIFICATION AND LABELLING AND PACKAGING FOR THE REPRESENTATIVE PRODUCT(S)

The product, D495 SS is consisting of pure active substance, without any co-formulants. Hence, the hazards for the products are identical to those of the active substance and the classification and labelling is the same. Please see section 2.1.

The hazard statement code H280 should be assigned to the product D495 SS.

Commercial products are provided in containers made of stainless steel and there are no indications of corrosion or other effects on such containers during storage under recommended conditions.

# 3. SUMMARY OF THE HUMAN HEALTH RISK ASSESSMENT

## 3.1 SUMMARY OF THE ASSESSMENT OF EFFECTS ON HUMAN HEALTH

Table 3.A.5 Summary of the assessment of effects on human health

| Endpoint | Brief description |
|---|---|
| **Toxicokinetics** | A pulmonary uptake of 75-80% for humans has been reported. Due to the physicochemical properties of EtO and the described working condition, inhalation is the most relevant route of exposure for EtO.<br>Ethylene oxide is considered to be rapidly and fully distributed through the body. The half-life in human blood has been calculated to be 48 minutes; thus no accumulation of ethylene oxide is to be expected.<br>At a steady state in humans 20-25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound.<br>Two major pathways of ethylene oxide metabolism have been established: conjugation with glutathione by glutathione transferase and hydrolysis to ethylene glycol (probably chemical rather than via epoxide hydrolase).<br>The major amount of ethylene oxide is metabolized by hydrolysis, while ca. 20 % are converted to glutathione conjugates. |
| **Acute toxicity** | Ethylene oxide is toxic if swallowed and toxic if inhaled.<br>Clinical signs of toxicity associated with inhalation exposure were frequent movement and preening, nasal discharge, lacrimation, diarrhoea, gasping and occasionally salivation, vomiting of frothy colourless/yellow mucus and convulsions with laboured breathing.<br><br>In addition, EtO may cause drowsiness or dizziness and is classified for this property (STOT SE 3: H336). |
| **Corrosion and irritation** | Based on weight of evidence from available animal and human data EtO is corrosive to skin. Considering ethylene oxide is classified as corrosive to skin, it is explicitly considered to cause serious eye damage.<br>EtO is also classified as an active substance that causes respiratory irritation |
| **Sensitisation** | There is a lack of evidence for a potential to cause skin sensitisation. The available data on asthmatic symptoms do not present evidence that justifies classification for specific respiratory sensitisation to ethylene oxide. Hence, no classification is warranted for these hazard classes. |
| **Repeated dose toxicity** | The subchronic repeated dose inhalation toxicity studies (rat, mice, dog, monkey, rabbit and guinea pig) were all published studies with a reliability score of 2 or 3. Despite the lack of full OECD guideline studies in 2 species, the available studies indicate that ethylene oxide affects different species, at concentrations in the same dose range as low as 50 ppm.<br><br>In the different species mentioned above, adverse effects seen were such as; increased mortality, neurotoxicity, genotoxicity (e.g. increased chromosomal aberrations and sister chromatid exchange), anemia and various effects/histopathological |

| | |
|---|---|
| | changes in organs such as lungs, liver, kidney, testis, adrenals. In addition, signs of anemia were seen in rat, mice and dog.<br><br>Case reports on operators that had an occupational and subchronic exposure to ethylene oxide, indicate that ethylene oxide affects humans at concentrations ranging from 36-700 ppm. The workers showed primarily neurological effects such as headaches, nausea, speech disorders, impairment of short-term memory, vertigo, incoordination, sensorimotor neuropathy, paraesthesia, distal limb weakness, cutaneous sensory loss and polyneuropathy<br><br>Due to limitations of the available data, is not possible to derive a reliable sub-chronic NOAEL for risk assessment. Ethylene oxide is classified as a genotoxic carcinogen, hence a subchronic NOAEL is not considered relevant for the human risk assessment. |
| **Genotoxicity** | Ethylene oxide has been shown to be genotoxic in virtually all systems studied, these include tests in yeast and fungi as well as in bacterial and mammalian systems and also in transgenic mice. Human data confirm the genotoxic properties of ethylene oxide. Ethylene oxide is a direct alkylating agent that reacts with nucleophiles without the need for metabolic transformation. |
| **Carcinogenicity** | The studies provide conclusive evidence that ethylene oxide was carcinogenic in two species (rat and mice).<br>Despite limited evidence in humans for the carcinogenicity of ethylene oxide, IARC consider ethylene oxide to be carcinogenic to humans (Group 1). |
| **Reproductive toxicity** | Ethylene oxide causes adverse effects on fertility and there are also indications on the developmental effects of ethylene oxide. Based on this it was concluded that EtO may damage fertility and that it is suspected of damaging the unborn child (H360Fd). |
| **Neurotoxicity** | EtO causes damage to the nervous system through prolonged or repeated exposure and is classified as STOT RE 1, H372 (Causes damage to nervous system through prolonged or repeated exposure) |
| **Immunotoxicity** | No information provided. |
| **Disruption of the endocrine system** | Based on the information available, no final conclusion on the ED properties of ethylene oxide could be made. The eCA is of the opinion that additional testing is not justified in this particular case because of the following reasons:<br><br>1. The already known severe hazard profile of this substance: ethylene oxide fulfils the exclusion criteria of Article 5, paragraph 1a, b and c of BPR given the following classification of the active: Carc. 1B, Muta. 1B and Repr. 1B.<br><br>2. As a consequence of these properties, strict RMMs should be in place to avoid or minimise occupational exposure as far as technically feasible if EtO is to be used. As a non-threshold mode of action is assumed for tumour formation, a conservative semi-quantitative risk assessment is already performed. It should furthermore be kept in mind that there is no agreed methodology to |

| | |
|---|---|
| | consider endocrine disruption in the risk assessment. Consequently, no impact on the risk assessment would be expected if the substance were to be additionally considered as an ED.<br><br>3. Handling of ethylene oxide is not easy due to its physicochemical and human health related hazardous properties. It is not desirable to expose either laboratory personnel or laboratory animals to ethylene oxide, unless it is strictly necessary.<br><br>4. Due to the unspecific alkylating properties of the substance, the available test systems for determining the endocrine activity would be likely to give equivocal results (if tests were performed). If mechanistic studies were nevertheless conducted, it would be difficult to conclude that the mechanism would be solely a (non-) ED MoA. |
| **Other effects** | |

## 3.2 REFERENCE VALUES

Table 3.A.6 Reference values

| | Study | NOAEL/ LOAEL | Overall assessment factor | Value |
|---|---|---|---|---|
| **DMEL (inhalation) for professionals** | NTP TR 326, Mouse inhalation, Long term duration (Comparable to OECD guideline 451) | $BMDL_{10}$ calculation for alveolar/ broncheolar adenomas and carcinomas in female mice resulted in a value of 35.5 ppm.<br><br>Corrected dose descriptor: **28,65 ppm**.<br>[*Differences between exposure conditions mice (6h/day, 5 days/week) and human (8h/day, 5 days/week)*]<br>and<br><br>[Difference between activity level: at rest (5.3 $m^3$) versus light activity (10 $m^3$)]<br>and<br><br>[*Differences between occupational (48 weeks/year for 40 years) and lifetime (52 weeks/year for 75 year) exposure conditions*] | High to low extrapolation: 10,000<br><br>(*linearised approach 1:100,000; basis for dose descriptor was $BMDL_{10}$*) | 28,65 ppm/10,000 = 0.00287 ppm ≈ **3 ppb** |
| **DMEL (inhalation) general public living** | NTP TR 326, Mouse inhalation, Long term duration | $BMDL_{10}$ calculation for alveolar/broncheolar adenomas and carcinomas in female | High to low extrapolation: 100,000 | 6.3 ppm/100,000 = 0.000063 ppm ≈ |

| | | | | |
|---|---|---|---|---|
| **in the surrounding area of the plant** | (Comparable to OECD guideline 451) | mice resulted in a value of 35.5 ppm.<br><br>Corrected dose descriptor:<br>**6.3 ppm**.<br>[*Differences between exposure conditions mice (6h/day, 5 days/week) and human (24h/day, 7 days/week)*] | (*linearised approach 1:1 000,000; basis for dose descriptor was* $BMDL_{10}$) | **0.06 ppb** |

## 3.3 RISK CHARACTERISATION

Due to the highly specialised use of Ethylene oxide (EtO) and the high level of RMM applied at the treatment facilities (described in chapter B.3.3.), the exposure to industrial workers involved in EtO disinfection is not possible to model in a realistic way through the use of the existing exposure models. Monitoring of exposure to personnel at EtO disinfection plants is mandatory under occupational law (EN 689:2018), the exposure assessment was therefore performed using submitted monitoring data from EtO disinfection plants in several European states. The whole data set can be found in Doc. III-B.6.6, and some key statistics are presented here:

Table 3.A.7 Exposure monitoring statistics

| **Exposure monitoring statistics** | |
|---|---|
| CAS no. | 75-21-8 |
| IUPAC name | Oxirane, Ethylene oxide |
| | |
| **Overall descriptive statistics** | |
| Number of measured values | 49 |
| Arithmetic mean | 0.190 ppm (0.343 mg/m$^3$) |
| Standard deviation | 0.21 ppm (0.38 mg/m$^3$) |
| Max-value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 3** | |
| Number of measured values | 15 |
| National OEL-value | 0.5 ppm (0.84 mg/m$^3$) |
| Arithmetic mean | 0.018 ppm (0.033 mg/m$^3$) |
| Standard deviation | 0.0054 ppm (0.0097 mg/m$^3$) |
| Max. value | 0.0285 ppm (0.051 mg/m$^3$) |
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 1, 2 and 9** | |

| | |
|---|---|
| Number of measured values | 34 |
| OEL-value | 1 ppm (1.8 mg/m$^3$) |
| Arithmetic mean | 0. 266 ppm (0.48 mg/m$^3$) |
| Standard deviation | 0.214 ppm (0.38 mg/m$^3$) |
| Max. value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.055 ppm (0.1 mg/m$^3$) |
| | |
| **Overall distribution:** | |
| 10% Percentile | 0.014 ppm (0.025 mg/m$^3$) |
| 50% Percentile | 0.11 ppm (0.21 mg/m$^3$) |
| 75% Percentile | 0.278 ppm (0.5 mg/m$^3$) |
| 90% Percentile | 0.5 ppm (0.91 mg/m$^3$) |
| 95% Percentile | 0.8 ppm (1.45 mg/m$^3$) |

The identified work tasks involved in the sterilisation process are listed below as scenarios, but they have not been assessed individually.

Table 3.A.8 Summary of identified tasks

| **Summary table: Exposure scenarios** | | | |
|---|---|---|---|
| **Scenario number** | Scenario (e.g. mixing/ loading) | Primary or secondary exposure Brief description of scenario | **Exposed group (e.g. professionals, non-professionals, bystanders)** |
| **1.** | Pre-treatment | Transfer of products into the pre-treatment area | **Industrial** |
| **2.** | Mixing and loading | Loading of products into the treatment tank | **Industrial** |
| **3.** | Sterilisation | Sterilisation | **Industrial** |
| **4.** | Post treatment | Transfer of the treated products from the treatment tank to the aeration chamber | **Industrial** |
| **5.** | **Post treatment** | **Warehouse** | **Industrial** |

The submitted exposure figures were compared directly to the DMEL for EtO.

Table 3.A.9 Conclusion of risk characterisation for industrial user

| **Task/ Scenario** | **Tier/ PPE** | **DMEL (ppb)** | **Measured exposure (8-hour TWA) (ppb)** | **Measured exposure/ DMEL (%)** | **Acceptable (yes/no)** |
|---|---|---|---|---|---|
| **Overall max. value** | | 3 | 888 | 29600% | No |
| **Overall average** | | 3 | 190 | 6333% | No |
| **Max value country 9** | | 3 | 888 | 29600 | No |
| **Average country 9** | | 3 | 324 | 10792 | No |
| **Min. value country 9** | | 3 | 56 | 1850 | No |
| **Max value country 3** | | 3 | 28.5 | 950% | No |
| **Average country 3** | | 3 | 18 | 600% | No |
| **Min value country 3** | | 3 | 12 | 400% | No |

Actual exposure monitoring data from 4 EtO sterilisation plants in Europe was compared

directly with the DMEL-value. Even the minimum value from the submitted data set results in an exceedance of the DMEL-value of 400%. Thus, no acceptable risk for industrial workers involved in EtO disinfection could be demonstrated, based on an elevated lifetime cancer risk of $1 \times 10^{-5}$.

Table 3.A.10 Conclusion of risk characterisation for professional user

*Not applicable*

Table 3.A.11 Conclusion of risk characterisation for non-professional user

*Not applicable*

Table 3.A.12 Conclusion of risk characterisation for indirect exposure

The primary use of EtO is industrial disinfection of single use medical equipment before these are placed on the market. Once placed on the market, these items are no longer regulated by the BPR, but by the Medical Devise Regulation (MDR; (EU) 2017/745). The accepted residual levels, and thus, secondary exposure of EtO and ethylene chlorohydrin (ECH) under MDD is defined in the ISO-standard ISO 10993-7:2008. Secondary exposure to the general public from medical devise was therefore not further assessed.

Ethylene oxide from the treatment chamber and the aeration rooms is exhausted to the atmosphere via a catalytic converter which converts ethylene oxide to carbon dioxide and water with an efficacy of 99.5% - 99.9%. A $PEClocal_{air}$ has been calculated to be 0.0001251 mg/m3 (equivalent to 0.068 ppb) as an average air concentration 100 m from the source of emission (Section B.3.3). This distance is considered to represent the average distance between the emission source and the border of the industrial site.

Several studies of EtO in air in the surrounding area of EtO sterilisation plants, mainly from the U.S. have been published (See. A.4.1.2.4.). Some of these reported monitoring data exceeds the estimated $PEClocal_{air}$. Although significant variation is seen in the reported monitoring data, both with regards to the possible atmospheric background level of EtO and to the air concentrations caused by emissions from EtO sterilisation plants, these reports clearly indicate that EtO emissions from treatment plants should be considered, particularly where the treatment plants are located close to residential areas or in urban areas. No monitoring data from the surroundings of European sterilisation plants have been submitted.

In order to assess the risk level for the general public living in proximity to a sterilisation facility, thus being subject to secondary exposure to atmospheric emissions of EtO, an increased lifetime cancer risk level of $1 \times 10^{-6}$ was regarded as a tolerable risk level. An exposure duration of up to 24 hour per day, 7 days per week was assumed.

Based on these calculations, secondary exposure to atmospheric emissions of EtO results in an unacceptable risk to the general public.

Table 3.A.13 Conclusion of risk characterisation for general public

| Scenario | Tier | DMEL | Estimated exposure | Estimated exposure/ DMEL (%) | Acceptable (yes/no) |
|---|---|---|---|---|---|
| **General public, secondary exposure** | | 0.06 ppb | 0.07 ppb | 116.7% | No |

# 4. SUMMARY OF THE ENVIRONMENTAL RISK ASSESSMENT

## 4.1 FATE AND BEHAVIOUR IN THE ENVIRONMENT

From the intended use of ethylene oxide in PT 2, the only compartment to which there is direct release is the atmosphere. In chapter B.3, a PEC for air is calculated. There is no release of liquid waste containing ethylene oxide or degradants to STPs. The only possibility for exposure of other environmental compartments comes from deposition from air to water and soil, as summarised in the table below. This possibility for indirect release is addressed qualitatively, by assessing the likelihood of deposition from air based on physical-chemical properties and what is known about degradation and distribution of ethylene oxide between environmental compartments.

Relevant degradation products are ethylene glycol, formed from hydrolysis of the active substance in freshwater, and ethylene chlorohydrin, formed from hydrolysis of the active substance in saltwater.

Table 4.A.14 Summary table on compartments exposed and assessed

| Compartment | Exposed (Y/N) | Assessed (Y/N) |
|---|---|---|
| **Air (atmosphere)** | Y<br>Direct exposure of the active substance ethylene oxide.<br>No relevant degradation products identified for this compartment | Y<br>The exposure of the active substance has been quantitatively assessed (a $PEClocal_{air}$ has been calculated) |
| **Surface water and sediment** | Y[b)]<br>Potential indirect exposure of the active substance via the atmosphere.<br>Relevant degradants potentially formed in these compartments: ethylene glycol, ethylene chlorohydrin | Y<br>The exposure of the active substance and degradants has been qualitatively assessed, based on environmental fate information and physico-chemical properties. |
| **Soil** | Y<br>Potential indirect exposure of the active substance via the atmosphere.<br>Relevant degradants potentially formed in this compartment: ethylene glycol, ethylene chlorohydrin | |
| **STP** | N<br>No release to STPs from the intended use | N |

The atmosphere is hence the most relevant environmental compartment. No relevant degradants have been identified for this compartment. For the aquatic compartment, ethylene glycol should be regarded as a relevant degradant, based on information from open literature on a high degree of hydrolysis of ethylene oxide to ethylene glycol. Furthermore, from the hydrolysis of ethylene oxide in saltwater, ethylene chlorohydrin is also produced.

Table 4.A.15 Summary table on relevant metabolites/degradants

| Metabolite/degradant/ transformation or reaction product | Compartment | % Active Substance |
|---|---|---|
| **Ethylene glycol** | Surface water (freshwater), | Not quantified. Based on general knowledge from open literature that |

| | | |
|---|---|---|
| | hydrolysis | ethylene oxide hydrolyses to ethylene glycol in significant amounts. Ethylene glycol is therefore regarded as a relevant degradant. |
| **Ethylene chlorohydrin** | Surface water (saltwater), hydrolysis | Not quantified. Based on general knowledge from open literature that in saltwater, ethylene oxide hydrolyses to both ethylene chlorohydrin and ethylene glycol. |

The table below lists some relevant properties of the active substance for the environmental fate and behaviour assessment.

Table 4.A.16 Summary table on relevant physico-chemical and fate and behaviour parameters of the active substance

| Parameter | Value | Unit | Remarks |
|---|---|---|---|
| Molecular weight | 44.05 | g/mol | - |
| Log Octanol/water partition coefficient (Log $K_{ow}$) | -0.30 | Log 10 | - |
| Organic carbon/water partition coefficient ($K_{oc}$) | 3.237 | L/kg | QSAR estimation, MCI methodology, (KocWin v. 2.00) |
| Henry's Law Constant | 14-20 | Pa/$m^3$/mol | at 20ºC |
| Vapour pressure | 146 | kPa | at 20ºC |
| Biodegradability (ready / not ready) | Readily bio-degradable | - | Based on OECD 301C test (MITI, 1995) |
| $DT_{50}$ for biodegradation in sewage | ≈ 20 days | d | Modified BOD test (Conway *et al.*, 1983), value should be considered purely indicative |
| $DT_{50}$ for hydrolysis in surface water (fresh water) | 34.5-40.2 | d | at 12ºC and pH 7.4 |
| $DT_{50}$ for degradation in air | 38-578 | d | Different sources give different results, based on e.g. OH radical concentrations and assumptions of 12-h or 24-h days. Ethylene oxide has the potential to persist for a long time in the atmosphere. |
| Bioconcentration (BCF), aquatic | 3.16 | L/kg | QSAR estimation (BCFBAF v.3.01) |
| Bioconcentration (BCF), terrestrial | 0.85 | L/kg | QSAR estimation, Guidance on the BPR Vol. IV Part B, eqn. 104d. |

The hydrolysis degradants ethylene glycol and ethylene chlorohydrin are expected to biodegrade in the environment at a comparable rate to ethylene oxide or faster. Ethylene glycol is however regarded as hydrolytically stable.

Experimental log Kow values of ethylene glycol and ethylene oxide are -1.36 and 0.03, respectively. The log Koc for ethylene glycol and ethylene chlorohydrin is 1 L/kg and 1.904 L/kg, respectively (based on QSAR MCI methodology).

## 4.2 EFFECTS ASSESSMENT

Based on the available ecotoxicity data on fish, daphnids and algae, fish are the most sensitive towards ethylene oxide with 96-h $LC_{50}$ values (experimental and estimated)

between 80 and 90 mg/L.

For daphnids, available data indicate 48-h $EC_{50}$ values of 212 and 420 mg/L (experimental and estimated, respectively). No data on ethylene oxide toxicity towards algae are available, but the estimated 96-h $EC_{50}$ is 800 mg/L, and a 96-h $EC_{50}$ (algae) for propylene oxide, which is considered suitable for read-across to ethylene oxide, is 240 mg/L.

Only the basic set of ecotoxicity data is available. No long-term studies on aquatic organisms, or studies on the effects towards terrestrial organisms are provided.

Furthermore, PNEC values have not been established due to the limited exposure to the environment following the intended use – please see the elaborated reasoning given in chapter A.4.4.

Regarding ethylene glycol as a relevant hydrolysis degradant, the available information on the toxicity towards fish, invertebrates and algae indicates that it has lower aquatic acute toxicity towards aquatic organisms than ethylene oxide has. The lowest aquatic acute toxicity effect concentration is the ECOSAR estimated 96-h $EC_{50}$ value for green algae, of 3500 mg/L.

Based on the available information for ethylene chlorohydrin, it shows aquatic acute toxicity in the same order of magnitude as ethylene oxide. Fish seem to be the most sensitive towards ethylene chlorohydrin, with 96-h $LC_{50}$ values between 67 and 112 mg/L.

## 4.3 EXPOSURE ASSESSMENT

The only PEC value which has been calculated is the $PECloca l_{air}$, due to the direct emissions to air from the intended use of ethylene oxide. According to the applicant, the calculation is based on a realistic worst case assumption, namely a high capacity plant, a catalytic efficiency of 99.9 % and year round emission.

The possibility of exposure to other compartments, indirectly via deposition from air, is assessed qualitatively. In conclusion, the high vapour pressure of ethylene oxide (149 kPa at 20 °C) and hence rapid volatilisation makes partitioning to other compartments highly unlikely.

Table 4.A.17 Summary table on calculated PEC values

| **Scenario** | **$PECloca l_{air}$ [mg/m³]** |
|---|---|
| Emission from industrial sterilisation of single use medical devices | $1.25 \times 10^{-4}$ |

## 4.4 RISK CHARACTERISATION

The only direct emission to the environment will be to the atmosphere. A $PECloca l_{air}$ has been calculated, at $1.25 \times 10^{-4}$ mg/m³. In the human health assessment, the $PECloca l_{air}$ is compared with the DMEL for the general public living in the surrounding areas of European sterilisation plants. The $PECloca l_{air}$ exceeds the DMEL, and hence unacceptable risk is identified. Risks to terrestrial vertebrates with regard to inhalation effects and exposure from nearby sterilising facilities can therefore not be excluded. This aspect is however considered covered by the human health risk assessment. The eCA is of the opinion that requesting additional data specifically for the terrestrial risk assessment will not add anything to the overall outcome of the risk assessment.

It is highly unlikely that ethylene oxide will be deposited to water or soil from the atmosphere, due to the high vapour pressure and hence rapid volatilisation. It is in other

words not likely that the ethylene oxide emitted to the atmosphere from the intended use will impact organisms in other environmental compartments. Based on the effects assessment, any limited amounts of ethylene oxide potentially reaching water or soil from the atmospheric release would be expected to give concentrations which are far from harmful to organisms.

Any potential liquid waste containing ethylene oxide is not released to STPs and subsequently to surface waters, but handled specifically as toxic waste and disposed of without emissions to the environment.

## 4.5 ASSESSMENT OF EXCLUSION CRITERIA, SUBSTITUTION CRITERIA AND POP

Table 4.A.18 Assessment of exclusion criteria, substitution criteria and POP

| Exclusion criteria | Conclusion |
|---|---|
| Conclusion on CMR | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as Carc. 1B., Muta. 1B and Repr. 1B. |
| Conclusion on ED assessment | A final conclusion is not possible and not needed in this special case. |
| Conclusion on PBT and vP/vB criteria | Ethylene oxide does not fulfill the the P/vP or B/vB criteria. Ethylene oxide does fulfill the T criterion. |
| Conclusion on substitution criteria | The substitution criteria in BPR Article 10(1)(a) and (e) is met. |
| Conclusion on LRTAP/POP assessment | Based on the available data, ethylene oxide persists in the atmosphere for a long time. However, the high vapour pressure makes it unlikely that ethylene oxide will deposit to other environmental compartments upon release to the atmosphere and have any impact on organisms. |

# A. Assessment of intrinsic properties and effects of the active substance

## A.1. General substance information

### A.1.1. Identity of the substance

Table A.1 Summary table on substance identity

| Summary table on substance identity | |
|---|---|
| Common name (ISO name, synonyms) | Ethylene oxide |
| Chemical name (EC name, CA name, IUPAC name) | Oxirane |
| EC number | 200-849-9 |
| CAS number | 75-21-8 |
| Other CAS numbers (e.g. deleted, related, preferred, alternate) | - |
| Molecular formula | $C_2H_4O$ |
| Molecular weight or molecular weight range | 44.05 g/mol |
| Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate) | - |
| Description of the manufacturing process and identity of the source (for UVCB substances only) | *Please see confidential Annex of the CAR* |

Table A.2 Structural formula

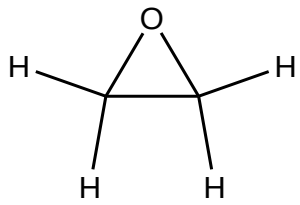
| Structural formula |
|---|
|  |



Table A.3 Origin of the natural active substance or precursor(s) of the active substance

Not relevant. Active substance is not from a natural source.

Table A.4 Method of manufacture

| Method of manufacture |
|---|
| ***Details of the manufacturing process are reported in the confidential annex of the CAR*** |

## A.1.2. Composition of the substance (reference specifications)

Table A.5 Main constituents

| Constituent (chemical name) | Typical concen-tration (%(w/w)) | Concen-tration range (%(w/w)) | Current CLH in Annex VI Table 3.1 (CLP) | | Remarks |
|---|---|---|---|---|---|
| | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | |
| Ethylene oxide | >99.9% | >99.9% | Flam. Gas 1<br>Press. Gas<br>Carc. 1B<br>Muta. 1B<br>Repr. 1B<br>Acute Tox. 3<br>Acute Tox. 3<br>STOT SE 3<br>STOT SE 3<br>STOT RE 1<br><br>Skin Corr. 1<br>Eye Dam. 1 | H220<br><br>H350<br>H340<br>H360Fd<br>H331<br>H301<br>H335<br>H336<br>H372 (nervous system)<br>H314<br>H318 | U* |

* Note U (of CLP as amended by Commission Delegated Regulation (EU) 2016/918 of 19 May 2016): When put on the market gases have to be classified as 'Gases under pressure', in one of the groups compressed gas, liquefied gas, refrigerated liquefied gas or dissolved gas. The group depends on the physical state in which the gas is packaged and therefore has to be assigned case by case. The following codes are assigned: Press. Gas (Comp.), Press. Gas (Liq.), Press. Gas (Ref. Liq.), Press. Gas (Diss.). Aerosols shall not be classified as gases under pressure (See Annex I, Part 2, Section 2.3.2.1, Note 2).

Table A.6 Impurities

Please see the confidential annex of the CAR for further information.

Table A.7 Additives

Not relevant, as the active substance does not contain any additives.

Purity of tested batches (Table A.8a/9a):

The purity, and impurities, of the batches used for ecotoxicological og toxicological testing should normally be reported in Table A.8a. However, the eCA considers that this table is not feasible to use in the case of ethylene oxide. Please see the following explanation.

For many of the studies used in the ecotoxicological and toxicological testing the levels of impurities are not given. When available, information on the purity, and the impurities, of the batches used for testing is stated in the study summaries in DocIIIA. The eCA has evaluated the studies and concluded that they can be used for the risk assessment of ethylene oxide as an active substance, despite the lack of details in the study reports. The following aspects were taken into consideration:

Ethylene oxide is produced by passing pure oxygen and pure ethylene over a fixed bed catalyst. The production process for ethylene oxide seems to be mainly unchanged since the 1930s (Rebsdat, 2012). No other additives or reagents are used. Thereafter, the produced ethylene oxide can be filtered and purified by a simple condensation/evaporation process, which allows for a very high purity of produced substance. It is therefore expected that the purity of the ethylene oxide used for testing is high. The reference specification

set for ethylene oxide in this CAR states a purity above 99.9%. Thusly, allowing an insignificant amount of impurities in the active substance as manufactured. Furthermore, none of the impurities observed are expected to be more hazardous than ethylene oxide itself. Hence, they are not expected to have any impact on the ecotoxicological or toxicological tests.

Another aspect is that many of the studies are tests performed on vertebrates. According to article 62(1) and Annex II (8-9) of the BPR testing on vertebrates should be seen as a last resort, and such testing should not be repeated for the purpose of this regulation. The eCA considers that the lack of data on the purity of ethylene oxide used for ecotoxicological and toxicological testing does not justify the need to perform new tests on vertebrates.

## A.1.3. Physical and chemical properties of the active substance

Table A.8 Physical and chemical properties of the active substance

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| **Aggregate state at 20°C and 101.3 kPA** | Gas | Not available | | **-** |
| **Physical state (appearance) at 25°C and 101.3 kPA** | Gas | Not available | | **Merck 2001** |
| **Colour at 20°C and 101.3 kPA** | Colourless | Not available | | **Verschueren 1983** |
| **Odour at 20°C and 101.3 kPA** | Sweet, olefinic, ether like | Not available | | **Verschueren 1983** |
| **Melting / freezing point** | -112.5°C | Not available | | **Lide, 2005, CRC handbook** |
| **Boiling point at** | 11°C | Not available | | **Verschueren 1983** |
| **Relative density** | 0.8821 g/cm$^3$ at 10°C | Not available | | **Lide, 2005, CRC handbook** |
| **Granulometry** | - | Not available | Not relevant for a gas | **-** |
| **Vapour pressure** | 146 kPa at 20°C | Not available | | **Verschueren 1983** |
| **Henry's law constant** | 14 Pa m$^3$ mol$^{-1}$ | Calculated | Calculated from activity coefficient and vapor pressure presented by Conway et al., 1983 | **BUA 1995 Conway et al., 1983** |
| **Surface tension** | | | Ethylene oxide is too hazardous to be experimentally determined for surface tension. Based on chemical structure, no surface | **Waiver** |

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| | | | activity is predicted.<br><br>Surface tension will not be used for (eco)toxicological evaluation. The biocidal products containing ethylene oxide is gaseous, hence surface tension is not relevant. | |
| **Water solubility at 20 °C** | 1000 g/L at 25ºC | Not available | | **Merck 2001** |
| **Partition coefficient (n-octanol/water) and its pH dependency** | Log $P_{OW}$: -0.30 | Not available | | **Hansch and Leo, 1979** |
| **Thermal stability and identity of breakdown products** | | - | Ethylene oxide is not an endothermic molecule and cannot decompose exothermically into its elements. However it can decompose or polymerise exothermically into various distributions of more stable products. | **Britton, LG (1990)** |
| **Reactivity towards container material** | Ethylene oxide reacts with water, strong acids, alkalis, and oxidizers; chlorides of iron, tin, and aluminium; and oxides of iron and aluminium. Based on experience, suitable materials for ethylene oxide are: Normalised steel and carbon steel, tempered steel, aluminium alloys, | Not available | | **ATSDR MMG 2007a** |

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| | stainless steel. | | | |
| **Dissociation constant** | | Not available | Ethylene oxide does not contain any dissociation groups | **-** |
| **Viscosity** | | Not available | Viscosity is required for liquid substances determined at 20 and 40 °C. Ethylene oxide is a gas at 20 and 40 °C, therefore determination of viscosity by test method OECD 114 is not possible. | **Waiver** |
| **Solubility in organic solvents, including effect of temperature on solubility** | Soluble in alcohol and ether | Not available | | **Merck 2001** |
| **Stability in organic solvents used in biocidal products and identity of relevant degradation products** | **Ethylene oxide is not solved in organic solvents** | **-** | **-** | **-** |

## A.1.4. Physical hazards and respective characteristics

Ethylene oxide has a harmonized classification. Please refer to section 2.

Table A.9 Physical hazards and respective characteristics

| Hazard class / characteristics | Guideline and Method | Parameter(s) | Results / Waiver | Reference |
|---|---|---|---|---|
| Explosives | Not available | Not available | Not applicable to gases | - |
| Flammable gases | Not available | Not available | Yes. Flammable limits of 3 to 100% in ambient air<br>Auto ignition temperature 457ºC | Britton, LG (1990) |
| Flammable aerosols | - | - | - | - |
| Oxidising gases | Not available | Not available | No oxidizing properties[1)] | - |
| Gases under pressure | Not available | Not available | Yes. According to the harmonized classification under CLP, please also see the Note U[2)]. | - |
| Flammable liquids | - | - | Not applicable to gases | - |
| Flammable solids | - | - | Not applicable to gases | - |
| Self-reactive substances and mixtures | - | - | Not applicable to gases | - |
| Pyrophoric liquids | - | - | Not applicable to gases | - |
| Pyrophoric solids | - | - | Not applicable to gases | - |
| Self-heating substances and mixtures | - | - | Not applicable to gases | - |
| Substances and mixtures which in contact with water emit flammable gases | - | - | Not applicable to gases | - |
| Oxidising liquids | - | - | Not applicable to gases | - |
| Oxidising solids | - | - | Not applicable to gases | - |
| Organic peroxides | - | - | Ethylene oxide is not an organic peroxide | - |
| Corrosive to metals | Not available | Not available | Not corrosive to metals[3)] | ATSDR MMG 2007a |
| Auto-ignition temperature (liquids and gases) | Not available | Not available | Auto ignition temperature 457ºC | Britton, LG (1990) |
| Relative self-ignition temperature for solids | - | - | Not applicable to gases | - |
| Dust explosion hazard | - | - | Not applicable to gases | - |

1) Flammable gases cannot normally be classified as oxidizing. Furthermore, the applicant has submitted a justification in DOCIIIA 3.16
2) Note U (of CLP as amended by Commission Delegated Regulation (EU) 2016/918 of 19 May 2016): When put on the market gases have to be classified as 'Gases under pressure', in one of the groups compressed gas, liquefied gas, refrigerated liquefied gas or dissolved gas. The group depends on the physical state in which the gas is packaged and therefore has to be assigned case by case. The following codes are assigned: Press. Gas (Comp.), Press. Gas (Liq.), Press. Gas (Ref. Liq.), Press. Gas (Diss.). Aerosols shall not be classified as gases under pressure (See Annex I, Part 2, Section 2.3.2.1, Note 2).
3) Flammable gases cannot be classified as corrosive to metals.

## A.1.5. Assessment of physical hazards according to the CLP criteria

Not applicable. Plese see harmonized classification for ethylene oxide.

## A.1.6. Analytical methods for detection and identification

Table A.10 Analytical methods

| Analyte (type of analyte e.g. active substance, metabolite/ degradant etc.) | Compartment | Linearity | Specificity | Recovery Rate (%) | Limit of quanitification (LOQ), Maximum Residue Limits or other limits | | | Analytical technique used in the analytical method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fortification range / Number of measure-ments | Mean | RSD | | |
| **Ethylene oxide** | In active substance as manufactured | - | - | - | - | - | - | - | Please see waiver below the table. |
| **Impurities** | In active substance as manufactured | - | - | - | - | - | - | - | Please see waiver below the table. |
| **Ethylene oxide** | Soil | - | - | - | - | - | - | - | Waiver. Based on the proposed use of ethylene oxide as a gaseous sterilising agent, environmental exposure is expected to be extremely minimal. |
| **Ethylene oxide** | Water | - | - | - | - | - | - | - | Waiver. Ethylene oxide is intended for |

| Analyte (type of analyte e.g. active substance, metabolite/ degradant etc.) | Compartment | Linearity | Specificity | Recovery Rate (%) | Limit of quanitification (LOQ), Maximum Residue Limits or other limits | | | Analytical technique used in the analytical method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fortification range / Number of measure-ments | Mean | RSD | | |
| | | | | | | | | | use as a gaseous sterilising agent; no application is envisaged for use in water. |
| **Ethylene oxide** | Air | From 5.4 µg/mL to 43.2 µg/mL measured in external standards. Correlation coefficient not reported. | No significant interference observed | Not reported | 97.9 % | Not reported | 17.5 µg/m$^3$ (9.72 ppb) | Gas chromatography with a DB-5 capillary column, 60 m x 0.32 mm id, df = 1.0 µm and an electron capture detector (ECD). | Shah, Y. (2007) |
| **Ethylene oxide** | Air | From 2.4 µg/mL to 33.5 µg/mL measured in spiked (external) standards. Correlation coefficient not reported. | Acetone and isopropanol can both be brominated by the chemistry on the Ethylene oxide monitor (3 M Brand 3550/3551) | Not reported | 85 % | Not reported | 0.75 ppm with a 15 min sample or 0.02 ppm with an 8 hr sample. | Gas Chromatograph with a J&W DB225 capillary column (15 m x 0.25 mm i.d., 0.25 µm df) and an electron capture detector (ECD). | 3M Company (1997) |
| **Ethylene oxide** | Body tissues | Not reported | Not reported | Not reported | Not reported | Not reported | Not reported. | Gas chromatography | Brown, C.D., Wong, B.A., |

| Analyte (type of analyte e.g. active substance, metabolite/ degradant etc.) | Compartment | Linearity | Specificity | Recovery Rate (%) | Limit of quanitification (LOQ), Maximum Residue Limits or other limits | | | Analytical technique used in the analytical method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fortification range / Number of measure-ments | Mean | RSD | | |
| | | | | | | | Lowest mean results obtained were 0.10 ppm in tissue | with a 10 ft 10% SP1000 packed column and flame ionisation detector (FID). | Fennell, T.R. (1996) |
| **Ethylene oxide** | Body fluids and tissues [Blood] | Not reported | CO2 may interfere when low resolution GC-MS is used | Not reported | Not reported | Not reported | Not reported. Lowest results obtained were 0.06 ppm in blood. | Gas chromatography with a 30 m x 0.53 mm i.d., 1µm df DB wax capillary column and flame ionisation detector (FID). | Fennell, T.R., Snyder, R.W., Parkinson, C., Murphy, J., James, R.A. (2004) |
| **Ethylene oxide** | Body fluids and tissues [Blood] | Not reported | Not reported | Not reported | Not reported | Not reported | 4 µg/L EtO in blood | Gas chromatography with 2.4 m x 2 mm i.d., packed steel GC column and flame ionisation detection (FID) | Brugnone, F., Perbellini, L., Faccini, G.B., Pasini, F., Bartolucci G.B. and DeRosa, E. (1986) |
| **Ethylene oxide** | On medical devices* | 0.01 ppm to 5.00 ppm | Not reported | 5 measurements: 0.01 ppm, 0.05 ppm, 0.10 ppm, 1.00 ppm and 5.00 ppm | 0.01 ppm: 30.7<br><br>0.05 ppm: 113.8 | Not reported | 0.050 ppm (inter-laboratory test) | Gas chromatography with capillary column and flame ionisation detector (FID). | T. Harper, L. Cushinotto, N. Blaszko, J. Arinaga, F. Davis, C. Cummins, M. |

| Analyte (type of analyte e.g. active substance, metabolite/ degradant etc.) | Compartment | Linearity | Specificity | Recovery Rate (%) | Limit of quanitification (LOQ), Maximum Residue Limits or other limits | | | Analytical technique used in the analytical method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fortification range / Number of measure-ments | Mean | RSD | | |
| | | | | | 0.10 ppm: 102.4<br><br>1.00 ppm: 99.3<br><br>5.00 ppm: 110.1 | | | | DiCicco (2008) |
| **Ethylene oxide** | On medical devices* | 1.0 ng to 100.0 ng | Not reported | Not reported | Not reported | 1.47 to 8.99 % | Not reported | Gas chromatography with capillary column and flame ionisation detector (FID). | M. DiCicco, B. Lang, T.I. Harper (2009) |
| **Ethylene oxide** | On medical devices* | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Not applicable | Gas chromatography with capillary column and FID or ECD detector. GC-MS can be used to confirm identity. | European Committee for Standardization (2008) |
| **Ethylene chlorohydrin (ECH) and Ethylene glycol (EG) (reaction products of Ethylene oxide)** | On medical devices* | ECH: 2-15 ppm<br><br>EG: 4-20 ppm | Ethylene oxide interferes with the detection of ethylene glycol at levels of above 2ppm. | 5 measurements: 10 ppm for both ECH and EG | ECH<br>PVC: 96 %<br>SR: 104 %<br>PP: 100 %<br>PE: 94 %<br><br>EG:<br>PVC: 98 %<br>SR: 102 %<br>PP: 103 % | The inter analytical variance for the determination of ECH and EG in polymers is about 2% (n=5). | ECH: 1 ppm<br><br>EG: 2 ppm | Gas chromatography with packed GC column and flame ionisation detector (FID). | E. De Rudder, E. De Graeve, R. Van Severen, P. Braeckman (1986) |

| Analyte (type of analyte e.g. active substance, metabolite/ degradant etc.) | Compartment | Linearity | Specificity | Recovery Rate (%) | Limit of quanitification (LOQ), Maximum Residue Limits or other limits | | | Analytical technique used in the analytical method | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fortification range / Number of measure-ments | Mean | RSD | | |
| | | | | | PE: 100 % | | | | |

*Residues in medical devices are not a part of this evaluation, as this is considered to be covered by other legislation. Therefore, the methods for detection of residues in medical equipment are included for information purposes only. The methods have not been evaluated by the eCA.

eCa's reasoning for non-submission of method for detection of ethylene oxide in the produced active substance:

The applicant has not provided a validated method for detection of ethylene oxide itself in the produced batches. Instead, various quality control methods are used to determine the impurities present in the produced batches. This is normally not an acceptable approach, but based on the following argumentation, the eCA has chosen to accept this.

Ethylene oxide is produced as a gas at room temperature, but condensates at around 10°C. It is produced from pure gaseous starting materials by passing the two gases (oxygen and ethylene) over a fixed bed catalyst. Very few impurities are expected from the production process, and the few formed can be removed by distillation (condensation/evaporation) of the active substance. Hence, a high degree of purity is expected.

Handling of ethylene oxide is not easy. Ethylene oxide is flammable, explosive, carcinogenic, mutagenic, reprotoxic and toxic, and special precautions must be taken when handling the substance. Most industrial facilities using ethylene oxide have specialised equipment and well-trained personnel. Although most routine laboratories do have skilled personnel, it is, according to the applicant, very difficult to find laboratories that have the proper equipment and training for handling of ethylene oxide. This makes it nearly impossible to find external laboratories willing to perform analysis on ethylene oxide. The eCA agrees that it is not desirable to expose laboratory personnel to ethylene oxide, unless it is strictly necessary. Furthermore, it is difficult to transport the samples from the factory to the laboratory. Specialised transport containers must be used, and great care must be taken to avoid accidents.

Semi-quantitative methods, namely GC-MS and IR are submitted, but there are no calibrations and validations available. Accurate analysis of ethylene oxide is a twofold problem, in that there are problems with both the availability of high purity standards and there are difficulties in handling the gas. The GC-MS spectra do not show any additional (unexplainable) peaks but do determine the purity of ethylene oxide. The GC-MS and IR confirm that the produced batches are ethylene oxide.

As all the analysis are done "in-house" on ethylene oxide manufacturing plants, they are not GLP compliant. Based on the nature of the active substance, the eCA accepts this.

Thusly, the eCA considers that the combination of quality control methods and the identity confirmation provided by the GC-MS/IR is sufficient for the purpose of setting a reference specification.

This view was found acceptable by the BPC working group for analytical methods and physicochemical properties (WG-IV-2020).

# A.2. Effects against target organisms

## A.2.1. Intended uses

### Function and field of use envisaged

Ethylene oxide is a gaseous sterilising agent. The field of use envisaged is the industrial sterilisation of single use medical devices as part of the production process, before they are made available on the market. Medical devices are regulated through Regulation (EU) No 2017/745, which repeals Directive (EC) No 93/42. According to this legislation, products specifically intended for the cleaning, disinfection or sterilisation of medical devices are also regarded as medical devices themselves. However, as sterilising agents used as part of the pre-market production process are not considered as medical devices, this use is regarded as a biocidal use.

The single use medical devices envisaged to be sterilised by ethylene oxide in the production phase at the industrial site, are those which cannot be sterilised by other means such as radiation, heat or steam.

The sterilisation occurs in large industrial units that take in unsterilised devices and process them under highly controlled conditions. During the sterilisation process, ethylene oxide is introduced into a sealed, stainless steel chamber which contains the products for sterilisation. On completion, ethylene oxide from the chamber is exhausted to the atmosphere via a catalytic converter which converts ethylene oxide to carbon dioxide and water, typically with efficiency greater than 99.9 %. In some cases, acid scrubbers convert the ethylene oxide to ethylene glycol with subsequent release to a liquid waste handling system, with efficiency typically in the range of 99.5 to 99.9 %. The sterilised products are then typically transferred to an aeration cell and are subjected to high rates of air exchange at temperatures close to the sterilisation temperature for approximately 12 hours. This process serves to further remove any residual ethylene oxide from the product and packaging. On completion of initial aeration, products are transferred to a secondary aeration area to further reduce residual ethylene oxide levels.

Table A.11 Summary table of intended uses

| Summary table of intended use(s) | |
|---|---|
| Product Type | 2 (disinfectants and algaecides not intended for direct application to humans or animals) |
| Product description | Sterilising agent for the sterilisation of single-use medical devices before they are made available on the market |
| Target organisms (including development stage) | Bacteria (including spores), fungi and viruses<br>(These are the target organisms intended by the applicant. However, please note that efficacy of the active substance is only demonstrated for bacteria.) |
| Description of use(s) | Industrial sterilisation of single use medical devices, which cannot be sterilised by other means, before these are made available on the market. |
| Mode of action | Ethylene oxide is a broad-spectrum sterilising agent. It is highly reactive and reacts with proteins, amino acids and the nucleic acids. |
| Objects to be protected | The intention of the sterilisation of single use medical devices is the protection of human health |
| Concentration of product in the in-use | Nominally 100 % (the representative biocidal product is composed of 100 % ethylene oxide) |

| | |
|---|---|
| formulation/product | |
| Concentration of active substance in the in-use formulation/product | Nominally 100 % (the representative biocidal product is composed of 100 % ethylene oxide) |
| Application rate(s) | The following application rate, i.e. the concentration of ethylene oxide in the sterilisation chamber, has been substantiated by the provided ISO 11135:2014 studies:<br><br>300 mg/L (based on approx. mean concentration from the two studies)<br><br>Please note that other application rates may be used, as long as they are sufficiently validated according to ISO 11135:2014. |
| Frequency of application | Items are sterilised once to ensure their sterility before they are placed on the market. |
| Field of use (indoors/outdoors) | Indoors in closed systems |
| Category(ies) of user(s) | Industrial users |
| Instruction for use | The treatment process is a highly specialised industrial process. The instructions for use will depend on the facility and the type of items to be sterilised. Further information regarding the operational conditions in the sterilisation plants can be found in chapter B.3.1.<br>Several factors aside from the a.s. concentration affect the sterilisation process and must be carefully determined when validating a process, in accordance with ISO 11135:2014. This includes temperature, humidity, pressure and contact time. Two ISO validation studies have been provided by the applicant, where all these parameter values are specified for two sterilisation processes (see text below, and Docs IIIB5.10/06 and /07). In addition, another example of a validated set of parameters has also been provided by the applicant, which can be seen in Doc IIIB5. |

## A.2.2. Summary on efficacy

## A.2.2.1 Efficacy

Ethylene oxide has been used as a sterilant for medical devices for many decades. The sterilisation of single use medical devices before these are made available on the market, must comply with the ISO standard ISO 11135:2014 (Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices), describes requirements that, if met, will provide an ethylene oxide sterilization process intended to sterilize medical devices, which has appropriate microbicidal activity.

For ethylene oxide, two ISO 11135:2014 validation studies have been submitted and assessed (Doc III B5.10/06 and Doc III B5.10/07), after a decision at the ad hoc follow-up after WG-III-2020 on the need for further efficacy data to clarify the innate biocidal activity of the active substance.

**Principles of the ISO 11135:2014 standard[1]**

Ethylene oxide concentrations, and parameters such as temperature, humidity and pressure, vary between sterilisation processes, depending on a.o. the medical devices to be sterilised and their packaging. The ISO 11135:2014 standard requires that each sterilisation process is set up in order to achieve the required sterility, and that this is in fact validated. For each change in the devices, packaging or other parts of the configuration, the process must be validated again. Several factors aside from the a.s. concentration affect the sterilisation process and must be carefully determined when validating a process, in accordance with ISO 11135:2014. This includes temperature, humidity, pressure and contact time.

A validation should demonstrate that the sterilisation process is efficient and reproducible for a certain sterilisation load (objects to be sterilised). A validation includes installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) which includes the microbiological performance qualification (MPQ), and shall be performed for each new / changed sterilisation process or sterilisation load (with regard to objects, packaging, load configuration, equipment or process parameters), unless there is equivalence to a previous validation.

The sterilisation load used shall represent the most challenging of the objects that are to be routinely sterilised. The location(s) within that load which is/are the most difficult to sterilise must be determined, and a process challenge device (PCD) containing biological indicators (BI's, target microorganisms) shall be placed here.

The rate of microbiological inactivation shall be determined using one of the approaches listed in annexes A or B to the ISO standard. The approach in Annex B is the "overkill approach", and is labelled a conservative determination of lethal rate. The half-cycle overkill approach has been used in the two provided studies for ethylene oxide, i.e. three consecutive experiments run at the half length of the full cycle, which must result in total inactivation of a microbial population of not less than $10^6$.

**The ISO 11135:2014 validation studies provided for ethylene oxide**

Sterilisation of the medical devices (PCDs) is shown in each of the two studies, with a SAL of $10^{-6}$, for a specified sterilisation process. A recommended target organism (BI) for ethylene has been used, i.e. *Bacillus atrophaeus*.

The following table shows the sterilisation parameters for the two validated processes:

| **Parameter** | **Value (in the sterilisation chamber)** | |
|---|---|---|
| | **Doc III B5.10/06** | **Doc III B5.10/07** |
| Ethylene oxide concentration | Half cycles:<br>291-318 mg/L<br>292-328 mg/L<br>300-332 mg/L<br><br>Full cycle:<br>329-334 mg/L | Half cycles:<br>269-295 mg/L<br>280-290 mg/L<br>280-300 mg/L<br><br>Full cycle:<br>280-300 mg/L |
| Temperature | 55 ± 3 °C | 60 ± 3 °C |
| Humidity | 40-95 % | 40-95 % |
| Pressure | 2.0 ± 0.2 bar | 2.0 ± 0.2 bar |

[1] The standard can be found here: https://www.iso.org/standard/56137.html

| | | |
|---|---|---|
| Contact time | 120 min<br><br>(60 min for the half cycles) | 140 min<br><br>(70 min for the half cycles) |

The information in the study reports all in all shows compliance with ISO 11135:2014. Please see the study summaries (Docs III) for the full evaluation of both the studies.

It should be noted that the goal of ISO 11135:2014 validation studies is to ensure that the sterilisation process as a whole gives the required level of sterilisation. The ISO 11135:2014 standard does not require using a control to ensure that the effect is caused by the active substance alone, i.e. a sterilisation cycle with all parameters in place except for the active substance. There is however a control checking for growth in the BIs (inclubation without exposure to a sterilisation process), to ensure that the achieved effect on the BIs is caused by the sterilisation process.

Furthermore, the gas used in the two validation studies is a mixture of ethylene oxide and carbon dioxide. The eCA's view is nevertheless that the sterilisation effect will be caused by ethylene oxide, not carbon dioxide. The reasoning is that concentrations of carbon dioxide in the sterilisation chamber is not high enough to cause a biocidal effect, and furthermore complete sterilisation by gaseous carbon dioxide is not known from literature. Further support for this reasoning can be found in the study summaries.

The eCA's opinion is that the submitted ISO 11135:2014 data are sufficient for showing the innate biocidal activity of ethylene oxide in the context of active substance evaluation. However, more detailed information or studies needs to be submitted at the product authorisation stage.

**Other information on the efficacy of ethylene oxide**

In addition, the applicant has provided open literature data (published articles) where the effects of various factors on the efficacy and resistance of ethylene oxide have been investigated.

Based on a literature search, some other relevant article references are also listed below. The review article by Russell (1990) concludes as follows: i) The higher the concentration of ethylene oxide, the more rapid is its sporicidal activity. However, the rate of kill is relatively slow and it is important that sufficient time is allowed when providing suitable conditions for sterilisation. ii) Activity is temperature dependent, increasing with increasing temperature. However, at each temperature, a critical concentration is reached where an increase in concentration will no longer affect the sporicidal activity. iii) Of all the factors influencing ethylene oxide activity, moisture is the most critical. The relationship between moisture and activity is complex, and conflicting results had been obtained at the time this review article was published.

Medical devices are carefully regulated through Regulation (EU) No 2017/745 on medical devices, which repeals Directive (EC) No 93/42 on medical devices. In order for medical devices to be designated as sterile, compliance with stringent measures such as those given in harmonised international standards are required. The eCA considers that the available data and references on the efficacy of ethylene oxide listed below are useful for understanding the basis of its biocidal activity, but the ISO 11135:2014 standard is key for the efficacy assessment. Ethylene oxide is a sufficiently effective biocidal agent for the intended biocidal use, i.e. for the sterilisation of single-use medical devices, when used in compliance with this ISO standard.

Table A.12 Experimental data on the efficacy of the active substance against target organism(s)

| Function and field of use | Test substance | Test organism(s) | Test method | Test system / concentrations applied / exposure time | Test results: effects | Remarks | Ref. |
|---|---|---|---|---|---|---|---|
| **Sterilant** | Gas mixture: 6 % ethylene oxide and 94 % carbon dioxide | *Bacillus atrophaeus ATCC 9372* | Validation of sterilisation acc. to ISO 11135:2014, in commercial sterilisation chamber on a medical device sterilisation facility. The "overkill approach" of ISO 11135:2014 was applied to validate the sterilisation efficacy: Three consecutive experiments (half-cycles) which must result in total inactivation of a microbial popu-lation of not less than $10^6$. | 3 sterilisation half-cycles (i.e. half of the length of the sterilisation process which is to be validated) as follows:<br><br>Ethylene oxide concentration in sterilisation chamber (in each of the half-cycles):<br>291-318 mg/L<br>292-328 mg/L<br>300-332 mg/L<br><br>Exposure time: 60 min<br>Temperature: 55 ± 3 °C<br>Humidity: 40-95 %<br>Pressure: 2.0 ± 0.2 bar | Some modifications to the sterilisation process had to be made in order to meet the required sterilisation level. After these modifications, no microbial growth occurred in any of the half-cycles. | RI: 1 | [redacted] (2018a),<br><br>Doc IIIB5.10/ 06 |
| **Sterilant** | Gas mixture: 6 % ethylene oxide and 94 % carbon dioxide | | | 3 sterilisation half-cycles (i.e. half of the length of the sterilisation process which is to be validated) as follows:<br><br>Ethylene oxide concentration in sterilisation chamber (in each of the half-cycles):<br>269-295 mg/L<br>280-290 mg/L<br>280-300 mg/L<br><br>Exposure time: 70 min<br>Temperature: 60 ± 3 °C<br>Humidity: 40-95 %<br>Pressure: 2.0 ± 0.2 bar | | RI: 1 | [redacted] (2018b),<br><br>Doc IIIB5.10/ 07 |
| **Sterilant** | 100 % ethylene | *Bacillus* | Evaluation of the influence on | Biological indicators were exposed in the | The initial time taken to reduce the spore population | RI: 2 | R.R. Reich & |

| Function and field of use | Test substance | Test organism(s) | Test method | Test system / concentrations applied / exposure time | Test results: effects | Remarks | Ref. |
|---|---|---|---|---|---|---|---|
| | oxide liquid | *stearo-termophilus, Bacillus subtilis* | storage conditions of biological indicators on EtO sterilization | test chamber<br><br>Ethylene oxide concentration: 1,200 mg/L<br><br>Temperature: 55 ± 1 ºC<br><br>Relative humidity: 60 ± 10 % | of *B. subtilis* by 90% was 1.8 minutes. Storage at ambient, refrigerated and freezer conditions and as well as conditions of 20ºC and 20 and 44% relative humidity had no significantly observable effect on ethylene oxide resistance. | The study is not considered reliable for the purpose of showing the efficacy of ethylene oxide, but a reliability of 2 is considered acceptable for the purpose of indicating that the efficacy of ethylene oxide towards organisms used as biological indicators are influenced by temperature and heat during storage of these organisms.<br><br>The eCA does not consider this study essential for the assessment of efficacy, but it is acceptable as information on the influence of temperature and humidity during storage of biological indicators of ethylene oxide efficacy. | L.L. Morien (1982),<br><br>Doc IIIB5.10/ 01 |
| **Sterilant** | Ethylene oxide | *Bacillus subtilis* var. *niger* | Spores hermetically sealed in various polymeric films were exposed to ethylene oxide at a concentration of 900 mg/L relative humidity of 40% and a temperature of 130ºF | EtO exposure occurred in a test chamber | In order of ethylene oxide resistance the study found that order polyethylene=poly vinyl chloride nylon< cellophane/polyethylene laminate< phenoxy < mylar polyethylene laminate. As the thickness of the polymeric film increased beyond a certain level the resistance to ethylene oxide increased. Spore strips that have been desiccated prior to ethylene oxide exposure demonstrated a high | RI: 2<br><br>The study is not considered reliable for the purpose of showing the efficacy of ethylene oxide, but a reliability of 2 is considered acceptable for the purpose of indicating how factors such as the type of polymeric materials (in which organisms to be killed are sealed) and the humidity affects the efficacy of ethylene oxide towards *B. subtilis var niger*. | J.E. Doyle, A.W. McDaniel , K.L. West, J.E. Whitbourne, R.R. Ernst (1970),<br><br>Doc IIIB5.10/ 02 |

| Function and field of use | Test substance | Test organism(s) | Test method | Test system / concentrations applied / exposure time | Test results: effects | Remarks | Ref. |
|---|---|---|---|---|---|---|---|
| | | | | | increase in ethylene oxide resistance. Introduction of moisture assists the sterilisation process as it helps heat the materials that are being processed. The load reaches higher temperatures more quickly and the ethylene oxide sterilises at a faster rate. Consequently it is recommended that pre-humidification remains a necessary part of ethylene oxide sterilization processes. | The eCA does not consider this study essential for the assessment of efficacy, but it is acceptable as information on the influence of polymeric materials and humidity on the efficacy of ethylene oxide. | |
| **Sterilant** | Liquid Ethylene Oxide | *Bacillus subtilis* var. *niger* | Spores on glass beads were exposed to EtO in a test chamber | EtO exposure occurred in a test chamber. Dose rate was 440, 800 and 1500 mg/L | The concentration dependence of the ethylene oxide sterilisation reactions is the result of the physical phenomena of adsorption and diffusion and does not reflect a specific chemical reaction per se. | RI: 2<br><br>The study is not considered reliable for the purpose of showing the efficacy of ethylene oxide, but a reliability of 2 is considered acceptable for the purpose of investigating factors which affect the concentration dependende of ethylene oxide efficacy towards *B. subtilis var. niger*.<br><br>The eCA does not consider this study essential for the assessment of efficacy, but it is acceptable as general background information on the mechanisms affecting ethylene oxide efficacy. | R.R. Ernst and J.J. Shull (1962),<br><br>Doc IIIB5.10/ 03 |
| Sterilant | 20 wt% Ethylene oxide and | *Bacillus subtilis* var. *globigii* | This study looked at the sterilisation of spore strips (to | The test with spore strips used a 40 L chamber with a | The study found that the minimum sterilising conditions were an EtO | RI: 2<br><br>The study is not considered | M. Furuhashi and T. |

| Function and field of use | Test substance | Test organism(s) | Test method | Test system / concentrations applied / exposure time | Test results: effects | Remarks | Ref. |
|---|---|---|---|---|---|---|---|
| | 80 wt% Carbon dioxide | | test different conditions) and medical devices (to validate the identified efficient conditions) in commercial EtO sterilisation bags of craft paper on one side and laminated polypropylene and polyester film on the other side. | mechanism for addition and removal of samples without affecting the EtO concentration, humidity or temperature. The test with medical devices used a 800 L steriliser | concentration of 400-500 mg/L a sterilising temperature of 55-60ºC with a sterilising time of 2 hours. | reliable for the purpose of showing the efficacy of ethylene oxide, but a reliability of 2 is considered acceptable for the purpose of investigating factors which affect the efficacy of ethylene oxide.<br><br>The eCA does not consider this study essential for the assessment of efficacy, but it is acceptable as general background information on the mechanisms affecting ethylene oxide efficacy. | Miyame (1982),<br><br>Doc IIIB5.10/ 05 |

**Further references**

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*Page, B.E.J. (1993) Special consideration for ethylene oxide: product residues. In Sterilization Technology (eds. Morrisey, R.F. & Phillips, G.B.) 402-420. New York: Van Nostrand Reinhold*

*Sintim-Damoa, K. (1993) Other gaseous methods. In Sterilization Technology (eds. Morrisey, R.F. & Phillips, G.B.) 335-347. New York: Van Nostrand Reinhold*

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

*Russell, A.D. (1998) Assessment of sporicidal activity. International Journal of Biodeterioration and Biodegradation 41: 281-287. Elsevier*

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

## A.2.2.2 Mode of action

Ethylene oxide is a highly reactive alkylating agent and reacts with proteins, amino acids and the nucleic acids. Reaction with the phosphated guanine component of DNA appears to result in bacterial cell to lose their ability to replicate (Michael & Stumbo, 1970). See also Marletta & Stumbo 1970, Winarno & Stumbo 1971 for further details on the mode of action of ethylene oxide.

## A.2.2.3 Resistance

Many factors will affect the efficacy of ethylene oxide (see above), resulting in micro-organisms being more resistant under certain conditions, e.g. low humidity. It should be noted that this is not resistance towards ethylene oxide, but factors associated with the conditions under which the ethylene oxide is applied. No reports of true ethylene oxide resistance are known to the eCA. It is expected that the development of resistance towards a substance with such a basic mode of action towards several vital molecules within the cell is unlikely.

## A.2.3. Conclusion on efficacy

The innate biocidal activity of ethylene oxide towards bacterial spores is sufficiently documented, through validation studies conducted in accordance with ISO standard 11135:2014. Further information/studies, including efficacy towards other target organisms, needs to be submitted at the product authorisation stage. The ethylene oxide sterilisation of medical devices before these are placed on the market, must be in conformity with ISO 11135:2014. The terilisation depends on several factors, such as ethylene oxide concentrations, temperature, pressure, humidity and the composition and configuration of the load of medical devices which are to be sterilised. Each sterilisation process must be validated by the user, in accordance with ISO 11135:2014.

## A.3. Assessment of effects on Human Health

Handling of ethylene oxide is not easy. The active substance is included in the 14th ATP to CLP and fulfils the exclusion criteria of Article 5, paragraph 1a, b and c of BPR given the following classification: Carc. 1B, Muta. 1B and Repr. 1B. Furthermore, EtO is a highly reactive gas (i.e. the substance readily reacts with diverse compounds and is explosive at concentrations higher than 3% in air). Based on both the human health hazardous properties and its flammable and explosive properties, special precautions must be taken when handling the substance. Specialised equipment and well-trained personnel are a prerequisite for using ethylene oxide in industrial facilities. Although most routine laboratories do have skilled personnel, it is, according to the applicant, very difficult to find laboratories that have the proper equipment and training for handling of ethylene oxide. The eCA agrees that it is not desirable to expose either laboratory personnel or laboratory animals to ethylene oxide, unless it is strictly necessary. Furthermore, even the transport of samples from the factory to the laboratory is difficult. Specialised transport containers must be used, and great care must be taken to avoid accidents.

Many of the studies provided by the applicant are open literature studies of older date and with deviation from the standards given today. However, due to the hazardous properties and reactivity of EtO, the eCA are of the opinion that additional testing is not justified, and that the data provided by the applicant are acceptable. In order to give a broader overview of the active in the CAR, the eCA have evaluated and included some additional articles available for the active [e.g. original articles cited in the RAC opinion, the CLH report (2016) and/or in the WHO (2003) evaluation].

As a consequence of the identified hazardous human health properties of EtO, strict RMMs must be in place to avoid or minimise occupational exposure as far as technically feasible if EtO is to be used. In regard to the risk assessment, a non-threshold mode of action is assumed for tumour formation, and a conservative semi-quantitative risk assessment must be performed. Based on this, AELs normally derived for acute, subchronic and chronic effects will not be relevant for this active. A DMEL (by inhalation) is the only relevant threshold to be used in the risk assessment for EtO.

### A.3.1. Toxicokinetics

Table A.13 Summary table of toxicokinetic studies

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| Non-guideline, non-GLP, in vivo uptake of ethylene oxide by | Sprague-Dawley rats, 2 males/group for inhalation and 1 male/group for | Ethylene oxide, 99.7% pure, three exposure levels of | A first-order elimination pattern of ethylene oxide from a closed chamber was observed. | Non-guideline and non-GLP study, an uptake was studied by | Filser J.G. et al. (1984) |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| inhalation (from a closed chamber) or intraperitoneal injection.<br><br>Reliability 3, supporting study. | intraperitoneal exposure | ca. 100-1000 ppm (graphic representation only); 835 nL gas/g bw for intraperitoneal injection | Following intraperitoneal injection, the course of ethylene oxide in the atmosphere of a closed chamber followed Bateman exponential function. The following first-order rate constants λ1,2 were obtained:<br>λ2 (inhalation) = -0.78 h-1 (n = 3, SD = ±5%)<br>λ1 (IP) = -5.53 h-1 (n = 3, SD = ± 1.7%)<br>λ2 (IP) = -0.504 h-1 ( n =- 3, SD = ± 13%)<br>Within the entire dose range studied, no indication of non-linear (saturation) kinetics was observed | measuring the concentrations of ethylene oxide in a closed chamber; no other parameters studied. Exposure levels are presented only graphically. The study is not sufficient as a stand-alone, but can be used as a part of weight of evidence approach. | A6.2/01 |
| Non-guideline, non-GLP, in vivo 3 or 4 hours inhalation exposure of rats, ethylene oxide concentration was determined in blood, muscle, brain and testes; elimination kinetics were studied by sacrificing rats 2-20 minutes post-exposure.<br><br>Reliability 2, key study. | Fischer 344 rats, 5/sex/group | Ethylene oxide, > 99.9% pure, 100 and 300 ppm (nominal); 99 ± 2 and 327± 2 ppm (analytical), 4 h whole body exposure with interim sacrifice of 1 rat after 3 hours | Elimination time course followed overall first-order kinetics, with $t_{1/2}$ (mean ± SD) of 13.8 ± 3.0 min for males and 10.8 ± 2.4 min for females at both exposure concentrations. Saturation state was not reached. The concentrations in blood and tissues after 3 and 4 hours were practically the same, indicating that steady state was reached. Peak ethylene oxide concentrations at steady state were equivalent in all tissues except testes, where concentrations were significantly lower (ca. 20% of other tissues). | Non-guideline and non-GLP study, concentration of ethylene oxide following inhalation exposure determined in several tissues; elimination kinetics studied by determining ethylene oxide concentrations in blood and tissues. The study is considered reliable and can be used in the assessment of toxicokinetic behaviour of ethylene oxide as a part of | Brown CD et al. (1996)<br>A6.2/02 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| | | | The rates of disappearance of ethylene oxide were essentially identical for blood and other tissues. | weight of evidence. | |
| Non-guideline, non-GLP, in vivo 3 or 4 hours inhalation exposure of mice, ethylene oxide concentration was determined in blood, muscle, brain and testes. Elimination kinetics were studied by sacrificing mice 2-20 minutes post-exposure.<br><br>Reliability 2, key study. | B6C3$F_1$ mice, 5/sex/group | Ethylene oxide, > 99.9% pure, 100 and 300 ppm (nominal); 99 ± 2 and 327± 2 ppm (analytical), 4 h whole body exposure with interim sacrifice of 1 mouse after 3 hours | Elimination time course followed overall first-order kinetics, with $t_{1/2}$ (mean ± SD) of 3.12 ± 0.2 min for males and 2.4 ± 0.2 min for females at 100 ppm; at 300 ppm, $t_{1/2}$ increased to 5.4 ± 0.5 min and 5.6 ± 0.2 min in males and females, respectively. Saturation kinetics was observed at the higher exposure concentration. Steady state was achieved after 3 hours. Peak ethylene oxide concentrations at steady state were equivalent in all tissues except testes, where concentrations were significantly lower (ca. 50% of other tissues). The rates of disappearance of ethylene oxide were essentially identical for blood and other tissues. | Non-guideline and non-GLP study, concentration of ethylene oxide following inhalation exposure determined in several tissues. The study is considered reliable and can be used in the assessment of toxicokinetic behaviour of ethylene oxide as a part of weight of evidence. | Brown CD et al. (1996) A6.2/02 |
| Non-guideline, non-GLP, *in vitro* metabolism of ethylene oxide in cytosol and microsomes from rats and mice. | Cytosolic and microsomal fractions were prepared from male and female rat and mouse liver, kidney, testes (male only), lung and brain tissue | Ethylene oxide, > 99.9% pure, incubation with 10-50 µL ethylene oxide in headspace for 2 hours | 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 | Non-guideline and non-GLP study, *in vitro* metabolism in cytosolic and microsomal fractions prepared from rats and mice. | Brown CD et al. (1996) A6_2(02) |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| Reliability 2, key study. | | | 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. The higher values in mice are consistent with the more rapid elimination in this species *in vivo* compared with rats. | | |
| Non-guideline, non-GLP study, dosimetry *in vivo* by measuring ethylene oxide concentrations in blood after 2, 3 and 4 hours of inhalation exposure. Glutathione concentrations were measured in liver, lung, kidneys and testes to assess the role of glutathione depletion in the saturable metabolism<br><br>Reliability 2, key study. | B6C3F1 mice, 13 males/concentration (4 males/concentration were used for blood measurements at 2, 3 and 4 hours; glutathione concentration was also determined in 4 mice/concentration after 4 hours exposure); the last mouse was used for the determination of breathing parameters | Ethylene oxide, > 99.9% pure, 0, 50, 100, 200, 300 and 400 ppm (nominal), 0, 55 ± 11, 104 ± 6, 204 ± 7, 301 ± 5 and 400 ± 11 ppm (analytical); nose-only 4 hours exposure, with interim blood measurements at 2, 3 and 4 hours | For ethylene oxide concentrations ≤ 200 ppm, blood concentrations remained essentially constant throughout the sampling period, indicating that the steady state was reached before 2 hours. Above 200 ppm blood concentrations increased with exposure duration. Terminal (4-hours) blood concentrations were linearly correlated with exposure concentrations between 50-200 ppm; above 200 ppm the blood concentration increased sublinearly. Glutathione levels in liver and lungs were significantly decreased in comparison to | Non-guideline, non-GLP study, male mice only; determination of ethylene oxide concentrations in blood and glutathione concentration in tissues. The study is considered reliable and can be used in the assessment of toxicokinetic behaviour of ethylene oxide as a part of weight of evidence. | Brown CD et al. (1998)<br>A6_2(03) |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| | | | sham-exposed controls at exposure concentration ≥ 100 ppm, while significant depletion of glutathione in testes and kidneys occurred only at ≥ 300 ppm. | | |
| Non-guideline, non-GLP, determination of urinary metabolites of ethylene oxide following inhalation or intravenous exposure in rats<br><br>Reliability 2, key study. | Sprague-Dawley rats, 5 males/dose | Ethylene oxide, 99.7% pure, 6 hours exposure to 200 ppm (nominal; 189 ppm analytical) by inhalation or 20 and 60 mg/kg bw intravenously as aqueous solution | At 20 mg/kg bw ethylene oxide intravenously, 31% was eliminated as 2-hydroxyethylmercapturic acid and 6% as ethylene glycol. No N-acetyl-S-carboxymethyl-L-cysteine was detected. At 60 mg/kg bw, the percentage of 2-hydroxyethylmercapturic acid decreased to 23.7%, while the percentage of ethylene glycol remained the same (6.3%). Larger amounts of 2-hydroxyethylmercapturic acid were excreted during the 6-24 hours period. No differences in the qualitative pattern of urinary metabolite excretion were evident in animals exposed by inhalation. | Non-GLP non-guideline study; determination of urinary metabolites of ethylene glycol following inhalation and intravenous exposure. No other toxicokinetic parameters were determined. Only male rats were used. Despite the number of limitations, the study is considered reliable and can be used in the assessment of toxicokinetic behaviour of ethylene oxide, addressing metabolism of ethylene oxide, as a part of weight of evidence. | Tardif R et al. (1987) A6.2/04 |
| Non-guideline, non-GLP, determination of urinary metabolites of ethylene oxide following inhalation or intravenous exposure in | Swiss CD-1 mice, 10 males/dose | Ethylene oxide, 99.7% pure, 6 hours exposure to 200 ppm (nominal; 193 ppm analytical) by inhalation or 20 and 60 mg/kg bw | At 20 mg/kg bw ethylene oxide intravenously, 8.3% was eliminated as 2 – hydroxyethylmercapturic acid, 5.8% as S-2-hydroxyethyl-L-cysteine; 1.9% as S-carboxymethyl-L-cysteine and | Non-GLP non-guideline study; determination of urinary metabolites of ethylene glycol following inhalation and intravenous exposure. No other toxicokinetic | Tardif R et al. (1987) A6.2/04 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| mice.<br><br>Reliability 2, key study. | | intravenously as aqueous solution | 3.3% as ethylene glycol. No N-acetyl-S-carboxymethyl-L-cysteine was detected. At 60 mg/kg bw, the percentages of different metabolites did not change significantly (7.0% as 2-hydroxyethylmercapturic acid, 5.0% as S-2-hydroxyethyl-L-cysteine, 3.2% as S-carboxymethyl-L-cysteine and 2.6% as ethylene glycol). Nearly equal amounts of 2-hydroxyethylmercapturic acid were excreted during 0-6 and 6-24 hours periods. No differences in the qualitative pattern of urinary metabolite excretion were evident in animals exposed by inhalation. | parameters were determined. Only male mice were used. Despite the number of limitations, the study is considered reliable and can be used in the assessment of toxicokinetic behaviour of ethylene oxide, addressing metabolism of ethylene oxide, as a part of weight of evidence. | |
| Non-guideline, non-GLP, determination of urinary metabolites of ethylene oxide following inhalation or intravenous exposure in rabbits.<br><br>Reliability 2, key study. | Rabbits, strain unspecified, 3 males/dose (intravenous), 4 males/dose (inhalation) | Ethylene oxide, 99.7% pure, 6 hours exposure to 200 ppm (nominal; 201 ppm analytical) by inhalation or 20 and 60 mg/kg bw intravenously as aqueous solution | At 20 mg/kg bw ethylene oxide intravenously, only 2% were excreted as ethylene glycol. No other metabolites were detected. At 60 mg/kg bw, this amount increased to 2.8%, which were excreted in the period of 6-24 hours. No differences in the qualitative pattern of urinary metabolite excretion were evident in animals exposed by inhalation. | Non-GLP non-guideline study; determination of urinary metabolites of ethylene glycol following inhalation and intravenous exposure. No other toxicokinetic parameters were determined. Only male rabbits were used. Despite the number of limitations, the study is considered reliable and can be used in the assessment of | Tardif R et al. (1987) A6.2/04 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| | | | | toxicokinetic behaviour of ethylene oxide, addressing metabolism of ethylene oxide, as a part of weight of evidence. | |
| Non-guideline, non-GLP, pharmacokinetic modelling for ethylene oxide in mouse, rat and human, based on the results of *in vitro* ethylene oxide incubation experiments with cytosol and microsomal fractions to determine glutathione transferase and epoxide hydrolase activities.<br><br>Reliability 2, supporting study. | Pharmacokinetic modelling using blood and tissue from the following species:<br>F344 rats, B6C3$F_1$ mice and humans | Ethylene oxide, purity unspecified | The activity of glutathione transferase in human liver cytosol was low (Vmax ~ 8.9 nmol/min/mg protein), while the activities reported for mouse liver and rat liver were respectively approximately 25 and 5-fold higher. Epoxide hydrolase activity was similar in all three species (Vmax ~ 1.8 nmol/min/mg protein). The nonenzymatic hydrolysis reaction rate was significant.<br>In the PBPK modelling, epoxide hydrolase activity was modelled as an enzyme-mediated process with Michaelis-Menten kinetics, while glutathione transferase activity was modelled as an enzyme-mediated process with a ping-pong mechanism. The model could predict the blood, muscle, brain and testes concentrations of ethylene oxide in rat and mouse in agreement with previous studies, if an uptake of ethylene oxide was considered to be 43-60% and | Non-guideline and non-GLP study. Pharmacokinetic models for ethylene oxide were developed to describe the exposure-tissue dose relationship in rodents and humans. | Fennell TR et al. (2001)<br>A6.2/05 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| | | | 40%, respectively, and a diffusion-limited distribution to testes was incorporated. For human model, the model produced a good fit of the experimental data by setting the pulmonary uptake to approximately 78%. | | |

A3.1.1 Short summary and overall relevance of the provided toxicokinetic information

A number of *in vivo* and *in vitro* toxicokinetic studies with ethylene oxide were submitted by the applicant. None of these studies fulfilled all criteria required by the modern guidelines, but together they provide sufficient weight of evidence for the absorption, distribution, metabolism and elimination of ethylene oxide by respiratory route. No studies addressing oral or dermal route of exposure were submitted; however, as ethylene oxide is a gas, respiratory route of exposure is considered to be the most relevant one.

Toxicokinetic models for ethylene oxide have been developed and gradually improved. Fennell et al. (2001) used *in vitro* and *in vivo* toxicokinetic data generated by Brown et al. (1996, 1998) to refine a previously developed PBPK model for ethylene oxide in rats and extend the models to mouse and humans. The model describes exposure-tissue dose relationship in rodents and humans. In the model, ethylene oxide metabolism in both rodents and humans was described as enzymatically catalysed conjugation with glutathione via cytosolic glutathione S-transferase (cGST) via a ping-pong mechanism and enzymatic hydrolysis via microsomal epoxide hydrolase with Michaelis-Menten kinetics. Model simulations of blood time courses in rats and mice were performed and output was compared to experimental data. The initial model simulations overpredicted observed blood dosimetry in both the rat and the mouse, suggesting that the default assumption of 100% pulmonary uptake of ethylene oxide from the alveolar region of the rodent lung may be an overestimation. By reducing the uptake to 43% (nose only) to 60 % (closed chamber) for rats and 40% for mice, a good agreement of the simulated blood levels and the elimination kinetics of ethylene oxide was obtained with experimental values. The basis of the values used in the modelling was publications suggesting an uptake between 40-60% in rodents (e.g. Filser and Bolt, 1984). For humans, pulmonary uptake was set at ca. 78% of cardiac output, based on observations of Brugnone et al. (1985, 1986) indicating an alveolar retention of ca. 75-80%. The model simulation for humans resulted in a good fit of the experimental data from Brugnone et al. (1986).

At a steady state in humans 20-25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound. The half-life of ethylene oxide in human blood has been calculated to be 48 minutes (Fennell et al., 2001); thus, no accumulation of ethylene oxide in humans over a working week is to be expected. Ethylene oxide and its metabolites have been shown to be rapidly distributed throughout tissues.

Studies involving inhalation exposure showed that the uptake and elimination of ethylene oxide was linear in rats at concentrations up to 1000 ppm. In mice, metabolism was linear up to a concentration of 200 ppm, but at higher concentrations metabolism was saturated and blood concentrations of ethylene oxide were proportionately higher (Filser and Bolt, 1984; Brown et al, 1996; 1998). Ethylene oxide elimination was more rapid in mice compared with rats; the elimination half-life in rats was 13.8 ± 3.0 min and 10.8 ± 2.4 min for males and females, respectively, whereas in mice the elimination half-life was 3.12 ± 0.2 min and 2.4 ± 0.2 min for males and females, respectively, at 100 ppm. Even though metabolism of mice was saturated at higher concentrations, the elimination half-life at 300 ppm, 5.4 ± 0.5 min in males and 5.6 ± 0.2 min in females, was still lower than in rats. There were some statistically significant differences in the concentration of ethylene oxide in the blood of rats and mice exposed to 100 or 300 ppm at the end of a 4 hours exposure period, but the differences were relatively small. Concentrations of ethylene oxide in the muscle, brain and testes of rats and mice exposed to 100 and 330 ppm ethylene oxide for 4 hours were also similar (Brown et al., 1996). Peak ethylene oxide concentrations at steady state were equivalent in all tissues except testes, where concentrations were significantly lower (ca. 20% and 50% of other tissues in rats and mice, respectively). Therefore, in the PBPK-model by Fennell (2001) a diffusion limitation was incorporated for the testis for better agreement between model prediction and the observed values.

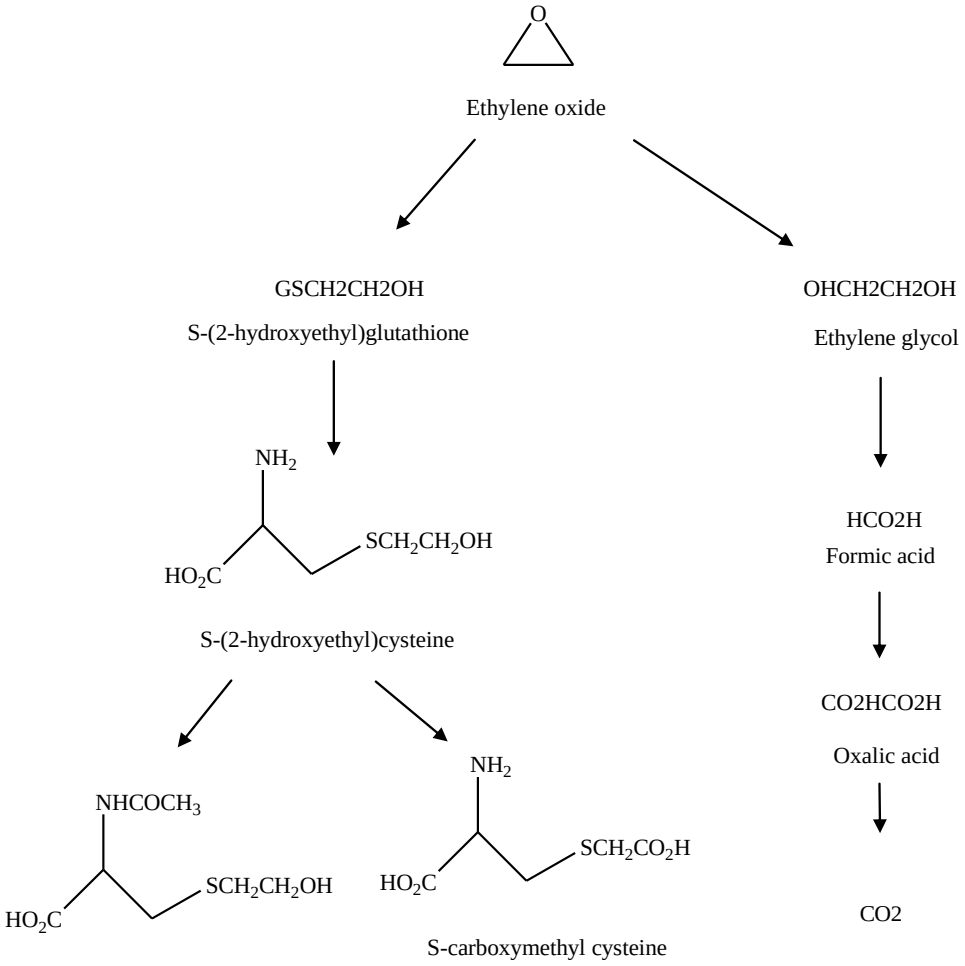
The metabolism of ethylene oxide was investigated *in vitro* using subcellular fractions

prepared from rat and mouse liver, kidney, lung, brain and testes. Three metabolites, S-(2-hydroxyethyl)glutathione, ethylene glycol and 2-chloroethanol, were identified, and metabolic activity was highest in liver, followed by kidney and testes. Lungs and male rat brain had relatively low activity. The metabolism of ethylene oxide was mainly catalysed by glutathione transferase, and $V_{max}$ values for this enzyme in rats and mice are consistent with the more rapid metabolism of ethylene oxide in mice (Brown et al., 1996). Four metabolites, 2-hydroxyethyl mercapturic acid, S-(2-hydroxyethyl)-cysteine, S-carboxymethyl cysteine and ethylene glycol, were identified in an *in vivo* study in which mice, rats and rabbits were exposed by inhalation and intravenous administration. Qualitative and quantitative differences in the metabolites were found amongst the species (Tardiff et al., 1987). Apart from 2-chloroethanol the metabolites identified in the *in vivo* and *in vitro* studies were formed by the same initial metabolic pathways, namely conjugation with glutathione and hydrolysis. 2-Hydroxyethyl mercapturic acid, S-(2-hydroxyethyl)-cysteine and S-carboxymethyl cysteine are then formed by further metabolism of the glutathione conjugate. 2-Chloroethanol is probably not a metabolite *in vivo*, but is formed because of the presence of chloride ions in the buffer solution used for the *in vitro* experiment.

In humans the major amount of ethylene oxide is metabolized by hydrolysis, only 20% are converted to glutathione conjugates and there is little change in metabolism with increasing exposure concentration. In mice and rats a higher portion of ethylene oxide is metabolized by GSH conjugation (80% and 60% respectively) resulting in a depletion of GSH at higher exposure concentrations (100 ppm and above) and non-linearity in metabolic elimination of ethylene oxide (CLH report, 2016; Fennell et al., 2001). Elimination of ethylene oxide metabolites in urine of rats exposed by inhalation to 100 ppm $^{14}C$ ethylene oxide for 6 hours, constituted ca. 60% of the dose, while 6% was recovered in faeces, 9% as $CO_2$ and 1% as unchanged ethylene oxide in the exhaled air (CHL report, 2016).

A proposed metabolic pathway is shown in below (next page).

Proposed metabolic pathway for ethylene oxide




Both *in vitro* and *in vivo* experiments showed that the saturation of metabolism in mice was not due to the saturation of the capacity of the enzymes involved in the biotransformation of ethylene oxide, but was probably due to depletion in glutathione levels in tissues (Brown et al., 1996, 1998).

A3.1.2 Values and conclusions used for the risk assessment

| **Value(s) used in the Risk Assessment – Oral absorption** | |
|---|---|
| Value(s) | Not derived |
| Justification for the selected value(s) | Not applicable, as ethylene oxide is a gas, thus exposure via oral route is not expected to occur. |

| **Value(s) used in the Risk Assessment – Dermal absorption** | |
|---|---|
| Value(s) | Not derived |
| Justification for the selected value(s) | Not derived.<br>Ethylene oxide is classified as corrosive to skin (Skin Corr. 1, H314) and as causing serious eye damage (Eye Dam. 1, H318). Based on this, a local risk assessment could be relevant. However, due to the physicochemical properties and described working condition, inhalation is the most relevant route of exposure for EtO. |

| | |
|---|---|
| | In addition, as the critical effect of ethylene oxide is the genotoxic carcinogenicity with a non-threshold mode of action, a risk assessment for local effects is not considered warranted.<br><br>It should be noted that the studies used for DMEL-setting was performed by chamber/whole body exposure and that dermal exposure also could be assumed to be covered in these studies. |

| **Value(s) used in the Risk Assessment – Inhalatory absorption** | |
|---|---|
| Value(s)* | 75-80% (human) |
| Justification for the selected value(s) | A pulmonary uptake of 75-80% for humans has been reported. Using these values in PBPK modelling, the simulated blood levels of EtO were in good agreement with the experimental values obtained.<br><br>Not to be used in the DMEL setting as the underlying data in the different species are considered not sufficiently consistent or robust to justify a correction of the DMEL value. |

| **Conclusion(s) used in the Risk Assessment – Distribution** | |
|---|---|
| Conclusion | Ethylene oxide is considered to be rapidly and fully distributed through the body. |
| Justification for the conclusion | Based on the available toxicokinetic studies, in which concentration of ethylene oxide in blood and several tissues, including brain, testes and muscles, was studied. |

| **Conclusion(s) used in the Risk Assessment – Metabolism** | |
|---|---|
| Conclusion | Two major pathways of ethylene oxide metabolism have been established: conjugation with glutathione by glutathione transferase and hydrolysis to ethylene glycol. |
| Justification for the conclusion | Based on the *in vitro* toxicokinetic studies in which cytosol and microsome fractions were exposed to ethylene oxide, and *in vivo* studies in which metabolites of ethylene oxide were identified in urine of the exposed animals. |

| **Conclusion(s) used in Risk Assessment – Elimination** | |
|---|---|
| Conclusion | At a steady state in humans 20-25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound. The major amount of ethylene oxide is metabolized by hydrolysis, while ca. 20 % are converted to glutathione conjugates. The half-life of ethylene oxide in human blood has been calculated to be 48 minutes; thus, no accumulation of ethylene oxide in humans over a working week is to be expected. |
| Justification for the conclusion | Based on the available *in vivo* toxicokinetic studies. |

## A.3.2. Acute toxicity / STOT SE

### A.3.2.1 Acute oral toxicity

Table A.14 Summary table of animal studies on acute oral toxicity

| **Method, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/group** | **Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other)** | **Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)** | **Value LD50** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|---|
| Pre-dates OECD guidelines and GLP<br>Reliability 3 | Rat, Wistar, male, number not explicitly stated, but the article mentions that in most cases 10 animals per dose were used. | Ethylene oxide (1% solution in water), oral gavage, dose levels not specified | 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. | 330 mg/kg bw (95% CI 290-360 mg/kg bw) | Predates modern guidelines. Clinical signs of toxicity, body weight changes and time at which animals died, were not reported. Gross pathology was apparently performed, as digestive tract irritation and effects on kidneys, liver and bile are reported. No information is available on dose levels, purity and batch of the test material. | Smyth et al. (1941)<br>A6.1.1 |
| Pre-dates OECD guidelines and GLP. | Guinea pig, strain not specified, male/female, | Ethylene oxide (1% solution in water), oral | Not reported | 270 mg/kg bw (95% CI 190-380 mg/kg bw) | Predates modern guidelines and GLP; no necropsy | Smyth et al. (1941)<br>A6.1.1 |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LD50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| Reliability 3 | number not explicitly stated, but the article mentions that in most case 10 animals per dose were used. | gavage, dose levels not specified | | | performed; clinical signs of toxicity and body weight changes not reported. No information on the test animals strain is provided. Gross pathology was apparently performed, as digestive tract irritation and effects on kidneys, liver and bile are reported. No information is available on the purity and batch of the test material. | |
| Predates OECD guidelines and GLP; similar to OECD guideline 401.<br><br>Reliability 3 | Rat, strain and sex unspecified, number of animals unspecified | Ethylene oxide, oral feed, dose levels unspecified | Not reported | 330 mg/kg bw | The study is cited in the CLH report (2016) and was mentioned in the aggregated CSR provided by ECHA during substance evaluation; the original paper could not be recovered.<br>Predates modern guidelines and GLP; | Bruhin H. (1961), additional study cited in CLH report (2016) |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity) Vehicle, Dose levels, Type of administration (gavage, in diet, other) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LD50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | | no further details are available. | |
| Predates OECD guidelines and GLP; no information on testing method available.<br><br>Reliability 3 | Mouse, male/female, strain and number of animals unspecified | Ethylene oxide, oral, no further details available | Not reported | 280 mg/kg bw (f)<br>365 mg/kg bw (m) | The study is cited in the CLH report (2016) and in the WHO (2003) evaluation; original paper could not be recovered.<br>Predates modern guidelines and GLP; no further details are available. | Woodard G (1971), additional study cited in CLH (2016) and WHO (2003) |
| Predates OECD guidelines and GLP; no information on testing method available.<br><br>Reliability 3 | Guinea pig, strain, sex and number of animals unspecified | Ethylene oxide, oral, no further details available | Not reported | 270 mg/kg bw | The study is cited in the CLH report (2016) and in the WHO (2003) evaluation; original paper could not be recovered.<br>Predates modern guidelines and GLP; no further details are available. | Woodard G (1971), additional study cited in CLH (2016) and WHO (2003) |

No human data on acute oral toxicity of ethylene oxide are available.

A3.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Limited information on acute oral toxicity of ethylene oxide is available. The applicant has submitted the report on acute oral toxicity study in rats and guinea pigs of Smyth et al., 1941. In addition, several other reports were recovered from public literature. The rat LD50 of 330 mg/kg bw was reported by Smyth (1941) and confirmed by Bruhin (1961). The LD50 in guinea pigs was 270 mg/kg bw (Smyth et al., 1941, Woodard, 1971), while in mice the reported LD50 was 280 mg/kg bw in females an 365 mg/kg bw in males (Woodard, 1971).

As the acute toxicity value expressed as LD50 is between 50 mg/kg bw and 300 mg/kg bw, the Committee for Risk Assessment (RAC) agreed to classify ethylene oxide as Acute Oral 3, H301 based on available information.

A3.2.1.2 Conclusion on acute oral toxicity related to risk assessment

| **Value used in the Risk Assessment – Acute oral toxicity** | |
|---|---|
| Value | LD50 = 50-300 mg/kg bw |
| Justification for the selected value | Ethylene oxide was evaluated by the RAC, with the conclusion to classify it for acute oral toxicity as Acute Tox. 3, H301 (toxic if swallowed), based on the lowest LD50 values of 270 mg/kg bw reported in two studies with guinea pigs, and an LD50 of 280 mg/kg bw reported in a study with female mice. This conclusion is followed by the eCA. For Acute Tox. 3 the LD50 values are in the range of 50-300 mg/kg bw. |

| **Data waiving** | |
|---|---|
| Information requirement | Acute oral toxicity.<br>A study with low reliability has been provided by the applicant. No further requirements needed. |
| Justification | 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.<br>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.<br>No data gaps on acute oral toxicity were identified. |

### A.3.2.2 Acute dermal toxicity

| Data waiving | |
|---|---|
| Information requirement | No information available, not required. |
| Justification | 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.<br>Moreover, the available inhalation animal studies are usually done with a whole body exposure, so that dermal exposure is also covered by these studies. |

### A.3.2.3 Acute inhalation toxicity

Table A.15 Summary table of animal studies on acute inhalation toxicity

| **Method, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/group** | **Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only)** | **Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations)** | **Value LC50** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|---|
| Predates modern guidelines and GLP, but similar to OECD guideline 403.<br><br>Reliability 2, key study. | Rat, male, strain unspecified (white), 10/dose | Ethylene oxide, gas, 882, 1343, 1648, 1843, 1992 and 2298 ppm, equal to 1590, 2420, 2970, 3320, 3590 and 4140 mg/m$^3$ (analytical concentrations), whole body | Frequent movement and preening, clear nasal discharge, lacrimation, diarrhoea, gasping (increasing in intensity during exposure) and, occasionally, salivation.<br>Pathology: Irritation in the upper respiratory passages, moderate congestion and petechial haemorrhages in the tracheal | LC50 (4-hr): 1460 ppm (95% CI = 620-2550 ppm) (2630 mg/m$^3$) | Predates modern guidelines and GLP; no information on the test animal strain, test substance purity and batch provided. No information on the concentrations at | Jacobson KH et al. (1956)<br>A.6.1.3 |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LC50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | (exposure chambers) | mucosa. The maximum pulmonary lesions consisted of a very minor degree of patchy scattered oedema sometimes involving the peribronchial zones. Gross distension of the stomach | | which clinical signs were observed, and individual data not available. Only one sex tested. | |
| Predates modern guidelines and GLP, but similar to OECD guideline 403.<br><br>Reliability 2, key study. | Mouse, female, strain unspecified (white), 10/dose | Ethylene oxide, gas, 533, 860, 882, 960, 1343 and 1365 ppm, equal to 960, 1550, 1590, 1730, 2420 and 2460 mg/m$^3$ (analytical concentrations), whole body (exposure chambers) | Frequent movement and preening, nasal discharge, lacrimation, gasping and, occasionally, salivation. Gross distension of the stomach | LC50 (4-hr): 835 ppm (95% CI = 623-1040 ppm) (1501 mg/m$^3$) | Predates modern guidelines and GLP; no information on the test animal strain, test substance purity and batch provided. No information on the concentrations at which clinical signs were observed, and individual data not available. Only one sex tested. | Jacobson KH et al. (1956)<br>A.6.1.3 |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LC50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| Predates modern guidelines and GLP, but similar to OECD guideline 403.<br><br>Reliability 2, key study. | Dog, male, Beagle, 3/dose | Ethylene oxide, gas, 327, 710, 1393 and 2830 ppm, equal to 590, 1280, 2510 and 5100 mg/m$^3$ (analytical concentrations), whole body (exposure chambers) | 2800 ppm: lacrimation, clear nasal discharge, salivation, vomiting of a frothy colourless/yellow mucus, diarrhea and convulsions with laboured breathing.<br>1400 ppm: similar signs except for diarrhea, convulsions and observable changes in respiration<br>Pathology: Moderate congestion in the lungs, dilatation of perivascular lymphatic spaces and perivascular oedema.<br>Gross distension of the stomach | LC50 (4-hr): 960 ppm (95% CI not specified) (1730 mg/m$^3$) | Predates modern guidelines and GLP; no information on the test animal strain, test substance purity and batch provided. Only one sex tested. | Jacobson KH et al. (1956)<br>A.6.1.3 |
| Comparable to OECD guideline 403, public literature stated to be GLP.<br><br>Reliability 2, supportive study. | Rat, Sprague-Dawley, male/female, 5/sex/dose | Ethylene oxide, gas, 99.9% pure; 1850, 2026 and 2182 ppm (males); 1443, 1637 and 1850 ppm (females); whole body (exposure chambers) | Clinical signs during 4hr exposure of all doses: periocular/perinasal/perioral wetness, gasping, audible respiration, hypoactivity, absence tail/toe pinch reflex.<br>No rats died, but one male rat died after 1 hour postexposure to the highest level (2182 ppm).<br>Postexposure observations (during 5 of 14 days): Periocular/perinasal/perioral encrustation, unkempt fur, | LC50 (4-hr): 1972 (95% CI = 1887-2061) ppm (equal to 3550 (95% CI: 3397-3710) mg/m3) (male);<br><br>1537 (95% CI = 1391- | The study was cited in the CLH report (2016) and the RAC opinion (2017); the original publication was recovered and assessed by the eCA. | Snellings WM et al. (2011); additional study cited in CLH report (2016) and the RAC opinion (2017). |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LC50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | gasping, audible respiration, decreased respiration rate, hypoactivity, tremors. Deaths occurred within three days postexposure. No clinical signs observed in survivors (all groups) after postexposure day 5. Histological examination of lungs: 2182 ppm males and 1850 ppm females; Congestion of lungs (moderate, marked or severe), mild haemorrhage and mild interstitial pneumonitis; in addition, pulmonary edema, emphysema and alveolar histiocytosis (2182 ppm in males) 1850 ppm males; No histopathological changes seen. 1443 ppm female (n=1) congestion of lungs. | 1698) ppm (equal to 2767 (95% CI: 2504-3056) mg/m3 (female);<br><br>1741 (95% CI = 1655-1831) ppm (equal to 3134 (95% CI: 2979-3296) mg/m3) (combined sexes) | | |
| Comparable to OECD guideline 403, GLP.<br><br>Reliability 2, supportive study. | Rat, Sprague-Dawley, male/female, 5/sex/dose | Ethylene oxide, gas, 99.9% pure; 4827, 5546 and 6161 ppm (males); 3966, 4202 and 4827 ppm (females); whole body (exposure | Clinical signs during 1hr exposure: 4827, 5546, and 6161 ppm males; absent startle reflex, in addition, gasping at the highest dose. 3966 and 4202 ppm females; no clinical signs. | LC50 (1-hr): 5748 (95% CI = 5276-6262) ppm (equal to 10346 (95% | The study was cited in the CLH report (2016) and the RAC opinion (2017); the original publication was recovered and assessed by the | Snellings WM et al. (2011); additional study cited in CLH report (2016) and |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LC50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | chambers) | 4827 ppm females; absent startle reflex.<br><br>Postexposure observation (during 3 out of 14 days):<br>At all doses and both sexes; decreased respiration rate, hypoactivity and unkempt fur. In addition;<br>4827 ppm males; Perioral/perinasal, and encrustation.<br>5546 and 6161 ppm males; Ataxia and tremors.<br>3966 ppm females; Ataxia and tremors. 4202 ppm females; Periocular/perinasal encrustation, ataxia and tremors<br>4827 ppm females; Perinasal wetness, pericular/perioral/perinasal and encrustation. | CI = 9497-11272) mg/m3 (male);<br><br>4439 (95% CI = 4034-4884) ppm (equal to 7990 (95% CI = 7261-8791) mg/m3 (female);<br><br>5029 (95% CI 4634-5459) ppm (equal to 9052 (95% CI = 8341-9826) mg/m3 (combined sexes) | eCA. | the RAC opinion (2017). |
| Comparable to OECD guideline 403, non-GLP. | Mouse, B6C3F1, male/female, 5/sex/dose | Ethylene oxide, gas, > 99% pure; target concentrations 100, 200, 400, 800 and | Clinical signs:<br>100-400 ppm; no signs described.<br>800 ppm; lacrimation and dyspnoea. | LC50 (4-hr): 660 ppm (95% CI = 509- | No LC50 was derived for males. The study was conducted as a part | NTP TR 326 (1987) |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD) Actual and nominal concentration, Type of administration (nose only / whole body / head only) | Signs of toxicity (nature, onset, duration, severity, reversibility, include concentrations) | Value LC50 | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| Reliability 2, supportive study. | | 1600 ppm; actual concentrations 96, 201, 409, 816 and 1542 ppm (males) and 96, 201, 409, 816 and 1542 ppm (females); whole body (exposure chambers) | 1600 ppm; severe dyspnoea, incoordination, semi-consciousness and diarrhoea | 856 ppm) (females) | of 2-year carcinogenicity study by NTP and is discussed in detail in Section 3.9; no Doc IIIA summary of the acute toxicity part was submitted by the applicant. | |

**Human data on acute inhalation toxicity**

The following information on human data on acute toxicity of ethylene oxide was taken from the SCOEL report (2012) and the CHL report (2016).

*There are numerous reports of casuistics of acute inhalation toxicity of ethylene oxide, mostly involving occupational intoxications. However, there are usually no details of the ethylene oxide concentrations or doses involved. Depending on the exposure conditions, the first symptoms appeared either during exposure or within a few minutes to several hours after the end of exposure. The main symptoms were headaches, nausea and generally persistent periodic vomiting. Dyspnoea, irritation of the eyes and upper respiratory mucosa, heart damage, excitation, stupor, vertigo and loss of consciousness were also observed. Clinical-pathological investigations revealed spontaneous nystagmus, impaired hearing, bilirubinuria, ventricular bigeminy, bradycardia, increased ventricular activation time. Permanent health impairment as a result of an acute ethylene oxide intoxication has not been described. The symptoms of systemic intoxication (e.g. headaches, vomiting) often appear before the local effects (irritation). However, when materials sterilised with ethylene oxide were used for an extracorporeal bypass, the high residue levels caused haemolysis, increased levels of free haemoglobin and renal complications in the patients. Deaths of three children from toxic shock after open heart operations were ascribed to the ethylene oxide residues in the PVC parts of the heart-lung machines which had been sterilised with this substance.*

A3.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

As ethylene oxide is a gas, inhalation is considered to be the main exposure route.

The applicant has submitted the report of Jacobson et al. (1956) on acute inhalation toxicity study with male rats, female mice and male dogs. In addition, several other reports in different species (rats, mice) were recovered in public literature. In the study by Snellings et al. (2011) four hours whole-body exposure of groups of 5 male and 5 female Sprague-Dawley rats to 99.9% pure ethylene oxide at concentrations of 1850, 2026 and 2182 ppm (males) and 1443, 1637 and 1850 ppm (females) caused ocular and nasal irritation, irregular breathing, absence of certain reflexes, ataxia, and tremors during exposure. No rats died during any of the 4-hour exposure periods, but one high-dose male rat died 1 hour after the 4hr- exposure period. All remaining deaths occurred within three days postexposure. All female rats and 4 out of 5 male rats of the highest exposure concentration groups (1850 and 2182 ppm, respectively) died. For all groups, no clinical signs were observed in survivors after postexposure day 5. Histological examination of lungs was conducted for 1850 and 2182 ppm males and 1443 and 1850 ppm females. The principal gross findings observed in animals that died included diffuse or multifocal discoloration of the lungs and hyperinflation of the lungs. Treatment-related microscopic lesions were seen in all the lungs of males of the 2182 ppm group and all the females of the 1850 ppm group that died, all by day 2 postexposure. The principal lung lesion observed in all the animals that died (both sexes) was moderate to severe pulmonary congestion. Other lesions which may indicate a toxic effect on the lungs included mild hemorrhage, pulmonary edema, and emphysema, which were noted in some of the animals that died. The 4-hr LC50 was 1972, 1537 and 1741 ppm in males, females and combined sexes, respectively (equal to 3550, 2767 and 3134 mg/m$^3$, respectively). These findings were supported by the results described in Jacobson et al. (1955): male rat LC50 of 1460 ppm (2630 mg/m$^3$).

In the NTP study (1987) performed with male and female B6C3F1 mice, groups of five female and five male mice were exposed to 100, 200, 400, 800 and 1600 ppm ethylene oxide for four h. No deaths were observed at 100, 200 and 400 ppm. 5/5 male mice and 4/5 female mice exposed to 800 ppm died 1-3 days after exposure. At 1600 ppm, all male (5/5) and female (5/5) mice died within 4 h after exposure. No clinical signs were described

at 100-400 ppm. In the groups exposed to 800 ppm ethylene oxide, lacrimation and dyspnoea were observed. The clinical findings reported at the highest dose included severe dyspnoea, incoordination, semi-consciousness and diarrhoea. An LC50 value of 660 ppm (95% CI 509-856 ppm) was calculated (female mice) based on the results.

Overall, the reported LC50 in rats were in the range of 1460-1972 ppm (Jacobson, 1956, and Snellings et al, 2011), in mice in the range of 660-835 ppm (Jacobson, 1956, and NTP, 1987), and 960 ppm in dogs (Jacobson, 1956).

For classification as Acute Tox. 3; H331, the LC50 needs to be between 500 and 2500 ppm. All LC50 derived from the different studies were well between these limits. The Committee for Risk Assessment (RAC) agreed to classify ethylene oxide as Acute Inhal. 3, H331 as a minimal classification based on available information.

No data gaps were identified; the applicant submitted sufficient information for each data requirement. Additional studies considered by RAC were in the public domain.

A3.2.3.2 Conclusion on acute inhalation toxicity related to risk assessment

| **Value used in the Risk Assessment – Acute inhalation toxicity** | |
|---|---|
| Value | LC50 (4-hr) = 660 (95% CI = 509-856 ppm) (mouse, female) |
| Justification for the selected value | Ethylene oxide was evaluated by the RAC, which has concluded to classify it for acute inhalation toxicity as Acute Tox. 3, H331, based on the LC50 values, calculated for 4-h exposure, in the available inhalation studies, varying between 660 ppm (female mice) and 1972 ppm (male rats). According to the CLP criteria, classification is required where the LC50 is ≤ 20000 ppm. For classification as Acute Tox. 3; H331, the LC50 needs to be between 500 and 2500 ppm. All LC50 derived from the different studies were well between these limits. |

## A.3.2.4 Specific target organ toxicity – single exposure Category 1 and 2 (STOT SE 1 and 2)

According to the 14th ATP to CLP, ethylene oxide is not classified as STOT SE 1 and 2.

## A.3.2.5 Specific target organ toxicity – single exposure Category 3 (STOT SE 3)

A3.2.5.1 Overall conclusion on acute toxicity related to risk assessment

| Value used in the Risk Assessment – Acute systemic toxicity | |
|---|---|
| **Value** | |
| Justification for the selected value | As ethylene oxide is corrosive to skin, it is also expected to cause respiratory tract irritation. This is consistent with clinical signs observed in acute and repeated dose inhalation toxicity studies.<br>Moreover, RAC concluded that the criteria for classification for specific target organ toxicity, based on transient, narcotic effects, are fulfilled. |
| Classification according to CLH | According to the 14$^{th}$ ATP to CLP, ethylene oxide is classified as STOT SE 3:<br>H335; May cause respiratory irritation<br>H336; May cause drowsiness or dizziness |

## A.3.3. Skin corrosion and irritation

No *in vitro* skin corrosion and irritation studies with ethylene oxide were provided by the applicant for this endpoint. The BPD indicates that an OECD 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. However, none of the studies/cases, could be regarded as fulfilling the data requirements according to BPD on its own. The eCA are of the opinion that a new study is not needed and should not be performed.

Table A.16 Summary table of in vitro studies on skin corrosion/irritation*

| **Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/group** | **Test substance (including purity), Vehicle, Dose levels Duration of exposure** | **Results Average score for erythema/eschar and oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility, other adverse local/systemic effects, histopathological findings** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|
| Pre-dates modern guidelines and GLP, 1-60 minutes exposure of rabbit skin to 10% and 50% aqueous solution of ethylene oxide under occlusive conditions.<br><br>Reliability 3 | Ethylene oxide, 10% and 50% aqueous solution, 1-60 minutes, observation period 6-7 days. | Rabbit, strain, sex and number of animals unspecified | Hyperemia and edema resulted when the duration of skin contact was 6 minutes or longer. The severer exposures resulted in scar formation. The intensity of response was roughly proportional to the length of exposure time and concentration. | The study is cited in CHL report (2016), and submitted by applicant for repeated dose toxicity; no Doc IIIA summary of the skin irritation part was provided by the applicant; no information on the batch of the test substance; no details on test conditions and scoring system; exposure under occlusive conditions instead of semi-occlusive | Hollingsworth RL et al., 1956 |

Table A.17 Summary table of animal studies on skin corrosion/irritation*

| **Type of data/ report, Reliability, Key/supportive study** | **Test substance (including purity), Vehicle** | **Relevant information about the study** | **Main effects, Observations** | **Reference** |
|---|---|---|---|---|
| Volunteer study.<br><br>Reliability 3. | Ethylene oxide, 1% aqueous solution | 2 hours exposure of large skin areas | Severe blistering after 12-14 hours; the first symptoms were systemic effects like nausea, vomiting and headaches, observed a few hours after the exposure.<br>The study is cited in the CHL report (2016); the original study could not be recovered. | Sexton RJ, 1949; additional study cited in the CLH report (2016) and the RAC opinion (2017), the original study could not be recovered. |
| Volunteer study.<br><br>Reliability 3 | Ethylene oxide, 1-90% aqueous | Not specified. | 50% solution produced the most severe skin reaction, which was attributed to the rapid | Sexton RJ, 1950; additional study cited in the CLH report (2016) and |

| Type of data/ report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| | solution | | evaporation of the more concentrated solutions, which prevented more prolonged skin contact.<br>The study is cited in the CHL report (2016); the original study could not be recovered. | the RAC opinion (2017), the original study could not be recovered. |
| Volunteer study.<br><br>Reliability 2 | Ethylene oxide, | Patch test (with sterilized materials).<br>1-8 hours exposure to different types of permeable materials (fabric, rubber and plastic) impregnated with ethylene oxide, applied to backs of 12 volunteers | The greatest irritation was observed in the slow-airing PVC blocks retaining > 2000 ppm ethylene oxide (2108-2943 ppm). The most severe acute reactions were six instances of mild edema in addition to erythema (maximal score 2+). The reactions began after 2-4 hours contact and became maximal by 8 hours. In all but one subject all reactions disappeared within 72 hours. The non-woven fabric patches produced no response even at ethylene oxide levels of 5100 ppm. | Shupack et al., 1981; additional study cited in the CLH report (2016) and the RAC opinion (2017). The original study was recovered and assessed by the eCA. |

No human data on skin corrosion/irritation were submitted by the applicant. The above mentioned studies summarized in the table were cited in the CLH report (2016) and the RAC opinion (2017); the study of Shupack et al., 1981 was recovered and assessed by the eCA. The above human data retrieved from public literature are also supported by other human data on skin irritative/corrosive properties of ethylene oxide, as reported in the SCOEL report (2012) and the CHL report (2016).

Case reports of patients whose intact skin or wounds had contact with gauze or other hospital supplies that had been sterilized with ethylene oxide indicated that the observed skin reactions included erythema, blister formation, scaling, crusted ulcerations and second degree burns (Alomar, 1981; Hanifin 1971; reviewed in ATSDR, 1990). Inadequate ventilation after sterilization with ethylene oxide resulted in documented problems. Severe burns are reported in nineteen hospitalized women after contact with reusable surgical gowns and drapes, sterilized with ethylene oxide (Biro, 1974). Substantial tissue burns subsequent to the insertion of breast implants sterilized with ethylene oxide are described by Cardenas-Camarena, 1998.

A3.3.1 Overall conclusion on skin irritation and corrosivity related to risk assessment

One skin study of limited reliability was recovered from public literature, which was cited in the CHL report (2016) (Hollingsworth et al., 1956; this study was submitted by the applicant for the repeated dose toxicity data requirement, no Doc IIIA summary for skin irritation

was provided by the applicant). Exposure of rabbits (strain, sex and number of animals unspecified) to 10% and 50% aqueous solutions of ethylene oxide for a period of 1-60 minutes caused scar formation when exposure was longer than 6 minutes. In human volunteer studies in the public domain, severe blistering was observed following 2 hour exposure to 1% aqueous solution of ethylene oxide (Sexton, 1949).

| Conclusion used in the Risk Assessment – Skin irritation and corrosivity | |
|---|---|
| Value/conclusion | Corrosive to skin |
| Justification for the value/conclusion | Based on weight of evidence from available animal and human data. |
| Classification according to CLH | Skin Corr. 1, H314 Causes severe skin burns and eye damage. |

| Data waiving | |
|---|---|
| Information requirement | Skin irritation/corrosion |
| 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 irritant to human skin.<br><br>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.<br><br>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.<br><br>The performance of a new skin irritation study is considered not warranted by the eCA. |

## A.3.4. Serious eye damage and Eye irritation

No *in vitro* studies on eye irritating properties of ethylene oxide are available.

Table A.18 Summary table of in vitro studies on serious eye damage and eye irritation*

| **Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/group** | **Test substance (including purity), Vehicle, Dose levels Duration of exposure** | **Results Average score for corneal opacity, iritis, conjunctival redness and conjunctival oedema (24, 48, 72 h) per animal, observations and time point of onset, reversibility** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|
| Pre-dates modern guidelines and GLP, repeated instillation of ethylene oxide solutions (0.1-20%) into rabbit eyes. The eyes were examined 6 hours, 1, 2, 7 and 14 days after the start of treatment using a biomicroscope and after 6 hours and 14 days by histopathology. The study was designed to determine the maximal non-damaging concentration of ethylene oxide.<br><br>Reliability 3. | Rabbit, New Zealand white, male/female, 6/group | Ethylene oxide, obtained from Eastman, Inc., purity not specified; 0.05 mL of 0.1, 1.0, 5.0 and 20% solution; every 10 minutes during a 6 hour period (36 applications in total). | The maximum non-damaging concentration of ethylene oxide for ocular tissues after 6 hours instillation varied from 0.1% for conjunctiva to > 20% for the lens and retina. Opacity was the pathologic change found for the lens and cornea. The changes for conjunctiva were manifested as congestion, swelling and discharge. Once induced, corneal and lenticular damage was irreversible based on biomicroscopic examination. The iris and anterior chamber usually returned to normal around day 7. At concentrations > 1% conjunctivitis persisted through day 14. Purulent discharge beginning around day 7 was noted in eyes exposed to very high concentrations of ethylene oxide. | The study design is not in agreement with modern guideline requirements, but indicates irreversible eye effects of ethylene oxide at certain concentration after 6 hours of repeated instillation. | McDonald TO et al., 1973<br>A.6.1.4/01 |

No human data on eye irritating properties of ethylene oxide are available.

A3.4.1 Overall conclusion on eye irritation and corrosivity related to risk assessment

One eye irritation study with rabbits was submitted by the applicant. The study predates the modern guidelines and GLP, and was designed to determine the highest non-irritating concentration of ethylene oxide solutions when instilled into rabbit eyes. Following repeated instillation every 10 minutes for 6 hours of 0.05 mL of ethylene oxide aqueous solutions into rabbit eyes, the maximum non-damaging concentration varied from 0.1% for conjunctiva to > 20% for the lens and retina. Opacity was the pathologic change found for the lens and cornea. The changes for conjunctiva were manifested as congestion, swelling and discharge. Once induced, corneal and lenticular damage was irreversible based on biomicroscopic examination. The iris and anterior chamber usually returned to normal around day 7. At concentrations > 1% conjunctivitis persisted through day 14. Purulent discharge beginning around day 7 was noted in eyes exposed to very high concentrations of ethylene oxide.

As ethylene oxide is corrosive to skin, it is explicitly considered to cause serious eye damage.

| **Conclusion used in Risk Assessment – Eye irritation and corrosivity** | |
|---|---|
| Value/conclusion | Causes serious eye damage |
| Justification for the value/conclusion | Considering ethylene oxide is classified as corrosive to skin, it is explicitly considered to cause serious eye damage. This is confirmed by an available eye irritation study with rabbits, although of limited reliability. |
| Classification according to CLH | Eye Dam. 1; H318 Causes serious eye damage |

| **Data waiving** | |
|---|---|
| Information requirement | No study provided. This is not considered to be a data gap. |
| Justification | 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*.<br>Therefore there is no data gap for eye irritation. |

## A.3.5. Skin sensitisation

No reliable animal studies on skin sensitisation with ethylene oxide were submitted by the applicant. No public available animal data were recovered from literature.

The following study with human volunteers was recovered from public literature by the eCA.

Table A.19 Summary table of animal studies on skin sensitisation*

| Type of data/report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study (e.g. description of the test subjects (general population/selected/un selected dermatitis patients/workers), exposure data (induction dose, daily and overall exposure) | Main effects, Observations | Reference |
|---|---|---|---|---|
| Volunteer study, patch test with sterilized materials like rubber, PVC.<br><br>Reliability 2. | Ethylene oxide | 1-8 hours exposure to different types of permeable materials (fabric, rubber and plastic) impregnated with ethylene oxide, applied to the backs of 12 volunteers | Three weeks after application of the patches, recurrent skin reaction was observed in one volunteer. In the initial experiment, he showed mild reactions (up to 1+) to two PVC blocks. Those reactions disappeared completely after 48 hr. The delayed reactions at those sites were intensified, the more intense of the two exhibiting not only erythema and edema, but also crusting of the skin (4+ reaction). This persisted for several weeks. | Shupack et al., 1981; additional study cited in the CLH report (2016) and the RAC opinion (2017). The original study was recovered and assessed by the eCA. |

A3.5.1 Short summary and overall relevance of the provided information on skin sensitisation

In addition to Shupack et al. (1981), a number of case reports and clinical surveillance data were described in the CLH report (2016). The majority of them referred, however, to parentheral routes of exposure to ethylene oxide (e.g. during dialysis). Severe allergic-type reactions and ethylene oxide IgE antibodies among dialysis patients have been reported. These data have also been evaluated by RAC. RAC has concluded that, as these reports focused on situations in which individuals were exposed to ethylene oxide parenterally (e.g. via sterilized medical equipment), they are not relevant for the assessment of skin sensitisation. Furthermore, RAC concluded that, although ethylene oxide has been extensively used for sterilization purposes for decades, the number of case reports is very low, and each of them includes only one individual with skin reactions after exposure. The reports did not conclusively establish the relationship between the observed

reactions and ethylene oxide exposure. As ethylene oxide is a corrosive substance, it is possible that the observed eczema may have also occurred due to irritation. Thus, based on the available data RAC has concluded that classification of ethylene oxide as sensitising to skin is not warranted.

| **Conclusion used in Risk Assessment – Skin sensitisation** | |
|---|---|
| Value/conclusion | Not sensitising to skin |
| Justification for the value/conclusion | RAC considers that there is a lack of evidence for a potential to cause skin sensitisation. RAC therefore concludes that no classification is warranted for ethylene oxide for this hazard class. |
| Classification according to CLH | No classification. |

| **Data waiving** | |
|---|---|
| Information requirement | Skin sensitisation |
| 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.<br><br>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.<br><br>The performance of a skin sensitisation study is considered not warranted by the eCA. |

## A.3.6. Respiratory sensitisation

No animal data on respiratory sensitisation are available.

Table A.20 Summary table of human data on respiratory sensitisation

| Type of data/report, Reliability Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| Case report, reliability 2 | Ethylene oxide | Accidental exposure 4 hours/day for 4 days | Symptoms after 4 days exposure: coughing, shortness of breath, wheezing. Persistence of symptoms for years after removal of exposure. Absence of IgE antibodies. Reactive airways dysfunction syndrome. | Deschamps D (1992); additional study cited in the CLH report (2016) and the RAC opinion (2017). The original study was recovered and assessed by the eCA. |

A3.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No studies on respiratory sensitization have been submitted by the applicant. One case report has been recovered from public literature (Deschamps, 1992). A worker exposed to ethylene oxide 4 hours/day for 4 days developed persistent nonimmunologic asthma which persisted at least 3 years after exposure. One year after the accident pulmonary function tests showed bronchial obstruction and bronchial hyperreactivity, with the forced vital capacity being 93% of the predicted value and forced expiratory volume in 1 s (FEV1) being 74% of the predictive value. The concentration of ethylene oxide during exposure was likely to have been above 700 ppm. Immunologic tests showed no formation of IgE antibodies to ethylene oxide.
Another case study (Dugue, 1991) is cited in the CLH report (2016) on ethylene oxide; however, the original study (in French) could not be recovered. A nurse involved in the cold sterilisation of dialysis equipment showed work related asthmatic symptoms. Increase airway reactivity occurred after challenge with ethylene oxide.
It is evident that ethylene oxide causes respiratory symptoms by inhalation. The substance is also classified for respiratory tract irritation (STOT SE 3). Available case reports do not allow distinguishing between respiratory hypersensitivity and respiratory irritation. No classification for respiratory sensitization is proposed by ethylene oxide by the RAC.

A3.6.2 Overall conclusion on respiratory sensitisation related to risk assessment

| Conclusion used in the Risk Assessment – Respiratory sensitisation | |
|---|---|
| Value/conclusion | Not sensitising |
| Justification for the value/conclusion | Substances shall be classified as respiratory sensitizers if there is evidence in humans that they may cause specific respiratory hypersensitivity and/or if there are positive results from animal tests. The available human data on EtO presents a few cases of asthmatic symptoms and bronchial hyper reactivity. High exposures to irritant gases/vapours, such as ethylene oxide, may result in irritant induced asthma or RADS. These are not, however, caused by specific sensitisation. The available data on asthmatic symptoms do not present evidence that justifies classification for specific respiratory sensitisation to ethylene oxide. RAC supported the proposal for no classification for respiratory sensitisation. |
| Classification according to CLH | Not classified. |

## A.3.7. Repeated dose toxicity/STOT RE

## A.3.7.1 Short term repeated dose toxicity

A3.7.1.1 Short-term oral toxicity

No short-term oral toxicity animal studies were submitted by the applicant or recovered from public literature.

No human data on short-term oral toxicity of ethylene oxide are available.

| Data waiving | |
|---|---|
| Information requirement | Short-term oral toxicity |
| Justification | 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. |

A3.7.1.2 Short-term dermal toxicity

No short-term dermal toxicity animal studies were submitted by the applicant or recovered from public literature.

No human data on short-term dermal toxicity of ethylene oxide are available.

| Data waiving | |
|---|---|
| Information requirement | Short-term dermal toxicity |
| Justification | 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. |

A3.7.1.3 Short-term inhalation toxicity

Table A.21 Summary table of inhalatory short-term animal studies (usually 28-day studies)*

| **Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/ group** | **Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body/ head only), Duration of exposure** | **NOAEL, LOAEL** | **Results (all dose levels including severity and magnitude of all effects, including target organs)** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|---|
| 14-day inhalation dose range finding study, non-guideline, non-GLP.<br><br>Reliability 3, | Mouse, B6C3F$_1$, male/female, 5/sex/group | Ethylene oxide >99%, target concentrations 0, 50, 100, 200, 400 or 800 ppm, whole-body inhalation exposure for 6 hours/day, 5 days/week for 2 weeks. | No reliable NOAEL/<br>LOAEL can be set since it is a dose range finding study | 800 ppm: all animals showed hunched and listless behaviour and died before the end of the exposure period.<br>No adverse treatment-related effects were noted in other groups. | Dose range finding study, with minimal parameters assessed. | NTP TR 326 (1987)<br>A.6.3.3/01 |

No human data on short-term inhalation toxicity were submitted by the applicant. A number of case reports describing accidental exposure to ethylene oxide for a period up to several months were described in the CLH report (2016) and in the RAC opinion (2017); the original studies were not recovered. These case reports were primarily focused on induction of neurological changes in exposed individuals and are briefly summarized below based on the summaries given in the CLH report (2016) and the RAC opinion (2017).

Table A.22 Summary table of human data on short-term inhalation toxicity*

| **Type of data/ report, Reliability, Key/supportive study** | **Test substance (including purity)** | **Relevant information about the study** | **Main effects, Observations** | **Reference** |
|---|---|---|---|---|
| Case report.<br><br>Reliability 2 | Ethylene oxide, concentration probably > 700 ppm (based on odour) | Peripheral neuropathy<br>4 operators were exposed to ethylene oxide due to a leaking sterilizer up to 2 months | Case 1: headache, nausea, vomiting, lethargy, motor seizures at 20-30 min intervals; patient was fully recovered 2 months later.<br>Case 2: headache, limb weakness, fatigability, shift to work without exposure resulted in significant improvement.<br>Case 3: headache, altered memory and thinking, fatigability, cramps; further work under condition of lower exposure (50 ppm) resulted in no improvement of nerve conduction studies.<br>Case 4: asymptomatic but nerve conduction studies showed sensorimotor polyneuropathy; further work under condition of lower exposure (50 ppm) resulted in no improvement of nerve conduction studies. | Gross JA (1979); additional article cited in CLH report (2016); original study not recovered |
| Case report.<br><br>Reliability 4 | Ethylene oxide, concentration probably > 700 ppm (based on odour) | 2 operators were exposed to ethylene oxide several months for about 1.5 hour/day when opening the sterilizer. | Sensorimotor neuropathy (axonal sural nerve degeneration). Symptoms improved after termination of exposure. | Kuzuhara S et al (1983); additional article cited in CLH report (2016); original study not recovered |
| Case report.<br><br>Reliability 2 | Ethylene oxide, up to 500 ppm | Exposure of one individual 2-3 times daily for a period of 5 months | Polyneuropathy (distal weakness of lower extremities and transitory reduced nerve conduction velocity, nerve fibre degeneration). Improvement in re-examination 1 year after exposure | Schroeder J.M.<br>(1985); additional article cited in CLH report (2016); original study not recovered |
| Summary table of human data on short-term inhalation toxicity | | | | |
| Type of data/ report, Reliability**, Key/supportive study | Test substance (including purity) | Relevant information about the study | Main effects, Observations | Reference |

| Value used in Risk Assessment – Short-term inhalation toxicity | |
|---|---|
| Value/conclusion | The derivation of a reliable NOAEL is not possible based on available data. |
| Justification for the value/conclusion | Only limited information on short-term inhalation toxicity of ethylene oxide is available. Available information is not considered sufficient for a reliable NOAEL derivation. As ethylene oxide is a genotoxic carcinogen with a non-threshold mode of action, AELs normally derived for acute, subchronic and chronic effects will not be relevant for this active. A DMEL (by inhalation) is the only relevant threshold to be used in the risk assessment for EtO. |

| Data waiving | |
|---|---|
| Information requirement | No further requirement. |
| Justification | Adequate sub-chronic toxicity study in rodent is available. |

A3.7.1.4 Overall conclusion on short-term repeated dose toxicity related risk assessment

| Value used in the Risk Assessment – Short-term repeated dose systemic toxicity | |
|---|---|
| Value | Not derived. |
| Justification for the selected value | Based on very limited information on short-term inhalation toxicity of ethylene oxide, no reliable NOAEL can be derived. |
| Classification according to CLH | Not classified. |

| Value/conclusion used in the Risk Assessment – Short-term repeated dose local effects | |
|---|---|
| Value/conclusion | Not derived. |
| Justification for the selected value/conclusion | Based on very limited information on short-term inhalation toxicity of ethylene oxide, no reliable NOAEL can be derived. |
| Classification according to CLH | Not classified. |

## A3.7.2 Sub-chronic repeated dose toxicity

A3.7.2.1 Sub-chronic oral toxicity

No animal data on subchronic oral toxicity of ethylene oxide were submitted by the applicant or recovered from public literature by the eCA.

No human data on subchronic oral toxicity of ethylene oxide are available.

| Data waiving | |
|---|---|
| Information requirement | Subchronic oral toxicity |
| Justification | 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. |

A3.7.2.2 Sub-chronic dermal toxicity

No animal data on subchronic dermal toxicity of ethylene oxide were submitted by the applicant or recovered from public literature by the eCA.

No human data on subchronic dermal toxicity of ethylene oxide are available.

| **Data waiving** | |
|---|---|
| Information requirement | Subchronic dermal toxicity |
| 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.<br>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. |

A3.7.2.3 Sub-chronic inhalation toxicity

Table A.23 Summary table of inhalatory sub-chronic animal studies (usually 90-day studies)*

| **Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/ group** | **Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure** | **NOAEL, LOAEL** | **Results (all dose levels including severity and magnitude of all effects, including also target organs)** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|---|
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Rat, strain unknown, male/female, 5, 10 or 20/group | Ethylene oxide, 97.0-98.6% pure, analytical concentrations 49, 113, 204, 357 and 841 ppm (equal to 0.09, 0.20, 0.37, 0.64 and 1.51 mg/L), 7 hours/day, 5 days/week, duration of treatment:<br>841 ppm: Up to 8 exposures<br>357 ppm: 7-123 exposures<br>204 ppm: 122-157 exposures<br>113 ppm: 122-157 exposures<br>49 ppm: 127-131 exposures | NOAEL 49 ppm (0.09 mg/L),<br>LOAEL 113 ppm (0.20 mg/L) | 49 ppm: exposure of 20 rats/sex/group 127-131 times had no effects on general appearance and behaviour, mortality, growth, final body and organ weights, and gross and histopathological examination of the tissues.<br>113 ppm: exposure of 20 rats/sex/group 122-157 times caused growth depression in both sexes (statistically significant in males) and a statistically significant increase in the lung weights of both sexes (> 10%).<br>204 ppm: exposure of 20 rats/sex/group 122-157 times caused mortality, growth depression, marked increase in the lung weights in both sexes, moderate increase in the kidney and slight increase in the liver weights of females. Slight decrease in testes weight was | No information on the batch of the test substance; the number of exposures varies and deviates from the guideline; small group sizes preclude statistical evaluation of the results; only limited information on experimental procedure and evaluated parameters is available. | Hollingsworth RL et al. (1956)<br>A.6.4.3/01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | not statistically significant. Histopathologically, slight tubular degeneration in testes, very slight cloudy swelling of a few convoluted tubules of male rat kidneys, and haemorrhage, congestion, emphysema and atelectasis of lungs in females were observed. Terminal blood urea nitrogen values were within the normal range. Qualitative urine tests on females for blood, sugar, albumin and sediment were all negative.<br>357 ppm: exposure of 10 rats/sex/group 7 times caused mortality, moderate loss of body weight and severe lung injury. Exposure of 10 rats/sex/group 33 to 42 times caused nearly 100% mortality, markedly subnormal growth, impairment of sensory and motor function of the nervous system at the level of the lumbar and sacral region; paralysis and subsequent atrophy of the hind limb muscles were observed. The neurotoxic effects were reversible following 100-132 days recovery period. Blood urea nitrogen values and haematological values were within the normal range.<br>841 ppm: exposure of 10 rats/sex/group 8 times caused 100% mortality; irritation of respiratory tract was evident at | | |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | gross necropsy.<br>Exposure of 5 rats/sex/group for 2 days caused mortality. Slight haemorrhage and congestion of the lungs, light coloration of the livers, enlargement of the kidneys and pale coloration and enlargement of adrenals were observed at gross necropsy. Histopathologically, slight to moderate interstitial edema, congestion and alveolar haemorrhage in lungs, slight fatty degeneration of livers, slight congestion and clouded swelling of the convoluted tubules in the kidneys, and numerous fat vacuoles in adrenal cortex were noted. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Mouse, strain unknown, female, 5 or 10/group | Ethylene oxide, 97.0-98.6% pure, analytical concentrations 49, 204, 357 and 841 ppm (equal to 0.09, 0.20, 0.37, 0.64 and 1.51 mg/L), 7 hours/day, 5 days/week, duration of | NOAEL 49 ppm (0.09 mg/L),<br>LOAEL 204 ppm (0.37 mg/L) (females only) | 49 ppm: exposure of 10 female mice 127-131 times had no effects on general appearance and behaviour, mortality, growth, final body and organ weights, and gross and histopathological examination of the tissues.<br>204 ppm: exposure of 10 female mice 122-157 times | No information on the batch of the test substance; the number of exposures varies and deviates from the guideline; small group sizes preclude statistical evaluation of the results; only limited | Hollingsworth RL et al. (1956)<br>A.6.4.3/01 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | treatment:<br>841 ppm: Up to 8 exposures<br>357 ppm: 7-123 exposures<br>204 ppm: 122-157 exposures<br>113 ppm: 122-157 exposures<br>49 ppm: 127-131 exposures | | caused slight increase in mortality compared to controls. Qualitative urine tests for blood, sugar, albumin and sediment were all negative. No further details are reported.<br>357 ppm: exposure of 10 female mice 7 times caused mortality, moderate loss of body weight and severe lung injury. Exposure of 10 female mice 33 to 42 times caused 100% mortality and markedly subnormal growth.<br>841 ppm: exposure of 5 female mice 8 times caused 100% mortality. Irritation of the respiratory tract was observed at gross necropsy. Mice appeared to be the most susceptible animal species tested at this concentration. | information on experimental procedure and evaluated parameters is available. | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Rabbit, strain unknown, male/female, 1 or 2/sex/group | Ethylene oxide, 97.0-98.6% pure, analytical concentrations 49, 204, 357 and 841 ppm (equal to 0.09, 0.20, 0.37, 0.64 and 1.51 mg/L), 7 hours/day, 5 days/week, duration of | NOAEL 113 ppm (0.20 mg/L), LOAEL 204 ppm (0.37 | 49 ppm: exposure of 2 rabbits/sex/group 127-131 times had no effects on general appearance and behaviour, mortality, growth, final body and organ weights, and gross and histopathological | No information on the batch of the test substance; the number of exposures varies and deviates from the guideline; small | Hollingsworth RL et al. (1956)<br>A.6.4.3/01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | treatment:<br>841 ppm: Up to 8 exposures<br>357 ppm: 7-123 exposures<br>204 ppm: 122-157 exposures<br>49 ppm: 127-131 exposures | mg/L) | examination of the tissues.<br>113 ppm: exposure of 2 rabbits/sex/group 122-157 times did not result in adverse effects.<br>204 ppm: exposure of 2 rabbits/sex/group 122-157 times had no substantial effect on mortality or growth. Slight to marked paralysis in the rear legs was seen after 196 days on experiment. Apparently no changes were observed at gross and microscopic examinations. Terminal blood urea nitrogen values and haematological values were within the normal ranges. Qualitative urine tests on female animals for blood, sugar, albumin and sediment were all negative.<br>357 ppm: exposure of 1 rabbit/sex/group 33 to 59 times resulted in the death of male rabbit after 48 exposures. The growth was markedly subnormal. Impairment of sensory and motor function of the nervous system at the level of the lumbar and sacral region was noted; paralysis and subsequent atrophy of the muscles of the hind lims were observed. The effects were reversible following a recovery period of 100-132 days. Blood urea nitrogen values and haematological values were within the normal range.<br>841 ppm: exposure of 1 | group sizes preclude statistical evaluation of the results; only limited information on experimental procedure and evaluated parameters is available. | |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | rabbit/sex/group 8 times caused 100% mortality. Irritation of the respiratory tract was observed at gross necropsy. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Guinea pig, strain unknown, male/female, 5 or 8/sex/group | Ethylene oxide, 97.0-98.6% pure, analytical concentrations 49, 204, 357 and 841 ppm (equal to 0.09, 0.20, 0.37, 0.64 and 1.51 mg/L), 7 hours/day, 5 days/week, duration of treatment:<br>841 ppm: Up to 8 exposures<br>357 ppm: 7-123 exposures<br>204 ppm: 122-157 exposures<br>49 ppm: 127-131 exposures | NOAEL 113 ppm (0.20 mg/L), LOAEL 204 ppm (0.37 mg/L) | 49 ppm: exposure of 8 guinea pigs/sex/group 127-131 times had no effects on general appearance and behaviour, mortality, growth, final body and organ weights, and gross and histopathological examination of the tissues.<br>113 ppm: exposure of 8 guinea pigs/sex/group 122-157 times did not result in adverse effects.<br>204 ppm: exposure of 8 guinea pigs/sex/group 122-157 times had no substantial effect on mortality or growth. Slight increase in the lung weight of male guinea pigs was observed. The observed apparent slight decrease in the testes weight was not statistically significant. Qualitative urine tests on female guinea pigs for blood, sugar, albumin and sediment | No information is available on the batch of the test substance; the number of exposures varies and deviates from the guideline; small group sizes preclude statistical evaluation of the results; only limited information on experimental procedure and evaluated parameters is available. | Hollingsworth RL et al. (1956)<br>A.6.4.3/01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | were all negative.<br>357 ppm: exposure of 8 guinea pigs/sex/group 123 times resulted in moderate growth depression in males and slight growth depression in females. A slight lung weight increase relative to body weight was seen in males. Appreciable degeneration of the tubules of the testes with replacement fibrosis in males and slight fatty degeneration in the adrenal cortex in females were seen at histopathological examination. Terminal blood urea nitrogen values and haematological values were within the normal ranges. Qualitative urine tests on female animals for blood, sugar, albumin and sediment were all negative.<br>841 ppm: exposure of 8 guinea pigs/sex/group 8 times caused 100% mortality. Irritation of the respiratory tract was observed at gross necropsy. Exposure of 5 guinea pigs for 3 times caused slight haemorrhages and congestion of the lungs, light coloration of the livers, enlargement of the kidneys and pale coloration and enlargement of the adrenals. Microscopic examination of the tissues revealed slight to moderate interstitial edema, congestion and alveolar haemorrhage in lungs, slight fatty degeneration of livers, slight congestion and | | |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | clouded swelling of the convoluted tubules in the kidneys, and numerous fat vacuoles in adrenal cortex were noted. There was evidence of regeneration in the livers from animals killed three days after the last exposure. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Monkey, strain unknown, male/female, 1 or 2/sex/group | Ethylene oxide, 97.0-98.6% pure, analytical concentrations 49, 204, 357 and 841 ppm (equal to 0.09, 0.20, 0.37, 0.64 and 1.51 mg/L), 7 hours/day, 5 days/week, duration of treatment:<br>841 ppm: Up to 8 exposures<br>357 ppm: 7-123 exposures<br>204 ppm: 122-157 exposures<br>49 ppm: 127-131 exposures | NOAEL 113 ppm (0.20 mg/L), LOAEL 204 ppm (0.37 mg/L) | 49 ppm: 1 monkey/sex/group were exposed, but were killed after the experiment run for 54 days because of an epidemic of amebic dysentery.<br>113 ppm: exposure of 2 female monkeys 122-157 times did not result in adverse effects.<br>204 ppm: exposure of 2 female monkeys 122-157 times had no substantial effect on mortality or growth. During the course of the experiment the monkeys developed less active knee jerk reflexes, and withdrawal from superficial pain stimuli in the hind feet and skin of the legs and back was abolished. There was a positive Babinski's reflex. Deep pain reflexes were elicited in the feei, toe pads etc. Partial paralysis and some evidence of | No information is available on the batch of the test substance; the number of exposures varies and deviates from the guideline; small group sizes preclude statistical evaluation of the results; only limited information on experimental procedure and evaluated parameters is available. | Hollingsworth RL et al. (1956)<br>A.6.4.3/01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | muscular atrophy of the rear extremities were noted. Apparently no effects were noted at gross and microscopic examination of the tissues. Terminal blood urea nitrogen values and haematological values were within the normal ranges. Qualitative urine tests on female animals for blood, sugar, albumin and sediment were all negative.<br>357 ppm: exposure of 1 female monkey 123 times resulted in subnormal growth. Impairment of sensory and motor function of the nervous system at the level of the lumbar and sacral region was noted; paralysis and subsequent atrophy of the muscles of the hind lims were observed. The effects were reversible following a recovery period of 100-132 days. Terminal blood urea nitrogen values and haematological values were within the normal ranges.<br>Exposure of 1 monkey/sex/group 38-41 times and 2 male monkeys 94 times caused growth depression and the same characteristic response of impairment of function of the nervous system, paralysis and muscular atrophy of the hind limbs. Knee jerk reflexes were very poor or non-existent. Pain perception in the hind quarters and about the | | |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | genitalia was very poor. The cremasteric reflex was elicited. The extensor reflex of the palms of the hind feet was non-existent. Three of the monkeys were killed 1 or 2 days after the last exposure, and the results of micropathologic studies were negative.<br>841 ppm: exposure of 1 female monkey 8 times resulted in death. Irritation of the respiratory tract was observed at gross necropsy. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Rat, strain unknown, 20 males/group for 6 and 26 weeks exposure; satellite groups for pathological examinations (5 animals killed every other week): 15 males/group for 6 weeks exposure and | Ethylene oxide, gas, purity unspecified; analytical concentrations:<br>6 weeks exposure:<br>406±42 ppm<br><br>26 weeks exposure:<br>102±24 ppm | 6 w: LOAEL 406±42 ppm<br><br>26 w: NOAEL 102±24 ppm | 6 weeks:<br>13/20 rats in the treatment group died. Reddish nasal discharge, diarrhoea, tendency toward the side position and laboured breathing were noted. 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. There was a progressive loss of body weight. Surviving rats regained weight over a period of several | Single concentrations tested; exposure durations deviating from the guideline; limited parameter investigated. | Jacobson KS (1956)<br>A.6.4.3/02 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | 60 males/group for the 6 months exposure | | | months after the end of exposure. At gross pathology, haemosiderosis in the spleen was seen in some animals.<br>26 weeks:<br>3/20 exposed and 3/20 control rats died. No clinical signs of toxicity were noted. Slight, but probably not significant decrease in body weight was observed compared to controls. No treatment-related changes were seen at gross necropsy. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3. | Mouse, strain unknown, 30 females/group for 6 and 26 weeks exposure; satellite groups for pathological examinations (5 animals killed every other week): 15/group for 6 weeks exposure and | Ethylene oxide, gas, purity unspecified; analytical concentrations:<br>6 weeks exposure:<br>406±42 ppm<br>26 weeks exposure:<br>98±16 ppm | 6 w: LOAEL 406±42 ppm<br>26 w: LOAEL 102±24 ppm | 6 weeks:<br>24/30 exposed mice died. No clinical signs are reported. Slightly increased loss in body weight was seen compared to controls, but was probably not significant. No pathological changes were seen at gross necropsy.<br>26 weeks:<br>8/30 exposed mice died, vs. 4/30 in controls. No clinical signs of toxicity and no effects on body weights were noted. No treatment-related changes were | Single concentrations tested; exposure durations deviating from the guideline; limited parameter investigated. | Jacobson KS (1956)<br>A.6.4.3/02 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | 60/group for the 6 months exposure | | | seen at gross necropsy. | | |
| Predates modern guidelines and GLP.<br><br>Reliability 3 | Dog, Beagle, 3 males/group for 6 and 26 weeks exposure. | Ethylene oxide, gas, purity unspecified; analytical concentrations:<br>6 weeks exposure:<br>292±26 ppm<br>26 weeks exposure:<br>100±13 ppm | 6 weeks exposure: LOAEL 292 ±26 ppm<br>26 weeks exposure: LOAEL 100±13 ppm | 6 weeks:<br>Vomiting, occasional slight tremors and transient weakness in the hindlegs were seen in two of the three animals. There were no significant changes in body weights, ECGs, blood calcium, blood urine, icteric indices and rectal temperature. Significant decrease in erythrocytes, haemoglobin and haematocrit was observed in 2 dogs; in the third there was a decrease in haemoglobin and no change in erythrocytes or haematocrit. Congestion of the lungs and moderate focal alveolar collapse were seen at necropsy. There was an abundance of fat in the Mm. semimembranosus and semitendinosus, and bundles of fibres were squeezed and distorted out of shape by large fat globules. In other areas fat completely replaced the muscle | Single concentrations tested; exposure durations deviating from the guideline; limited parameter investigated. | Jacobson KS (1956)<br>A.6.4.3/02 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | fibres.<br>26 weeks:<br>No mortalities, clinical signs of toxicity or body weight changes were observed. There were no significant changes in body weights, ECGs, blood calcium, blood urine, icteric indices and rectal temperature. 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 differences decreases in these parameters in another dog. The dogs were not sacrificed at the end. | | |
| Non-guideline, non-GLP.<br><br>Reliability 3. | Rat, Wistar, 28 males/dose | Ethylene oxide, purity unspecified, 500 ppm, whole body exposure for 6 hours/day, 3 days/week for 2, 6 and 13 weeks | LOAEL 500 ppm | There were no mortalities. Rats in the exposed group had an ataxic gait after 6-9 weeks of exposure, but no further effects were noted. 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 | Only one concentration tested, limited weekly exposure no gross necropsy and histopathology performed; only haematological parameters evaluated. | Mori K (1990) A.6.4.3/03 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | weeks erythrocyte counts had recovered slightly but haemoglobin and haematocrit were reduced and mean cell volume and reticulocytes were still increased. Glutathione reductase activity in erythrocytes was reduced at all 3 time points and glutathione content was reduced slightly at 13 weeks. The glutathione stability test gave a positive result. The only other significant change was in acetyl cholinesterase activity which was increased at 13 weeks. Exposure to ethylene oxide induced macrocytic normochromic anemia. | | |
| Non-guideline, non-GLP.<br><br>Reliability 3. | Rat, Wistar, 24 males/dose | Ethylene oxide, purity unspecified, analytical concentration 500 ± 10 ppm, whole body exposure for 6 hours/day, 3 times/week for 2, 6 or 13 weeks | LOAEL 500 ppm | 13-week exposure resulted in significant reduction of haemoglobin, haematocrit and erythrocyte count, but there were no differences in MCV or MCHC. Reticulocytes were increased. Exposure to ethylene oxide for 13 weeks caused therefore a normocytic and normochromic anaemia. Exposure to ethylene oxide caused alterations in haem- | Only one concentration tested, limited weekly exposure, no gross necropsy and histopathology performed; only haematological parameters evaluated. | Fujishiro K et al. (1990) A.6.4.3/04 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | porphyrin metabolism. Hepatic microsomal CYP-450 and prohaem decreased significantly, while the activity of hepatic ALA-synthase and ferrochelatase increased. Coproporphyrin greatly increased in the urine and tended to increase in the liver of the exposed animals. Uroporphyrin increased by 37% in liver, but protoporphyrin in the liver and erythrocytes did not increase significantly. | | |
| Non-guideline, non-GLP.<br><br>Reliability 3. | Rat, Wistar, male, number/group not specified. | Ethylene oxide, 500 ppm, whole body exposure for 6 hours/day, 3 days/week, 1 day, 4 and 12 weeks | LOAEL 500 ppm | Exposure to ethylene oxide for 4 and 12 weeks inhibited creatine kinase activity in serum, brain, spinal cord and muscle. ASAT and LDH activities were not influenced by the treatment. There was also a 20% reduction in serum triglycerides. Other biochemical parameters were unaffected. The inhibition of creatine kinase activity was not counteracted by dithiothretiol, which is a protective agent for sulphydryl groups. | Only one concentration tested; limited weekly exposure, no gross necropsy and histopathology performed; only enzyme activities and clinical chemistry parameter evaluated. | Matsuoka M et al. (1990) A.6.4.3/05 |
| Comparable to OECD 413, GLP. | Mouse, $B6C3F_1$, | Ethylene oxide, > 99.9% pure, target concentrations, | NOAEL 10 ppm; | There were no mortalities, clinical signs of toxicity or | Some limitations in parameters and | Snellings WM et al. (1984) |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| Reliability 2. | 30/sex/group | 10, 50, 100 and 250 ppm, overall mean analytical concentrations (coefficient of variation) 10 (6%), 48 (4%), 104 (4%) and 236 (4%) ppm, whole body exposure for 6 hours/day, 5 days/week, for 10 (males) or 11 (females) weeks | LOAEL 50 ppm. | changes in body weights. Statistically significantly reduced locomotor activity in females and hunched posture during gait in males were noted at 50 ppm; at higher concentrations these effects occurred in both sexes; abnormal righting, toe pinch and tail pinch reflexes were noted at 250 ppm. Erythrocyte count and haemoglobin were significantly reduced in males, while erythrocyte count, packed cell volume, haemoglobin and MCH were reduced in females at 250 ppm. At 250 ppm, increased relative liver weight, reduced absolute testes weight and reduced relative spleen weight were observed; at 100 ppm, reduced relative spleen weight was seen in females only. There were no effects on clinical chemistry and urinalysis parameters, and no effects were seen at gross pathology and histopathology of selected tissues. | tissues examined and shorter exposure period in comparison to OECD guideline 413. | A.6.4.3/06 |
| Comparable to OECD 413, non- | Mouse, $B6C3F_1$, | Ethylene oxide, > 99% pure, nominal concentrations 0, | NOAEL 50 ppm; | All mice in two highest dose groups died. At 600 ppm, all | Comparable to OECD guideline | NTP TR 326 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| GLP.<br><br>Reliability 2. | 10/sex/group | 50, 100, 200, 400 and 600 ppm, whole-body exposure for 6 hours/day, 5 days/week for 14 weeks | LOAEL 100 ppm | mice had anorexia, dyspnea, decreased activity and were bloated and listless. 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. There were no toxicological relevant effects on organ weights. Haematological and clinical chemistry examinations were not performed. | 413; some limitations in parameter and tissues examined in comparison to OECD guideline 413. | (1987)<br>A.6.4.3/07 |
| Non-guideline, non-GLP, exposure of male cynomolgus monkeys to two different concentrations of ethylene oxide for 24 months.<br><br>Reliability 3. | Monkey, Cynomolgus, 12 males/group | Ethylene oxide, 99.7% pure, nominal concentrations 0, 50 and 100 ppm for 7 hours/day, 5 days/week, for 24 months | LOAEL 50 ppm | One monkey from each ethylene oxide exposure group died, but deaths were considered to be unrelated to treatment. Significantly decreased weight gain was observed at 100 ppm starting from week 25. Exposure to ethylene oxide caused no differences in RBC or leucocytes, no changes in | Only male animals tested. | Lynch D et al. (1984), Setzer et al. (1996)<br>A.6.4.3/08 |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | urinalysis, clinical chemistry, electrocardiographic indices or encephalography. Incidence of cataracts was increased in exposed monkeys (2/11 and 3/12 at 50 and 100 ppm, respectively, vs. 0/11 in controls). Axonal dystrophy and demyelination in distal portion of medulla were observed at both exposure concentrations (examination conducted on 2 monkeys/exposure group). Furthermore, a decreased nerve conduction velocity were observed in 2/12 monkeys at 100 ppm. All ethylene oxide exposed groups showed statistically reduced sperm count and sperm motility, while drive range (progressive sperm motility) was significantly elevated. Exposure to ethylene oxide at both concentrations resulted in statistically significant increases in sister chromatid exchange rates and incidences of chromosome aberrations in a dose-response manner. | | |
| Non-guideline, | C75BL/6J | Ethylene oxide, 255 ppm, 6 | LOAEL | Decrease in the number of | The study was cited | Popp DM et |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| non-GLP, exposure of male mice to a single concentration of ethylene oxide for different time periods (up to 10 weeks)<br><br>Reliability 2 with restrictions, indicated in the CLH report (2016) | mice, 4 males/group | hours/day, 5 days/week for 1, 2, 4, 8 and 14 days, 4, 6, 8 and 10 weeks | 255 ppm | erythrocytes (maximal observed decrease of -9.3% from controls), the quantity of haemoglobin (maximal observed decrease of -9.1% from controls), the haematocrit value (the maximal observed decrease of –5.5% from controls), the bone marrow cell density, and the number of lymphocytes was observed. Bone marrow populations and the leukocytes in the peripheral blood were perturbed from their normal homeostatic level after the first day of exposure. Differential analyses of leukocytes show that granulocytes were elevated while lymphocytes were lost from the circulation. In the bone marrow granulocytes depleted and lymphocytes increased. An accommodation occurred after continued exposure, which resulted in a persistent depression of lymphocytes in both the bone marrow and the peripheral blood. Highly condensed and pycnotic nuclei were observed | in the CLH report (2016) and the RAC opinion (2017); original study was not recovered. Only male animals tested, one concentration tested, only haematological parameter were evaluated. | al. (1986), additional study cited in the CLH report (2016) and RAC opinion (2017). |

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), form (gas, vapour, dust, mist) and particle size (MMAD), Actual and nominal concentration, Type of administration (nose only / whole body / head only), Duration of exposure | NOAEL, LOAEL | Results (all dose levels including severity and magnitude of all effects, including also target organs) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | in the lymphocytes that remained in the peripheral blood, indicating some cell death. The presence of highly vacuolated granulocytes, eosinophils, lymphocytes and monocytes in the peripheral blood suggests that these cells were affected by ethylene oxide dissolved in the serum. Alterations in the cell cycle data of the bone marrow indicate immediate accommodation to functional cell loss by physiological recruitment from a G0 stem-cell pool. | | |

A number of subchronic repeated dose toxicity studies were submitted by the applicant and summarized in Doc IIIA. Five subchronic inhalation toxicity studies have been conducted in rats, four in mice, two in monkeys and one each in guinea pigs, rabbits and dogs. All studies were published studies, none of the studies had a reliability score of 1, all had a reliability score of 2 or 3. In addition, one more study (Popp et al., 1986) was cited in the CLH report (2016) and the RAC opinion (2017). The original study was not recovered; the summary of the study was added to the table by the eCA based on information provided in the CLH report (2016).

In a study with varying periods of exposure (Hollingsworth et al., 1956) adverse findings in rats consisted of increased mortality at 841 ppm and 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 effects on the testes, kidneys and lung and decreased body weight gain. At 113 ppm, body weight gain was also decreased and lung weight increased. In rats after inhalation exposure for 24-31 weeks, a NOAEL of 49 ppm was established. A NOAEL of 100 ppm was established in another study in which rats were exposed by inhalation for 6 months (Jacobson, 1956). Only one exposure concentration was used in this study, so a LOAEL was not established. In the same study exposure to 400 ppm ethylene oxide for 6 weeks was associated with increased mortality in rats, loss of body weight and clinical signs of toxicity consisting of reddish nasal discharge and diarrhoea as well as signs of neurotoxicity. Three other studies were conducted at a single exposure level of 500 ppm (Mori et al, 1990; Fujishiro et al., 1990 and Matsuoka et al, 1990). These studies confirmed that exposure of rats to high concentrations of ethylene oxide caused neurotoxic effects and also caused anaemia. Again, as only a single concentration of ethylene oxide was used, LOAELs were not established, but these studies were conducted at much higher concentrations of ethylene oxide then those summarised previously. Taken together these studies show that repeated exposure to concentrations of ethylene oxide ≥ 204 ppm caused deaths, the lowest non-lethal dose exposure was to 113 ppm at which there was an increase in lung weight and a decrease in bodyweight gain. As in most studies only one exposure concentration was tested and not all parameters were examined according to modern guidelines, a reliable NOAEL for subchronic toxicity in rats could not be established.

An ethylene oxide concentration of 841 ppm caused deaths in mice (Hollingsworth, 1956). Increased mortality was also found in mice exposed to 357 ppm, but this was considered secondary to lung irritation and severe lung injury; mortality was also slightly increased at 204 ppm but no other effects were reported. In a further study mortality and signs of hemosiderosis were noted in mice exposed to 400 ppm ethylene oxide for 6 weeks, but during a 6 months exposure to 100 ppm ethylene oxide the only finding was a slight increase in mortality (Jacobson, 1956). In the NTP study (NTP TR 326, 1987) 14-week exposure to ethylene oxide was associated with clinical signs of toxicity consisting of anorexia, dyspnea, decreased activity bloating and listlessness 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. In the other study with an exposure period of 11 weeks (Snellings et al., 1984) 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 at 250 ppm. 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, although there were no associated neuro-muscular histopathology changes. There were also changes in absolute and/or relative weights of the liver in females, testes in males and spleen in both sexes. Absolute and relative spleen weights were also decreased in females exposed to 100 ppm, but in contrast with the findings of the NTP study there were no treatment related effects on histopathology.

Although the two most recent studies provide a more reliable assessment of the toxicity of ethylene oxide to mice, the results of all studies show that repeated exposures to ≥ 204 ppm ethylene oxide caused mortalities. There appears to be some inconsistency in the findings of the studies conducted by Snellings et. al. and the NTP but this was probably because the protocols used were slightly different. Haematology, clinical chemistry and urinalysis were not included in the NTP study and only liver weight was reported; investigations in the other study focussed on clinical signs of toxicity and there was limited histopathology. Based on the signs of neurotoxicity (reduced locomotor activity, abnormal posture during gait) observed at 50 ppm in the study of Snellings et al. (1984), the NOAEL in mice is set at 10 ppm.

Signs of neurotoxicity were the principal adverse findings in monkey exposed to 204 and 357 ppm ethylene oxide, exposure to the higher concentration of 841 ppm caused deaths. Body weight gain was reduced at 357 ppm (Hollingsworth, 1956) and also at 100 ppm (Lynch et al, 1984b). Some effects consistent with neurotoxicity were found in monkeys exposed to 100 and 50 ppm (axonal dystrophy and demyelination in distal portion of medulla, examination performed on 2 monkeys/group; at 100 ppm, 2/12 monkeys showed decreased nerve conduction velocity) (Lynch et al., 1984b). Both sperm count and motility were however significantly reduced in animals from both treatment groups, and there were 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. Ten years after the end of exposure, examination of 3 or 4 monkeys per group showed a statistically significant increase in cataracts. The NOAEL was < 50 ppm.

Signs of neurotoxicity were also noted in dogs exposed to 300 ppm ethylene oxide for 6 weeks, and vomiting was 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 anaemia. There were no clinical signs of neurotoxicity in dogs exposed to 100 ppm ethylene oxide for 26 weeks but again the haematology results were consistent with anaemia. A NOAEL was not established.

Mortality was increased at 357 ppm in rabbits and there were clinical signs of neurotoxicity but no associated histopathology at either 357 or 204 ppm. The only microscopic effect reported in these two groups was in the lungs of male rabbits exposed to 204 ppm. The NOAEL was 113 ppm.

Effects on lungs, liver, kidney 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. The NOAEL was 204 ppm.

Since many of the studies were not guideline studies, and had in many cases rather deviating study designs, it is difficult to compare the studies. In the Hollingworth study (1956), there is no major species difference in the subchronic toxicity of ethylene oxide to rats, mice, guinea pigs, rabbits or monkeys where a similar study design was used for all those species. Out of available subchronic toxicity data, two studies with mice (NTP, 1987, and Snellings et al., 1984b) are considered to be most reliable. In the NTP study (1987), the NOAEL was 50 ppm. In the study of Snellings et al., 1984b, first signs of neurotoxicity (reduced locomotor activity, hunched posture during gait) were evidenced in the neuromuscular screening test from 50 ppm, therefore the NOAEL is considered to be 10 ppm. It should be noted that both studies had some limitations compared to OECD guidelines.

The TNsG states that subchronic toxicity should usually be studied in two species, one rodent and one non-rodent. Where testing in two species is required the testing may be

waived only if it is scientifically justified; in case residues are found in the food chain waiving is not possible. For the subchronic inhalation toxicity test, it is not indicated in the TNsG, that the preferred 2nd species is the dog.

As indicated above, the available subchronic toxicity studies were difficult to compare, and none of them was fully according to OECD guidelines. Despite the lack of full OECD guideline studies in 2 species, the available studies indicate that ethylene oxide affects different species, at concentrations in the same dose range as low as 50 ppm. Ethylene oxide is classified as a genotoxic carcinogen, hence a subchronic NOAEL is not considered relevant for the human risk assessment. It is considered not justified to set a data gap for subchronic toxicity for on animal welfare reasons.

With regard to local effects, ethylene oxide is classified as corrosive (Skin Corr. 1, H314) and as causing respiratory tract irritation (STOT SE 3, H335). In the NTP study with mice (1987), rhinitis of the nasal cavity was observed starting from 200 ppm. A moderate increase in the lung weight was seen in rats exposed to 113 ppm; at 204 ppm, haemorrhage, congestion, emphysema and atelectasis of lungs was observed in exposed female rats. Based on the results of the NTP (1987) study, the NOAEC for local effects via repeated respiratory exposure is considered to be 100 ppm.

**Human data**

No individual robust study summaries of human data on subchronic toxicity were submitted by the applicant. The assessments of SCOEL (2012) and the CLH report on ethylene oxide (2016) were used as a starting point for the evaluation by the eCA. All the studies mentioned in those reports were publicly available studies. The majority of reported studies focused on neurotoxicity of ethylene oxide and included case reports on operators occupationally exposed to ethylene oxide, with different exposure durations (from several minutes up to 20 years); however, most of them were identified as supporting studies in the CLH report, and only a few could be considered as subchronic exposure. These studies are summarised in Section 3.11 on neurotoxicity and are briefly cited here.

In the SCOEL, a study of Garry et al. (1979) is cited, in which neurological symptoms were seen in 12 people who had been exposed occupationally to ethylene oxide for about six months: headaches (6 persons), nausea (5), speech disorders and impairment of short-term memory (5), vertigo (3) and incoordination (2). Measurements carried out in the air of the room during one sterilisation cycle revealed a maximal ethylene oxide concentration of 36 ppm.

Kuzuhara et al. (1983, cited in CLH, 2016) reported 2 cases of workers that were exposed to ethylene oxide for several months for about 1.5 hour/day. The estimated peak exposure was probably ~700 ppm, as the workers could detect the odour when opening the sterilizer. Sensorimotor neuropathy (axonal nerve degeneration) was observed, which involved paresthesia, distal limb weakness and cutaneous sensory loss. The symptoms improved after the termination of exposure but returned when the subjects returned to work.

Schröder et al. (1985, cited in CLH, 2016) reported a case of a worker in sterilization factory who showed weakness in the lower extremities and a progressive gait unsteadiness after 5 months of exposure (up to 500 ppm 2-3 times daily). Nerve conduction studies were markedly abnormal (decreased sensory and motor conduction velocity) indicating moderate to severe polyneuropathy. Re-examination one year later showed markedly improved conditions. Nerve biopsy (sural nerve) showed moderate decrease of large myelinated fibres associated with an increase of small myelinated fibres.

| Value used in Risk Assessment – Sub-chronic inhalation toxicity | |
|---|---|
| Value/conclusion | The derivation of a reliable NOAEL for risk assessment is not possible based on available data. The available studies indicate that ethylene oxide affects different species, at concentrations in the same dose range as low as 50 ppm. |
| Justification for the value/conclusion | Out of available subchronic toxicity data, two studies with mice (NTP, 1987, and Snellings et al., 1984b) are considered to be most reliable. In the NTP study (1987), the NOAEL was 50 ppm.<br><br>In the study of Snellings et al., 1984b, first signs of neurotoxicity (reduced locomotor activity, hunched posture during gait) were evidenced in the neuromuscular screening test from 50 ppm.<br><br>The studies have limitations; hence it is difficult to conclude on an overall sub-chronic NOAEL by inhalation.<br><br>Ethylene oxide is classified as a genotoxic carcinogen, hence a subchronic NOAEL is not considered relevant for the human risk assessment. It is considered not justified to set a data gap for subchronic toxicity for on animal welfare reasons. |

| Value/conclusion used in the Risk Assessment – Sub-chronic repeated dose local effects | |
|---|---|
| Value/conclusion | The derivation of a reliable NOAEL for risk assessment is not possible based on available data. |
| Justification for the selected value/conclusion | In the NTP study with mice (1987), rhinitis of the nasal cavity was observed starting from 200 ppm. A moderate increase in the lung weight was seen in rats exposed to 113 ppm; at 204 ppm, haemorrhage, congestion, emphysema and atelectasis of lungs was observed in exposed female rats. Based on the results of the NTP (1987) study, the NOAEC for local effects is considered to be 100 ppm. |
| Classification according to CLH | Not classified |

## A3.7.3 Long-term repeated dose toxicity

Combined chronic toxicity/carcinogenicity studies are reported in Section 3.9 on carcinogenicity. No separate long-term toxicity studies were conducted.

## A3.7.4 Specific target organ toxicity – repeated exposure (STOT RE)

EtO is classified according to CLH as STOT RE 1; H372 (nervous system). Information is provided in Section A.3.12 Neurotoxicity.

## A.3.8. Genotoxicity / Germ cell mutagenicity

### A3.8.1 In vitro

Table A.24 Summary table of in vitro genotoxicity studies

| **Method, Guideline, GLP status, Reliability, Key/supportive study** | **Test substance (including purity), Vehicle, Doses** | **Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains)** | **Results (including cytotoxicity and +/-S9 mix)** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|
| *In vitro* gene mutation in bacterial cells<br>Predates modern guidelines and GLP, similar to OECD 471.<br><br>Reliability 2.<br>Key study. | Ethylene oxide, 0, 0.88, 4.4 and 8.8 mg/plate | *S. typhimurium* TA98, TA100, TA1535 and TA1537 | Positive in TA100 and TA1535, negative in TA98 and TA1537 | Purity 99.7%.<br>Deviations: lack of metabolic activation and cytotoxicity not measured. | Pfeiffer EH and Dunkelberg H (1980)<br>A6.6.1/01 |
| *In vitro* cytogenicity in mammalian cells<br>Similar to OECD 473, non-GLP.<br><br>Reliability 2.<br>Key study | Ethylene oxide, 0, 3500, 6900, 13800 and 27700 ppm for chromosomal aberrations; 0, 457, 1372, 4115 and 12344 for micronucleus frequency | Chinese hamster lung fibroblasts V79<br>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 | Positive for chromosomal aberrations and micronucleus induction<br><br>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. | Purity 100%.<br>Deviations: lack of metabolic activation, cytotoxicity not measured, no positive control. | Zhong B-Z et al (1992)<br>A6.6.2/01 |

| Method, Guideline, GLP status, Reliability, Key/supportive study | Test substance (including purity), Vehicle, Doses | Relevant information about the study (e.g. organism (e.g. bacteria), cell type, strains) | Results (including cytotoxicity and +/-S9 mix) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| *In vitro* gene mutation in mammalian cells<br>Predates modern guidelines and GLP, similar to OECD 476.<br><br>Reliability 2.<br>Key study | Ethylene oxide, 0.088 to 0.33 mg/mL | Chinese hamster ovary cells | Positive with and without metabolic activation (S9) | Deviations: purity unknown, only graphically presentation of the result and no tabled data | Tan E-L et al (1981)<br>A6.6.3/01 |
| *In vitro* gene mutation in mammalian cells<br>Similar to OECD 476, non-GLP.<br><br>Reliability 2.<br>Key study | Ethylene oxide, 0, 625, 1250, 5000 and 7500 ppm in V79 cells and 0, 625, 1250 and 5000 ppm in SHE cells | Chinese hamster lung cells (V79) and Syrian hamster embryo cells (SHE) | Positive | Purity 10% EtO in nitrogen (dose levels presented as EtO).<br>Deviations: lack of metabolic activation, only four concentrations tested. | Hatch GG et al. (1986)<br>A6.6.3/02 |

| Conclusion used in Risk Assessment – Genotoxicity in vitro | |
|---|---|
| Conclusion | Ethylene oxide has been shown to induce both gene mutations and chromosomal alterations in prokaryotic and eukaryotic cells. |
| Justification for the conclusion | The studies summarised above are representative of a large number of genotoxicity studies in the scientific literature. There are too many studies to summarise in this section, but there are extensive reviews (IARC, 2008; CICADS, 2003). Ethylene oxide is active in a wide range of *in vitro* systems. Ethylene oxide has been shown to be genotoxic in virtually all systems studied. See overall conclusion in 3.8.3. |

## A3.8.2 In vivo

Table A.25 Summary table of in vivo genotoxicity studies*

| Method, duration of study, Guideline, GLP status, Reliability, Key/supportive study | Test substance (including purity), Vehicle, Doses | Relevant information about the study (e.g. species and strain, sex, no per group, route, frequency of application, sampling times, duration of exposure) | Main effects, Observations (specify regarding dose and sampling time) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| Micronucleus test in bone marrow<br>Similar to OECD 474/475, non-GLP.<br><br>Reliability 2.<br>Key study. | Ethylene oxide, 0, 30, 60, 120 or 150 mg/kg bw | Mouse, Swiss albino, single intraperitoneal exposure, sacrifice 24 hours post-administration with 2 h colchicine treatment at the end to arrest metaphases | Positive; increased number of mononucleated PCE at 120 and 150 mg/kg bw; dose dependent increases in chromosomal aberrations and sister chromatid exchanges in the bone marrow cells. | Deviation: purity unknown. | Farooqi Z et al. (1993)<br>A6.6.4/01 |
| Dominant lethal test<br>Similar to OECD 478, non-GLP.<br><br>Reliability 2.<br>Key study. | Ethylene oxide, 99.7% pure, 0, 165, 204, 250 and 300 ppm | Mouse, C3H x 101, T-stock, (SEC x 10)$F_1$ and (SEC x C57BL)$F_1$, respiratory exposure 6 hours/day on weekdays for 6 weeks and then daily beginning the 7th week for 2.5 weeks. | Positive. 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. | Purity: 99.7%. Deviations: no positive control, treated males were mated towards the end of the exposure period rather than at intervals during the exposure period. The deviations from the guideline protocol are not significant because a positive result was obtained in the study. | Generoso WM et al. (1990)<br>A6.6.6/01 |

### Summary of human data on genotoxicity

Many studies have been carried out to evaluate the effect of human exposure to ethylene oxide on the incidences of chromosomal aberrations (including micronuclei) and sister chromatid exchange in peripheral blood lymphocytes of workers exposed occupationally to ethylene oxide. These include workers at hospital and factory sterilisation units and those working at ethylene oxide manufacturing and processing plants; the studies are in the public domain. WHO (2003) refers to 25 references on human data, evaluated in IARC (1994, 2008). Also EU (2012,

SCOEL report) summarised human data. The results of the studies show that ethylene oxide induces chromosomal damage in exposed humans. In general, the degree of damage is correlated with the level and duration of exposure.

These human data on genotoxicity indicate that increases in chromosomal aberrations in peripheral blood lymphocytes and sister chromatid exchange in peripheral blood cells have been consistently reported in studies of workers exposed to concentrations of ethylene oxide of 5 ppm (9.2 mg/m$^3$). At lower concentrations no consistent results were found.

In some studies, increases in the frequency of sister chromatid exchange have been observed to persist after exposure had ceased.
In some studies, the frequency of micronuclei in peripheral blood was increased in workers exposed to relatively high (3.7–60.4 mg/m$^3$) levels of ethylene oxide. However, in the majority of the studies involving exposures to lower levels, no effect on the frequency of micronuclei was observed.

In one study increases in single-strand DNA breaks in peripheral mononuclear blood cells obtained from individuals exposed to (4-h TWA) ethylene oxide concentrations of 0.1 – 0.49 mg/m$^3$, 0.5–2.0 mg/m$^3$, and >2 mg/m$^3$, respectively.

The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is the formation of adducts, chromosomal aberrations or micronuclei. Taking these studies together, it is clear that occupational ethylene oxide exposure can lead to genotoxic damage in exposed humans. Chromosomal aberrations could be established at exposure levels of 5 ppm (9.2 mg/m$^3$) and above. At exposures of 1 ppm, no clear cytogenetic changes could be established so far.

| **Conclusion used in Risk Assessment – Genotoxicity *in vivo*** | |
|---|---|
| Conclusion | Ethylene oxide has been shown to induce both gene mutations and chromosomal alterations *in vivo*. |
| Justification for the conclusion | The studies summarised above are representative of a large number of genotoxicity studies in the scientific literature. There are too many studies to summarise in this section but there are extensive reviews (IARC, 2008; CICADS, 2003). Ethylene oxide is active in a wide range of *in vivo* systems. Ethylene oxide has been shown to be genotoxic. See overall conclusion in 3.8.3. |

A3.8.2.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The applicant has submitted several publications on the genotoxicity *in vitro* and *in vivo*, and these studies are representative of a large number of genotoxicity studies in the scientific literature. There are too many studies to summarise but there are extensive reviews. E.g., WHO (2003) concluded the following:

*„Owing to the consistency of the results, only a brief summary of studies conducted is provided here. Ethylene oxide is a potent alkylating agent that has been genotoxic in virtually all studies in which it was examined (reviewed in IARC, 1994). In in vitro testing, it induced DNA*

*damage and gene mutations in bacteria, yeast, and fungi and gene conversion in yeast. In mammalian cells, observed effects include gene mutations, micronucleus formation, chromosomal aberrations, cell transformation, unscheduled DNA synthesis, sister chromatid exchange, and DNA strand breaks. The results of in vivo studies on the genotoxicity of ethylene oxide have also been consistently positive (see IARC, 1994) following ingestion, inhalation, or injection. In vivo exposure to ethylene oxide induced gene mutation in normal and transgenic mice and in rat, sister chromatid exchange in lymphocytes from rabbit, rat, and monkey, in bone marrow cells from mouse and rat, and in rat spleen. In vivo exposure to ethylene oxide also induced heritable mutations or effects in germ cells in rodents (IARC, 1994). Ethylene oxide induced dominant lethal effects in mice and rats and heritable translocations in mice.*

*Human data confirm the genotoxic properties of ethylene oxide, as chromosomal aberrations, sister chromatid exchange, micronuclei, and/or single-strand DNA breaks in peripheral blood have been consistently reported in studies of workers exposed to ethylene oxide*."

The following table is copied from IARC evaluation (2012):

**Comparison of the evidence for key events – cytogenetic, genetic, and related changes – induced by ethylene oxide in humans, human cells, and experimental animals**

| End-point | In-vivo exposure | | In-vitro exposure |
|---|---|---|---|
| | Animals | Humans | Human cells |
| Haemoglobin-adduct formation | Strong | Strong | Strong |
| DNA-adduct formation | Strong | Weak[a] | Strong |
| Mutations in reporter genes in somatic cells | Strong | Weak[a] | Strong |
| Mutations in cancer-related genes in tumours | Strong | NR | not applicable |
| Increased levels of cancer-related proteins in tumours | Strong | NR | not applicable |
| Cytogenetic alterations in somatic cells | | | |
| Sister chromatid exchange | Strong | Strong | Strong |
| Structural chromosomal aberrations | Strong[b] | Strong | Moderate |
| Micronucleus formation | Strong[b] | Strong | NR |

a Possibly due to a lack of adequate studies
b Positive responses were seen only at exposure concentrations above those used in the rodent cancer-bioassays
NR, not reported
From IARC (2008)

| Conclusion used in the Risk Assessment – Genotoxicity | |
|---|---|
| Conclusion | Ethylene oxide has been shown to have genotoxic and mutagenic activity in numerous assays in both somatic and germ cells, and prokaryotic and eukaryotic organisms, including humans. |
| Justification for the conclusion | The studies summarised above are representative of a large number of genotoxicity studies in the scientific literature. There are too many studies to summarise in this section but there are extensive reviews (IARC, 2008; CICADS, 2003), and consistent results. Ethylene oxide has been shown to be genotoxic in virtually all systems studied, these include tests in yeast and fungi as well as in bacterial |

| | |
|---|---|
| | and mammalian systems and also in transgenic mice. Human data confirm the genotoxic properties of ethylene oxide. Ethylene oxide is a direct alkylating agent that reacts with nucleophiles without the need for metabolic transformation. |
| Classification according to CLH | Muta. 1B; H340 May cause genetic defects |

## A.3.9. Carcinogenicity

Table A.26 Summary table of carcinogenicity studies in animals

| **Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/ group** | **Test substance (including purity), Vehicle, Dose levels, Duration of exposure** | **NOAEL, LOAEL** | **Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels)** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|---|
| Comparable to OECD guideline 453, non-GLP, *in vivo* inhalation exposure of rats. Reliability 3 | Fischer 344 rats, 80 males/ group | Ethylene oxide, 99.7% pure, 0, 50 and 100 ppm (nominal); 0, 50.9 ± 2.2, 101.3 ± 3.5 ppm (analytical), 7 hours/day, 5 days/week for 24 months (486 exposure days) | LOAEL 50 ppm, lowest dose tested | Increased mortality and statistically significantly reduced body weights were observed at both tested concentrations. Skeletal muscle atrophy in absence of sciatic nerve degeneration was observed at 100 ppm. Increased incidences of mononuclear cell leukemia at 50 ppm (the lack of statistical significance at the higher exposure may have been due to reduced survival (19% vs 49% in controls), peritoneal mesothelioma at 100 ppm and mixed cell brain glioma at 50 and 100 ppm were observed in | Only male rats were tested; the report does not specify which 34 tissues were examined histopathologically; no interim analyses were performed, and only two dose levels were tested. Food consumption was not reported. A pulmonary infection outbreak occurred after 8 and 16 months of exposure. Consequently, rats were treated with tetracycline at several stages during the study. | Lynch et al. (1984a, b) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | NOAEL, LOAEL | Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | exposed rats.<br>The following numbers of rats with tumors were reported:<br>Brain: 0/76, 2/77, 5/79 at 0, 50 and 100 ppm, p < 0.05 (high dose)<br>Mononuclear cell leukemia: 24/77, 38/79, 30/76, p = 0.03 (low dose)<br>Peritoneal mesothelioma: 3/78, 9/79, 21/79, p = 0.002 (high dose); although the low dose was not statistically significant, an effect of treatment cannot be excluded. | | |
| Comparable to OECD guideline 451, non-GLP, *in vivo* inhalation exposure of rats.<br>Reliability 2 | Fischer 344 rats, 120/sex/dose | Ethylene oxide, > 99.9% pure, 0 (two control groups to assess normal intravariability), 10, 33 or 100 ppm (nominal), 10, 33 and 101 ppm (coefficient of variation 5%) (analytical); 6 hours/day, 5 days/week, for approximately 2 years (24 months females, 25 months males); interim necropsy at 6 and 12 months (10/sex/dose), and 18 months | LOAEL 10 ppm | Statistically significant increase in mortality at 100 ppm from the 22-23rd month onwards.<br>Statistically significant reduced body weight gain at 33 (females only) and 100 ppm (both sexes).<br>Dose-related increased prevalence of mononuclear cell leukemia at all exposure concentrations; incidence statistically significantly increased only in females at 100 ppm. | Not all experimental details are reported.<br>A occurred at 15 months.<br>The EtO treatment were stopped for 2 weeks during the outbreak this month, after which mortality normalised. | Snellings W.M. et al. (1984), Garman et al. (1985), Garman et al. (1986) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | NOAEL, LOAEL | Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | (20/sex/dose) | | 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. Statistically significant increase in the number of female rats with multiple neoplasms was observed starting from 10 ppm. Increased incidence of primary brain tumors (both sexes, not statistically significant) and peritoneal mesothelioma (males only) at 33 and 100 ppm; statistically significant positive trend, when adjusted for mortality, between ethylene oxide exposure and tumor induction for both tumor types. Statistically significantly increased incidence of subcutis fibroma in males at 100 | | |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | NOAEL, LOAEL | Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | ppm.<br>The numbers of rats with tumors were as follows:<br>Males:<br>Brain (gliomas): 1/181, 0/92, 3/85, 6/87, p < 0.05 (trend)<br>Mononuclear cell leukemia: 13/97, 9/51, 12/39, 9/30, p < 0.05 (trend)<br>Peritoneal mesothelioma: 2/97, 2/51, 4/39, 4/30, p < 0.005 (trend)<br>Subcutaneous fibroma: 3/97, 9/51, 1/39, 11/30, p , 0.01 (high dose)<br>Females:<br>Brain (gliomas): 0/187, 1/94, 2/90, 2/78, p < 0.05 (trend)<br>Mononuclear cell leukemia: 11/116, 11/54, 14/48, 15/26, p < 0.005 (trend) | | |
| Comparable to OECD guideline 451, non-GLP, *in vivo* inhalation exposure of mice. Reliability 2 | B6C3F1 mice, 50/sex/dose | Ethylene oxide, > 99% pure, 0, 50 and 100 ppm (nominal); 0, 49.7 ± 3.6 and 99.3 ± 7.8 ppm (analytical); 6 hours/day, 5 days/week for 102 weeks | LOAEL 50 ppm | No effect on survival and mean body weights.<br>Positive trends for alveolar/bronchiolar carcinomas and adenoma and papillary cystadenomas of the harderian gland in both sexes; and malignant lymphomas, uterine | Only two concentrations tested. No interim sacrifice. Several parameters were not reported, like food and water consumption, differential blood count. These deviations are considered not to affect the carcinogenicity evaluation | NTP TR 326 (1987) A6.7/03 |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | NOAEL, LOAEL | Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | adenocarconimas and mammary gland tumors in females.<br>The numbers of mice with tumors were as follows:<br>Males:<br>Lung (one or more pulmonary adenoma/total mice at risk): 11/50, 19/50, 26/50 at 0, 50 and 100 ppm, p = 0.002 (trend)<br>Harderian gland (papillary cystadenomas): 1/43, 9/44, 8/42 at 0, 50 and 100 ppm, p < 0.03 (trend)<br>Females:<br>Lung (pulmonary adenomas): 2/49, 5/48, 22/49, p < 0.001 (trend)<br>Harderian gland (papillary cystadenomas): 1/46, 6/46, 8/47, p < 0.04 (trend)<br>Lymphoma: 9/49, 6/49, 22/49, p = 0.023 (trend)<br>Uterus (adenocarcinomas): 0/49, 2/47, 5/49, p < 0.03 (trend)<br>Mammary gland (carcinomas): 1/49, 8/48, 6/49, p = 0.012 (low dose only) | | |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | NOAEL, LOAEL | Results (Please indicate any results that might suggest carcinogenic effects, as well as other toxic effects, for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| Non-guideline, non-GLP, *in vivo* inhalation exposure of mice. Reliability 3 | A/J mice, female, 30/dose | Ethylene oxide, ≥99.7% pure, two independent experiments: 0, 70 and 200 ppm [0, 128 or 366 mg/m3]; and 0 and 200 ppm (nominal); 5 hours/day, 5 days/week for 6 months; intraperitoneously urethane-injected mice served as positive controls | LOAEL 70 ppm | The survival was not affected by exposure. The numbers of animals with pulmonary adenomas were 8/30, 16/28 and 25/29 at 0, 70 and 200 ppm, respectively (statistically significantly, $p < 0.001$ (trend)) in the first experiment and 8/29 and 12/28 (non-significant) at 0 and 200 ppm in the second experiment. The tumor multiplicity significantly increased ($p < 0.05$) in each experiment, with a dose-dependent trend in the first experiment. | Additional study included in the IARC evaluations (1994; 2008; 2012); original study was not recovered. Non-guideline, non-GLP study, only female mice tested, maximum 2 exposure concentrations, 6 months exposure only. | Adkins et al. (1986); additional study included in IARC (1994; 2008; 2012). |

The applicant has submitted the reports on two carcinogenicity studies conducted in the rat (Lynch et al., 1984 and Snellings et al., 1984) and one in the mouse (NTP, 1987). In addition, one more mouse study (Adkins et al., 1986) was evaluated by IARC (1994, 2008, 2012) and found to be adequate, and therefore added by the eCA, although mice were exposed only for 6 months. Other conducted studies were not considered to be adequate by IARC and therefore not further considered for evaluation.

In a combined chronic/carcinogenicity study male Fischer rats were exposed to 0, 50 or 100 ppm for 7 hours per day, 5 days per week for 2 years (Lynch et al., 1984). Survival was reduced in both treatment groups and there was also a reduction in body weight gain at both 50 and 100 ppm. 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 were reduced at 50 and 100 ppm, with a dose response evident for the brain weight, while absolute and relative adrenal weights were increased at both concentrations, albeit in the absence of a dose response. There was an increase in the incidence of mononuclear cell leukemia 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 result of an infection and were considered not related to exposure to ethylene oxide.

In the other study conducted in the rat (Snellings et al., 1984) exposure levels were 0, 10, 33 and 100 ppm for 6 hours per day, 5 days per week for approximately 2 years. 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 peritoneal mesotheliomas in males and brain tumours in both sexes at 33 and 100 ppm and subcutis fibroma in males at 100 ppm. Dose-related increased prevalence of mononuclear cell leukemia at all exposure concentrations; incidence statistically significantly increased only in females at 100 ppm. 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. Also, statistically significant increase in the number of rats with multiple neoplasms was observed starting from 10 ppm. This study was also affected by an infection in both test and control animals, but it was considered not to have compromised the study.

In the NTP (1987) study, mice were exposed to 0, 50 or 100 ppm 6 hours per day, 5 days per week for 2 years. In both male and female mice, a dose related increase in the incidence of benign or malignant neoplasms in the lung and benign neoplasms in the Harderian gland was noted. Ethylene oxide also caused an increase in malignant tumours of the uterus, mammary gland and lymphomas in female mice.

Groups of 30 female strain A/J mice were exposed by inhalation to 0, 70 or 200 ppm ethylene oxide for 6 h per day on 5 days per week for up to 6 months in two independent experiments (Adkins et al, 1986); in the second experiment, the 70-ppm group was omitted. At the end of the 6th month, the survivors were killed and examined for pulmonary adenomas. The survival was not affected by exposure. The numbers of animals with pulmonary adenomas among survivors (and tumour multiplicity) in the first experiment were: untreated controls, 8/30 (0.46 ± 0.38 [± SD] adenomas/mouse); low-dose, 16/28 (0.86 ± 0.45); high-dose, 25/29 (2.14 ± 0.49); and urethane-treated, 19/19 (20.1 ± 1.77); those in the second experiment were: untreated controls, 8/29 (0.22 ± 0.38); ethylene oxide-treated, 12/28 (0.73 ± 0.98); and urethane-treated, 19/19 (23.5 ± 6.49). The tumour multiplicity increased significantly in each experiment ($p < 0.05$); in the first experiment, it also increased significantly in a dose-dependent manner ($p < 0.001$).

In conclusion, the studies provide conclusive evidence that ethylene oxide was carcinogenic in two species.

Table A.27 Summary table of human carcinogenicity data

| **Type of data/ report, Reliability, Key/supportive study** | **Test substance (including purity), Vehicle** | **Relevant information about the study** | **Main effects, Observations** | **Reference** |
|---|---|---|---|---|
| Cohort study, | Ethylene | 18 235 workers employed at 14 industrial plants | Lymphohematopoetic cancers: | Steenland et al. (1991); |

| Type of data/ report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| reliability 2 | oxide | that used ethylene oxide for sterilization since 1943 with ≥ 3 months exposure to ethylene oxide, followed to 1998. Adjusted for age, race (white/non-white), date of birth (within 5 years); in an internal case–control analysis (excluding 1 small plant), log cumulative exposure to ethylene oxide lagged by 15 years significantly related to mortality from LH cancers in men (p = 0.02), but not in women; duration of exposure, peak exposure and average exposure less predictive of mortality from LH cancer; similar pattern observed for lymphoid-cell tumours | All cohort members: SMR (95% CI) = 1.00 (0.79-1.24), 79 cases<br><br>Lymphoid cancers (Non-Hodgkin, lymphoma, myeloma, lymphocytic leukemia):<br>Men with 15-year lag, cumulative exposure ≥ 13,500 ppm-days: SMR (95% CI) = 3.76 (1.03-13.64), p-trend = 0.13<br><br>Hodgkin lymphoma, all cohort members: SMR (95% CI) = 1.24 (0.53-2.43), 6 cases<br><br>Non-Hodgkin lymphoma, all cohort members: SMR (95% CI) = 1.00 (0.72-1.35), 31 case<br><br>Multiple myeloma, all cohort members, SMR (95% CI) = 0.92 (0.54-0.87), 13 cases<br><br>Leukemia, all cohort members, SMR (95% CI) = 0.99 (0.71-1.36) (29 cases) | Stayner et al. (1993); Steenland et al. (2004) A6.12.4/01 |
| Cohort study, reliability 2 | Ethylene oxide | 278 men intermittently exposed to ethylene oxide in a chlorohydrin unit in West Virginia since 1949, followed to 1988. Primarily exposed to ethylene chlorohydrin, ethylene dichloride and bischloroethyl ether. | Lymphohematopoetic cancers:<br>All cohort members, relative risk (95% CI) = 2.94 (1.27-5.80), 8 cases | Benson & Teta (1993); additional study included in the IARC evaluations (1994, 2008, 2012) |
| Cohort study, reliability 2 | Ethylene oxide | 1896 men potentially exposed to ethylene oxide since 1940 at 2 chemical plants in West Virginia, but who never worked in chlorohydrin unit, followed to 1988 | Lymphohematopoetic cancers:<br>All cohort members, relative risk (95% CI) = 0.6 (0.2-1.2), 7 cases<br>Lymphosarcoma and reticulosarcoma, relative risk | Teta et al. (1993) A6.12.4/03 |

| Type of data/ report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| | | | (95% CI) = 1.0 (0.1-3.56) (2 cases)<br>Leukemia, relative risk (95% CI) = 1.1 (0.4-2.5), 5 cases | |
| Cohort study, reliability 2 | Ethylene oxide | 2063 men employed between 1940 and 1988 in West Virginia, followed up to 2003 | Lymphohematopoetic cancers, SMR (95% CI) = 0.89 (0.59-1.29), 27 cases<br>Non-Hodgkin lymphoma, SMR (95% CI) = 1.05 (0.54-1.83), 12 cases<br>Leukemia, SMR (95% CI) = 0.93 (0.47-1.67), 11 cases | Swaen et al. (2009); additional study included in the IARC evaluations (1994, 2008, 2012) |
| Meta-analysis, reliability 2 | Ethylene oxide | Meta-analysis of data from nearly 33000 workers from Germany, Italy, Sweden, the UK and the USA | Leukemia, meta-SMR (95% CI) = 1.08 (0.61-1.93), 35 deaths<br>Non-Hodgkin lymphoma, meta SMR (95% CI) = 1.34 (0.96-1.89), 33 deaths | Teta et al. (1999); additional study included in the IARC evaluations (1994, 2008, 2012) |
| Cohort study, reliability 2 | Ethylene oxide | 1011 women potentially exposed to ethylene oxide in sterilization units at 8 hospitals in the UK during 1964–1986, followed to 31 December 2000 | Breast cancer, all cohort members, relative risk (95% CI) = 0.84 (0.42-1.51), 11 cases | Gardner et al. (1989), Coggon et al., 2004; additional study included in the IARC evaluations (1994, 2008, 2012) |
| Cohort study, reliability 2 | Ethylene oxide | 2170 workers employed for ≥ 12 months in 1964–1985 at 2 plants in Sweden where medical equipment was sterilized with ethylene oxide, followed for cancer incidence to 1990 | Breast cancer, all cohort members, relative risk (95% CI) = 0.46 (0.15-1.08), 5 cases | Hagmar et al. (1991, 1995); additional study included in the IARC evaluations (1994, 2008, 2012) |
| Cohort study, reliability 2 | Ethylene oxide | 7576 women worked for ≥ 1 year at 13 plants, followed for breast cancer incidence to 1998. Recognized to be an underestimate because of incomplete ascertainment of cases. | Breast cancer, all cohort members, relative risk (95% CI) = 0.87 (0.7-0.97), 319 cases<br>Breast cancer, excluding carcinoma in situ, all cohort members, relative risk (95% CI) = 0.94 (0.84-1.05), 299 cases<br>Breast cancer, >14620 ppm-days | Steenland et al., 2003 A6.12.4/02 |

| Type of data/ report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| | | | with 15 year lag, relative risk (95% CI) = 1.74 (1.16-2.65), 48 cases<br>Breast cancer, excluding carcinoma in situ, >14620 ppm-days with 15 years lag, relative risk (95% CI) = 1.87 (1.12-3.10) | |
| Cohort study, reliability 2 | Ethylene oxide | 1132 workers employed during 1974–80 at a sterilizing plant in New York State that used ethylene oxide, followed for cancer incidence to 1957 | Breast cancer, all cohort members, relative risk (95% CI) = 1.72 (0.99-3.00), 12 cases (based on SEER rates for 1978-1981)<br>Breast cancer, all cohort members, relative risk (95% CI) = 1.57 (0.90-2.75), 12 cases (based on SEER rates for 1981-1985) | Norman et al., 1995; additional study included in the IARC evaluations (1994, 2008, 2012) |

The number of epidemiological data and case reports on carcinogenicity of ethylene oxide in humans is very extensive. Only two studies were submitted by the applicant, the study of Steenland et al. (1991 and subsequent updates from 2003 and 2004) and the meta-analysis by Teta et al. (1999). As ethylene oxide was evaluated by IARC (1976, 1985, 1987, 1994, 2008 and 2012), the most recent IARC evaluation was used as a starting point for the assessment. Only the studies which were considered by IARC to be adequate and relevant for the assessment, were included in the summary table above. IARC has considered the study by NIOSH of more than 18,000 employees at 14 industrial facilities where ethylene oxide was used to sterilize medical supplies or food spices, or to test the sterilizing equipment (Steenland et al., 1991; Stayner et al., 1993) to be the most conclusive epidemiological investigation. Initial mortality results for the NIOSH cohort were reported by Steenland et al. (1991). There were 343 deaths from cancer, vs. 380.3 expected; standarised mortality ratio (SMR) (95% CI) = 0.90 (0.81–1.00). The SMRs were not statistically significantly increased for lymphatic and haematopoietic cancers combined, for lymphosarcoma-reticulosarcoma, Hodgkin lymphoma, leukaemia, non-Hodgkin lymphoma or myeloma. No significant trend in mortality was observed in relation to duration of exposure, but the SMR for leukaemia (1.79, based on five deaths) and non-Hodgkin lymphoma (1.92, based on five deaths) were higher after allowance for a latency of more than 20 years. Among the sterilizer operators, mortality ratios were 2.78 (two deaths observed) for leukaemia and 6.68 (two deaths) for lymphosarcoma/reticulosarcoma.

In a further analysis of the same study (Stayner et al., 1993), an exposure–response analysis was conducted with the use of previously derived quantitative estimates of individual exposure to ethylene oxide (Greife et al., 1988). Analysis was limited to 13 of the facilities studied, since exposure information at one facility was inadequate. Mortality from lymphatic and haematopoietic cancer was greatest in the

group with the highest category of cumulative exposure to ethylene oxide (> 8500 ppm–days) (13 deaths; SMR, 1.24; 95%CI: 0.66–2.13), but the trend across three categories of cumulative exposure was weak (χ2, 0.97; p = 0.32). A similar pattern was observed for non-Hodgkin lymphoma, but not for leukaemia.

A significant positive trend in risk with increasing cumulative exposure to ethylene oxide was observed for all neoplasms of the lymphatic and haematopoietic tissues (p < 0.05). Moreover, this trend was strengthened (p < 0.01) when the analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukaemia and non-Hodgkin lymphoma). The exposure–response relationship between cumulative exposure to ethylene oxide and leukaemia was positive but non-significant (p = 0.23).

Extending the mortality assessments through 1998, the SMR (95% CI) from cancer overall was 0.98 (0.92-1.03), and statistically significant excess was found only for bone cancer (SMR (95% CI) = 2.82 (1.23-5.56), 6 deaths). Mortality from lymphatic and haematopoietic cancer was associated with log-cumulative exposures to ethylene oxide lagged by 15 years in men only (p = 0.02 for trend). A similar pattern was observed for lymphoid cell tumors, with SMR (95% CI = 3.76 (1.03-13.64), p = 0.13 for trend).

In the cohort of ethylene oxide-production workers in West Virginia, including 2063 men employed between 1940 and 1988 and followed up through 2003, no indications were found for excess cancer risks associated with ethylene oxide exposure, also for lympho-haematopoetic cancers (Greenberg et al., 1990; Teta et al., 1993; Swaen et al., 2009).

A meta-analysis of the data on nearly 33,000 workers from Germany, Italy, Sweden, the United Kingdom and the USA (Teta et al., 1999) showed no association between excess cancer risk and ethylene oxide exposure for leukemia (meta-SMR (95% CI) = 1.08 (0.61-1.93), 35 deaths) and non-Hodgkin lymphoma (meta-SMR (95% CI) = 0.96-1.89, 33 deaths). This includes the NIOSH study data from 1991 but not the follow up data that were published in 2004.

Four cohort studies were assessed by IARC that examined association between breast cancer and ethylene oxide exposure. Three studies (Gardner et al., 1989 on hospital-based sterilization workers in the UK; Hagmar et al., 1991 on Swedish sterilant workers, and Steenland et al., 2003 on NIOSH cohort) showed no overall excess risk, while borderline significant increase was seen in the study of Norman et al., 1995. Increased relative risks for breast cancer at the highest level of cumulative exposure to ethylene oxide were seen in the NIOSH study (Steenland et al., 2003), if 15 year lag was considered (11620 ppm–days, OR (95% CI) = 1.87 (1.12–3.10)).

There was no consistent evidence of an association between ethylene oxide exposure and excess cancer risk for other cancer types (IARC, 2012 and references therein).

A.3.9.1 Overall conclusion on carcinogenicity related to risk assessment

The carcinogenic properties of ethylene oxide were not reviewed in the latest CLH report (2016), but it was concluded that ethylene oxide

was already classified as Carc. Cat. 1B. The most recent IARC evaluation (2012) concluded that there is limited evidence in humans for a causal association of ethylene oxide with lymphatic and haematopoietic cancers (specifically lymphoid tumours, i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia), and breast cancer.

There is sufficient evidence in experimental animals for the carcinogenicity of ethylene oxide.

There is strong evidence that the carcinogenicity of ethylene oxide, a direct-acting alkylating agent, operates by a genotoxic mechanism. A dose-related increase in the frequency of ethylene oxide-derived haemoglobin adducts has been observed in exposed humans and rodents, and a dose-related increase in the frequency of ethylene oxide-derived DNA adducts has been demonstrated in exposed rodents. Ethylene oxide consistently acts as a mutagen and clastogen at all phylogenetic levels, it induces heritable translocations in the germ cells of exposed rodents, and a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.

IARC considers ethylene oxide to be carcinogenic to humans (Group 1).

| **Conclusion used in Risk Assessment – Carcinogenicity** | |
|---|---|
| Value/conclusion | No NOAEL could be established in the carcinogenicity studies. A calculated $BMDL_{10}$ of 35.5 ppm for alveolar/broncheolar adenomas and carcinomas in female mice from the long-term mouse inhalation study (NTP TR 326) was used in the risk assessment. Please refer to C.1.2. Reference values for more information. |
| Justification for the value/conclusion | Ethylene oxide is classified as a genotoxic carcinogen. Hence, a chronic NOAEL is not considered relevant for the human risk assessment. |
| Classification according to CLH | Carc. 1B; H350 May cause cancer |

## A.3.10. Reproductive toxicity

### A3.10.1 Sexual function and fertility

Table A.28 Summary table of animal studies on adverse effects on sexual function and fertility

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| Predates modern guidelines and GLP, similar to OECD 415, reliability 2 | Rat, Fischer 344, 30 males and females per treatment or each of two negative control group | Ethylene oxide, purity unknown, inhalation - concentration: 0, 10, 33 and 100 ppm<br><br>Exposure: Prior to mating, 12 weeks, 6 hours/day, 5 days/week; During cohabitation, 1-2 weeks, 6 hours/day, 7 days/week; Gestation: 19 days, 6 hours/day, 7 days/week; Parturition + 5 | NOAEC parental: 100 ppm<br><br>NOAEC offspring: 33 ppm<br><br>NOAEC reproduction: 33 ppm | 100 ppm: decreased fertility indices, increased gestation length, decreased number of born pups, decreased number of implantation sites | Only one generation was investigated. Litters were reduced to 10 pups per group irrespective of sex on day 4. The following parameters were examined: body weights of all F0 animals every two weeks until the cohabitation period; litter weights on PND 4 and 14; pup body weights and sex on PND21; male and female fertility indices; gestation duration (number of days from observation of vaginal plug to parturition); number of implantation sites; ratio of number of pups born to the number of | Snellings WM et al. (1982b) A6.8.2/01 |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| | | days: no exposure;<br>Post parturition: 16 days, 6 hours/day, 7 days/week | | | implantation sites; number of litters; number of pups born dead or alive and offspring survival indices. No information on test substance batch, food consumption, organs weights, histopathology, oestrus cycle, sperm and developmental parameters. | |
| Predates modern guidelines and GLP, reliability 2 | Rat, Sprague-Dawley, 32-45 females/dose | Ethylene oxide, >99% pure, target concentrations 150 ppm, 7 hours per day<br>Group 1: no exposure<br>Group 2: GD 7-16<br>Group 3: GD 1-16<br>Group 4: three weeks before mating + GD 1-16 | LOAEC maternal 150 ppm<br>LOAEC developmental: 150 ppm | Increased resorptions in group 4 (statistically significant), reduced fetal body weight in all groups, reduced crown-rump length in all groups, reduced skeletal ossification in all groups (primarily skull and sternebrae).<br>Maternal toxicity: increased absolute and relative kidney | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA. The study was designed to evaluate effects of ethylene oxide exposure before mating (treatment of preovulatory oocytes) or during the egg fertilization or early pronuclear stage of the zygote. Dead embryos | Hardin BD et al. (1983) (publishing data from Hackett PL, 1982) ; additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | | | and spleen weights in all groups, reduced body weight in group 4 | were classified as resorption bodies (death near the time of implantations), midgestation death (characterized by presence of placenta and embryonic mass that lacked eye pigment) and late fetal death (day 11 or later post-mating). | |
| No guideline study, no GLP, reliability 4 | Hybrid mice, (C3H x C57BL)F1 and (SEC x C57BL)F1, females | Ethylene oxide, purity unknown, Experiment 1): Concentrations 0 or 1200 ppm, 1.5 hours/day for 4 consecutive days before mating Experiment 2): Concentrations 300 ppm, 6 hours/day for 10 exposures over a 14-day premating period. Experiment 3): | LOAEC (fertility, f) 300 ppm | 300 ppm: reduced number of implants, increased percentage of resorptions 1200 ppm: increased percentage of resorptions, induced loss of conceptuses was 15.7% at 1200 ppm and 58.2% at 300 ppm. Midgestation and late fetal deaths significantly increased following exposure 1 and 6 | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA. The study was designed to evaluate effects of ethylene oxide exposure before mating (treatment of preovulatory oocytes) or during the egg fertilization or early pronuclear stage of the zygote. Dead embryos | Generoso WM et al. (1987); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | single exposure to 1200 ppm for 1.5 hours starting 1, 6, 9 or 25 hours after mating Dams killed at GD 17 | | hours post-mating, effects no longer visible in females exposed 9 and 25 hours post-mating. | were classified as resorption bodies (death near the time of implantations), midgestation death (characterized by presence of placenta and embryonic mass that lacked eye pigment) and late fetal death (day 11 or later post-mating). | |
| Predates modern guidelines and GLP, reliability 2<br><br>Male fertility | Rat, 20 males/group | Ethylene oxide, purity 97.0 - 98.6%, inhalation, 204 ppm (370 mg/m3), 176 days, 7 hours/day | LOAEC (fertility, m) 204 ppm | Reduced testes weights (not statistically significant), small testes, slight degeneration of few tubules | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The study is described in more detail in Table A.23 under Sub-chronic inhalation toxicity (A3.7.2.3). | Hollingsworth RL et al. (1956). The study was submitted by the applicant as a repeated dose toxicity study. |
| Predates modern guidelines and GLP, reliability 2<br><br>Male fertility | Guinea pig, 8 males/group | Ethylene oxide, purity 97.0 – 98.6%, inhalation, 357 ppm (640 mg/m3), 176 days, 7 hours/day | LOAEC (fertility, m) 204 ppm | 357 ppm: growth depression, degeneration of tubules of testes 204 ppm: decreased testes weights (not statistically | The study is cited in the RAC opinion (2017), the CLH report and in the WHO (2003) evaluation. The study is described in more detail in Table A.23 under Sub-chronic | Hollingsworth RL et al. (1956). The study was submitted by the applicant as a repeated dose |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|
| | | and 204 ppm (370 mg/m3), 176 days, 7 hours/day | | significant) | inhalation toxicity (A3.7.2.3). | toxicity study. |
| No guideline study, no GLP, reliability 2<br>Male fertility | Wistar rat, 6 males/group in exposed groups, 12 males/group in controls | Ethylene oxide, purity unknown, inhalation, chamber exposure, concentrations 50, 100, 250 ppm for 6 hours/day, 5 days/week for 13 weeks | LOAEC (fertility, m)<br>50 ppm | 50 and 100 ppm: abnormal sperm heads, teratic type<br>250 ppm: increased number of total abnormal sperm heads (teratic and immature heads), slight testicular Degeneration (reduced seminiferous tube diameter, focal vacuolation of the seminiferous epithelium, germ cell loss), reduced epididymal weight.<br><br>No effects on body weight after 13 weeks exposure. | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA.<br>The study was designed to evaluate effects of ethylene oxide exposure on spermatogenesis and involved evaluation of testicular and epididymal weights, histopathology, lactase dehydrogenase X activity in testis, and sperm counts and sperm head abnormalities in the epididymis.<br><br>Food intake of control and low dose groups was restricted according to the intake of the high | Mori K et al. (1991); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| | | | | | dose group. Purity unknown. | |
| No guideline study, no GLP, reliability 2<br>Male fertility | Wistar rat, 6-8 males/group | Ethylene oxide, purity unknown, inhalation, chamber exposure, concentrations 0, 500 ppm for 6 hours/day, 3 days/week for 2, 4, 6 or 13 weeks | LOAEC (fertility, m)<br>500 ppm | 2 weeks: mild degeneration of germ cells (anomaly of nuclear shapes, pycnosis of mature spermatids),<br>4 weeks: conspicuous degeneration, nuclear vacuolization of immature round spermatids; increased GST activity<br>6 weeks: exfoliation of germ cells, nearly complete disappearance of mature spermatids; increased GST activity<br>13 weeks: marked reduction in germ cells in about 50% of seminiferous tubules, which | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA.<br>The study was designed to evaluate the effects of repeated exposure to ethylene oxide on organ weights, morphological changes of the testis, plasma testosterone concentration and glutathione metabolism.<br>Not according to modern guidelines nor GLP.<br><br>Pair-feed to minimize differences due to food-intake. | Mori K et al. (1989); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| | | | | contained only Sertoli cells, increased GST activity<br><br>Statistically significantly reduced testicular and epididymal weights were seen following 4, 6 and 13 weeks of exposure; the decrease was time-dependent.<br>No effects on the body weights were seen. No effects on plasma testosterone concentration. | | |
| No guideline study, no GLP, reliability 2<br>Male fertility | B6C3F1 mice, 30/sex/group | Ethylene oxide, purity >99.9%, inhalation, chamber exposure concentrations 0, 10, 50, 100, 250 ppm, 6 hours/day, 5 | NOAEC (fertility, m) 100 ppm<br>LOAEC (fertility, m) 250 ppm<br><br>NOAEC (fertility, f) 250 ppm | No effects on survival or body weight<br>250 ppm: absolute testicular weights depressed, normal histology | The study is cited in the CLH report (2016) and in the WHO (2003) evaluation.<br>The study is described in more detail in Table A.23 under Sub-chronic inhalation toxicity (A3.7.2.3). | Snellings WM et al. (1984)<br>A 6.4.3/06. |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| | | days/week, for 10 weeks (males) or 11 weeks (females) | | | | |
| Mouse sperm morphology test | Mice, Swiss Webster, male, 10 males/dose (exposed groups), 5 males/dose in controls. | Route of exposure: inhalation, chamber exposure, 0, 200, 400 ppm for 6 hours/day, 5 days/week, sacrifice 1, 3, 5 weeks after the beginning of exposure, representing treatments of spermatozoa, spermatid and preleptotene spermatogonial cells | LOAEC (fertility, m) 200 ppm | Statistically significant increase ($p<0.01$) in the percentage of abnormal sperms heads starting from 200 ppm at each sacrifice interval. | The study is cited in the RAC opinion (2017), the CLH report and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA. The study was designed to evaluate effects of ethylene oxide exposure on different stages of spermatogenesis. One thousand sperm cells were scored per each animal. Only the head morphology was examined.<br><br>Ip-injection of cylcophosphamide was used as positive control | Ribeiro LR et al. (1987); additional study cited in the CLH report (2016) and RAC opinion (2017) |
| No guideline study, no GLP, | Cynomolgus monkey, 12 | Ethylene oxide, purity 99.7%, | LOAEC (fertility, m) 50 ppm | 50 and 100 ppm: significant reduction | The study is cited in the RAC opinion (2017), the | Lynch DW et al. (1984a) |

| Method, Duration of study, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels Duration of exposure | NOAELs, LOAELs (e.g. maternal/parental toxicity, effects on sexual function and fertility) | Results (for all dose levels, specify critical effects on sexual function and fertility for parental animals (and offspring if relevant), report e.g. incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
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| reliability 2<br>Male fertility | males/dose | 0, 50, 100 ppm,<br>7 hours/day, 5 days/week for 24 months | | in sperm counts and motility, while drive range (progressive sperm motility) was significantly elevated.<br><br>100 ppm: decreased body weight from week 25 onwards | CLH report (2016) and in the WHO (2003) evaluation. The study is described in more detail in Table A.23 under Sub-chronic inhalation toxicity (A3.7.2.3). | A.6.4.3/08 |

A3.10.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

As ethylene oxide is a gas, inhalation is considered to be the main exposure route.

The applicant has submitted the report of Snellings et al. (1982b) on reproductive toxicity study with Fischer 344 rats. Inhalation exposure levels were 10, 33 and 100 ppm and exposure started 12 weeks before mating and continued until day 21 after parturition. Two concurrent control groups exposed only to air were used. The following parameters were examined: body weights of all F0 animals every two weeks until the cohabitation period; litter weights on PND 4 and 14; pup body weights and sex on PND21; male and female fertility indices; gestation duration (number of days from observation of vaginal plug to parturition); number of implantation sites; ratio of number of pups born to the number of implantation sites; number of litters; number of pups born dead or alive and offspring survival indices. The major effect observed was the significantly ($p < 0.001$) lower median number of pups born at 100 ppm exposure group compared to the medians of both control groups. The medians for the 33 ppm, 10 ppm, and the two air-control groups were 9 or 10 pups, whereas the median was 4 for the 100 ppm exposure group. Also, the median number of implantation sites per pregnant female was lower in the 100 ppm group than in control groups. The ratio of the number of foetuses born to the number of implantation sites per female was also decreased. There were no treatment-related effects on body weight gain of pups or parental animals. No pups were found dead at parturition and there were no statistically significant effects on the survival rate of the F1a generation.

In addition, several other reports in different species (rats, mice, guinea pig, monkey) were recovered in public literature and evaluated by the eCA. The CLH report (2016) and the RAC opinion (2017) on the classification and labelling of ethylene oxide were used as a starting point in the evaluation. The studies by Generoso et al. (1987) and Hardin et al. (1983) confirmed the effects on fertility observed by Snellings et al (1982b). In the study of Hardin et al. (1983), which is described in detail in Section 3.10.1, statistically significantly increased incidence of resorptions was seen in rats exposed to 150 ppm ethylene oxide 3 weeks before mating and during GD 1-16. The effect occurred in the presence of maternal toxicity which was manifested as reduced body weight, as well as increased absolute and relative kidney and spleen weights. The study of Generoso et al. (1987) was designed to evaluate effects of ethylene oxide exposure before mating (treatment of preovulatory oocytes) or during the egg fertilization or early pronuclear stage of the zygote. Dead embryos were classified as resorption bodies (death near the time of implantations), midgestation death (characterized by presence of placenta and embryonic mass that lacked eye pigment) and late fetal death (day 11 or later post-mating). Single exposure of rats for 1.5 hours to 1200 ppm ethylene oxide 1 and 6 hours post-mating significantly increased the number of midgestation and late fetal deaths (97 and 81 vs 4 and 7 in controls); this effect was no longer visible in rats exposed 9 or 25 hours post-mating. In rats exposed to 300 ppm 6 hours/day for 10 exposures over a 14-day premating period reduced number of implantations and increased percentage of resorpions were seen, which was explained by the authors by the dominant-lethal mutations induced by ethylene oxide in ovarian oocytes.

In addition, there were several studies describing specific effects of ethylene oxide on spermatogenesis and sperm morphology. An old study by Hollingsworth et al. (1956), which is discussed in more details under the section of Sub-chronic inhalation toxicity (A3.7.2.3) in Table A.23, described slight degeneration of testes in rats after sub-chronic exposure to 204 ppm ethylene oxide. In guinea pigs exposed to 357 ppm, more appreciable testicular degeneration was observed. No information on sperm counts or reproductive performance is available. The effects were accompanied by depressed growth of the exposed animals.

In a more recent study, effects on sperm morphology have been described by Mori et al.

(1991) after exposure to 50, 100 and 250 ppm ethylene oxide for 13 weeks. At 250 ppm, a statistically significant decrease in epididymal weights (1.06 vs. 1.32 g), but no change in the weight of testis was observed. No differences in the body weights were seen between control and treated groups. Food intake of the control group was restricted to the level of that of high dose group. The number of abnormal sperm was increased statistically significantly ($p < 0.01$) at 250 ppm but not at lower exposure levels. When these were subdivided to immature (with sperm heads resembling spermatocytes) and teratic type (e.g. with amorphous or pycnomorphous sperm head) of sperm, the number of immature sperm was increased at 250 ppm ($p < 0.01$) and the number of teratic type sperm heads was increased in all treated groups ($p < 0.05$), but not in relation to the concentration of ethylene oxide. Histopathology showed slight degenerations (reduced diameter, focal vacuolisation, germ cell loss) in the seminiferous tubules at 250 ppm. At lower doses seminiferous tubules remained normal.

In an earlier study by Mori et al. (1989), inhalation exposure of rats to 500 ppm of EtO for 2, 4, 6 or 13 weeks resulted in time dependent decrease in the relative weights of the testes and the epididymis of the exposed group while body weight gain of the exposed group was not different from control. Light microscopic examination revealed degeneration and exfoliation of germ cells. At 2 weeks, disorder of the arrangement and mild degeneration of seminiferous tubules were observed. At 4 weeks, the degeneration of mature spermatids became conspicuous and the nuclear vacuolisation of immature round spermatids was also observed. At 6 weeks, all types of germ cells including spermatogonia and spermatocytes degenerated and exfoliated, and mature spermatids almost completely disappeared. At 13 weeks, germ cell reduction was prominent in approximately half of the seminiferous tubules and they contained only Sertoli cells. Some seminiferous tubules were reported to show germ cell recovery at 13 weeks compared with 6 weeks. Plasma testosterone concentration was not affected. Exposure to ethylene oxide caused changes in glutathione redox cycle, with both glutathione reductase and glutathione peroxidase activities decreased by 54% and 16%, respectively, after 2 weeks of exposure. Glutathione reductase activity showed a slight recovery at 6 and 13 weeks, while glutathione peroxidase activity fully recovered at 4 weeks and was increased by 17.7% at 13 weeks. The authors stipulated that the decrease in glutathione reductase activity was due to the alkylation of the enzyme molecules by ethylene oxide. On the contrary, glutathione S-transferase activity, which was measured by interaction with two different substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-epoxy-3-(p-nitrophenoxy)propane, was increased at 6 and 13 weeks by 63% and 72.8% with CDNB and 54.6% and 81.9% with 1,2-epoxy-3-(p-nitrophenoxy)propane. Glutathione S-transferase was considered to be the major enzyme detoxifying ethylene oxide in the testes, and its increase in activity is considered to play an important role in protecting against ethylene oxide-induced toxicity. It should be noted that despite changes in glutathione redox cycle, the glutathione concentration following exposure to 500 ppm was not affected in the exposed animals in the study of Mori et al. (1989) compared to untreated controls, which is inconsistent with the opinion of RAC (2017) that glutathione depletion in rodents occurs starting from 200 ppm. Thus the exact mechanism by which the changes in the glutathione redox cycle play a role in the testicular damage is not clear. It should also be noted that adverse effects on sperm parameters were seen at lower dose levels (50-100 ppm) at which no glutathione depletion would yet be expected to occur.

Ribeiro et al. (1987), exposed mice (10 males/group) for 5 days, 6 hours/day to 200 and 400 ppm EtO with subsequent sacrifice 1, 3 and 5 weeks after the beginning of exposure to target the three stages of germ cell development (spermatozoa, spermatid and preleptotene spermatogonial cells) and evaluated the frequency of abnormal sperm cells. One thousand sperm cells from each animal were scored; only the head morphology was examined. Statistically significant increases in the number of abnormal sperm were observed at all time points at both doses, suggesting that ethylene oxide can influence all stages of spermatogenesis. The authors explained it by the mutagenic potential of ethylene oxide.

In the study of Lynch et al. (1984) with Cynomolgus monkeys, which is also reported in a section on subchronic inhalation toxicity, a decrease in the number and mobility of spermatozoa has been observed after exposure to 50 ppm and 100 ppm ethylene oxide by inhalation exposure for 7 h/d, 5 d/wk, for 24 months. Exposure to 100 ppm resulted also in significantly decreased body weight.

The effects on reproduction were observed in the absence of clear general toxicity in several studies. The Committee for Risk Assessment (RAC) agreed to classify ethylene oxide as Repr. 1B; H360Fd, i.e. Cat 1B for fertility and Cat 2 for development. Their argumentation was as follows: *Since these effects have been seen in the absence of clear signs of general toxicity in several studies, RAC considers that the available evidence is sufficient to meet the criteria of Category 1B for fertility.*

A3.10.1.2 Overall conclusion on sexual function and fertility related to risk assessment

| **Conclusion used in Risk Assessment – Effects on fertility** | |
|---|---|
| Value/conclusion | Ethylene oxide causes adverse effects on fertility. |
| Justification for the value/conclusion | In the one-generation study in rats, decreased number of implantations and born foetuses per implantation site (indicating post-implantation losses) was observed without any signs of parental toxicity at 100 ppm. These findings are supported by other studies in mice and rats, showing increased incidences of resorptions and/or decreased incidences of implantations. Additional support for the fertility effects comes from the studies reporting specific effects on spermatogenesis and sperm morphology in rats, mice, guinea pigs and monkey. |

## A.3.10.2 Developmental toxicity

Table A.29 Summary table of animal studies on adverse effects on development*

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| Predates modern guidelines and GLP, similar to OECD 414 (1981), reliability 2 | Rat, Fischer 344,<br>22 mated females per treatment or negative control group,<br>11 mated females per positive control group | Ethylene oxide, >99.9% pure, target concentrations 10, 33 and 100 ppm, gestation days 6 - 15, 6 hours per day | NOAEC maternal 100 ppm, highest dose tested,<br><br>NOAEC developmental 33 ppm, LOAEC developmental 100 ppm | 100 ppm: no maternal clinical signs or mortality. | Statistically significant reduction in fetal body weight. Slightly (non-significantly) elevated incidence in vertebral variations (11% at 100 ppm vs. 5-75 in controls); renal pelvic dilatation occurred in 29% of the 100 ppm pups vs. 20-28% in controls. | The study is submitted by the applicant and cited in the RAC opinion (2017). Parameters evaluated: number and position of the implantation sites; viable and dead foetuses; early and late resorptions; number of corpora lutea; body weights, sex, crown-to-rump length and gross developmental defects for all foetuses. Skeletal and visceral evaluations were performed on each half of the high-exposure, two air control and two | Snellings WM et al. (1982a), IIIA 6.8.1/01 |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | | positive control groups.<br>Deviations: no information is available on the batch of the test substance; only clinical signs and demeanour of maternal animals examined; no body weight measured during gestation. | |
| Predates modern guidelines and GLP, reliability 2 | Rat, Sprague-Dawley, 32-45 females/dose | Ethylene oxide, >99% pure, target concentrations 150 ppm, 7 hours per day<br>Group 1: no exposure<br>Group 2: GD 7-16<br>Group 3: GD 1-16<br>Group 4: three weeks before mating + GD 1- | LOAEC maternal 150 ppm<br>LOAEC developmental: 150 ppm | Maternal toxicity: increased absolute and relative kidney and spleen weights in all groups, reduced body weight in group 4 | Increased resorptions in group 4 (statistically significant), reduced fetal body weight in all groups, reduced crown-rump length in all groups, reduced skeletal ossification in all groups (primarily skull and sternebrae). | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation; the original article was recovered and assessed by the eCA. All fetuses were examined for external, visceral and skeletal effects; internal head examination was | Hardin BD et al. (1983) (publishing data from Hackett PL, 1982); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | 16 | | | | performed on the half of the foetuses. Histopathology was performed on maternal animals (organs and tissues not specified). | |
| Predates modern guidelines and GLP, reliability 2 | Rabbit, New Zealand white, 23-30 females/ dose | Ethylene oxide, >99% pure, target concentrations 150 ppm, 7 hours per day<br>Group 1: no exposure<br>Group 2: GD 7-19<br>Group 3: GD 1-19 | NOAEC maternal 150 ppm<br><br>NOAEC developmental150 ppm | No effects | No effects | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation; the original article was recovered and assessed by the eCA. All fetuses were examined for external, visceral and skeletal effects; internal head examination was performed on the half of the foetuses. Histopathology was performed on maternal animals (organs and tissues | Hardin BD et al. (1983) (publishing data from Hackett PL, 1982); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | | not specified). | |
| No guideline study, no GLP, reliability 2 | Rat, Sprague-Dawley | Ethylene oxide, purity unknown,<br><br>Experiment 1): concentrations 0, 400, 800, 1200 ppm, 0.5 h/d, GD 6-15<br>Experiment 2): concentrations 0, 200, 400, 800, 1200 ppm, 0.5 h, 3 times per day, GD 6-15 | The following was concluded based on findings in experiment 2:<br>NOAEC maternal: 800 ppm<br>NOAEC developmental: 400 ppm<br>LOAEC developmental: 800 ppm | Experiment 1):<br>No effects on maternal weight gain.<br><br>Experiment 2):<br>1200 ppm: reduced maternal weight gain. | Experiment 1):<br>1200 ppm dilated renal pelvis and ureter (doubtful toxicological significance due to the wide variations in renal development).<br><br>Experiment 2):<br>1200 ppm and 800 ppm: decreased fetal body weights | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation; original paper was recovered and evaluated by the eCA. Exposure was only 0.5 hours, either once or three times per day during GD 6-15. Maternal weights were recorded on GD 0, 6, 11, 16 and 21. Number of implantation sites and resorption sites and live and dead foetuses were recorded. Live foetuses were examined for external alterations and sex determination; | Saillenfait AM et al. (1996); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | | skeletal and visceral examinations were conducted on each half of foetuses. | |
| Guideline or GLP unknown. Reliability 4 | Mice, C57BL/6J | Ethylene oxide, purity unknown, Experiment 1): concentrations 2100 ppm per hour (1400 ppm x 1.5 h or 700 ppm x 3 h or 350 ppm x 6 h)<br><br>2700 ppm per hour (1800 ppm x 1.5h or 1543 ppm x 1.75 h or 1350 ppm x 2h, 900 ppm x 3h or 450 ppm x 6h for 1.5, 3 or 6h Single exposure on GD 7 | LOAEC maternal 350 ppm x 6 hours (58% females with clinical symptoms at 30 minutes – depressed movement or arousal, crusty eyes, labored breathing)<br><br>LOAEC developmental: 350 ppm x 6 hours (15% with malformations, mostly eye disorders, vs 75 in controls) | Maternal effects: reduced body weight in all groups, death in high exposure groups (> 1350 ppm x 3 h). | Fetal effects: increased resorptions, increased incidence of fetal deaths, decreased fetal weights, increased incidence of eye malformations, decreased crown-rump length, some effects seen already at lowest concentration. | The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation. The original study was recovered and assessed by eCA. The study was designed to test the applicability of Haber's law in the toxicity of ethylene oxide and involved a single exposure on GD 7 only. The following parameters were recorded: resorptions, crown-rump length; sex; viability; external abnormalities; gross necropsy was | Weller et al. (1999); additional study cited in the CLH report (2016) and RAC opinion (2017) |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | | performed on all pups. | |
| No guideline study, no GLP, reliability 4 | Mice, female | Ethylene oxide, purity unknown Test 1): Concentration: 0, 1200 ppm, 1.5 h single exposure at 1, 6, 9, or 25 h after mating Test 2): Concentration: 0, 1800 ppm, 1.5 h single exposure at 6 h after mating, killed on GD 11 to 15. | LOAEC developmental 1200 ppm | | Exposure 1h after mating: reduced number of live fetuses, increased incidence of abnormal foetuses. Exposure 6h after mating: reduced number of live fetuses, increased incidence of abnormal foetuses | The study is cited in the RAC opinion (2017) and the CLH report (2016). Not according to modern guidelines nor GLP. Abstract only (cited in US EPA, 2010), no further details are available. | Rutledge JC et al. (1989); additional study cited in the CLH report (2016) and RAC opinion (2017) |
| No guideline study, no GLP, reliability 2 | CD-1 mice, female | Ethylene oxide, purity unknown, intravenous administration once daily at 0, 75 150 mg/kg bw/day, in 4 periods | NOAEL maternal 75 mg/kg bw/day LOAEL maternal 150 mg/kg bw/day<br><br>NOAEL developmental 75 mg/kg bw/day LOAEL | Maternal: 150 mg/kg: clinical signs, reduced body weight; mortality in tests I, III and IV; | Developmental: 150 mg/kg: reduced number of live foetuses, decreased fetal body weight, malformed fetuses | The study is cited in the RAC opinion (2017) and the CLH report; the original paper was recovered and assessed by eCA. The study was designed to span the entire period of | LaBorde JB et al. (1980); additional study cited in the CLH report (2016) and RAC opinion |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | Test I: GD 4-6, Test II: GD 6-8, Test III: GD 8-10, Test IV: GD 10-12. | developmental 150 mg/kg bw/day | reduced body weight gain in period I, II and IV. | | implantation and major organogenesis. Clinical examinations and gross necropsy were conducted on maternal animals. The following parameters were further recorded: number of live and dead fetuses, number of resorptions; live fetal weights and sex. Al foetuses were examined for external and internal abnormalities and skeletal malformations and variations; head examination was performed on all foetuses. Histopathological evaluation was conducted on one | (2017) |

| Method, Duration of exposure, Route of exposure, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/ group | Test substance (including purity), Vehicle, Dose levels, | NOAELs, LOAELs (e.g. maternal, teratogenicity, embryotoxicity, offspring, parental, reproductive toxicity) | Results, maternal/ parental (e.g. corrected body weight gain, for all dose levels) | Results, developmental (e.g. pup survival, structural abnormalities, altered growth, functional deficiencies, incidences and severity of the effects for all dose levels) | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|---|---|
| | | | | | | fetus per litter. | |

No robust study summaries of human data on developmental toxicity of ethylene oxide have been submitted by the applicant. The following studies were recovered from public literature, using the CLH report (2016) and the RAC opinion (2017) on ethylene oxide as a starting point, and summarized by the eCA.

Table A.30 Summary table of human data on adverse effects on development

| Type of data/report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| Questionnaire/hospital records<br>Female Finnish hospital sterilizing staff in 1980; nursing auxiliaries as unexposed controls. | Ethylene oxide<br>Concentration not measured in the study, but typical 8-h TWA in Finnish sterilization unit range from 0.1- 0.5 ppm, with peak exposures up to 250 ppm | A total of 1443 pregnancies (545 workers exposed during pregnancy). | Increased frequency of spontaneous abortion based on questionnaires:<br>16.7% in exposed versus 5.6% in non-exposed ($p < 0.01$) (11.3% for the sterilising staff vs 10.6% for the nursing auxiliaries).<br><br>Based on the hospital discharge register: 22.6% in exposed vs. 9.25 in non-exposed ($p < 0.05$).<br>Adjustment for age, parity, decade of pregnancy, smoking habits, and intake of coffee and alcohol did not affect the differences. | Hemminki K et al. (1982). The study is cited in the RAC opinion (2017), the CLH report and in the WHO (2003) evaluation. The original study was recovered and assessed by the eCA. |
| Cross-sectional study<br>Questionnaire<br>Female dental assistants working fulltime | Ethylene oxide, exposure concentrations and timing unknown | Exposure to ethylene oxide and occurrence of spontaneous abortion and pre- and post-term delivery is based on selfreporting.<br>A total of 1320 pregnancies (32 reported exposed during pregnancy). | Age adjusted relative risk:<br>spontaneous abortion: 2.5 (95% CI = 1.0–6.3);<br>pre-term births (21-37 weeks): 2.7 (95% CI = 0.8–8.8);<br>post-term births (≥42 weeks): 2.1 (95% CI = 0.7–5.9)<br>After adjustment for unscavenged nitrous oxide: relative risk of all three adverse pregnancy outcomes 2.5 (95% CI = 1.0-6.1); after further adjustment for smoking relative risk 2.1 (95% CI = 0.7-5.7) | Rowland AS et al. (1996). The study is cited in the RAC opinion (2017), the CLH report (2016) and in the WHO (2003) evaluation, no further details are available. |
| Cross-sectional study<br>Questionnaire<br>Hospital sterilizing units in a province in South Africa, 68.8% fo medical facilities in Gauteng; 96.5% | Ethylene oxide | Information on exposure was based on walk-through surveys, questionnaire collected data and measurements at the time of the study. | Prevalence odds ratio (POR) for pregnancies highly exposed to ethylene oxide vs. low exposed pregnancies:<br>spontaneous abortion: 20.8 (95% CI = 2.1-199); relative risk 16.6 | Gresie-Brusin DF et al. (2007). The study is cited in the RAC opinion (2017) and the CLH report (2016). The study was reported as an abstract |

| Type of data/report, Reliability, Key/supportive study | Test substance (including purity), Vehicle | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| participation rate. | | A total of 98 pregnancies.<br><br>. | (95% CI = 2.0-140.4, p = 0.004) pregnancy loss: 8.6 (95% CI = 1.8-43.7); relative risk 6.2 (95% CI =2.0-19.9, p = 0.003)<br>No association between exposure to ethylene oxide and stillbirth, low birth weight and combined adverse reproductive outcome. | only, which was recovered and assessed by the eCA |
| Hospital records/central statistical data in Finland<br>Paternal exposure to ethylene oxide (n=10) | Ethylene oxide | Exposure status based on occupational titles and industry.<br>99 186 pregnancies (10 pregnancies were assigned to paternal ethylene oxide exposure, resulting in 3 spontaneous abortions). | Increased risk of spontaneous abortion (odds ratio = 4.7; 95% CI = 1.2–18.4)<br>Other potential confounding factors (e.g. previous abortions and alcohol and tobacco consumption) were not considered in the analysis. | Lindbohm M-L et al. (1991). The study is cited in the RAC opinion (2017) and the CLH report (2016). The original study was recovered and assessed by the eCA. The study focussed on the assessment of paternal exposure to various mutagenic agents, including ethylene oxide. |

### A3.10.2.1 Short summary and overall relevance of the provided information on adverse effects on development

As ethylene oxide is a gas, inhalation is considered to be the main exposure route.

The applicant has submitted the report of Snellings et al. (1982a) on developmental toxicity study with rats. In this study, rats were exposed to different levels of ethylene oxide (10, 33 and 100 ppm) for 6 hours/day during GD 6–15. The study included two air-exposed control groups and one positive control (aspirin) group. The general study protocol was overall comparable with OECD guideline 414 (1981). The following parameters were examined: number and position of the implantation sites; viable and dead foetuses; early and late resorptions; number of corpora lutea; body weights, sex, crown-to-rump length and gross developmental defects for all foetuses. Skeletal and visceral evaluations were performed on each half of the high-exposure, two air control and two positive control groups. Examination of maternal weights during the gestation period was not performed, and only appearance and demeanor of maternal animals were examined.

Exposure to 100 ppm 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 the observed effects on fetal body weights and vertebral variations were specific developmental effects or related to maternal toxicity.

In addition, several other reports in different species (rats, mice, rabbit) were recovered in public literature. Out of those, the report of Hardin et al. (1983, published data from Hackett, 1982) is considered the most relevant, as it confirms the results for the rat observed in Snellings et al. (1982a) and indicates that the rabbit is less sensitive than the rat. The study was cited in the RAC opinion (2017) and recovered from public literature and assessed by the eCA. Two different species, rats and rabbits, were exposed to a single concentration of ethylene oxide (150 ppm) during different periods (group 1: filtered air only, group 2: exposure during GD 7-16 (rats) or 7-19 (rabbits); group 3: exposure during GD 1-16 (rats) or 1-19 (rabbits), and group 4: exposure 3 weeks before mating and during GD 1-16 (rats only)). Rats exposed 3 weeks before mating and during gestation (group 4) showed reduced body weights, and in all exposed rat groups absolute and relative kidney and spleen weights were increased. Statistically significantly increased incidence of resorptions was seen in group 4 (exposure 3 weeks before mating and during GD 1-16). Small reductions in rat foetal body weight and crown-rump length were seen in all exposed groups. Reduced skeletal ossification was also observed, primarily of the skull and sternebrae. No adverse effects on development or embryotoxicity were seen in ethylene oxide-exposed rabbits.

The study of Neeper-Bradley et al. (1993) also utilized a protocol comparable to the OECD guideline 414 (1981); however, only the study abstract was available, and the original study could not be recovered. The study abstract was cited in the CLH report (2016) and the RAC opinion (2017). The study reported concentration-dependent reductions in fetal weights in mice after gestational exposure to 50, 125 and 225 ppm ethylene oxide (4%, 5% and 10% reduction vs controls, respectively). Increased incidences of skeletal variations (n=12, primarily unossified or poorly ossified areas) were noted in the 225 ppm group; in the 125 ppm group, three variations were observed. Reduced maternal body weight gain and increased relative liver weights were reported in two higher exposure groups; reduced food consumption was also observed at the highest dose.

Also, additional studies were cited in the RAC Opinion (2017), CLH report (2016) and/or the WHO evaluation (2003), as indicated in the table above. None of these studies were in line with OECD guideline 414 but revealed fetal deaths and malformations at high dose levels, accompanied by slight to severe maternal toxicity. Single high dose exposures during the critical periods of organogenesis have resulted in foetal deaths and malformations, especially eye disorders. It is not possible to conclude that these malformations would have been in all cases secondary to maternal toxicity. Since ethylene oxide is a well-established mutagen, it can be hypothesised that malformations at high doses in developing embryos could be caused by a genotoxic mechanism. On the other hand, it should be noted that at these high doses, GSH depletion may play a role in the foetotoxicity and teratogenicity of EtO. There are only limited data available on the foetotoxicity of ethylene oxide in humans but in the few available studies suggestions on the increased incidence of spontaneous abortions have been obtained. Biases related to questionnaire based studies and/or the effects of confounders (e.g. other concurrent exposures) cannot be totally excluded.

The Committee for Risk Assessment (RAC) agreed to classify ethylene oxide as Repr. 1B; H360Fd, i.e. Cat 1B for fertility and Cat 2 for development. Their argumentation was as follows: *applying a weight of evidence approach, it can be concluded that there are indications on the developmental effects of ethylene oxide. However, malformations have been mainly seen at high dose levels in which GSH depletion may play a role. At lower dose levels, in the absence of maternal toxicity decreased foetal weights were observed. Additionally, in one study skeletal variations were observed. These can be considered to support Category 2. Classification for developmental effects*

A3.10.2.2 Overall conclusion on effects on development related to risk assessment

| **Conclusion used in Risk Assessment – Effects on development** | |
|---|---|
| Value/conclusion | There are indications on the developmental effects of ethylene oxide. |
| Justification for the value/conclusion | At lower dose levels decreased foetal weights were observed which at least in one study were reported to occur in the absence of maternal toxicity. In addition, one study reported increased incidence of skeletal variations, but at the dose levels at which maternal toxicity was also observed. At higher dose levels variations and malformations were observed. Due to limited reporting it could not be concluded that these malformations would have been in all cases secondary to maternal toxicity. |

| **Data waiving** | |
|---|---|
| Information requirement | Teratogenicity study in rabbits is not provided by the applicant. |
| Justification | In the public available study of Hardin et al. (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.<br><br>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. |

## A.3.10.3 Effects on or via lactation

No effects were observed on or via lactation. However, as ethylene oxide is widely distributed through the body and is presumed to be able to penetrate placenta, its presence in the breast milk cannot be excluded.

A3.10.3.1 Overall conclusion on effects on or via lactation related to risk assessment

| Conclusion used in Risk Assessment – Effects on or via lactation | |
|---|---|
| Value/conclusion | No effects on or via lactation were reported in the available studies. However, as ethylene oxide is widely distributed through the body and can penetrate placenta, its presence in the breast milk cannot be excluded. |
| Justification for the value/conclusion | Based on the available reproductive toxicity and toxicokinetic studies. |

A3.10.5 Overall conclusion on reproductive toxicity related to risk assessment

| Conclusion used in the Risk Assessment – Reproductive toxicity | |
|---|---|
| Value | Ethylene oxide is toxic to male reproductive organs, affects pregnancy outcomes and has developmental effects at 100 ppm and above. |
| Justification for the selected value | Ethylene oxide is toxic to male reproductive organs and affects pregnancy outcomes (reduced number of implantation) in female animals at concentrations of 100 ppm and above. Developmental toxicity (increased number of resorptions, reduced number of pups born, reduced fetal body weights, reduced length and variations in ossification, skeletal malformations (cervical/thoracal) and malformation of the eye) occurred in the same order of magnitude. Additionally some effects (increased spontaneous abortions) were also reported in humans, though these data in humans have some deficiencies (insufficient information on exact exposure). The available knowledge on differences in metabolism among different species, including man, is considered insufficient to conclude that the reproductive toxicity seen in several animal species is not relevant for humans, but human data does not provide conclusive evidence on the effects of ethylene oxide on fertility (criteria for Category 1A for fertility effects are not fulfilled). |
| Classification according to CLH | Repr. 1B; H360Fd May damage fertility and the unborn child, |

## A.3.11. Aspiration hazard

Not relevant.

## A.3.12. Neurotoxicity

No individual study summaries on neurotoxicity studies in animals have been submitted by the applicant. In general, signs of neurotoxicity were reported in some of the subchronic toxicity studies summarised under the section of Sub-chronic inhalation toxicity (A3.7.2.3) in Table A.23 and discussed in detail there. Neurotoxicity was noted amongst the clinical signs when rats were exposed to 357 ppm ethylene oxide (Hollingsworth et al., 1956) and in rats exposed to 400 ppm for 6 weeks (Jacobson et al., 1956) and 500 ppm for 13 weeks (Mori et al., 1990 and Matsuoka et al., 1990). In mice, signs of possible neurotoxicity appeared at 50 ppm (hunched posture during gait in males, reduced locomotor activity in females) and were apparent when animals were exposed to 250 ppm for 10-11 weeks (Snellings et al., 1984). In monkeys exposed to 100 and 50 ppm axonal dystrophy and demyelination in distal portion of medulla was observed (examination performed on 2 monkeys/group); at 100 ppm, 2/12 monkeys showed decreased nerve conduction velocity (Lynch et al., 1984b). In another old study with monkeys, neurotoxicity was detected at 204 ppm (Hollingsworth et al., 1956. Neurotoxicity was apparent in dogs exposed to 300 ppm ethylene oxide for 6 weeks (Jacobson et al., 1956).

Table A.31 Summary table of human data on neurotoxicity

| **Type of data/report, Reliability, Key/supportive study** | **Test substance (including purity)** | **Relevant information about the study** | **Main effects, Observations** | **Reference** |
|---|---|---|---|---|
| Clinical study, reliability 4 (no original study report available, study cited in SCOEL (2012)) | Ethylene oxide, 36 ppm | Occupational exposure of 12 individuals to ethylene oxide for about 6 months; maximal ethylene oxide concentration of 36 pm was measured in the room during one sterilisation cycle | Headaches (6 persons), nausea (5), speech disorders and impairment of short-term memory (5), vertigo (3) and incoordination (2). | Garry VF et al. (1984), cited in SCOEL (2012) |
| Cross-sectional study.<br><br>Reliability 4 | Ethylene oxide, 8 h TW 4.7 ppm, but peak exposures possible | Comparison of 25 workers exposed for 1-11 years, with 4 excluded after the review of the questionnaire, with 24 unexposed control workers; evaluation with a self-administered questionnaire and neuropsychological screening. The outcome could have been biased due to the awareness of | There were significantly more subjects judged as impaired in the exposed group (5/22) versus control group (1/23) suggesting CNS dysfunction and cognitive impairment. | Klees JE et al. (1990), cited in CLH (2016); original study not recovered, only abstract was available |

| Type of data/report, Reliability, Key/supportive study | Test substance (including purity) | Relevant information about the study | Main effects, Observations | Reference |
|---|---|---|---|---|
| | | exposure among subjects. | | |
| Survey<br><br>Reliability 2 | Ethylene oxide, mean concentration per cycle 3.4 ppm, cycle duration from 2.77 to 11.75 minutes; peak concentrations (> 260 ppm) probably occurred due to odour detection | Survey on 165 sterilizer workers from 27 hospitals | The main symptoms were headaches, skin and eye irritation, dry mouth, sore throat, skin rash, runny nose, loss of sense of smell, shortness of breath, nausea, numbness in fingers and drowsiness. | Bryant HE et al. (1989), cited in US EPA (2010); original study was recovered and evaluated by the eCA. |
| Clinical study (measurement of nervous system function).<br><br>Reliability 2 | Sterilizer using 12% ethylene oxide and 88% chlorodifluoromethane, or 100% ethylene oxide; estimated average exposure < 1 ppm, but with peak exposures up to 250 ppm (measured) | Clinical studies of 8 hospital workers and 8 control workers, exposed for 5-20 years | Exposed group performed more poorly in eight psychometric tests, but 7/8 were not statistically significant. Dose-response-relationship between years of exposure and slowing if sural nerve conduction velocity. No significant abnormalities in blood chemistry. | Estrin WJ et al. (1987)<br>Estrin WJ et al. (1990), cited in CLH (2016), original study was not recovered; only the abstract was available. |
| Case report, 2 subjects.<br><br>Reliability 4 | Ethylene oxide, several months of exposure for ~1.5 hour/day; peak exposures were probably ~ 700 ppm due to odour detection | Case report on 2 workers at ethylene oxide sterilization plant | Sensorimotor neuropathy (axonal nerve degeneration) was observed, which involved paresthesia, distal limb weakness and cutaneous sensory loss. The symptoms improved after the exposure termination, but returned when the subjects returned to work. | Kuzuhara S et al. (1983), cited in CLH (2016) ; original study not recovered, only abstract was available |
| Case report, 1 subject.<br><br>Reliability 4 | Ethylene oxide, 5 months of exposure, 2-3 times daily, up to 500 ppm | Case report on 1 worker | Polyneuropathy (distal weakness of lower extremities and transitory reduced nerve conduction velocity, nerve fibre degeneration) Improvement in reexamination | Schröder JM et al. (1985) cited in CLH (2016); original study not recovered, only abstract was available |

No individual robust study summaries of human data on neurotoxicity were submitted by the applicant. The assessments of SCOEL (2012) and the CLH report on ethylene oxide (2016) were used as a starting point for the evaluation by the eCA. A number of case reports on operators occupationally exposed to ethylene oxide, including different exposure durations (from several minutes up to 20 years), were included in the CLH report; however, most of them involved only several subjects, the exposure concentration could not be definitely established, and consequently they were identified as supporting studies in the CLH report. Furthermore, several cross-sectional studies and surveys were reported. The studies which were considered the most relevant by the CLH report and the SCOEL, are summarized in the table above and cited below.

Klees (1990) describes a group of 25 hospital workers chronically exposed to ethylene oxide (8h TWA of 4.7 ppm) compared to 24 unexposed workers. After review of a self-administered questionnaire 4 exposed workers were excluded from the study. Subjects were tested with a neuropsychological screening battery (memory scale, fingertapping, grip strength, etc.) by examiners blinded to exposure status. Results were reviewed independently by two neuropsychologists. Neuropsychological function was classified as either normal, impaired or disagreement (between the two neuropsychologists). Disagreement occurred in 7/23 controls and 10/22 exposed workers. Exposed subjects were significantly more frequently classified as impaired (5/22) compared to controls (1/23). These findings give some evidence that central nervous system dysfunction and cognitive impairment may result from chronic ethylene oxide exposure.

In the SCOEL, a study of Garry et al. (1979) is cited, in which neurological symptoms were seen in 12 people who had been exposed occupationally to ethylene oxide for about six months: headaches (6 persons), nausea (5), speech disorders and impairment of short-term memory (5), vertigo (3) and incoordination (2). Measurements carried out in the air of the room during one sterilisation cycle revealed a maximal ethylene oxide concentration of 36 ppm.

Bryant et al. (1989) published a survey on 165 sterilizer workers from 27 hospitals which had been exposed to ethylene oxide during sterilization cycles. Mean concentration per cycle was 3.4 ppm, with the cycle duration from 2.77 to 11.75 minutes. Detection of ethylene oxide odour suggested peak concentrations > 260 ppm. The main symptoms were headaches, skin and eye irritation, dry mouth, sore throat, skin rash, runny nose, loss of sense of smell, shortness of breath, nausea, numbness in fingers and drowsiness.

In a clinical study (Estrin, 1987) 8 workers chronically exposed to ethylene oxide (or ethylene oxide + chlorodifluoromethane) were evaluated with a computerized psychometric test battery (8 subtests), nerve conduction studies, P-300 event-related potential and EEG spectral analysis. Exposed group performed more poorly (but not significant) in the psychometric test battery. A significant relationship was only found between decreasing performance on the continuous performance test and years of exposure. Nerve conduction studies indicate a significant reduction in sural velocity with increased years of exposure. P-300 and EEG spectral analysis showed no significant results.

Kuzuhara et al. (1983) reported 2 cases of workers that were exposed to ethylene oxide for several months for about 1.5 hour/day. The estimated peak exposure was probably ~700 ppm, as the workers could detect the oudor when opening the sterilizer. Sensorimotor neuropathy (axonal nerve degeneration) was observed, which involved paresthesia, distal limb weakness and cutaneous sensory loss. The symptoms improved after the exposure termination, but returned when the subjects returned to work.

Based on the available human and animal data, RAC has agreed to classify ethylene oxide as STOT RE 1, H372 (Causes damage to nervous system through prolonged or repeated exposure).

| Conclusion used in Risk Assessment – Neurotoxicity | |
|---|---|
| Value/conclusion | Ethylene oxide is considered neurotoxic and is classified as STOT RE 1, H372 (Causes damage to nervous system through prolonged or repeated exposure) |
| Justification for the value/conclusion | Based on available animal and human data. |

| Data waiving | |
|---|---|
| Information requirement | No study provided. This is not considered to be a data gap. |
| Justification | According to the TNsG of BPD (98/8/EC), indications that the active have neurotoxic effects, may require further in-depth investigation. However, based on the available human and animal data, RAC has concluded to classify ethylene oxide as STOT RE 1, H372 (Causes damage to nervous system through prolonged or repeated exposure).<br>The eCA thus considers that performance of further neurotoxicity studies is not warranted. |

## A.3.13. Immunotoxicity

No studies on immunotoxicity of ethylene oxide were submitted by the applicant. Not considered necessary.

## A.3.14. Endocrine disruption

The assessment of the endocrine disrupting potential of ethylene oxide was performed in accordance with the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No. 528/2012 and (EC) No. 1107/2009 (adopted 5 June 2018).

No regulatory studies relevant for investigating the endocrine disrupting (ED) properties of ethylene oxide were submitted by the applicant. The available data package for ethylene oxide consists only of studies from open literature, such as repeat dose toxicity studies and several reproductive and developmental toxicity studies, as well as a limited number of epidemiological studies. The majority of the animal studies predates modern guidelines and are non-compliant with modern data requirements. Furthermore, most of the studies were performed according to non-standard experimental protocols, e.g. examining the influence of particular exposing regimes only on some specific parameters of interest. Therefore, most of the studies on ethylene oxide have significant deviations from the relevant guideline studies. Nevertheless, the data package on ethylene oxide is extensive, and the studies from open literature has been used together in a weight of evidence evaluation. The lines of evidence for adverse effects and potential endocrine activity of ethylene oxide are reported in the tabulated form in the table below. A summary of the relevant information from the available epidemiological and animal studies is also provided in the text below.

**Human data**
In accordance with Section 4.4 of the ECHA/EFSA Guidance, available epidemiological data which may be illustrative of the endocrine disrupting properties of the substance should be

provided and used in the assessment. The available epidemiological studies which may be relevant for the assessment of the endocrine disrupting properties of ethylene oxide mainly consists of studies addressing reproductive and developmental toxicity. The available epidemiological studies have also been evaluated by RAC (2017), who concluded that the human data does not provide conclusive evidence on the effects of ethylene oxide on fertility. RAC stated that studies based only on questionnaires may be affected by recall and reporting bias; studies evaluating spontaneous abortions are especially vulnerable to these biases since individual recognition of early spontaneous abortions is likely to vary. Furthermore, the database is limited, and the role of confounders in different studies cannot be completely ruled out. RAC concluded that the main evidence on the adverse effects on fertility and development of ethylene oxide comes from animal data. For these reasons, the assessment of potential endocrine disrupting properties of ethylene oxide has been based on the available animal data on ethylene oxide, and not the data from the epidemiological studies. Nevertheless, findings from the relevant epidemiological studies are summarised and discussed in the text below.

In the study of Rowland et al. (1996), a questionnaire was administered to 1320 women whose most recent pregnancy was conceived while working full-time as dental assistants. Thirty-two women reported exposure to ethylene oxide; unexposed women comprised the control group. No further information on exposure was available. The age-adjusted relative risk of spontaneous abortion among ethylene oxide-exposed women was 2.5 (95% CI = 1.0–6.3); the relative risks of preterm births (21–37 weeks) and post-term births (≥ 42 weeks) were 2.7 (95% CI = 0.8–8.8) and 2.1 (95% CI = 0.7–5.9), respectively. Using a logistic model, women exposed to ethylene oxide were 2.7 times (95% CI = 1.2–6.1) more likely to have any of the three adverse pregnancy outcomes after adjusting for age, but when the results were adjusted for smoking, nitrous oxide exposure and high amalgam use, a relative risk of 2.1 with a 95% CI of 0.7–5.7 was obtained.

In the study of Hemminki et al., 1982 an increased frequency of spontaneous abortions was reported among sterilising staff employed in Finnish hospitals in 1980. According to the questionnaire-based data from sterilising staff performing such tasks during pregnancy, the frequency was 16.1%, whereas in the control group it was 7.8%. Supporting the questionnaire data, frequencies of 22.6% (exposed) vs. 9.2% (non-exposed) were obtained when the frequency data was obtained from hospital discharge registers. The 8 h TWA exposure levels in Finnish hospitals ranged from 0.1–0.5 ppm (with peaks up to 250 ppm) at that time. Adjustment for age, parity, decade during which the pregnancy occurred, smoking habits, and intake of coffee and alcohol did not affect the difference. The increased frequency of spontaneous abortion correlated with exposure to ethylene oxide but not with exposure to glutaraldehyde or formaldehyde.

Gresie-Brusin (2007) also reported a questionnaire-based study among female workers in sterilising units in South Africa. A significantly increased risk of spontaneous abortions (prevalence odds ratio (POR) = 20.8, 95% CI = 2.1–199) and pregnancy losses (POR = 8.6, 95% CI = 1.8–43.7) was described in females exposed to high levels of ethylene oxide compared to those with low exposure. The study was based on 98 exposed women with singleton pregnancies. No associations were found between exposure to ethylene oxide and stillbirth.

An increased risk of spontaneous abortion (odds ratio = 4.7; 95% CI = 1.2–18.4) was also reported by Lindbohm et al. (1991) who evaluated the effects of paternal occupational exposure to different mutagenic agents. However, this was based only on 10 pregnancies which were assigned to
paternal ethylene oxide exposure, resulting in 3 spontaneous abortions. Other potential confounding factors, such as previous abortions and alcohol and tobacco consumption, were not considered in the study.

## Animal data

Relevance and reliability of the data

There are no regulatory studies available investigating the endocrine disrupting (ED) properties of ethylene oxide. All the available studies are from open literature and predates modern data requirements. Most of the available studies have significant deviations from standard guidelines. Nevertheless, the data package on ethylene oxide is extensive, and RAC considered the available evidence sufficient to meet the CLP criteria of Category 1B for effects on fertility and sexual function.

The most relevant studies for information on endocrine disruption by EATS-modalities for ethylene oxide are the reproductive and developmental toxicity studies by Snellings et al. (1982b and 1982a, respectively), in addition to the studies by Mori et al. (1989 and 1991), Ribeiro et al. (1987) and Lynch et al. (1984). The last four studies were included in the justification for the classification Category 1B for fertility by RAC (2017). The study by Hollingsworth et al. (1956) also provides some information on EATS-mediated parameters. However, since this study is of old age with limited reporting it will only be considered as supportive.

The two studies of Snellings et al. (1982b and 1982a) are the only available studies that were conducted according to protocols comparable with modern guidelines. In the one-generation reproductive toxicity study on Fisher 344 rats by Snellings et al. (1982b), the following parameters were examined: body weights of all F0 animals every two weeks until the cohabitation period; litter weights on PND 4 and 14; pup body weights and sex on PND21; male and female fertility indices; gestation duration (number of days from observation of vaginal plug to parturition); number of implantation sites; ratio of number of pups born to the number of implantation sites; number of litters; number of pups born dead or alive and offspring survival indices. In the developmental toxicity study on Fisher 344 rats by Snellings et al. (1982a), the following parameters were examined: number and position of the implantation sites; viable and dead foetuses; early and late resorptions; number of corpora lutea; body weights, sex, crown-to-rump length and gross developmental defects for all foetuses. Skeletal and visceral evaluations were performed on each half of the high-exposure, two air control and two positive control groups. Maternal body weight during the gestation period was not examined; only appearance and clinical signs of toxicity of maternal animals were examined.

The study of Neeper-Bradley et al. (1993) also utilized a test protocol comparable to the OECD guideline 414 (1981). However, only the study abstract was available, and the original study could not be recovered. The study abstract was cited in the CLH report (2016) and the RAC opinion (2017). According to the abstract, the following parameters were examined in the maternal animals: clinical signs daily, body weights on days 0, 6, 9, 12, 15, 18 and 21 of gestation; food consumption at 3-day intervals throughout gestation; at necropsy, liver and lung weights, gravid uterine weights, number of corpora lutea, number of implantation sites; number of early and late resorptions; dead and live foetuses. All foetuses were weighted and examined externally for malformations, variations and sex determinations. Approximately one half of foetuses from each litter was examined for visceral and craniofacial malformations and variations, while another half were examined for skeletal malformations and variations.

The lines of evidence for adverse effects and potential endocrine activity of ethylene oxide are presented in the table below, and the results from the animal studies have been summarised and discussed in the text below the table.

Table A.32 Lines of evidence for adverse effects and potential endocrine activity of ethylene oxide.

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| EATS-mediated parameter | Thyroid histopathology | Lynch et al., 1984b, IIIA 6.7/01 | Rat | 24 months | > 100 | No adverse effects observed | Sufficient: no adverse effects seen at any exposure concentration | Overall negative evidence of adversity | T |
| | | Snellings et al., 1984b, IIIA 6.7/02 | Rat | 24 (M) or 25 (F) months | > 100 | No adverse effects observed | Sufficient: no adverse effects seen at any exposure concentration | | |
| | | NTP TR 326, 1987, IIIA 6.7/03 | Mouse | 102 weeks | > 100 | No adverse effects observed | Sufficient: no adverse effects seen at any exposure concentration | | |
| EATS-mediated parameter | Testicular weight | Hollingsworth et al. (1956), IIIA 6.4.3/01 | Guinea pigs | 123 exposures, 7 hours/day, 5 days/week | 204 | Decrease in testes weight, not statistically significant | Supportive: old study (1956), limited reporting, no information on dose response, not statistically significant effect | Overall positive evidence of adversity | EAS |
| | | Hollingsworth et al. (1956), IIIA | Rat | 122-157 exposures, 7 hours/day, | 204 | Decrease in testes weight, not statistically significant | Supportive: old study (1956), limited reporting, no information on dose response, not | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | 6.4.3/01 | | 5 days/week | | | statistically significant effect | | |
| | | Snellings et al. (1984a), IIIA 6.4.3/06 | Mouse | 10 weeks | 50 | Reduced absolute testes weight at three higher dose levels, no clear dose-response | Supportive: no clear dose-response, absence of corroborating histopathological findings | | |
| | | Mori et al., 1989, cited in the CLH report (2016) | Rat | 4, 6 or 13 weeks | 500 | Statistically significantly reduced relative testes weight at a single tested dose, in a time-dependent manner | Sufficient: statistically significantly decrease was observed in a time-dependent manner | | |
| EATS-mediated parameter | Epididymal weight | Mori et al., 1991, cited in the CLH report (2016) | Rat | 13 weeks | 250 | Statistically significantly reduced epididymal weight at the highest dose level; evidence of dose response | Sufficient: changes observed in a dose-dependent manner, statistical significance reached at the highest dose level | Overall positive evidence of adversity | EAS |
| | | Mori et al., 1989, cited in the CLH report (2016) | Rat | 4, 6 or 13 weeks | 500 | Statistically significantly reduced relative epididymal weight at a single tested dose, in a time-dependent manner | Sufficient: a decrease was observed in a time-dependent manner | | |
| EATS-mediated parameter | Testicular histopathology | Hollingsworth et al. (1956), IIIA 6.4.3/01 | Guinea pig | 123 exposures, 7 hours/day, 5 days/week | 357 | Appreciable degeneration of the tubules of the testes with replacement fibrosis | Supportive: old study (1956), limited reporting, no information on dose-response | Overall positive evidence for adversity | EAS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | Hollingsworth et al. (1956), IIIA 6.4.3/01 | Rat | 122-157 exposures, 7 hours/day, 5 days/week | 204 | Slight tubular degeneration in testes | Supportive: old study (1956), limited reporting, no information on dose response, effect described as "slight" | | |
| | | Snellings et al. (1984a), IIIA 6.4.3/06 | Mouse | 10 weeks | > 250 | No histopathological findings in testis at any dose level | Not supportive: no histopathological findings at any dose level | | |
| | | Mori et al., 1991, cited in the CLH report (2016) | Rat | 13 weeks | 250 | Slight degeneration in seminiferous tubules at the highest dose level | Supportive: no information on dose-response (effect observed at the highest dose only), effect described as "slight" | | |
| | | Mori et al., 1989, cited in the CLH report (2016) | Rat | 13 weeks | 500 | Testicular atrophy and severe degeneration of the seminiferous tubules. | Sufficient: although some tubules were almost intact, others were severely affected with diminished germ cells (no information on dose response -single dose tested) | | |
| EATS-mediated parameter | Sperm motility | Lynch et al., 1984a, IIIA 6.4.3/08 | Monkey | 24 months | 50 | Statistically significantly reduced sperm motility in the absence of clear dose-response | Sufficient: effect observed in both exposed groups, although in the absence of clear dose-response | Positive evidence of adversity | EAS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| EATS-mediated parameter | Sperm morphology | Lynch et al., 1984a, IIIA 6.4.3/08 | Monkey | 24 months | > 100 | No increase in abnormal sperm heads | No effects seen at both dose levels | Overall positive evidence of adversity | EAS |
| | | Ribeiro LR et al., 1987, cited in the CLH report (2016) | Mouse | 5 days | 200 | Statistically significant increase in the percentage of abnormal sperm heads | Sufficient: statistically significant effects observed at both tested dose levels with an evidence of dose-response | | |
| | | Mori et al., 1991, cited in the CLH report (2016) | Rat | 13 weeks | 50 | Statistically significantly increased number of abnormal sperm heads of teratic type at all dose levels without clear evidence of dose response | Sufficient: statistically significant effect observed at all dose levels, although in the absence of clear dose-response; but correlated with other observed adverse effects | | |
| | | Mori et al., 1989, cited in the CLH report (2016) | Rat | 2, 4, 6 and 13 weeks | 500 | Degeneration of germ cells; mild at 2 weeks, conspicuous at 4 weeks; exfoliation of germ cells at 6 weeks | Sufficient: changes observed in time-dependent manner | | |
| EATS-mediated parameter | Sperm numbers | Lynch et al., 1984a, IIIA 6.4.3/08 | Monkey | 24 months | 50 | Statistically significantly reduced sperm count in a dose-response manner | Sufficient: effect observed in both exposed groups in a dose-response manner | Evidence of adversity | EAS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| In vivo mechanistic | Testosterone level | Mori et al., 1989, cited in the CLH report (2016) | Rat | 2, 4, 6 and 13 weeks | > 500 | No changes of testosterone concentration in plasma. | No effect was seen at any exposure duration. However, the plasma testosterone concentration showed great variations. | No evidence of adversity. However, this is only based on one study, and the results showed great variations. | |
| Sensitive to, but not diagnostic of, EATS | Adrenal weight | Hollingsworth et al. (1956), IIIA 6.4.3/01 | Rat | Up to 8 exposures, 7 hours/day, 5 days/week | 841 | Enlarged adrenals | Limited: old study, limited reporting, no information on dose response (effect seen at the highest dose level) | Overall positive evidence of adversity | Sensitive to, but not diagnostic of, EATS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | Lynch et al., 1984b, IIIA 6.7/01 | Rat | 7 hours/day, 5 days/week, 2 years | 50 | Increased absolute and relative adrenal weights | Sufficient; statistically significant effects seen at both exposure concentrations, albeit in the absence of dose response, and correlated with microscopic changes | | |
| Sensitive to, but not diagnostic of, EATS | Adrenal histopathology | Hollingsworth et al. (1956), IIIA 6.4.3/01 | Guinea pig | 123 exposures, 7 hours/day, 5 days/week | 357 | Slight fatty degeneration of adrenals in females only | Limited: old study, limited reporting, no information on dose response, effect seen in one sex only, effect described as "slight" | Overall positive evidence of adversity | Sensitive to, but not diagnostic of, EATS |
| | | Lynch et al., 1984b, IIIA 6.7/01 | Rat | 7 hours/day, 5 days/week, 2 years | 50 | Vacuolation and hyperplasia or hypertrophy of the cells of the zona fascicularis; in some rats the areas of hyperplasia and hypertrophy formed distinct nodules which compressed the adjacent cortical tissue | Sufficient; statistically significant effects seen at both exposure concentrations, albeit in the absence of dose response | | |
| Sensitive to, but not diagnostic of, EATS | Fertility index | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post- | 100 | Decreased fertility index by P generation | Limited: no information on dose-response (effect observed at the highest dose tested); not statistically significant effect | Limited evidence of adversity | Sensitive to, but not diagnostic of, EATS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | | | parturition starting from PND5 | | | | | |
| Sensitive to, but not diagnostic of, EATS | Fetal development | Rutledge et al., 1989, cited in the CLH report (2016) | Mouse | 1.5 hour exposure 1 and 6 hours mating | 1200 | Statistically significantly increased number of fetuses with anomalities: hydrops and eye effects; also defects in the limbs and tail occurred in females exposed 6 hours after mating. | Limited: study available only as an abstract (cited in CLH report), no information on dose response (single dose tested); very high exposure concentration. | Overall positive evidence of adversity (for skeletal variations only) | |
| | | LaBorde et al., 1980, cited in the CLH report (2016) | Mouse | 2 days (different periods: GD 4-6, GD 6-8, GD 8-10, GD 10-12) | 150 mg/kg bw/day (intravenous administration) | Significant increase in the number of malformed fetuses/litter after exposure at GD 6-8 and GD 10-12. | Sufficient; evidence of dose-response; however, the route of administration is irrelevant for occupational exposure | | |
| | | Weller et al., 1999, cited in the CLH report (2016) | Mouse | Single exposure on GD7: 2100 ppm/hour, equal to 1.5 hour x 1400, 3 h x 700 or 6 h x 350 | 2100/ hour | Significantly increased incidences of eye defects (microphthalmia, anophthalmia) at all exposure concentrations | Limited; no information on dose-response (study designed to evaluate C x t effects), very high exposure concentrations at which also maternal effects were seen (weight loss, mortality at the highest dose level) | | |
| | | Snellings et al., 1982a, IIIA | Rat | 9 days (GD6-15) | 100 | Slightly (non-significantly) elevated incidence in vertebral | Limited: no information on dose-response (effect observed at the highest dose level); not | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | 6.8.1/01 | | | | variations, renal pelvic dilation) | statistically significant effect | | |
| | | Neeper-Bradley et al., 1993, cited in the CLH report (2016) | Rat | 9 days (GD6-15) | 125 | Increased incidence of skeletal variations (primarily unossified or poorly ossified areas involving the head region, extremities and sternebrae) | Sufficient; effects observed in a dose-response manner | | |
| | | Saillenfait et al., 1996, cited in the CLH report (2016) | Rat | 9 (GD 6-15), 3 exposures/ day, 0.5 h/exposure | 1200 | Increased incidence of dilated renal pelvis and ureter | Limited: toxicological significance is doubtful in view of wide variations in the renal development; no information on dose response (effect seen at the highest tested dose level); very high exposure concentration | | |
| | | Hardin et al., 1983, cited in the CLH report (2016) | Rat | Group 1: no exposure<br>Group 2: GD 7-16<br>Group 3: GD 1-16<br>Group 4: three weeks before mating + GD 1-16 | 150 | Statistically significantly reduced crown-rump length and reduced skeletal ossification in al groups | Limited: no information on dose-response (single dose tested), although effects were seen at all exposure durations; signs of maternal toxicity observed (increased absolute and relative kidney and spleen weights in all groups, reduced body weight in group 4) | | |
| Sensitive to, but not diagnostic of, EATS | Fetal mortality | Rutledge et al., 1989, cited in | Mouse | 1.5 hour (single exposure 1, 6, 9 or 25 | 1200 | Reduced number of live fetuses | Limited: study available only as an abstract (cited in CLH report), no information on dose | Limited evidence of adversity: in two out of | Sensitive to, but not |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | the CLH report (2016) | | hours after mating) | | | response (single dose tested); very high exposure concentration. | three studies effects occurred only at very high exposure concentrations at which maternal toxicity was evident; also no information on dose-response is available. | diagnostic of, EATS |
| | | LaBorde et al., 1980, cited in the CLH report (2016) | Mouse | 2 days (different periods: GD 8-10, GD 10-12) | 150 mg/kg bw/day | Reduced number of live fetuses | Limited: no information on dose response (effects seen at the highest dose level); exposure route (intravenous) irrelevant for occupational exposure | | |
| | | Weller et al., 1999, cited in the CLH report (2016) | Mouse | Single exposure on GD7: 1 h x 2100, 1.5 hour x 1400, 3 h x 700 or 6 h x 350 | 2100/ hour (1400 x 1.5 hour) | Increased incidence of fetal deaths at 1400 ppm x 1.5 hour only | Limited; no information on dose-response (study designed to evaluate C x t effects), very high exposure concentrations at which also severe maternal effects were seen (mortality) | | |
| Sensitive to, but not diagnostic of, EATS | Fetal weight | LaBorde et al., 1980, cited in the CLH report (2016) | Mouse | 2 days (different periods: GD 4-6, GD 6-8, GD 8-10, GD 10-12) | 150 mg/kg bw/day | Significant reduction in mean fetal body weight at all treatment periods | Limited: no information on dose-response (effects observed at the highest dose level), route of administration (intravenous) not relevant for occupational exposure | Overall positive evidence of adversity; in two studies a dose-response effect is reported. However, at least in one study the effect occurred at a dose level at | Sensitive to, but not diagnostic of, EATS |
| | | Snellings et al., 1982a, IIIA 6.8.1/01 | Rat | 9 days (GD 6-15) | 100 | Statistically significantly reduced fetal body weight | Limited: no information on dose-response (effects observed at the highest dose level), maternal body weight gain during gestation not monitored. | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | Neeper-Bradley et al., 1993, cited in the CLH report (2016) | Rat | 9 days (GD 6-15) | 50 | Reduced fetal body weights in a concentration-dependent manner (reduction of 4, 5 and 10% relative to controls) | Sufficient: evidence of dose-response; no signs of maternal toxicity reported at the lowest dose level | which no maternal toxicity was evident. | |
| | | Saillenfait et al., 1996, cited in the CLH report (2016) | Rat | 9 (GD 6-15), 3 exposures/ day, 0.5 h/exposure | 3 x 800 | Statistically significantly reduced fetal body weight at 3 x 800 and 3x 1200 ppm; effect at 3 x 200 ppm was not considered toxicologically significant due to unusually high weight in controls | Sufficient; evidence of dose-response at two highest dose levels; signs of maternal toxicity (decrease in body weight and body weight gain) were seen at the highest dose level. | | |
| | | Hardin et al., 1993, cited in the CLH report (2016) | Rat | Group 1: no exposure<br>Group 2: GD 7-16<br>Group 3: GD 1-16<br>Group 4: three weeks before mating + GD 1-16 | 150 | Reduced fetal body weight | Limited: no information on dose-response (single dose tested), although effects were seen at all exposure durations; signs of maternal toxicity observed (increased absolute and relative kidney and spleen weights in all groups, reduced body weight in group 4) | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| Sensitive to, but not diagnostic of, EATS | Gestational interval | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post-parturition from PND5 | 100 | Statistically significantly increased gestation length (4 females 23 days, 2 females 25/26 days, 1 female undeterminable) | Limited: no information on dose-response (effects seen at the highest dose); the biological significance of the effect was considered unknown by the author, as the gestation length for the laboratory rat is reported to be 21 to 23 days | Limited evidence of adversity | Sensitive to, but not diagnostic of, EATS |
| Sensitive to, but not diagnostic of, EATS | Number of implantations, corpora lutea | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post-parturition from PND5 | 100 | Statistically significantly decreased number of implantation sites per pregnant female (6 vs 10 or 11 in controls). | Sufficient: statistically significant effect observed | Positive evidence of adversity | Sensitive to, but not diagnostic of, EATS |
| Sensitive to, but not diagnostic of, EATS | Number of pups at day 0 postpartum | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post-parturition from PND5 | 100 | Statistically significantly reduced median number of pups born on PND0 | Sufficient: statistically significant effect observed, consistent with other effects observed (lower number of implantation sites and lower median number of fetuses born per number of implantation sites) | Overall positive evidence of adversity | Sensitive to, but not diagnostic of, EATS |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | Number of fetuses born per implantation site | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post-parturition from PND5 | 100 | Statistically significantly reduced number of fetuses born per implantation site (indicating post-implantation loss) | Sufficient: statistically significant effect observed | | |
| Sensitive to, but not diagnostic of, EATS | Pre-implantation losses | Generoso et al., 1987, cited in the CLH report (2016) | Mouse | 14 days pre-mating period, 6 hours/day, 10 exposures in total | 300 | Significantly reduced number of implantations per female | Considered to be supporting evidence; however, it should be noted that at these high dose levels GSH depletion and reduced elimination of ethylene oxide could have occurred | Positive evidence of adversity | Sensitive to, but not diagnostic of, EATS |
| Sensitive to, but not diagnostic of, EATS | Post-implantation losses | Generoso et al., 1987, cited in the CLH report (2016) | Mouse | 1.5 hours, 4 days before mating | 1200 | Increased number of conceptus losses (defined as [1- (mean living embryos in test groups/mean living embryos in controls)] x 100 (15.7). | Limited: no information on dose-response (single dose tested), maternal toxicity observed (no further details provided) | Limited evidence of adversity, as in one study effects were reported at very high exposure concentration ; maternal toxicity was evident in both studies and no information on dose- | Sensitive to, but not diagnostic of, EATS |
| | | Generoso et al., 1987, cited in the CLH report (2016) | Mouse | 14 days pre-mating period, 6 hours/day, 10 exposures in total | 300 | Increased number of conceptus losses (defined as [1- (mean living embryos in test groups/mean living embryos in controls)] x 100 (58.2). | Limited: no information on dose-response (single dose tested), maternal toxicity observed (no further details provided) | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | response is available | |
| Sensitive to, but not diagnostic of, EATS | Resorptions | Weller et al., 1999, cited in the CLH report (2016) | Mouse | Single exposure to 2100 ppm/hour on GD7: 1.5 hour x 1400, 3 h x 700 or 6 h x 350 | 2100/ hour | Increased number of resorptions at 1400 ppm x 1.5 hour (24%) and 700 ppm x 3 hours (27%) | Limited: no information on dose response, very high exposure concentrations at which also maternal effects were seen (weight loss, mortality at the highest dose level) | evidence of adversity | Sensitive to, but not diagnostic of, EATS |
| | | Weller et al., 1999, cited in the CLH report (2016) | Mouse | Single exposure to 2700 ppm/hour on GD7: 1.5 h x 1800, 1.75 h x 1543, 2 h x 1350, 3 h x 900, 6 h x 450 | 2700/ hour | Increased number of resorptions at 450 ppm x 6 hours (28%), 900 ppm x 3 hours (22%) and 1800 ppm x 1.5 hours (14%) | Limited: no information on dose response, very high exposure concentrations at which also maternal effects were seen (weight loss, mortality at the highest dose level) | | |
| | | Generoso et al., 1987, cited in the CLH report (2016) | Mouse | 4 days before mating for 1.5 hours/day | 1200 | Statistically significantly increased number of resorptions (10.8% vs. 3.0 in controls) | Considered to be supporting evidence: no information on dose-response (single dose tested), maternal toxicity observed (no further details provided); it is possible that at such high dose levels GSH depletion and reduced elimination of ethylene oxide occurred. | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | Generoso et al., 1987, cited in the CLH report (2016) | Mouse | 14 days pre-mating period, 6 hours/day, 10 exposures in total | 300 | Statistically significantly increased number of resorptions (41.1% vs. 6.4 in controls) | Considered to be supporting evidence: no information on dose-response (single dose tested), maternal toxicity observed (no further details provided); it is possible that at such high dose levels GSH depletion and reduced elimination of ethylene oxide occurred. | | |
| | | Hardin et al., 1993, cited in the CLH report (2016) | Rat | Group 1: no exposure<br>Group 2: GD 7-16<br>Group 3: GD 1-16<br>Group 4: three weeks before mating + GD 1-16 | 150 | Statistically significant increased number of resorptions in group 4 | Considered to be supporting evidence: no information on dose-response (single dose tested); signs of maternal toxicity observed (increased absolute and relative kidney and spleen weights in all groups, reduced body weight in group 4); it is possible that at such high dose levels GSH depletion and reduced elimination of ethylene oxide occurred. | | |
| Sensitive to, but not diagnostic of, EATS | Sex ratio | Neeper-Bradley et al., 1993, cited in the CLH report | Rat | 9 days (GD 6-15) | > 225 | No changes in the sex ratio of the pups | Sufficient: no evidence of changes in sex ratio at any exposure concentration | Overall negative evidence of adversity (no effects reported in | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | (2016) | | | | | | any of the studies) | |
| | | Snellings et al., 1982a, IIIA 6.8.1/01 | Rat | 9 days (GD 6-15) | > 100 | No effects on the sex of fetuses reported | Sufficient: no evidence of changes in sex ratio at any exposure concentration | | |
| | | Snellings et al., 1982b, IIIA 6.8.2/01 | Rat | 12 weeks before mating, 1-2 weeks mating, 19 days gestation, 16 days post-parturition from PND5 | > 100 | No effects on the sex of pups reported | Sufficient: no evidence of changes in sex ratio at any exposure concentration | | |
| Sensitive to, but not diagnostic of, EATS | Tumour induction | NTP TR 326, 1987, IIIA 6.7/03 | Mouse | 102 weeks | 50 | Increased incidence of lung adenomas and carcinomas | Sufficient; ethylene oxide is a known carcinogenic substance | Sufficient evidence of adversity; dose-response effects were evident in all studies. | Sensitive to, but not diagnostic of, EATS |
| | | Adkins et al., 1986, cited in the IARC evaluation (1994, 2008, 2012) | Mouse | 6 months | 70 | Increased incidence of pulmonary adenomas | Sufficient; ethylene oxide is a known carcinogenic substance | | |

| Grouping | Line(s) of evidence | Reference | Species | Exposure duration | Effect level (ppm) | Observed effects (positive and negative) | Assessment of each line of evidence | Assessment of the integrated line of evidence | Modality |
|---|---|---|---|---|---|---|---|---|---|
| | | Lynch et al., 1984b, IIIA 6.7/01 | Rat | 24 months | 50 | Increased incidence of peritesticular mesotheliomas | Sufficient; ethylene oxide is a known carcinogenic substance | | |
| | | Snellings et al., 1984b, IIIA 6.7/02 | Rat | 24 (M) or 25 (F) months | 10 | Increased incidence of mononuclear cell leukemia | Sufficient; ethylene oxide is a known carcinogenic substance | | |

Effects on EAS-mediated parameters

The available studies show adverse effects on male reproductive organs, consisting of reduced testes and epididymal weights, testicular tubular degeneration, decreased sperm counts and motility and increased number of abnormal sperms.

Decreased testes and epididymal weights following exposure to ethylene oxide in different species were reported in several studies (mice in Snellings et al., 1984a; rats and guinea pigs in Hollingsworth et al., 1956; rats in Mori et al., 1989 and 1991). In the study of Snellings et al. (1984a) reduced absolute testes weights were seen starting from 50 ppm. However, there was no clear evidence of dose response, and the histopathological findings were normal. A statistically significant reduction in testes and epididymis weight was also reported by Mori et al. (1989) at 500 ppm. As only a single dose level was tested, there was no information on dose-response available, although the effect did occur in a time-dependent manner (exposure for 2, 4, 6 and 13 weeks). The body weight gain of the exposed group was not different from control group. Furthermore, light microscopic examination revealed degeneration and exfoliation of germ cells in this study. At 2 weeks, disorder of the arrangement and mild degeneration of seminiferous tubules were observed. At 4 weeks, the degeneration of mature spermatids became conspicuous and the nuclear vacuolisation of immature round spermatids was also observed. At 6 weeks, all types of germ cells including spermatogonia and spermatocytes degenerated, and exfoliated, and mature spermatids almost completely disappeared. At 13 weeks, germ cell reduction was prominent in approximately half of the seminiferous tubules and they contained only Sertoli cells. Some seminiferous tubules were reported to show germ cell recovery at 13 weeks compared with 6 weeks. It should be noted that plasma testosterone concentration was not affected in this study. Tubular degeneration of testes (with replacement fibrosis) was also reported in guinea pigs exposed to 357 ppm for a period of 176 days (Hollingsworth et al.,1956). Slight tubular degeneration was reported in the same study in rats exposed for 176-226 days to 204 ppm (7 hours/day exposure). Mori et al. (1991) reported slight degeneration in the seminiferous tubules in rats exposed to 250 ppm ethylene oxide (6 hours/day, 5 days/week) for 13 weeks. Plasma testosterone concentration was only measured in one rat study (Mori et al. 1989, mentioned above), there were no effects observed on testosterone levels in this study.

Reduced sperm count and sperm motility and significantly elevated drive range (progressive sperm motility) were reported by Lynch et al., 1984a in Cynomolgus monkeys exposed to 50 and 100 ppm ethylene oxide for 24 months. Ribeiro et al. (1987) studied the influence of ethylene oxide exposure on different stages of spermatogenesis by exposing mice to 200 or 400 ppm ethylene oxide for 5 days, 6 hours/day and sacrificing them 1, 3 or 5 weeks after the exposure. He reported that ethylene oxide induced concentration-related and statistically significant increases in the incidences of abnormal sperm at all stages of spermatogenesis (spermatozoa, spermatids and spermatogonial cells) in mice exposed for 5 days.

Overall, it is concluded that there is sufficient evidence of ethylene oxide causing adverse effects on male reproductive organs (testes and epididymis), in addition to adverse effects on spermatogenesis and sperm morphology. It should be noted that the effects on spermatogenesis and sperm morphology (as reported by Mori et al. 1989 and 1991, Ribeiro et al., 1987 and Lynch et al., 1984) were included in the justification for the classification Category 1B for fertility by RAC (2017). According to the ECHA/EFSA Guidance, the reported effects on the male reproductive organs and sperm parameters are indicative of effects mediated by an EAS modality.

Effects on parameters which are sensitive to, but not diagnostic of, EATS

A number of adverse effects on fertility and the development of offspring (which are regarded as sensitive to, but not diagnostic of, EATS) were reported in available studies. Adverse effects on fertility included reduced number of implantation sites and reduced

number of pups born on PND0, as well as statistically significantly lower number of foetuses born per implantation site, and significantly increased percentage of resorptions. Adverse effects on development included decreased fetal weights and crown-rump lengths, and fetal abnormalities, the latter being observed usually at very high exposure concentrations, at which GSH depletion may have played a role.

Decreased fertility index, increased gestation length, decreased number of born pups and decreased number of implantation sites were reported by Snellings et al. (1982b) in a one-generation study with rats exposed to 100 ppm ethylene oxide for 12 weeks prior to mating, 1-2 weeks during mating, 19 days during gestation and 16 days post-parturition starting from PND 5. Statistically significantly reduced fetal weight was also reported by Snellings et al. (1982a) in a developmental study with rats exposed during gestation days 6-15 to 100 ppm ethylene oxide. Hardin BD et al. (1983), publishing the data of Hackett PL, 1982, reported increased number of resorptions in rats exposed to 150 ppm ethylene oxide from GD1 to GD16, and reduced fetal body weights, reduced crown-rump length and reduced skeletal ossification in rats exposed on GD7-16, GD1-16 and three weeks before gestation and GD1-16. No effects were reported in rabbits in the same study.

Increased number of resorptions and/or increased incidence of fetal deaths were also reported in two inhalation studies (Weller et al., 1999 and Rutledge et al.,1989) with mice exposed to very high concentrations of ethylene oxide (> 1000 ppm). LaBorde et al. (1980) reported reduced number of live fetuses and decreased fetal body weights in mice following intravenous exposure to 150 mg ethylene oxide/kg bw/day at GD4-6, 6-8, 8-10 and 10-12.

Exposure to high concentrations of ethylene oxide also caused increased incidence in fetal malformations, in particular eye malformations. Increased incidence of eye malformations was reported by Weller et al. (1999) in mice exposed to 2100 ppm (1400 ppm x 1.5 h or 700 ppm x 3 h or 350 ppm x 6 h) and 2700 ppm per hour (1800 ppm x 1.5h or 1543 ppm x 1.75 h or 1350 ppm x 2h, 900 ppm x 3h or 450 ppm x 6 h) on GD 7. Rutledge et al. (1989) also reported increased incidence of abnormal foetuses following 1.5 h exposure to either 1200 or 1800 ppm ethylene oxide soon after mating (up to 25 hours). Exposure to lower concentrations of ethylene oxide caused slightly elevated incidence in skeletal variations (Snellings et al., 1982a, Neeper-Bradley et al., 1993).

There is limited information in the available studies on ethylene oxide in regard to effects on other endocrine endpoints, such as changes in sex ratio. Since the available scientific studies on ethylene oxide are of relatively old age and have limited reporting, it was not always possible to deduce from the reports whether no effects on these parameters were observed, or whether these parameters were simply not examined in the studies. However, at least Neeper-Bradley et al. (1993) has reported the absence of the effects on sex ratio following exposure of 25 pregnant CD rats to 0, 50, 125 and 225 ppm ethylene oxide for 6 hours/day on GD 6-15. Sex of the pups and foetuses, respectively, was also examined in the one-generation toxicity study of Snellings et al. (1982b) and developmental toxicity study of Snellings et al. (1982a), with neither of the studies reporting effects.

The RAC has proposed to classify the substance as toxic to reproduction (Repr. 1B, H360F) based primarily on the results of Snellings et al. (1982b), in which decreased number of implantations and born foetuses per implantation site (indicating post-implantation losses) were observed, and the supporting studies of Generoso et al. (1987) and Hardin et al. (1983) showing increased incidences of resorptions and/or decreased incidences of implantations. Regarding developmental effects, the RAC has proposed to classify ethylene oxide as Cat. 2 for development, based on the reported effects on fetal weights, skeletal variations and malformations, although the evidence is not completely clear on whether or not these malformations occurred only in the presence of maternal toxicity. Overall, it can be concluded that sufficient evidence of adversity is available for several reproductive and

developmental endpoints which are considered sensitive to, but not indicative of, EATS.

Effects on T-mediated parameters

In general, the available studies provide limited information regarding thyroid effects. Since the available studies on ethylene oxide are of relatively old age with limited reporting, it was not always possible to deduce from the reports whether no effects were seen in particular organs and tissues, or whether these organs and tissues were simply not examined. However, the absence of histopathological effects on the thyroid was clearly reported in the available 2-year chronic/carcinogenicity studies (Lynch et al., 1984b; Snellings et al., 1984b; NTP, 1987). Thyroid weights were apparently not examined in either of the studies. The available evidence suggests that ethylene oxide does not cause histopathological changes in the thyroid.

Evidence of EATS-mediated endocrine activity

In order to obtain information on the potential for endocrine activity by ethylene oxide, the systematic review methodology as described in the ECHA/EFSA Guidance was used to search for *in vitro* and *in vivo* studies containing relevant mechanistic information. The only relevant study identified was the study by Mori et al. (1989), where plasma concentration of testosterone had been measured. Furthermore, ethylene oxide was not found to be listed in the ToxCast inventory.

**Initial analysis of evidence**

In accordance with the ECHA/EFSA Guidance, an initial analysis of evidence needs to be performed in order to conclude on whether the EATS-mediated adversity or the EATS endocrine activity have been sufficiently investigated. In the present case, as the available studies predated modern guidelines and did not include the examination of all compulsory parameters according to the latest guideline updates, it can be concluded that neither the EATS-mediated adversity or the EATS-mediated endocrine activity have been sufficiently investigated for ethylene oxide. However, according to the flowchart on page 13 in the ECHA/EFSA Guidance, it is possible to proceed to the MoA-analysis because EAS-mediated adversity has been observed.

**Mode of action analysis**

Since EAS-mediated adversity has been observed for ethylene oxide, a mode of action (MoA) needs to be addressed. However, as stated on page 40 in the ECHA/EFSA Guidance *"In the case of adversity based on 'EATS-mediated' parameters, the underlying knowledge (i.e. by coherence analysis (Susser, 1991)) of the likely endocrine nature of the effects may be such that judgement can be reached on the biological plausibility of a link without recourse to a detailed MoA analysis.*" It is further stated that *" In such cases, the MoA analysis could be very simple; when an adverse effect is 'EATS-mediated', the biologically plausible link is already pre-established in the absence of information proving the contrary (i.e. a fully developed non-ED MoA). This is because, in the case of 'EATS-mediated' parameters, where the pattern of effects is deemed adverse, the biological plausibility that the adverse effects are caused via an EATS-mediated MoA is high, based on existing knowledge and theory (i.e. coherence analysis), and as such, it may not be necessary to generate further empirical data on the substance under evaluation to substantiate the link between the observed adverse effect(s) and an endocrine-mediated MoA."*

In the case of ethylene oxide, it should be noted that the observed effects could have been caused by alternative, non-ED modes of action, either by a genotoxic mechanism (considering that ethylene oxide is a well-established alkylating agent), or by depletion of the glutathione detoxification system.

Depletion of the glutathione detoxification system as MoA

Glutathione is present in high concentration in the testes of the adult rat and plays a major role in ethylene oxide detoxification in rodents. In the testes, it is also important in the generation of cysteine-containing proteins (Grosshans and Calvin, 1985) and selenoproteins which are prevalent in spermatozoa (Ganther, 1979). The adverse effects on testicular weight and histopathology and epididymal weight were seen mainly at high dose levels (200 ppm and above), at which the detoxification pathway of ethylene oxide may have been saturated due to depletion of the glutathione detoxification system. The only exception is the study of Snellings et al. (1984a), in which changes in only absolute testes weights in mice were seen at 50 ppm, but in the absence of a clear dose response and without an effect on relative testes weights. In rodents, conjugation with glutathione is the main elimination pathway for ethylene oxide. The RAC opinion (2017) states that in general, the depletion of the glutathione detoxification system in rodents occurs at dose levels exceeding 200 ppm, resulting in lower ethylene oxide elimination.

A hypothesis that protein and DNA alkylation (e.g. alkylation of enzymes) by ethylene oxide causes modification of the glutathione redox cycle was also expressed by EPA (EPA, 2010), based on the results of Mori et al. (1989). In this study, glutathione metabolism (including glutathione reductase, glutathione peroxide and glutathione S-transferase activities) was investigated in testes of rats exposed to 500 ppm ethylene oxide for 6 hours/day, 3 days/week for 2, 4, 6 and 13 weeks. Exposure to ethylene oxide caused changes in the glutathione redox cycle, with both glutathione reductase and glutathione peroxidase activities decreased by 54% and 16%, respectively, after 2 weeks of exposure. Glutathione reductase activity showed a slight recovery at 6 and 13 weeks, while glutathione peroxidase activity fully recovered at 4 weeks and was increased by 17.7% at 13 weeks. The authors stipulated that the decrease in glutathione reductase activity was due to the alkylation of the enzyme molecules by ethylene oxide. However, it should be noted that the glutathione S-transferase activity, which was measured by interaction with two different substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-epoxy-3-(p-nitrophenoxy) propane, was increased at 6 and 13 weeks by 63% and 72.8% with CDNB and 54.6% and 81.9% with 1,2-epoxy-3-(p-nitrophenoxy) propane. Glutathione S-transferase was considered to be the major enzyme detoxifying ethylene oxide in the testes, and its increase in activity is considered to play an important role in protecting against ethylene oxide-induced toxicity. It should also be noted that despite changes in the glutathione redox cycle, the glutathione concentration following exposure to 500 ppm was not affected in the exposed animals in the study of Mori et al. (1989) compared to untreated controls. Thus, it is the opinion of the eCA that the results from the study by Mori et al. (1989) does not provide clear evidence for the exact mechanism by which the changes in the glutathione redox cycle play a role in the testicular damage in rodents. It should also be noted that adverse effects on sperm parameters were seen at lower dose levels (50-100 ppm), at which no glutathione depletion would yet be expected to occur (these effects could rather be related to a genotoxic mechanism). Overall, it is the opinion of the eCA there is conflicting evidence for the effects of ethylene oxide on the depletion of the glutathione detoxification system and the role in the testicular damage.

Genotoxic insult as MoA

In the RAC Opinion (2017), it was stated that the effects on sperm quality during the specific stages of spermatogenesis may have been caused by a genotoxic insult by increasing the number of abnormal sperms, although it was stated that other mechanisms could not be excluded. This is in accordance with the harmonised classification of the active as Muta. 1B (germ cell mutagenicity). A genotoxic insult causing effects on the spermatogenesis and the number of sperms could also explain the reduced testis- and epididymis weight observed. Thus, considering the known properties and the harmonised classification of the substance, the observed effects on male reproductive organs could be caused by a genotoxic mode of action, although other mechanisms cannot be excluded.

## Summary

In summary, it can be concluded that there is sufficient evidence of ethylene oxide causing adverse effects on several endpoints which are considered to be EAS-mediated according to the ECHA/EFSA Guidance, namely testicular and epididymal weight, testicular histopathology, sperm numbers, sperm morphology and sperm motility. It should be emphasized that in the RAC Opinion (2017) ethylene oxide is proposed to be classified as toxic to reproduction and development (Repr. 1B, H360Fd), and the effects on spermatogenesis and sperm morphology were included in the justification for the classification of Category 1B for fertility.

Since EAS-mediated adversity has been observed for ethylene oxide, a mode of action needs to be addressed. However, as stated on page 40 in the ECHA/EFSA Guidance "In the case of adversity based on 'EATS-mediated' parameters, the underlying knowledge (i.e. by coherence analysis (Susser, 1991)) of the likely endocrine nature of the effects may be such that judgement can be reached on the biological plausibility of a link without recourse to a detailed MoA analysis."

However, the observed adverse effects could also have been caused by alternative, non-ED modes of action, either by a genotoxic mechanism, or by depletion of the glutathione detoxification system. Overall, it is the opinion of the eCA that there is conflicting evidence for the effects of ethylene oxide on the depletion of the glutathione detoxification system and the role this plays in the observed adverse effects on male reproductive organs. Considering the known properties and the harmonised classification of the substance (Muta. 1B), the observed effects on male reproductive organs could be caused by a genotoxic mode of action, although other mechanisms cannot be excluded. Based on the information available, no final conclusion on the ED properties of ethylene oxide could be made.
The eCA is of the opinion that additional testing is not justified in this particular case because of the following reasons:

1. The already known severe hazard profile of this substance: ethylene oxide fulfils the exclusion criteria of Article 5, paragraph 1a, b and c of BPR given the following classification of the active: Carc. 1B, Muta. 1B and Repr. 1B.

2. As a consequence of these properties, strict RMMs should be in place to avoid or minimise occupational exposure as far as technically feasible if EtO is to be used. As for the risk assessment, a non-threshold mode of action is assumed for tumour formation, and hence, a conservative semi-quantitative risk assessment is already performed. It should furthermore be kept in mind that there is no agreed methodology to consider endocrine disruption in the risk assessment. Consequently, no impact on the risk assessment would be expected if the substance was to be additionally considered as an ED.

3. Handling of ethylene oxide is not easy. EtO is a highly reactive gas (i.e. the substance readily reacts with diverse compounds and is explosive at concentrations higher than 3% in air). Based on both the human health hazardous properties and its flammable and explosive properties special precautions must be taken when handling the substance. Specialised equipment and well-trained personnel are a prerequisite for using ethylene oxide in industrial facilities. Although most routine laboratories do have skilled personnel, it is, according to the applicant, very difficult to find laboratories that have the proper equipment and training for handling of ethylene oxide. The eCA agrees that it is not desirable to expose either laboratory personnel or laboratory animals to ethylene oxide, unless it is strictly necessary. Furthermore, even the transport of samples from the factory to the laboratory is difficult. Specialised transport containers must be used, and great care must be taken to avoid accidents.

4. Due to the unspecific alkylating properties of the substance, the available test systems for determining the endocrine activity would be likely to give equivocal results (if tests were performed). If mechanistic studies were nevertheless conducted, it would be difficult to conclude that the mechanism would be solely a (non-) ED MoA.

In conclusion, even if additional testing would be performed, it seems unlikely that a clear conclusion on the ED properties of ethylene oxide would be possible nor that it would have an effect on the final risk assessment. Any additional animal testing should therefore be considered unnecessary, which in line with the BPR should be avoided as much as possible.

The active substance was also discussed at the ED EG 13. Following the reasoning provided by the eCA and a discussion among the experts, "there seemed to be consensus that further testing would not be warranted for this substance" (according to the minutes of the ED EG).

| **Conclusion used in Risk Assessment – Endocrine disruption** | |
|---|---|
| Conclusion | A final conclusion is not possible in this special case. |
| Justification for the conclusion | The data provided was insufficient to conclude on whether the EAS-mediated adverse effects was due to an ED mode of action. |

| **Data waiving** | |
|---|---|
| Information requirement | No further testing is warranted. |
| Justification | Based on the information available, no final conclusion on the ED properties of ethylene oxide could be made. The eCA is of the opinion that additional testing is not justified in this particular case because of the following reasons:<br><br>1. The already known severe hazard profile of this substance: ethylene oxide fulfils the exclusion criteria of Article 5, paragraph 1a, b and c of BPR given the following classification of the active: Carc. 1B, Muta. 1B and Repr. 1B.<br><br>2. As a consequence of these properties, strict RMMs should be in place to avoid or minimise occupational exposure as far as technically feasible if EtO is to be used. As a non-threshold mode of action is assumed for tumour formation, a conservative semi-quantitative risk assessment is already performed. It should furthermore be kept in mind that there is no agreed methodology to consider endocrine disruption in the risk assessment. Consequently, no impact on the risk assessment would be expected if the substance were to be additionally considered as an ED.<br><br>3. Handling of ethylene oxide is not easy due to its physicochemical and human health related hazardous properties. It is not desirable to expose either laboratory personnel or laboratory animals to ethylene oxide, unless it is strictly necessary. |

| | |
|---|---|
| | 4. Due to the unspecific alkylating properties of the substance, the available test systems for determining the endocrine activity would be likely to give equivocal results (if tests were performed). If mechanistic studies were nevertheless conducted, it would be difficult to conclude that the mechanism would be solely a (non-) ED MoA. |

## A.3.15. Further Human data

**Direct observations, e.g. clinical cases, poisoning incidents if available**

No studies on direct observations on the effects in humans following ethylene oxide exposure were submitted by the applicant. Relevant studies were recovered by the eCA based on the data presented in the RAC opinion (2017), CLH report (2016) and WHO evaluation (2003) of ethylene oxide and are summarized in the respective sections.

The applicant has presented a summary of available information on direct observations following exposure to ethylene oxide which was reviewed and adapted by the eCA, where considered necessary.

Ethylene oxide can cause irritation to the eyes and is classified as corrosive to skin (Skin Corr. 1, H314) and irritating to respiratory tract (STOT SE 3, H335). Mild irritation of the skin has been reported after contact with aqueous solutions containing as low as 1% ethylene oxide. Dermal injury is characterised by oedema and erythema followed by the formation of vesicles.

Severe allergic-type reactions and ethylene oxide IgE antibodies among dialysis patients have been reported. These data have been evaluated by RAC (2017). RAC has concluded that, as these reports focused on situations in which individuals were exposed to ethylene oxide parentherally (e.g. via sterilized medical equipment), they are not relevant for the assessment of skin sensitisation. Furthermore, RAC concluded that, although ethylene oxide has been extensively used for sterilization purposes for decades, the number of case reports is very low, and each of them includes only one individual with skin reactions after exposure. The reports did not conclusively establish the relationship between the observed reactions and ethylene oxide exposure. As ethylene oxide is a corrosive substance, it is possible that the observed eczema may have also occurred due to irritation. Thus, based on the available data RAC has concluded that ethylene oxide does not need to be classified as a skin or respiratory sensitizer.

Spontaneous abortions were reported amongst hospital staff using ethylene oxide for sterilisation (Hemminki, 1982; Gresie-Brusin, 2007), but this appears to have been associated with exposure to high peak concentrations of ethylene oxide. In another study of dental assistants (Rowland, 1996), exposure to ethylene oxide was reported to have caused a 2.1 fold increase in the incidence of either spontaneous abortion or pre- or post-term deliveries but the 95% confidence interval was 0.7-5.71.

Neurological effects were noted in 4 of 12 employees working at a damaged steriliser who were exposed to approximately 700 ppm ethylene oxide (Jay, 1982). The symptoms were headache, nausea, vomiting, apathy, followed by crampy attacks. Three further employees showed cataracts. Sensoripolyneuropathy has been reported in a number of cases following single or repeated exposure to ethylene oxide (WHO, 2003). These incidents again involved exposure to high concentrations of ethylene oxide and amelioration of the symptoms following the cessation of exposure was commonly observed.

Haematological effects have been reported but these have not been consistent. In one study there was a statistically significant increase in the percentage of lymphocytes and a reduction in the percentage of neutrophils in women exposed to ethylene oxide. In another study in males and females there were statistically significant increases in haematocrit, mean numbers of red blood cells, monocytes and eosinophils and reductions in absolute mean numbers of lymphocytes and platelets. In two other studies there were no significant haematological effects (WHO, 2003).

Chromosomal events in humans exposed to ethylene oxide have been extensively studied and reviewed by IARC (1994, 2008). Sister chromatid exchange, chromosomal aberrations and micronucleus formation have all been found in workers exposed to a wide range of concentrations of ethylene oxide. Sister chromatid exchange and chromosomal aberrations have been investigated in 33 and 24 groups of workers, respectively, and micronucleus formation in 4 groups. With regard to sister chromatid exchanges, statistically significant increases were commonly found in studies that evaluated individuals who were exposed to > 5 ppm ethylene oxide. Studies that failed to find significant increases in the frequency of sister chromatid exchange were primarily studies of workers exposed to ≤ 5 ppm ethylene oxide though increases were found in some studies in which exposure was ≤ 5 ppm. Amongst studies of chromosomal aberrations one study found significant increases in chromosomal aberrations in individuals exposed to ethylene oxide concentrations of approximately 1 ppm or less but in other studies there was no evidence of increased chromosome aberrations amongst workers exposed to approximately 1 ppm. There have been fewer investigations of the impact of ethylene oxide exposure on micronucleus formation, but the available evidence shows that although the incidence of micronucleus formation is increased at higher concentrations individuals exposed to levels ≤ 1 ppm gave negative results.

**Epidemiological studies on the general population, if available**

The applicant submitted three epidemiological studies on general population with ethylene oxide (Steenland, 1991; Steenland, 2004; Steenland, 2003). These data concern the assessment of the cancer risk from exposure to ethylene oxide and are summarised in Section 3.9.
Also, additional studies were recovered by the eCA based on the RAC opinion (2017), the CLH report (2016) and the WHO evaluation (20030 of ethylene oxide. These studies are summarized and discussed in the respective sections.

**Sensitisation/allergenicity observations**

A number of case reports and clinical surveillance data report allergic-type reactions to ethylene oxide. These reports were discussed in detail in Section 3.4. The majority of them referred, however, to parenteral routes of exposure to ethylene oxide (e.g. during dialysis). Severe allergic-type reactions and ethylene oxide IgE antibodies among dialysis patients have been reported. Although ethylene oxide has been extensively used for sterilization purposes for decades, the number of case reports is very low, and each of them includes only one individual with skin reactions after exposure. The reports did not conclusively establish the relationship between the observed reactions and ethylene oxide exposure. As ethylene oxide is a corrosive substance, it is possible that the observed eczema may have also occurred due to irritation. The reports were assessed by the RAC (2017) in its evaluation of classification and labelling of ethylene oxide, and it was concluded by the RAC that ethylene oxide does not need to be classified as a skin or respiratory sensitizer.

## A.3.16. Other data

## Mechanistic studies

### *In vitro* studies

Table A.A.33 Summary table of mechanistic studies *in vitro*

| **Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study** | **Species, Strain, Sex, No/Group** | **Test substance (including purity), Vehicle, Dose levels, Duration of exposure** | **Results** | **Remarks (e.g. major deviations)** | **Reference** |
|---|---|---|---|---|---|
| Non-guideline, non-GLP, investigation of the formation of products with nucleophilic sites in DNA after in vitro treatment of DNA and hemoglobin with ethylene oxide. The reactivities of human, mouse and rat hemoglobin towards ethylene oxide were compared. | *In vitro* heparinized blood from human, CBA mouse and Fischer rat strains; calf thymus DNA. | Ethylene oxide, $^{14}C$-labeled, specific activity 1200 GBq/mol, radiochemical purity 95.2%; treatment of erythrocytes with 16 kBq/mL for 30 min at 37 °C in sealed tubes; treatment of DNA with 50 kBq/mL at 37 °C for 3 hours. | In DNA N-7-(2-hydroxyethyl)guanine was the main product following ethylene oxide treatment, whereas O6-(2-hydroxyethyl)guanine and N-3-(2-hydroxyethyl) adenine were much lower (0.5% and 4.4% of alkylation of guanine-N-7). In haemoglobin, the main reaction products were 2-hydroxyethylations of cysteine, N-terminal valine, and the two imidazole nitrogens in histidine and carboxylic groups. The main differences between species was 12 and 170 times higher reactivity of cysteine in mouse and rat haemoglobin, respectively, than in human haemoglobin. The reactivities of nitrogens in valine and histidine were about the same in all three species. | Non-GLP, non-guideline study; *in vitro* analysis of DNA and haemoglobin protein adducts following ethylene oxide exposure. | Segerbäck D (1990) |

### *In vivo* studies

Table A.A.34. Summary table of mechanistic studies *in vivo*

| Method, Duration of study, Guideline, GLP status, Reliability, Key/supportive study | Species, Strain, Sex, No/Group | Test substance (including purity), Vehicle, Dose levels, Duration of exposure | Results | Remarks (e.g. major deviations) | Reference |
|---|---|---|---|---|---|
| Non-guideline, non-GLP, formation of N7-(2-hydroxyethyl)guanine (7-HEG) adducts in DNA from liver, brain, lung and splee of rats exposed to ethylene oxide for 4 weeks by inhalation | Fischer 344 rats, 10 males/dose | Ethylene oxide, 99% pure, 0, 3, 10, 33 or 100 ppm (nominal); 2.97 ± 0.34, 10.0 ± 0.5, 31.8 ± 3.2 and 99 ± 6 ppm (analytical), 6 hours/day, 5 days/week, 4 weeks | The amount of 7-HEG increased with concentration in all four examined tissues. The dose-response curves in all four tissues were linear in the whole concentration range. When 7-HEG was compared across tissues, brain and lung exhibited evidence for a slightly sublinear response between 33 and 100 ppm. There were significantly greater amounts of 27-HEG in brain and lung than in liver and spleen of rats at 100 ppm ($p < 0.005$). The number of 7-HEG adducts in rats exposed to 3 ppm ethylene oxide was 5.3-12.5 times than endogenous 7-HEG in unexposed controls. Statistically higher amounts of 7-HEG accumulated in tissues of rats than in mice exposed to the same concentrations of ethylene oxide. | Non-guideline non-GLP study, determination of N7-(2-hydroxyethyl)guanine (7-HEG) adducts in DNA of several tissues from rats exposed for 4 weeks. Only male rats were used. | Wu K-Y et al. (1999) |
| Non-guideline, non-GLP, formation of N7-(2-hydroxyethyl)guanine (7-HEG) adducts in DNA from liver, brain, lung and splee of mice exposed to ethylene oxide for 4 weeks by inhalation | B6C3F1 mice, 10 males/dose | Ethylene oxide, 99% pure, 0, 3, 10, 33 or 100 ppm (nominal); 2.97 ± 0.34, 10.0 ± 0.5, 31.8 ± 3.2 and 99 ± 6 ppm (analytical), 6 hours/day, 5 days/week, 4 weeks | The amount of 7-HEG increased with concentration in all four examined tissues. The dose-response curves in all four tissues were linear in the whole concentration range. When 7-HEG was compared across tissues, lung exhibited evidence for a slightly sublinear response between 33 and 100 ppm. The number of 7-HEG adducts in rats exposed to 3 ppm ethylene oxide was 1.3-2.5 times than endogenous 7-HEG in unexposed controls. | Non-guideline non-GLP study, determination of N7-(2-hydroxyethyl)guanine (7-HEG) adducts in DNA of several tissues from rats exposed for 4 weeks. Only male rats were used. | Wu K-Y et al. (1999) |

Three mechanistic studies addressing the interaction of ethylene oxide with DNA, one *in vitro* with heparinized blood and two *in vivo*, were submitted by the applicant.

Ethylene oxide reacted directly with DNA both *in vitro* and *in vivo*. The main product was N-7-(2-hydroxyethyl)guanine. Five other minor adducts were detected in the *in vitro* study, two were definitely identified as O6-(2-hydroxyethyl)guanine and N-3-(2-hydroxyethyl) adenine and two others were suspected to be N-7-(2-hydroxyethyl)adenine and N-1-(2-hydroxyethyl)adenine. The other product was not identified. Ethylene oxide also reacts directly with haemoglobin to form S-(2-hydroxyethyl)cysteine, N-(2-hydroxyethyl) valine, O-(2-hydroxyethyl)serine and both NK- and NT-(2-hydroxyethyl) histidine. Species differences in the relative proportions of the adducts formed were apparent in the study which was conducted with rat, mouse and human erythrocytes *in vitro*.

## A.4. Environmental effects assessment

**Reasoning behind a qualitative risk assessment**

The environmental part of the ethylene oxide dossier is based on a limited data set. The submitted data consists of open literature data from the period between 1979 and 1991, with the exception of one study from 2009, as well as QSAR estimations.

A qualitative environmental risk assessment has been performed for all relevant compartments except for the atmosphere, where a PEC has been calculated and discussed in light of monitoring data and data from the human health section. The assessment mainly entails a qualitative evaluation of the likelihood that an effect will occur under the expected conditions of exposure.

The limited data set as well as the lack of a quantitative assessment was considered acceptable by the eCA based on the following underlying provisions, guidance and agreements:

- BPR Article 6(2) regarding the possibility to waive data
- Guidance on the Biocidal Products Regulation, hereafter referred to as the Guidance on the BPR
  - Vol IV, part A, section 1.2 on guiding principles with regard to information requirements (ECHA, 2018)
  - Vol IV, parts B+C, section 4.4. on qualitative risk characterisation (ECHA, 2017c)
- The outcome of the early discussion at WG-I-2018 on the possibility for a qualitative approach to the environmental assessment of ethylene oxide. The principal argument for the chosen approach is the low exposure potential to the environment from the intended use of the substance.

It has been important for the eCA to ensure that the submitted information, though limited, is sufficient to assess whether ethylene oxide meets the criteria in BPR Article 5(1) or Article 10(1) with regard to the environment. Furthermore, in accordance with the conclusion at WG-I-2018, the eCA has aimed at a qualitative approach which is sufficiently justified by as thorough as possible descriptions of the environmental fate of ethylene oxide seen in context with the expected emissions from use of the substance.

**Intended use and foreseen exposure pathways**

Ethylene oxide as a biocidal active substance is intended for use as a sterilising agent for single use medical devices which cannot be sterilised by other means, before these are made available on the market. During the sterilisation process, ethylene oxide is introduced into a sealed, stainless steel chamber which contains the medical devices. Upon completion, ethylene oxide from the chamber is exhausted to the atmosphere via a catalytic converter which converts ethylene oxide to carbon dioxide and water, typically with an efficiency greater than 99.9%. The sterilisation chamber and its contents are then repeatedly flushed with nitrogen and air to remove the remaining ethylene oxide from the chamber. After completion of post sterilisation flushing, sterilised products are transferred to an aeration cell and are subjected to high rates of air exchange at temperatures close to the sterilisation temperature for approximately 12 hours. This process serves to further remove any residual ethylene oxide from the product and packaging, and the exhaust from this phase is also treated via a catalytic converter. Upon completion of the initial aeration, products are transferred to a secondary aeration area, where an optional additional period of elevated temperature storage can also be used to further reduce residual ethylene oxide levels if required. Atmospheric emissions of ethylene oxide in Europe are strictly regulated with maximum permissible concentrations in exhaust gas set by individual Member States.

In some sterilisation plants, acid scrubbers containing a solution of water and 5% sulphuric acid ($H_2SO_4$) are used as an alternative to the catalytic conversion system. Ethylene oxide from the sterilisation chamber is dissolved in the acid solution which converts ethylene oxide to ethylene glycol with an efficiency typically in the range of 99.5 to 99.9%. Sodium hydroxide is then added to neutralise the solution. According to the applicant, neutralised ethylene oxide free solution from the acid scrubbers is transported to waste water treatment plants as toxic waste water, where it undergoes specialist handling and treatment, thus minimising any potential environmental contamination.

Hence, based on this information from the applicant, the only relevant direct route of exposure is release to the atmosphere. Release to soil and surface water could potentially occur indirectly, via deposition from the atmosphere. In the aquatic compartment, ethylene oxide will hydrolyse to ethylene glycol, which should be regarded as a relevant transformation product. The hydrolysis of ethylene oxide in saltwater will produce ethylene chlorohydrin in addition to ethylene glycol. The possibility of indirect exposure to soil and to water via atmospheric release, and subsequently the formation of ethylene glycol and ethylene chlorohydrin in the aquatic (freshwater and saltwater) compartment, is investigated in the environmental assessment.

## A.4.1. Fate and distribution in the environment

## A.4.1.1 Degradation

A4.1.1.1 Abiotic degradation

**Hydrolysis of ethylene oxide**

The hydrolysis of ethylene oxide has been determined in fresh water and salt water, as described in the published article by Conway *et al.*, 1983 (Doc IIIA7.1.1.1.1/01). It is not considered that salt water is a relevant environmental compartment for the intended use of ethylene oxide, but a brief description of these results is also included.

The study has not been performed in accordance with a standard test guideline, and there are several deficiencies when compared to modern guideline-compliant studies. For example, testing has been done at only one pH value instead of three as required in the OECD 111 guideline, no information of recoveries is given and the statistical information is limited. However, the publication is still considered to give basic useful information on the hydrolysis of ethylene oxide at neutral pH and 25 °C.

For the fresh water experiment, solutions of sterile distilled water and sterile and non-sterile Kanawha River water containing approximately 65 mg/L ethylene oxide at pH 7.4 were prepared. For the salt water experiment, pH 7 buffered solutions containing approximately 200 mg/L ethylene oxide and 0 %, 1 % and 3 % NaCl were prepared. The solutions were incubated in the dark at 25 °C for 350 hours. Samples were taken at approx. day 0, 4, 5, 7, 10, 12 and 13 and analysed by gas chromatography. The analysis results indicate normal first-order kinetics in all cases, therefore rate constants and half-lives ($DT_{50}$ values) can be derived from linear regression. The $DT_{50}$ values derived at 25 °C for fresh water range from 12.2 to 14.2 days and the $DT_{50}$ values for salt water at 25 °C range from 9.3 to 11.0 days. In accordance with the Guidance on the BPR, Vol. IV Part B, equation 28, the $DT_{50}$ values for fresh water and salt water were recalculated to 12 °C and 9 °C, respectively.

The resulting hydrolysis half-lives are as follows:

- fresh water, 12 °C: 34.5–40.2 days
- salt water, 9 °C: 33.4–39.6 days

Although not specifically investigated in the study, it is well known from open literature that the hydrolysis of ethylene oxide in freshwater gives ethylene glycol:

$$C_2H_4O + H_2O \rightarrow HO{-}CH_2CH_2{-}OH$$

In the absence of data from the study, ethylene glycol should be regarded as a significant hydrolysis transformation product.

The Conway (1983) study also discusses the formation of ethylene chlorohydrin in salt water. However, as it is considered that the marine aquatic compartment is of low relevance for ethylene oxide used as intended by the applicant, these results have not been investigated further in the CAR.

**Hydrolysis of ethylene glycol**

Ethylene glycol is expected to be hydrolytically stable (Lyman *et al.*, 1982, as mentioned by WHO, 2000).

Table A.35 Summary table - Hydrolysis

| **Method, Guideline, GLP status, Reliability, Key/supporting study** | **pH** | **Temp. [°C]** | **Initial TS concentration, $C_0$[mg/L]** | **Half-life, $DT_{50}$ [d]** | **Remarks** | **Reference** |
|---|---|---|---|---|---|---|
| Guideline not stated<br>Non-GLP<br>RI: 2 | Fresh-water<br>7.4<br><br>Salt-water<br>7.0 | 25<br><br><br><br><br>25 | Freshwater<br>65<br><br>Saltwater<br>200 | Freshwater<br>12.2[a]<br>12.9[b]<br>14.2[c]<br><br>(12 °C: 34.5-40.2)<br><br>Saltwater<br>13.1[d]<br>11.0[e]<br>9.3[f]<br><br>(9 °C: 33.4-39.6) | No coefficient of correlation ($r^2$) given.<br><br>According to the authors of the publication, water temperature will have more effect than pH on the hydrolysis of ethylene oxide. | Conway et al. (1983)<br><br>Doc IIIA 7.1.1.1.1/ 01 |

[a] *Sterile distilled water;* [b] *Sterile river water;* [c] *Non-sterile river water;* [d] *0 % NaCl;* [e] *1 % NaCl;* [f] *3 % NaCl;* *n.a. = not available*

| **Value used in Risk Assessment** | |
|---|---|
| Value/conclusion | At a temperature of 12 °C, half-lives of approximately 35-40 days in fresh water have been observed. Ethylene glycol is a major hydrolysis transformation product, which should be considered hydrolytically stable. |
| Justification for the value/conclusion | See text above. |

**Phototransformation of ethylene oxide in water**

No studies are available on the phototransformation of ethylene oxide in water. However, the absorption spectrum of ethylene oxide (see Doc IIIA3.4) indicates that it does not absorb solar radiation in the wavelengths necessary to reach the earth's surface (absorption maxima at 159, 171 and 174 nm, no absorption above 182 nm). This is in accordance with what could be expected based on the molecular structure of ethylene oxide, given that it does not contain any double bonds and should therefore not absorb radiation with higher wavelengths. This is supported by the Hazardous Substances Data Bank, 2008[2], which state that ethylene oxide does not contain chromophores that absorb at wavelengths > 290 nm and is therefore not expected to be susceptible to direct photolysis by sunlight. It is hence not expected that photolysis in water will be a significant removal mechanism and a study is not considered necessary.

**Phototransformation of ethylene glycol and ethylene chlorohydrin in water**

Based on the molecular structures of ethylene glycol and ethylene chlorohydrin, they are also not expected to undergo sunlight-induced photolysis (WHO, 2000; Calvert and Pitts, 1966).

| Data waiving | |
|---|---|
| Information requirement | Phototransformation in water including identity of the products of transformation |
| Justification | Not necessary since photolysis due to sunlight is not expected to be a significant removal process for ethylene oxide or the relevant hydrolysis degradation products, since none of them are expected to absorb radiation with wavelengths > 290 nm. |

**Estimated photo-oxidation in air**

The half-life of ethylene oxide in the atmosphere has been estimated using both equation 31 in the Guidance on the BPR, Vol. IV part B, and the QSAR program AOPWIN v. 1.92 (US EPA, 2010a), which calculates half-lives based on the rate constants for the atmospheric gas-phase reactions between photochemically produced hydroxyl radicals and organic chemicals. The specific degradation rate constant with OH radicals ($k_{OH}$) was estimated in AOPWIN to be $0.2803 \cdot 10^{-12}$ $cm^3$ / (molecule · sec). Subsequently, the pseudo first order rate constant for degradation in air (24-h day) is calculated using equation 31 in the Guidance on the BPR, Vol IV part B:

$k_{deg, air} = k_{OH} \times OHCONC_{air} \times 24 \times 3600$ *($OHCONC_{air}$ = 5 × $10^5$ molecules/$cm^3$)*
$= 0.012/d$

Hence, based on the standard calculation between $k_{deg}$ and $DT_{50}$:

$DT_{50, air} = 57.2$ d

The estimated half-life of ethylene oxide in AOPWIN, using the same assumptions as those given in the Guidance on the BPR, Vol. IV part B, gives the same result, i.e. a $DT_{50}$ of 57.2 days. If an average 12-h daylight concentration in the atmosphere of $1.5 \times 10^6$ OH radicals/$cm^3$ is assumed, a $DT_{50}$ of is 38.2 days is estimated in AOPWIN (see Doc IIIA7.3.1/01).

An experimental $k_{OH}$ of $0.076 \times 10^{-12}$ $cm^3$ / (molecule × sec) is reported in AOPWIN, from

[2] Hazardous Substances Data Bank (2008) Ethylene oxide CASRN: 75-21-8, Environmental Fate & Exposure: Environmental Fate/Exposure Summary. Reviewed by SRP on 9/18/2008. National Library of Medicine, National Toxicology Information Program, Bethesda, MA 20894

Atkinson, R (1989). Using this experimental $k_{OH}$ and the equation above gives a $DT_{50}$ of 211 days.

A publication by Cupitt (1987), see Doc IIIA 7.3.2/03, was also submitted by the applicant, where estimated lifetimes (residence times) of ethylene based on average hydroxyl (OH) radical conditions and relevant atmospheric temperatures over the continental U.S. range from 217-578 days (see Doc IIIA7.3.2/03). Assuming an OH radical concentration of $1 \cdot 10^6$ and $5 \cdot 10^5$ molecules/cm$^3$ gave an estimated half-life of 217 and 578 days, respectively.

Reports of estimated atmospheric half-lives of ethylene oxide as summarised by WHO (2003), range from 38 days, as calculated with AOPWIN v. 1.92, to 382 days.

On this basis, it can be concluded that ethylene oxide is relatively persistent in the atmosphere and may have some potential for long-range atmospheric transport and atmospheric accumulation if released into the atmosphere in significant amounts.

Based on the high water solubility of ethylene oxide (1000 g/L at 25 °C), it could be expected that washout through precipitation could be a relevant removal pathway from the atmospheric compartment. However, the high vapour pressure (146 kPa at 20 °C) and hence rapid volatilisation is likely to counteract this effect (WHO, 2003). Winer *et al.* (1987), see Doc IIIA 7.3.2/01, and Dana *et al.* (1985) conducted a series of experiments to determine the atmospheric loss rates of ethylene oxide as a function of water vapour concentration, and found minimal losses (see Doc IIIA7.3.2/01 and 02). Atmospheric washout ratios ranged from 4 to 11, and the authors concluded that based on a comparison with washout ratios for readily wet deposited compounds, which range from $10^4$ to $10^6$, ethylene oxide is to a very low extent wet deposited from the atmosphere.

The Technical Agreements on Biocides, Environment, version 2.1 item ENV A5, states that for very volatile substances (with small scale use) there is no need to conduct a risk assessment for subsequent compartments following the release path via air. Ethylene oxide is considered a VOC according to several definitions, e.g. Directive 1999/13/EC and Directive 2004/42/EC, due to its high vapour pressure (146 kPa at 20 °C) and low boiling point (11 °C). The eCA considers that this is supportive of the assumption that there is no need for a more detailed environmental risk assessment than that presented in this CAR.

Based on the available information, the reaction between ethylene oxide and hydroxyl radicals leads to the formation of simpler molecules which in turn degrade, and does not lead to the formation of any transformation products of concern.

Table A.36 Summary table – Photo-oxidation in air*

| **Model** | **Estimated daily (24h) OH concentration [OH/cm³]** | **Overall OH rate constant [cm³/molecule sec]** | **Half-life [d]** | **Reference** |
|---|---|---|---|---|
| Guidance on BPR, vol. IV part B, eqn. 31<br><br>AOPWIN v. 1.92 | $5.0 \times 10^5$ | $2.8 \times 10^{-13}$ | 57.2 | ECHA (2017)<br><br>US EPA (2010) |

| **Value used in Risk Assessment** | |
|---|---|
| Value/conclusion | The estimated atmospheric half-life of ethylene oxide calculated in accordance with the Guidance on the BPR, Vol. IV part B, is 57.2 days. Other findings indicate that it could be significantly longer. Ethylene oxide should be regarded as relatively persistent in the atmosphere with a potential for long-range transport. |
| Justification for the value/conclusion | See text above. |

A4.1.1.2 Biotic degradation

A4.1.1.2.1 Biodegradability (ready/inherent)

**Biodegradability of ethylene oxide**

Experimental data

Two published scientific articles, one by Conway *et al.* (1983), see Doc IIIA7.1.1.2.1/01, and one by Bridié *et al.* (1979), see Doc IIIA7.1.1.2.1/02, were originally submitted as information on the biodegradability of ethylene oxide. After an ad hoc follow-up from WG-III-2020, it was concluded that more information was needed, in order to be able to conclude on the persistency of ethylene oxide. As a result of this, the applicant submitted an OECD 301C study (MITI, 1995, see Doc IIIA7.1.1.2.1/04).

In the **MITI (1995)** OECD 301C study (GLP), the biodegradation in an activated sludge mixture from sewage plant, river and lake sites was measured at a temperature of 25 ± °C and a pH 7 ± 1. The initial ethylene oxide concentration was 100 mg/L, and the concentration of active sludge was 30 mg/L (suspended solids concentration). The concentration of the reference substance aniline was 100 mg/L. An activity control, inoculum blank and abiotic control was included, but no toxicity control (this is not considered to affect the reliability of the study). The OECD 301C validity criteria were passed. The biodegradation after 28 days based on O2 consumption was 107 %. Ready biodegradation was hence shown in this test.

It should be noted that there are some details missing in the study report. For example, the mineral stock solutions were made in accordance with a Japanese guideline, and it is not known whether this gives solutions which are identical to those given in OECD 301C. Also, it is stated that the test flasks were improved for volatile substances, but it is not stated how. However, additional tests were performed to investigate volatilisation from the flasks, as described in Doc IIIA7.1.1.2.1/04. This study is hence considered reliable to support the conclusion that ethylene oxide is readily biodegradable.

In the study by **Conway *et al.* (1983),** biological oxygen demand (BOD) was measured (BOD) over 20 days. The lightly seeded dilution-bottle test generally followed the BOD test procedure described in "Standard Methods for the Examination of Water and Wastewater", 14$^{th}$ ed. (APHA, 1975), which to the eCA's understanding were highly regarded guidelines at that time. Some modifications to the BOD procedure were made, most importantly extending the test duration from 5 to 20 days. The resulting test setup has many similarities with the OECD 301D (closed bottle) test, but limited reporting complicates a full comparison. The publication only gives the biooxidation as a percentage of the theoretical oxygen demand, no information on the start and end test concentrations of ethylene oxide are given. However, taking into account the limited environmental exposure of ethylene oxide, the eCA considers the submitted study to be sufficient for the purpose of this risk assessment as long as the results are interpreted with caution.

Ethylene oxide was incubated with domestic sewage sludge inoculum in airtight bottles under aerobic conditions in the dark at 20 ± 1 °C for 20 days. Oxygen levels were read at day 5, 10, 15 and 20. The samples were re-aerated when necessary. Glucose/monosodium glutamate was used as a reference substance in the inoculum control, in line with the guideline.

The resulting bio-oxidation of ethylene oxide (expressed as a percentage of theoretical oxygen demand) after 5, 10, 15 and 20 days was 5, 22, 40 and 52 %, respectively.

The eCA is of the opinion that the findings in the study by Conway *et al.* (1983) indicate that biodegradation of ethylene oxide does occur, but that the results should be treated

with caution.

In the study by **Bridié *et al.* (1979)**, both the biological and chemical oxygen demand (BOD and COD) of ethylene oxide were determined. The BOD determination followed "Standard Methods for the Examination of Water and Wastewater", 13th ed. (APHA, 1971), and the COD determination followed the ASTM D 1252-67 standard potassium dichromate method (ASTM, 1974). The reporting is very limited, and the eCA is of the opinion that the findings should only be used as supporting information.

The measured COD was well in line with the theoretical oxygen demand (ThOD), i.e. the COD was 96 % of the ThOD. The bio-oxidation of ethylene oxide (the BOD expressed as a percentage of the ThOD) after 5 days of incubation was 3 %. This is in line with the Conway *et al.* (1983) study, where the 5-d value was 5 %. This supports the assumption that the level of biodegradation of ethylene oxide during the first 5 days of exposure is low.

QSAR data

An estimation of the ready biodegradation potential was performed using the model Biowin v. 4.10 (US EPA, 2010b). As described in the Biowin User Guide, the different Biowin models are based on their own set of biodegradation data for a high number of chemicals. All the models' estimates are based on structural fragment constants. Each defined structural fragment is assigned a constant reflecting the degree to which it affects the biodegradation of a chemical, and the total estimated biodegradation prediction depends on the combination of structural fragments in a given molecule. The results of Biowin 1, 2, 5, 6 and 7 are given as probabilities for fast/ready biodegradation. The value 0.5 is the turning point between not fast / not readily biodegradable and fast / readily biodegradable. Biowin 3 and 4 give results as time units, where 5 = hours, 4 = days, 3 = weeks, 2 = months and 1 = longer.

The Ready Biodegradability Prediction is YES if the Biowin 3 result is "weeks" or faster AND the Biowin 5 probability is ≥ 0.5. If this condition is not satisfied, the prediction is NO (not readily biodegradable). Also, according to the REACH guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11: PBT/vPvB, the following Biowin results would be supportive of (potential) persistence: Biowin 2 < 0.5 and Biowin 3 < 2.25, or Biowin 6 < 0.5 and Biowin 3 < 2.25.

The seven different Biowin models gave the following results for ethylene oxide, ethylene glycol and ethylene chlorohydrin (see below for information on experimental data for the two degradation products):

| **Biowin model** | **Ethylene oxide** | **Ethylene glycol** | **Ethylene chlorohydrin** |
|---|---|---|---|
| 1 (Linear Model Prediction) | 0.3792 – Does Not Biodegrade Fast | 1.0355 - Biodegrades Fast | 0.7566 - Biodegrades Fast |
| 2 (Non-Linear Model Prediction) | 0.2599 - Does Not Biodegrade Fast | 0.9874 - Biodegrades Fast | 0.7559 - Biodegrades Fast |
| 3 (Ultimate Biodegradation Timeframe) | 3.0931 - Weeks | 3.3819 - Days-Weeks | 3.0080 - Weeks |
| 4 (Primary Biodegradation Timeframe) | 3.7744 - Days | 4.0171 - Days | 3.7604 - Days |
| 5 (MITI Linear Model Prediction) | 0.6220 - Readily Degradable | 0.9486 - Readily Degradable | 0.7337 – Readily Degradable |
| 6 (MITI Non-Linear Model Prediction) | 0.8348 - Readily Degradable | 0.9734 - Readily Degradable | 0.8062 - Readily Degradable |
| 7 (Anaerobic Model Prediction) | 0.3388 - Does Not Biodegrade Fast | 1.1536 - Biodegrades Fast | 1.0062 - Biodegrades Fast |
| **Ready Biodegradability Prediction** | **YES** | **YES** | **YES** |

The overall Biowin prediction is hence that ethylene oxide and the two hydrolysis

degradants ethylene glycol and ethylene chlorohydrin are readily biodegradable. For ethylene oxide, this estimation is in line with the results of the OECD301C ready biodegradation test (Doc IIIA7.1.1.2.1/04).

**Biodegradability of ethylene glycol**

Experimental data

In the study by **Conway *et al.* (1983)** described above, degradation products were not searched for. However, based on knowledge on transformation pathways of ethylene oxide in the environment, the biodegradation of ethylene glycol was also determined as part of the study. The same test procedure as for ethylene oxide, i.e. a modified 20-day BOD test, was followed.

The resulting bio-oxidation of ethylene glycol after 5, 10, 15 and 20 days was 39, 73, 85 and 96 %, respectively. This is both a more rapid and a higher overall level of bio-oxidation compared to the results for ethylene oxide from the same study.

QSAR data

See table above. The overall Biowin prediction is that ethylene glycol is readily biodegradable. When comparing the results with those of ethylene oxide, the model predicts that ethylene glycol biodegrades faster than ethylene oxide. This is in line with the findings of Conway *et al.* (1983) and with some further references summarised by WHO (2000). Furthermore, according to a review article by Staples et al. (2001), ethylene glycol is not persistent in air, surface water, soil, or groundwater.

**Biodegradability of ethylene chlorohydrin**

Experimental data

In the study by **Conway *et al.* (1983)** described above, degradation products were not searched for. However, based on knowledge on transformation pathways of ethylene oxide in the environment, the biodegradation of ethylene chlorohydrin was also determined as part of the study. The same test procedure as for ethylene oxide, i.e. a modified 20-day BOD test, was followed.

The resulting bio-oxidation of ethylene chlorohydrin after 5, 10, 15 and 20 days was 0, 13, 46 and 57 %, respectively. This is comparable to the results for ethylene oxide from the same study.

QSAR data

See table above. The overall Biowin prediction is that ethylene chlorohydrin is readily biodegradable.

Table A.37 Summary table - biodegradation studies (ready/inherent)*

| Method, Guideline, GLP status, Reliability, Key/support-ive study | Test type | Test parameter | Inoculum | | | Additional substrate | Test sub-stance conc. | Degradation | | Remarks [positive control] | Reference (Doc III) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Type | Conc. | Adap-tation | | | Incub. period | Degree | | |
| ***Ethylene oxide*** | | | | | | | | | | | |
| **OECD 301C GLP, RI: 2 Key study** | BOD | Oxygen con-sumption | Activ-ated sludge | 30 mg/L | No | No | 100 mg/L | 28 d | 107 % | Reference substance: aniline (conc.: 100 mg/L) | **IIIA7.1.1.2 .1/04 (MITI, 1995)** |
| **APHA Standard Methods (1975), BOD test Non-GLP, RI: 2 Supportive information** | BOD | Oxygen con-sumption | Activ-ated sludge | n.s. | No | No | n.s. | 20 d | 5 d: 5 %<br><br>10 d: 22 %<br><br>15 d: 40 %<br><br>20 d: 52 % | The performance of the system was confirmed with the glucose/ glutamic acid check, in accordance with the guideline.<br><br>These results should be treated with caution, due to limited knowledge on the study conditions. | **IIIA7.1.1.2 .1/01 Conway et al. (1983)** |
| **APHA Standard Methods (1971), BOD test Non-GLP, RI: 3 Supportive information** | BOD | Oxygen con-sumption | Activ-ated sludge | n.s. | No | Yes[1] | n.s. | 5 d | 3 % | No information given on test methods or conditions. Supporting information only. | **IIIA7.1.1.2 .1/02 Bridié et al.** (1979) |

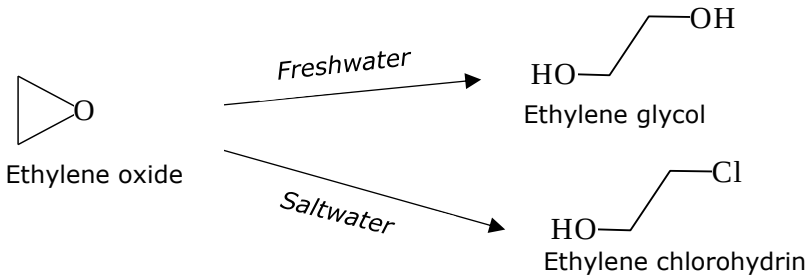
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Ethylene glycol*** | | | | | | | | | | | |
| **APHA Standard Methods (1975), BOD test Non-GLP, RI: 2 Supportive information** | BOD | Oxygen con-sumption | Activ-ated sludge | n.s. | No | No | n.s. | 20 d | 5 d: 39 %<br><br>10 d: 73 %<br><br>15 d: 85 %<br><br>20 d: 96 % | The performance of the system was confirmed with the glucose/ glutamic acid check, in accordance with the guideline.<br><br>These results should be treated with caution, due to limited knowledge on the study conditions. | **IIIA7.1.1.2 .1/01 Conway et al. (1983)** |
| ***Ethylene chlorohydrin*** | | | | | | | | | | | |
| **APHA Standard Methods (1975), BOD test Non-GLP, RI: 2 Supportive information** | BOD | Oxygen con-sumption | Activ-ated sludge | n.s. | No | No | n.s. | 20 d | 5 d: 0 %<br><br>10 d: 13 %<br><br>15 d: 46 %<br><br>20 d: 57 % | The performance of the system was confirmed with the glucose/ glutamic acid check, in accordance with the guideline.<br><br>These results should be treated with caution, due to limited knowledge on the study conditions. | **IIIA7.1.1.2 .1/01 Conway et al. (1983)** |

***1 Allylthiourea was added to each test solution at a concentration of 0.5 mg/L, to prevent nitrification***
***n.s. = not specified***

| Value used in Risk Assessment | |
|---|---|
| Value/conclusion | Ethylene oxide is readily biodegradable in accordance with OECD 301C. Ethylene glycol and ethylene chlorohydrin are expected to biodegrade in the environment at a rate which is equal to or faster than ethylene oxide. |
| Justification for the value/conclusion | See text above. |

A4.1.1.3 Rate and route of degradation including identification of metabolites and degradation products

To sum up the sections above, ethylene oxide is readily biodegradable, hydrolyses to a significant extent to ethylene glycol and additionally to ethylene chlorohydrin in freshwater. The available data for these two degradation products indicate that they are also are expected to be readily biodegradable, although ethylene glycol is hydrolytically stable. Neither ethylene oxide nor the two degradation products undergo photolysis. The following simple environmental degradation pathway, based on hydrolysis, can be outlined for ethylene oxide:




No further data is submitted on the degradation of ethylene oxide other than the data described above, with the justification that the exposure to environmental compartments other than air is foreseen to be negligible to non-existent. Separate waivers for all the endpoints are given in Doc IIIA 7, and are summarised in the data waiving boxes below.

| Data waiving | |
|---|---|
| Information requirement | Biological sewage treatment<br>– aerobic biodegradation<br>– anaerobic biodegradation<br>– STP simulation test |
| Justification | Further testing is not necessary due to the lack of direct exposure of the substance to STPs. The submitted data on biodegradation in the presence of domestic sewage is considered sufficient, with the conclusion that ethylene oxide is readily biodegradable. |

| Data waiving | |
|---|---|
| Information requirement | Biodegradation in freshwater – aerobic aquatic degradation Water/sediment degradation study |
| Justification | Further testing is not necessary due to the lack of direct exposure of the substance to freshwater and the high vapour pressure leading to rapid volatilisation / minimal deposition from air. |

| Data waiving | |
|---|---|
| Information | Biodegradation in seawater |

| | |
|---|---|
| requirement | Seawater/sediment degradation study |
| Justification | Direct exposure to seawater following use of ethylene oxide as a gaseous sterilant will not occur and any indirect exposure is expected to be negligible. |

| **Data waiving** | |
|---|---|
| Information requirement | Higher tier degradation studies in water or sediment |
| Justification | Further testing is not necessary due to the lack of direct exposure of the substance to freshwater and the high vapour pressure leading to rapid volatilisation / minimal deposition from air. |

| **Data waiving** | |
|---|---|
| Information requirement | Biotic degradation in soil<br>– Laboratory soil degradation studies<br>– Higher tier degradation studies in soil |
| Justification | Further testing is not necessary due to the lack of direct exposure of the substance to freshwater and the high vapour pressure leading to rapid volatilisation / minimal deposition from air. |

The eCA considers that this is acceptable, and that the conclusion that ethylene oxide is readily biodegradable, is valid based on the available information. Furthermore, the water and soil compartments are unlikely to be exposed to ethylene oxide from the emissions to air, based on the data given above in the preceding chapters and summarised in the following paragraphs. No emissions to STPs occur from the intended use.

Upon emission to air, ethylene oxide might persist for a long time in the atmosphere. The reported estimated atmospheric half-lives range from 38 to 578 days, with some differences in underlying assumptions of e.g. OH radical concentrations and atmospheric temperatures. Real-life local concentrations in air upon emission will depend on local conditions such as weather patterns, wind and convection.

Even though ethylene oxide has a long half-life in air, there are no indications that ethylene oxide is deposited to water or soil at any significant extent. The high water solubility (1000 g/L at 25 °C) could potentially result in wet deposition through e.g. precipitation, but the vapour pressure is high (146 kPa at 20 °C) and rapid volatilisation is expected to counteract this effect. The degradation in air is due to reaction with OH radicals.

The intended use of ethylene oxide does not give direct release to surface water or soil, also not via STPs, as no release of liquid waste from ethylene oxide sterilisation plants to STPs is foreseen (this is handled separately as toxic waste).

If ethylene oxide nevertheless theoretically should reach the water compartment, it is expected to both hydrolyse and biodegrade. The hydrolysis of ethylene oxide in freshwater results in the formation of ethylene glycol, and in saltwater hydrolysis also forms ethylene chlorohydrin.

Volatilisation (due to the high vapour pressure) is expected to be a significant removal process from both soil and water, and the duration of any potential indirect exposure is likely to be short-lived. The estimated Koc value for ethylene oxide is 3.237 L/kg (see section A.4.1.2.1), indicating that the compound is not strongly adsorbed to soil and is therefore unlikely to accumulate in this compartment.

In conclusion, air is the only compartment to which environmental release occurs. Emissions to other compartments are considered as negligible.

A4.1.1.3.1 Short summary and overall relevance of the provided information on degradation and conclusion on rapid degradation

Ethylene oxide is expected to be relatively persistent in air. In water, volatilisation is likely to be the most significant removal process, but ethylene oxide will both hydrolyse and biodegrade. Ethylene oxide is readily biodegradable.

## A4.1.2 Distribution

A4.1.2.1 Adsorption onto/desorption from soils

No data has been submitted on the adsorption/desorption of ethylene oxide. Due to the limited indirect exposure to soil from the intended use of ethylene oxide, this is considered acceptable. Based on the high vapour pressure of ethylene oxide, it is expected that the deposition from atmosphere to soil will be negligible.

In the absence of experimental data, a Koc value has been estimated using the KOCWIN program, version 2.0 (US EPA, 2010c). The estimates are based MCI method is generally considered as more robust than the log Kow method. Log Koc values > 4.5 indicate very strong sorption to soil, and negligible migration to ground water. Values < 1.5 indicate negligible sorption and rapid migration to ground water.

The Koc for ethylene oxide estimated with the molecular connectivity index (MCI) is 3.237 L/kg, and the Koc estimated from the log Kow of -0.30 is 4.662 L/kg.

For ethylene glycol and ethylene chlorohydrin, log Koc values were estimated using the same methodology. The log Koc estimated from MCI is 1 L/kg and 1.904 L/kg for ethylene glycol and ethylene chlorohydrin, respectively. The log Koc estimated from an experimental log Kow of -1.36 for ethylene glycol is 0.2239 L/kg. (The log Koc if based on the estimated log Kow of -1.20 is only slightly higher, at 0.2744 L/kg.) For ethylene chlorohydrin the log Koc estimated from an experimental log Kow of 0.03 is 3.39 L/kg.

Table A.38 Summary table – Estimated adsorption/desorption of ethylene oxide and the hydrolysis transformation products ethylene glycol and ethylene chlorohydrin

| Substance | log Koc (based on MCI) | log Koc (based on log Kow) | Reference |
|---|---|---|---|
| Ethylene oxide | 3.24 L/kg | 4.66 L/kg | USEPA (2010c) |
| Ethylene glycol | 1 L/kg | 0.22 L/kg | |
| Ethylene chlorohydrin | 1.90 L/kg | 3.39 L/kg | |

A4.1.2.2 Higher tier soil adsorption studies

No data submitted. Please see separate waivers in Doc IIIA 7.

| Data waiving | |
|---|---|
| Information requirement | Higher tier soil adsorption studies |
| Justification | Based on the negligible exposure to soil, no further testing is considered necessary. |

A4.1.2.3 Volatilisation

Regarding volatilisation, please see Part A, section 1.3 Physical and chemical properties of

the active substance. The high vapour pressure of ethylene oxide (146 kPa at 20 °C) will lead to rapid volatilisation. For further information, please see the discussion in the chapter on photo-oxidation in air.

## A.4.1.3 Bioaccumulation

No experimental data on aquatic or terrestrial bioconcentration or bioaccumulation has been provided. The eCA considers this acceptable, due to both the low log Kow (-0.30) which does not give rise to any concerns regarding bioaccumulation, and furthermore due to the limited exposure which makes it unnecessary to investigate this further.

However, estimations of $BCF_{fish}$ and $BCF_{earthworm}$ are presented.

**Estimated aquatic bioconcentration**

The calculation of $BCF_{fish}$ from the log Kow value described in the Guidance on the BPR, Vol. IV Part B, section 3.8.3.2, is only suitable for substances with a log Kow of between 2 and 6, or above 6.

The model BCFBAF vs. 3.01 (US EPA, 2012a) can estimate the BCF for substances with lower log Kow values, but it should be noted that as its predecessor model BCFWIN, all compounds with a log Kow < 1.0 are automatically assigned a log BCF of 0.50, i.e. at BCF of 3.16 L/kg. Correction factors for structural elements are not used for substances with such a low log Kow. However, even though simple, there is no reason to believe that this is an erroneous estimation for ethylene oxide. Based on the log Kow of -0.30, it is very likely that a true BCF would be well below any trigger values for a bioaccumulation potential.

The estimated $BCF_{fish}$ for ethylene oxide is hence 3.16 L/kg, and does not give rise to concern regarding bioaccumulation.

**Estimated terrestrial bioconcentration**

The $BCF_{earthworm}$ can be estimated in accordance with section 3.8.3.7, equation 104d, in the Guidance on the BPR, Vol. IV Part B. It is stated in the guidance that this equation is advised used for substances with a log Kow between 1 and 8, but that it is reasonable to assume that extrapolation to lower log Kow values is possible. The estimated $BCF_{earthworm}$ according to this calculation is

$$BCF_{earthworm} = (0.84 + 0.012 \times Kow) / RHO_{earthworm} = 0.85 \text{ L/kg}$$

where log Kow = -0.30 (Kow = 0.5) and a default $RHO_{earthworm}$ of 1 kg/L is assumed.

Neither the estimated $BCF_{fish}$ nor the estimated $BCF_{earthworm}$ give rise to concern regarding bioaccumulation.

## A.4.1.4 Monitoring data

The literature data describing monitoring data on ethylene oxide in air which are available to the eCA, are summarised in the following table. All these data are from the USA.

Table A.39 Monitoring data on ethylene oxide concentrations in outdoor air

| Reference | Ethylene oxide concentration in air | Remarks |
|---|---|---|
| Halik, T. (2018), Ethylene oxide air monitoring report. GHD Services Inc., Report No. 01 | 0.58 µg/m$^3$ = 0.32 ppb | Highest measured concentration in air outside Sterigenics facility |
| Holmes, J.R. (1993), Persistence of ethylene oxide in the atmosphere, California EPA, Research Note 93-6 | 0.03 µg/m$^3$ = 0.02 ppb | Remote coastal locations (according to the author believed to represent hemispheric background concentrations for ethylene oxide) |
| | 7.20 µg/m$^3$ = 4.00 ppb | Downwind of an ethylene oxide fumigation plant |
| | 0.05 µg/m$^3$ = 0.03 ppb | Los Angeles area, suburbs |
| | 1.44 µg/m$^3$ = 0.8 ppb | Los Angeles area, downtown |
| Olaguer, E. *et al.* (2019), Ethylene oxide exposure attribution and emissions quantification based on ambient air measurements near a sterilization facility. Int. J. Environ. Res. Public Health 2020, 17, 42 | 0.25 µg/m$^3$ = 0.14 ppb | Urban background concentration near facility |
| | 2.08 µg/m$^3$ = 1.16 ppb | Highest 24 h concentration occurred in a parking lot / residential neighbourhood immediately across the street from the sterilization facility and directly downwind from the site |
| | 0.30 µg/m$^3$ = 0.17 ppb | Background concentration which was by the author not thought to be contributed to by the facility. |
| WHO (2003), Concise International Chemical Assessment Document 54, Ethylene oxide | 0.04-956 µg/m$^3$ = 0.02-553 ppb | 24-h ambient air concentrations in Los Angeles, reported by Havlicek et. al (1992). |
| | 0.03-0.40 µg/m$^3$ = 0.02-0.22 ppb | Northern California |
| | 0.03-0.36 µg/m$^3$ = 0.02-0.20 ppb | Remote coastal regions, California |

## A.4.2. Effects on environmental organisms

Due to the negligible exposure of ethylene oxide to the aquatic and terrestrial compartment, a quantitative effects assessment for the environment has not been performed. A basic data set with information on the toxicity of ethylene oxide towards fish, daphnids, algae, and microorganisms is available, and a discussion of these data is provided in the following sections. Most of the data provided is from open literature and is several decades old. However, given the limited exposure of ethylene oxide to the environment, the eCA considers that the data submitted is sufficient. Taking into account the harmonised human health classification of ethylene oxide (i.e. fulfilment of the BPR exclusion criteria) and the outcome of the human health risk assessment, it is not expected that the generation of more data for the environmental risk assessment would have any impact on the outcome of the overall risk characterisation.

Furthermore, the eCA considers that new studies on ethylene oxide should not be generated unless strictly necessary. Ethylene oxide is classified as Carc. 1B, Muta. 1B and Repr. 1B. Furthermore, it is a highly reactive gas (i.e. the substance readily reacts with diverse compounds and is explosive at concentrations higher than 3 % in air). According to the applicant, it is difficult to find laboratories that have the proper equipment and training for the safe handling of ethylene oxide. Since the BPR exclusion criteria are met, a possible ethylene oxide approval will be subject to Article 5(2). Strict RMMs would have to be in place to avoid or minimise exposure as far as technically feasible. The generation of new studies for the environmental risk assessment would hence not have any impact on the outcome of the overall decision.

Since ethylene glycol should be considered a relevant degradation product for the aquatic compartment, data on ethylene glycol has also been included. The exposure to the aquatic compartment is as mentioned negligible, but since it can be expected that a significant fraction of any ethylene oxide present in water will hydrolyse to ethylene glycol, information has nevertheless been presented in order to give an as complete picture as possible in the qualitative assessment.

In saltwater, ethylene oxide hydrolyses to form ethylene chlorohydrin. Even though it is considered that the exposure to seawater is expected to be even lower than the exposure to freshwater, information on chlorohydrin has also been included where available, for the sake of completeness.

### A.4.2.1 Atmosphere

According to the Guidance on BPR, Vol IV. Part B, it should be considered whether there are indications of adverse effects on terrestrial vertebrates from e.g. inhalation. The eCA considers that this part is covered by the human health effects assessment. No data on plants is available.

Regarding abiotic effects, ethylene oxide is not anticipated to contribute to global warming due to that it does not absorb solar radiation above 182 nm. According to the Guidance on BPR, Vol. IV Part B, a potential greenhouse gas shows absorption bands between 800 and 1200 nm.

According to the information available to the eCA, ethylene oxide does not have any effects on ozone. Ethylene oxide has a long lifetime in the atmosphere, but it does not contain any halogen substituents. According to UNEP (2001), ethylene oxide can be used in sterilisation technology alone or combination with ozone-depleting substances such as CFCs, as the CFCs can suppress the flammability of ethylene oxide, but ethylene oxide itself does not deplete the ozone layer.

## A.4.2.2 Toxicity to sewage treatment plant (STP) microorganisms

### Inhibition of microbial activity (aquatic)

Two studies have been provided describing the influence on microbial activity of ethylene oxide – an activated sludge growth inhibition study by Conway *et al.* (1983), see Doc IIIA7.4.1.4/01, and an activated sludge, respiration inhibition test conducted in accordance with the Commission Regulation EC No 440/2008, C. 11 Biodegradation – Activated Sludge Respiration Inhibition Test guideline (BASF, 2009), see Doc IIIA74.1.4/02.

In the Conway study, activated sludge growth inhibition tests were performed using ethylene oxide and ethylene glycol. The initial concentrations of test substance are not known. No guideline has been referred to. Test substance was incubated for 16 hours at 22 ºC on a shaker in the presence of nutrients, buffers, growth substrate and sewage microorganisms. Toxicity was indicated when the resulting turbidity was less than 50% of the control. The resulting $IC_{50}$ values for ethylene oxide and ethylene glycol were 10-100 mg/L and 10 000 mg/L, respectively.

In the BASF study, the inhibition of microbial respiration rate was studied in activated sludge from the municipal wastewater treatment plant of Mannheim, Germany. Nominal concentrations of 1000, 500, 250, 125, 62.5 mg/L ethylene oxide were used. There were however no analytical measurements of the test concentrations throughout the test, but the nominal concentrations were recalculated based on measurements in the stock solution. The reference substance was 3,5-dichlorophenol. The resulting 3-h $EC_{50}$ (determined by Probit analysis) was > 713 mg/L.

Table A.40 Summary table – inhibition of microbial activity*

| **Method, Guideline, GLP status, Reliability, Key/ supportive study** | **Species/ Inocu-lum** | **Endpoint** | **Exposure** | | **Results** | **Remarks** | **Refer-ence** |
|---|---|---|---|---|---|---|---|
| | | | **De-sign** | **Dur-ation** | | | |
| *Ethylene oxide* | | | | | | | |
| Non-GLP Supportive study RI: 3 | Activated sludge (sewage) microorganisms | Growth inhibition | | 16 hours | $IC_{50}$: 10-100 mg/L | Toxicity was indicated when turbidity of the vessel was less than 50 % of the control. | Conway et al. (1983) IIIA7.4.1.4/01 |
| EC No 440/ 2008, C. 11 , Biodegrada-tion – Activat-ed Sludge Respiration Inhibition Test (similar to OECD 209) GLP Key study RI: 2 | Activated sludge (dom-estic) | Respi-ration inhibition | static | 180 min | $EC_{10}$: 130 mg/L<br>$EC_{20}$: 250 mg/L<br>$EC_{50}$ > 713 mg/L | Reference subst.: 3,5-dichloro-phenol | BASF (2009) Doc IIIA7.4.1.4/02 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ethylene glycol* | | | | | | | |
| Non-GLP Supportive study RI: 3 | Activated sludge (sewage) microorganisms | Growth inhibition | | 16 hours | $IC_{50}$: 10 000 mg/L | Toxicity was indicated when turbidity of the vessel was less than 50 % of the control. | Conway et al. (1983) IIIA7.4.1.4/01 |

## A.4.2.3 Aquatic compartment

A4.2.3.1 Freshwater compartment

**Acute/short-term toxicity (freshwater) – fish**

Two articles from the open literature were submitted to give information on the toxicity of ethylene oxide and potential degradation products toward fish, Conway *et al*., 1983 (IIIA7.4.1.1/01) and Bridié *et al*., 1979 (IIIA7.4.1.1/02).

Conway *et al.* (1983) performed fish toxicity tests in accordance with the 1975 version of the US-EPA guidance "Methods for Acute Toxicity tests with Fish, Macroinvertebrates and Amphibians". 96-h $LC_{50}$ values for fathead minnow (*Pimephales promelas*) were determined in dechlorinated tap water, for both ethylene oxide and the potential degradation products ethylene glycol and ethylene chlorohydrin. The initial concentration of test substance are not reported. The resulting 96-h $LC_{50}$ value for ethylene oxide was 84 mg/L. The 96-h $LC_{50}$ value for the two potential transformation products ethylene glycol and ethylene chlorohydrin were > 10 000 mg/L and ≈ 90 mg/L, respectively.

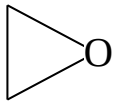
The publication by Bridié *et al.* (1979) lists 24-h $LC_{50}$ values for goldfish (*Carassius auratus*) of several chemicals including ethylene oxide and ethylene glycol. Static-tank acute toxicity tests were conducted in accordance with the guidelines laid down by the American Public Health Association in 1971, with the following resulting 24-h $LC_{50}$ values for ethylene oxide and ethylene glycol, respectively: 90 mg/L and > 5 000 mg/L.

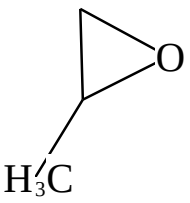
QSAR data

96-h $LC_{50}$ values for fish were estimated using the QSAR software ECOSAR v. 1.11 (USEPA, 2012), and are approximately 80 mg/L for ethylene oxide and 38 000 mg/L for ethylene glycol. Details are given below, in the summary table on acute/short-term toxicity to fish.

Read-across data: propylene oxide

The structure of ethylene oxide and propylene oxide only differ in that propylene oxide has an extra methyl group:

*Ethylene oxide:* 


*Propylene oxide:* 


This results in a slightly higher log Kow for propylene oxide (estimated at 0.37, compared to -0.30 for ethylene oxide). It can generally be assumed that a higher lipophilicity (higher

log Kow) gives a higher uptake in organisms and a higher toxicity. Based on this assumption, ethylene oxide could be expected to have a lower acute toxicity towards aquatic organisms than propylene oxide has. ECOSAR v. 1.11 predicts the 96-h $LC_{50}$ for fish to be 45 mg/L, compared to 80 mg/L estimated for ethylene oxide. However, ethylene oxide may be have a higher ability to induce long-term effects, due to e.g. mutagenicity. Nevertheless, the eCA considers that the information on the acute aquatic toxicity of propylene oxide is useful as a possibly conservative indication of the corresponding toxicity of ethylene oxide.

A study on the toxicity of propylene oxide towards aquatic organisms is available (Stephenson, 1986, see Doc IIIA7.4.1.3/01 – GLP, non-guideline). It was originally submitted to give information on algal toxicity, which is not available for ethylene oxide, but the study also describes the toxicity of propylene oxide towards rainbow trout (formerly *Salmo gairdneri*, now *Oncorhynchus mykiss*) in a 96-h toxicity test. Eight glass aquaria were filled with 11.5 L filtered dechlorinated water. Seven of them contained propylene oxide in concentrations between 10 and 1000 mg/L, and the eighth aquarium served as the control. Five fish (not fed during the test) were placed in each aquarium. The aquaria were sealed to prevent loss of propylene oxide by evaporation. At 24-h intervals, dead fish were removed and recorded, pH and dissolved oxygen concentrations were measured and the test solutions were renewed. The temperature during the test was approx. 18.5 °C, the pH was 8.0-8.4, and the dissolved oxygen concentrations were 8.0-10.2 mg/L. The resulting 96-h $LC_{50}$ (graphical interpretation, log/probit graph paper) was 52 mg/L.

Summary, acute toxicity towards fish

All the data described above are listed in the summary table on acute/short-term toxicity to fish further below. As can be easily seen in the table, the $LC_{50}$ values for fish both from the publications on ethylene oxide and QSAR estimations are between 80 and 90 mg/L. For propylene oxide, the $LC_{50}$ values for fish are slightly lower, but still within the same order of magnitude (estimated and experimental $LC_{50}$ of 45 and 54 mg/L, respectively).

**Acute/short-term toxicity (freshwater) – invertebrates**

Data on two invertebrate species are available, from the article by Conway *et al.* (1983), see Doc IIIA7.4.1.2/01. Toxicity tests with *Daphnia magna* were performed in accordance with the 1975 version of the US-EPA guidance "Methods for Acute Toxicity tests with Fish, Macroinvertebrates and Amphibians". 48-h $EC_{50}$ values were determined in dechlorinated tap water, for both ethylene oxide and the potential degradation products ethylene glycol and ethylene chlorohydrin. The initial concentration of test substance are not reported. The resulting 48-h $EC_{50}$ value for ethylene oxide was 212 mg/L. The 48-h $EC_{50}$ value for ethylene glycol and ethylene chlorohydrin were > 10 000 mg/L and 100 mg/L, respectively.

Conway (1983) also determined 48-h $EC_{50}$ values for brine shrimp (*Artemia salina*), see Doc IIIA7.4.1.2/02. According to the article, the test procedures used are as described by Price *et al.* (1974), with some modifications. The resulting 48-h $EC_{50}$ value for ethylene oxide was 745 mg/L. The 48-h $EC_{50}$ values for ethylene glycol and ethylene chlorohydrin were > 20 000 mg/L and 680 mg/L, respectively.

QSAR data

48-h $EC_{50}$ values for daphnids were estimated using the QSAR software ECOSAR v. 1.11 (US EPA, 2012b), and are approximately 420 mg/L for ethylene oxide and 16 000 mg/L for ethylene glycol. Details are given below, in the summary table on acute/short-term toxicity to invertebrates.

Cf. the read-across data from propylene oxide (see next paragraph), ECOSAR v. 1.11

predicts the propylene oxide 48-h $EC_{50}$ for daphnids to be approx. 190 mg/L.

Read-across data: propylene oxide

For a justification on the usefulness of propylene oxide data as an indication of the acute aquatic toxicity of ethylene oxide, please see the section above on acute toxicity towards fish. A study on the toxicity of propylene oxide towards aquatic organisms is available (Stephenson, 1986, see Doc IIIA7.4.1.3/01 – GLP, non-guideline). It was originally submitted to give information on algal toxicity, which is not available for ethylene oxide, but the study also describes the toxicity of propylene oxide towards *Daphnia magna* in a 48-h static toxicity test. Triplicate sets of 140 mL glass flasks with reconstituted freshwater contained propylene oxide in concentrations between 10 and 1000 mg/L, and three flasks served as controls. Ten daphnids were placed in each flask. The flasks were sealed to prevent loss of propylene oxide by evaporation. After 24 and 48 h, the numbers of immobilised daphnids (not swimming for 10 seconds after stirring the flask) were recorded. The temperature during the test was 18-22 °C, the pH was 8.0-8.1, and the dissolved oxygen concentrations were 9.0-9.2 mg/L. The resulting 48-h $EC_{50}$ (probit analysis after log transformation according to Finney (1971)) was 350 mg/L.

Summary, acute toxicity towards invertebrates

All the data described above are listed in the summary table on acute/short-term toxicity to invertebrates further below. As can be easily seen in the table, the $EC_{50}$ values for daphnids both from the publications on ethylene oxide and QSAR estimations, are within the same order of magnitude, at 212 mg/L and 420 mg/L, respectively. The $EC_{50}$ value for brine shrimp is reported to be 680 mg/L. For propylene oxide, the $EC_{50}$ values for daphnids are 190 and 350 mg/L (estimated and experimental, respectively).

**Acute/short-term toxicity (freshwater) – algae**

No experimental data on the toxicity of ethylene oxide to algae are available to the eCA.

QSAR data

96-h $EC_{50}$ values for algae were estimated using the QSAR software ECOSAR v. 1.11 (US EPA, 2012b), and are approximately 800 mg/L for ethylene oxide and 3 500 mg/L for ethylene glycol. Details are given below, in the summary table on acute/short-term toxicity to algae.

Cf. the read-across data from propylene oxide (see next paragraph), ECOSAR v. 1.11 predicts the propylene oxide 96-h $EC_{50}$ for algae to be approx. 310 mg/L.

Read-across data: propylene oxide

For a justification on the usefulness of propylene oxide data as an indication of the acute aquatic toxicity of ethylene oxide, please see the section above on acute toxicity towards fish. A study on the toxicity of propylene oxide towards aquatic organisms is available, which includes investigation of growth inhibition of algae in a 96-h growth experiment (Stephenson, 1986, IIIA7.4.1.3/01 – GLP, non-guideline).

Ten flasks were filled with 295 mL culture medium and propylene oxide in concentrations between 1.0 and 1000 mg/L., and six flasks served as controls. Each flask had an initial *S. capricornutum* concentration of 500 cells/mL. The flasks were sealed to prevent loss of propylene oxide by evaporation. After 48 and 96 h of incubation in a cooled orbital incubator under constant illumination, a temperature of 22-26 °C and a pH of 7.2-7.5, cell concentrations were determined with a Coulter Counter. The resulting 96-h $EC_{50}$ based on

cell concentration (probit analysis after log transformation according to Finney (1971)), was 240 mg/L.

Summary, toxicity towards algae

The data described above are listed in the summary table on acute/short-term toxicity to algae further below. The $EC_{50}$ value estimated by QSAR estimations and the $EC_{50}$ value for propylene oxide are within the same order of magnitude, i.e. 800 and 240 mg/L, respectively. Based on the QSAR estimations and the read-across to propylene oxide, algae do not represent the most sensitive trophic level. The lack of experimental data on the algal toxicity of ethylene oxide is therefore considered acceptable.

Table A.41 Summary table – acute/short-term toxicity to fish

| Method, Guideline, GLP status, Reliability, Key/ supportive study | Species | Endpoint/ Type of test | Test material | Exposure | | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Design | Dura-tion | LC/$EC_{50}$ | | |
| *Ethylene oxide* | | | | | | | | |
| EPA 660-3-75-009 (1975)<br>Non-GLP<br>RI: 2-3 | Fathead minnow (*Pimephales promelas*) | Mortality | Ethylene oxide | Static | 96 h | 84 mg/L *(95 % CL: 73-96 mg/L)* | Range finding study, 24-h $LC_{50}$<br>Static aerated test: 274 mg/L (95% CL 150-500 mg/L)<br>Static test sealed under oxygen: 86 mg/L (95% CL 50-150 mg/L) | Conway *et al.* (1983)<br>IIIA7.4.1.1/ 01 |
| Non-GLP<br>RI: 3 | Goldfish (*Carassius auratus*) | Mortality | Ethylene oxide | Static | 24 h | 90 mg/L | | Bridié *et al.* (1979b)<br>IIIA7.4.1.1/ 02 |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Fish | Mortality | Ethylene oxide | n.a. | 96 h | 80 mg/L | Log Kow used for estimation: -0.30<br>ECOSAR class: epoxides, mono | USEPA (2012) |
| *Ethylene glycol* [a)] | | | | | | | | |
| EPA 660-3-75-009 (1975)<br>Non-GLP<br>RI: 2-3 | Fathead minnow (*Pimephales promelas*) | Mortality | Ethylene glycol | Static | 96 h | > 10 000 mg/L | | Conway *et al.* (1983)<br>IIIA7.4.1.1/ 01 |
| Non-GLP<br>RI: 3 | Goldfish (*Carassius auratus*) | Mortality | Ethylene glycol | Static | 24 h | > 5 000 mg/L | | Bridié *et al.* (1979b)<br>IIIA7.4.1.1/ 02 |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Fish | Mortality | Ethylene glycol | n.a. | 96 h | 38 000 mg/L | Log Kow used for estimation: -1.20<br>ECOSAR class: neutral organics | USEPA (2012) |
| *Ethylene chlorohydrin* [b)] | | | | | | | | |
| EPA 660-3-75-009 (1975)<br>Non-GLP<br>RI: 2-3 | Fathead minnow (*Pimephales promelas*) | Mortality | Ethylene chloro-hydrin | Static | 96 h | 67 mg/L *(95 % CL: 49-84 mg/L)*<br>112 mg/L *(95 % CL: 90-131 mg/L)* | | Conway *et al.* (1983)<br>IIIA7.4.1.1/ 01 |

| Method, Guideline, GLP status, Reliability, Key/ supportive study | Species | Endpoint/ Type of test | Test material | Exposure | | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Design | Dura-tion | $LC/EC_{50}$ | | |
| *Propylene oxide* [c)] | | | | | | | | |
| Non-guideline<br>GLP<br>RI: 2 | Rainbow trout (*Salmo gairdneri*) | Mortality | Propylene oxide | Semi-static [d)] | 96 h | 52 mg/L | | Stephenson (1989)<br>IIIA7.4.1.3/ 01 |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Fish | Mortality | Propylene oxide | n.a. | 96 h | 45 mg/L | Log Kow used for estimation: 0.37<br>ECOSAR class: epoxides, mono | USEPA (2012) |

a) Ethylene glycol is a major degradation product (hydrolysis) in freshwater
b) Ethylene chlorohydrin is a degradation product in saltwater, included here for the sake of completeness only, as it was available in the submitted data.
c) Propylene oxide is structurally similar to ethylene oxide (has one methyl group extra) and is included for read-across purposes
d) The test solutions were renewed at 24-h intervals.

Table A.42 Summary table – acute/short-term toxicity to invertebrates

| Method, Guideline, GLP status, Reliability, Key/ supportive study | Species | Endpoint/ Type of test | Test material | Exposure | | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Design | Dura-tion | $LC/EC_{50}$ | | |
| *Ethylene oxide* | | | | | | | | |
| EPA 660-3-75-009<br>Non-GLP<br>RI: 2-3 | *Daphnia magna* | Mortality | Ethylene oxide | Static | 48 h | 212 mg/L | The $EC_{50}$ is a mean of triplicate values ranging from 137 to 300 mg/L | Conway *et al.* (1983)<br>IIIA7.4.1.2/ 01 |
| Price *et al.* (1974)<br>Non-GLP | Brine shrimp (*Artemia salina*) | Mortality | Ethylene oxide | Static | 48 h | 745 mg/L | The $EC_{50}$ is a mean duplicate values: 490 and 1000 mg/L | Conway et al. (1983)<br>IIIA7.4.1.2/ 02 |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Daphnid | Mortality | Ethylene oxide | n.a. | 48 h | 420 mg/L | Log Kow used for estimation: -0.30<br>ECOSAR class: epoxides, mono | USEPA (2012) |

| Method, Guideline, GLP status, Reliability, Key/ supportive study | Species | Endpoint/ Type of test | Test material | Exposure | | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Design | Dura-tion | $LC/EC_{50}$ | | |
| *Ethylene glycol* [a)] | | | | | | | | |
| EPA 660-3-75-009<br>Non-GLP<br>RI: 2-3 | *Daphnia magna* | Mortality | Ethylene glycol | Static | 48 h | > 10 000 mg/L | | Conway *et al.* (1983)<br>IIIA7.4.1.2/ 01 |
| Price *et al.* (1974)<br>Non-GLP | Brine shrimp (*Artemia salina*) | Mortality | Ethylene glycol | Static | 48 h | > 20 000 mg/L | | Conway et al. (1983)<br>IIIA7.4.1.2/ 02 |
| ECOSAR v. 1.11<br>estimate<br>RI: 0 | Daphnid | Mortality | Ethylene glycol | n.a. | 48 h | 16 000 mg/L | Log Kow used for estimation: -1.20<br>ECOSAR class: neutral organics | USEPA (2012) |
| *Ethylene chlorohydrin* [b)] | | | | | | | | |
| EPA 660-3-75-009<br>Non-GLP<br>RI: 2-3 | *Daphnia magna* | Mortality | Ethylene chloro-hydrin | Static | 48 h | 100 mg/L<br>*(95 % CL: 50-200 mg/L)* | | Conway *et al.* (1983)<br>IIIA7.4.1.2/ 01 |
| Price *et al.* (1974)<br>Non-GLP | Brine shrimp (*Artemia salina*) | Mortality | Ethylene chloro-hydrin | Static | 48 h | 680 mg/L | | Conway et al. (1983)<br>IIIA7.4.1.2/ 02 |
| *Propylene oxide* [c)] | | | | | | | | |
| Non-guideline<br>GLP<br>RI: 2 | *Daphnia magna* | Mortality | Propylene oxide | Static | 48 h | 350 mg/L | | Stephenson (1989)<br>IIIA7.4.1.3 /01 |
| ECOSAR v. 1.11<br>estimate<br>RI: 0 | Daphnid | Mortality | Propylene oxide | n.a. | 48 h | 190 mg/L | Log Kow used for estimation: 0.37<br>ECOSAR class: epoxides, mono | USEPA (2012) |

a) Ethylene glycol is a major degradation product (hydrolysis) in freshwater
b) Ethylene chlorohydrin is a degradation product in saltwater, included here for the sake of completeness only, as it was available in the submitted data
c) Propylene oxide is structurally similar to ethylene oxide (has one methyl group extra) and is included for read-across purposes

Table A.43 Summary table – acute/short-term toxicity to algae

| Method, Guideline, GLP status, Reliability, Key/ supportive study | Species | Endpoint/ Type of test | Test material | Exposure | | Results | Remarks | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Design | Dura-tion | $LC/EC_{50}$ | | |
| *Ethylene oxide* | | | | | | | | |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Green algae | Mortality | Ethylene oxide | n.a. | 96 h | 800 mg/L | Log Kow used for estimation: -0.30<br>ECOSAR class: epoxides, mono | USEPA (2012) |
| *Ethylene glycol* [a)] | | | | | | | | |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Green algae | Mortality | Ethylene glycol | n.a. | 96 h | 3 500 mg/L | Log Kow used for estimation: -1.20<br>ECOSAR class: neutral organics | USEPA (2012) |
| *Propylene oxide* [b)] | | | | | | | | |
| Non-guideline<br>GLP<br>RI: 2 | *Selenastrum capri-cornutum* | Growth rate (cell number) | Propylene oxide | Static | 96 h | 240 mg/L | | Stephenson (1986)<br>IIIA7.4.1.3/ 01 |
| ECOSAR v. 1.11 estimate<br>RI: 0 | Green algae | Mortality | Propylene oxide | n.a. | 96 h | 310 mg/L | Log Kow used for estimation: 0.37<br>ECOSAR class: epoxides, mono | USEPA (2012) |

a) Ethylene glycol is a major degradation product (hydrolysis) in freshwater
b) Propylene oxide is structurally similar to ethylene oxide (has one methyl group extra) and is included for read-across purposes

### A.4.2.4 Terrestrial compartment

The exposure of soil micro-organisms to ethylene oxide will be insignificant. No data has been submitted and this is considered acceptable by the eCA. Please see separate waivers in Doc IIIA 7.

### A.4.2.5 Groundwater

A groundwater assessment is not considered necessary, due to the lack of exposure to other compartments than the atmosphere, and subsequent limited partitioning to soil or water.

### A.4.2.6 Birds and mammals

No data for birds is available. Even though birds could be exposed in the atmospheric compartment and special attention should be given to this fact, the eCA is of the opinion that no further studies are needed. The intrinsic properties of the substance and the risk assessment on human health already give rise to concern. A new study on birds for the sake of the environmental assessment would not change this fact. Data on mammals are considered covered by the section on human health effects.

### A.4.2.7 Primary and secondary poisoning

An assessment of primary and secondary poisoning is not considered necessary. Ethylene oxide is not likely to bioaccumulate in the environment, based on the log Kow of -0.30. In addition, the exposure to organisms in soil or water is expected to be negligible from the intended use. No further assessment of secondary exposure via the food chain is therefore considered necessary.

## A.4.3. Endocrine disruption

An assessment of the endocrine disrupting properties of ethylene oxide for non-target organisms has not been conducted. It is not possible to conclude on the endocrine disrupting properties for human health, but additional testing is not justified in the particular case of ethylene oxide (please see section A3.14).

## A.4.4. Derivation of PNECs

PNECs have not been calculated, based on the negligible exposure to soil and surface water. No emission to STPs is foreseen, and the high vapour pressure and hence rapid volatilisation of ethylene oxide makes it highly unlikely that the substance will partition from air to other environmental compartments. Therefore, no PECs have been calculated for the aquatic compartment or for soil. The only PEC calculated is $PEClocal_{air}$, and derivation of a $PNEC_{air}$ is not standard in the environmental risk assessment. It is referred to the human health risk assessment for comparison of the $PEClocal_{air}$ with relevant toxicity threshold values.

## A.4.5. Overall summary of acute and chronic aquatic toxicity data and Comparison with the CLP criteria

Ethylene oxide does not have a harmonised classification for environmental endpoints. The evaluation of ethylene oxide as a biocidal active substance in PT 2 has not made any new information available to the eCA indicating that any criteria for environmental classification are met.

However, environmental classification and labelling has not been particularly investigated

*per se* in the context of this CAR. For substances already listed in Annex VI to CLP (as was the case for ethylene oxide prior to the eCA's biocidal evaluation), the mandatory requirement for a full evaluation of all hazard classes does not apply. This is in accordance with the document "Dossier submitter's assessment of hazard classes and evaluation by RAC – Summary of the paper presented by ECHA to the 12th Meeting of Competent Authorities for REACH and CLP (CARACAL) on the 13-14 March 2013, and the response provided on the 23 September 2013."

## A.5. Assessment of additional hazards

### A.5.1. Hazardous to the ozone layer

No data has been submitted for this purpose. Ethylene oxide is long-lived in the atmosphere, but it is indicated that reactions with OH radicals are the dominant removal pathway (Cupitt *et al.*, 1987, see Doc IIIA7.3.2/03). Winer *et al.* (1988) state that the reaction of ethylene oxide with ozone is too slow to represent a significant removal pathway. Furthermore, UNEP (2001) mentions that ethylene oxide is sometimes used in combination with ozone-depleting substances such as CFCs, but that ethylene oxide itself does not deplete the ozone layer.

There are no indications that ethylene oxide has ozone layer-depletion potential.

#### A.5.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Based on the information given above, there are no indications that ethylene oxide has ozone layer-depletion potential.

## A.6. Additional Labelling

Not relevant.

# A.7. Assessment of exclusion criteria, substitution criteria and POP

## A.7.1. Exclusion criteria

### A.7.1.1 Assessment of CMR properties

Table A.44 Assessment of CMR properties

| Criteria (BPR Article 5[1]) | Assessment |
|---|---|
| Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, carcinogen category 1A or 1B | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as Carc. 1B. |
| Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, mutagen category 1A or 1B | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as Muta. 1B. |
| Active substances which have been classified in accordance with Regulation (EC) No 1272/2008 as, or which meet the criteria to be classified as, toxic for reproduction category 1A or 1B | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as Repr. 1B. |
| **Conclusion on CMR properties** | The exclusion criteria in BPR Article 5(1)(a), (b) and (c) are met. |

### A.7.1.2 Assessment of endocrine disrupting properties

Table A.45 Assessment of endocrine disrupting properties

| Criteria (BPR Article 5) | Assessment |
|---|---|
| Active substances which, on the basis of the criteria specified pursuant to the first subparagraph of paragraph 3 are considered as having endocrine-disrupting properties that may cause adverse effects in humans and to the environment. | An assessment of the endocrine disrupting potential of ethylene oxide was performed in accordance with the scientific criteria set out in COMMISSION DELEGATED REGULATION (EU) 2017/2100. Based on the information available, no final conclusion on the ED properties of ethylene oxide could be made.<br><br>However, additional testing is not justified in this particular case because of the following reasons:<br>1. The already known severe hazard profile of this substance: ethylene oxide fulfils the exclusion criteria of Article 5, paragraph 1a, b and c of BPR given the following classification of the active: Carc. 1B, Muta. 1B and Repr. 1B.<br><br>2. As a consequence of these properties, strict RMMs should be in place to avoid or minimise occupational exposure as far as technically feasible if EtO is to be used. As a non-threshold mode of action is assumed |

| Criteria (BPR Article 5) | Assessment |
|---|---|
| | for tumour formation, a conservative semi-quantitative risk assessment is already performed. It should furthermore be kept in mind that there is no agreed methodology to consider endocrine disruption in the risk assessment. Consequently, no impact on the risk assessment would be expected if the substance were to be additionally considered as an ED.<br><br>3. Handling of ethylene oxide is not easy due to its physicochemical and human health related hazardous properties. It is not desirable to expose either laboratory personnel or laboratory animals to ethylene oxide, unless it is strictly necessary.<br><br>4. 4. Due to the unspecific alkylating properties of the substance, the available test systems for determining the endocrine activity would be likely to give equivocal results (if tests were performed). If mechanistic studies were nevertheless conducted, it would be difficult to conclude that the mechanism would be solely a (non-) ED MoA. |
| **Conclusion on ED properties** | A final conclusion is not possible and not needed in this special case. |

## A.7.1.3 PBT Assessment (following Annex XIII to Regulation (EC) No 1907/2006)

### Assessment of persistence (P/vP)

The REACH guidance on Information Requirements and Chemical Safety Assessment, Chapter R.11: PBT/vPvB assessment, version 3.0 (ECHA, 2017d) explains which information can be used to identify the possible fulfilment of the P criterion. This information includes results from screening tests on biodegradability (e.g. ready biodegradability tests), results from QSAR estimations, monitoring data, information on abiotic degradation and other suitable information.

At the discussion of the ethylene oxide draft CAR at the WG-III-2020, the need for an ad hoc follow-up regarding the persistence of ethylene oxide was identified, due to insufficient information for drawing a conclusion. Further information was provided by the applicant, and this chapter was revised.

The information listed and discussed below has been taken into account by the eCA in the P assessment of ethylene oxide and its degradation products (i.e. ethylene glycol which is the major degradation product in the environment, and ethylene chlorohydrin which is expected to be formed in saltwater).

<u>Ethylene oxide</u>

Ready biodegradability, key data for the P assessment:

- Experimental data: OECD 301C study (MITI, 1995). Result: ready biodegradability. Please see chapter A4.1.1.2 and Doc IIIA7.1.1.2.1/04 for further details.

Ready biodegradability, further information (supporting data for the P assessment):

- Experimental data: published article with results from a non-standard, 20 day BOD study (Conway *et al.*, 1983). Result: a biodegradation half-life of approx. 20 days (i.e. not readily biodegradable if compared with OECD 301 criteria, but below the P criterion trigger). The results should not be given too much weight, due to e.g. lacking information on test conditions (please see chapter A4.1.1.2 and Doc IIIA.7.1.1.2.1/01 for further details).
- QSAR data: Biowin estimates that ethylene oxide is readily biodegradable. Please see chapter A.4.1.1.2 for further details on the estimations.

Other degradation data (supporting data for the P assessment):

- Hydrolysis, experimental data: published article (Conway *et al.*, 1983), hydrolysis at one pH only, in freshwater and saltwater at 25 °C. Initial ethylene oxide concentration in freshwater and saltwater: 65 mg/L and 200 mg/L, respectively. Half-lives recalculated to 12 °C in sterile distilled water, sterile river water and non-sterile river water, respectively: 34.5 days, 36.5 days and 40.2 days. Saltwater half-lives recalculated to 9 °C range from 33.4 to 39.6 days.
- Hydrolysis, experimental data: simple test at 25 °C, with an initial ethylene oxide concentration of 100 mg/L, carried out in connection with the ready biodegradation test of MITI (1995). See Doc IIIA7.1.1.2.1/04. The half-life recalculated to 12 °C: 38.8 days.
- Some other data on biodegradation are available, see Doc IIIA7.1.1.2.1/02 and Doc IIIA7.1.1.2.1/03, but these have not been taken into account in the persistence assessment due to very little information on how the results were obtained.

Ethylene glycol

- Experimental data: published article with results from a non-standard, 20 day BOD study (Conway *et al.*, 1983). Result: 96 % biodegradation after 20 days (i.e. readily biodegradable if compared with OECD 301 criteria). The results should not be given too much weight, due to e.g. lacking information on test conditions (please see chapter A4.1.1.2 and Doc IIIA.7.1.1.2.1/01 for further details).
- QSAR data: Biowin estimates that ethylene glycol is readily biodegradable. Please see chapter A4.1.1.2 for further details on the estimations.
- Ethylene glycol is expected to be hydrolytically stable (Lyman et al., 1982, as mentioned by WHO, 2000).
- Literature search by the eCA on the biodegradation of ethylene glycol: According to a review article by Staples *et al.* (2001)[3], ethylene glycol is not persistent in air, surface water, soil, or groundwater.

Ethylene chlorohydrin

- Experimental data: published article with results from a non-standard, 20 day BOD study (Conway *et al.*, 1983 – Doc IIIA7.1.1.2.1/01). Result: 57 % biodegradation after 20 days (i.e. not readily biodegradable if compared with OECD 301 criteria, but a half-life below the P criterion trigger). The results should not be given too much weight, due to e.g. lacking information on test conditions (please see chapter A4.1.1.2 and Doc IIIA.7.1.1.2.1/01 for further details).
- QSAR data: Biowin estimates that ethylene chlorohydrin is readily biodegradable. Please see chapter A.4.1.1.2 for further details on the estimations.

[3] Staples, C.A., Williams, J.B., Craig, G.R., Roberts, K.M (2001): Fate, effects and potential environmental risks of ethylene glycol: a review. *Chemosphere Vol 43 (3), April 2001, pp 377-383* [link]

Summary and conclusion

The key data for the P assessment of ethylene oxide is the ready biodegradation study (MITI, 1995). The eCA considers this study sufficiently reliable to indicate that ethylene oxide is readily biodegradable.

Furthermore, other information is available which should not be used to draw a conclusion on its own but which is seen to support the conclusion that ethylene oxide biodegrades readily, or relatively fast, in the environment, i.e. QSAR estimations of ready biodegradability, and indications of a biodegradation half-life of approximately 20 days (Conway *et al.*, 1983).

Hydrolysis data based on very simple tests (at one pH and with little further information on test conditions) indicate half-lives under or directly at the 40 day P criterion trigger. These studies have shortcomings, nevertheless the results are consistent across several ethylene oxide concentrations (65 mg/L, 100 mg/L and 200 mg/L) in fresh- and saltwater. The ready biodegradation information for ethylene oxide, together with these hydrolysis data which one value is 40.2 days and the rest are below 40, in total point towards ethylene oxide not being persistent.

Furthermore, the information available on relevant degradation products do not give rise to concern regarding persistence. Even though ethylene glycol should be considered hydrolytically stable, it is expected to be readily biodegraded, when collectively taking into account experimental data, QSAR data and literature information. Ethylene chlorohydrin is also not expected to be persistent, based on experimental data and QSAR data.

It should also be noted that the conclusion of the REACH substance evaluation of ethylene oxide is that it is not persistent. This is based on the MITI (1995) and Conway (1983) studies, an OECD 301D study with the result ready biodegradable (Dow, 1978 – study report not available, REACH IUCLID study summary is the only available information and hence the reliability cannot be reviewed for this a.s. evaluation), and read-across to propylene oxide which is given as readily biodegradable (OECD 301C).

Table A.46 Assessment of P/vP criteria

| P Criteria | Assessment |
|---|---|
| T1/2 > 60 days in seawater, or | Hydrolysis half-life in saltwater (9 °C):<br>33.4-39.6 days<br><br>Hydrolysis degradant ethylene glycol hydrolytically stable, but available biodegradation information indicates not P. Available information on hydrolysis degradant ethylene chlorohydrin indicates not P. |
| T1/2 > 40 days in fresh- or estuarine water, or | Key data: Readily biodegradable (experimental data)<br><br>Supporting data:<br>Readily biodegradable (Biowin estimations)<br>Biodegradation half-life: approx. 20 days<br>Hydrolysis half-life in freshwater: 34.5-40.2 days<br><br>Hydrolysis degradant ethylene glycol hydrolytically stable, but available biodegradation information indicates not P. |
| T1/2 > 180 days in seawater sediment, or | No data available. |
| T1/2 > 120 days in freshwater- or estuarine | No data available. |

| | |
|---|---|
| sediment, or | |
| T1/2 <= 120 days in soil. | No data available. |
| **vP Criteria** | **Assessment** |
| T1/2 > 60 days in sea-, fresh- or estuarine water, or | See information above. This criterion is not met based on the available data. |
| T1/2 > 180 days in seawater-, freshwater- or estuarine sediment, or | See information above. This criterion is not met based on the available data. |
| T1/2 > 180 days in soil. | No data available. |
| **Conclusion on P / vP properties** | Based on the ready biodegradability of ethylene oxide, and also taking into account other supportive data pointing towards a low concern for persistence in the environment, the P / vP criteria are not met. |

**Assessment of bioaccumulation (B/vB)**

The following information on the bioaccumulation potential of ethylene oxide is currently available to the eCA:

- log Kow: -0.30
- Estimated $BCF_{fish}$: 3.16 L/kg
- Estimated $BCF_{earthworm}$: 0.85 L/kg
- The log Kow of the transformation product is also low and does not give rise to concern.

Neither the estimated $BCF_{fish}$ nor the estimated $BCF_{earthworm}$ give rise to concern regarding bioaccumulation.

Table A.47 Assessment of the B/vB criteria

| **B Criteria** | **Assessment** |
|---|---|
| BCF > 2000 | Estimated BCF values are 0.85 L/kg and 3.16 L/kg, i.e. far below the trigger value. This criterion is not met based on the available data. |
| **vB Criteria** | **Assessment** |
| BCF > 5000 | See information above. This criterion is not met based on the available data. |
| **Conclusion on B / vB properties** | Based on the available data, the B / vB criteria are not met. |

**Assessment of toxicity (T)**

Table A.48 Assessment of the T criteria

| T Criteria | Assessment |
|---|---|
| NOEC/$EC_{10}$ (long-term) < 0.01 mg/L for freshwater or seawater organisms, or | No data available |
| substance meets the criteria for classification as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2) according to the CLP Regulation, or | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as Carc. 1B and Muta. 1B |
| there is other evidence of chronic toxicity, as identified by the substance meeting the criteria for classification: specific target organ toxicity after repeated exposure (STOT RE category 1 or 2) according to the CLP Regulation. | Ethylene oxide is classified in accordance with Regulation (EC) No 1272/2008 and meets criteria to be classified as STOT RE 1 (nervous system). |
| **Conclusion on T properties** | Based on the harmonised classification of ethylene oxide, the T criterion is met. |

**Summary and overall conclusions on PBT or vPvB properties**

Based on the assessment described in the subsections above, ethylene does not fulfil the P or B criteria, but it does fulfil the T criterion.

Based on the available information, ethylene oxide is not considered a PBT / vPvB substance.

## A.7.2. Substitution criteria

Table A.49 Assessment of substitution criteria

| Substitution criteria (BPR, Article 10) | Assessment |
|---|---|
| One of the exclusion criteria listed in Article 5(1) is met but AS may be approved in accordance with Article 5(2) | Exclusion criteria listed in Article 5(1) (a), (b) and (c) are met. |
| The criteria to be classified, in accordance with Regulation (EC) No 1272/2008, as a respiratory sensitiser are met | Ethylene Oxide does not fulfil criterion (b) of Article 10(1) |
| The acceptable daily intake, acute reference dose or acceptable operator exposure level, as appropriate, is significantly lower than those of the majority of approved active substances for the same product-type and use scenario | To be considered when all PT 2 active substances are evaluated. |
| Two of the criteria for being PBT in accordance with Annex XIII to Regulation | Ethylene oxide does not fulfil criterion (d) of Article 10(1) |

| Substitution criteria (BPR, Article 10) | Assessment |
|---|---|
| (EC) No 1907/2006 are met | |
| There are reasons for concern linked to the nature of the critical effects which, in combination with the use patterns, amount to use that could still cause concern, such as high potential of risk to groundwater, even with very restrictive risk management measures | Ethylene Oxide fulfil criterion (e) of Article 10(1) |
| The AS contains a significant proportion of non-active isomers or impurities. | Ethylene oxide does not fulfil criterion (f) of Article 10(1) |
| **Conclusion on substitution criteria** | The substitution criteria in BPR Article 10(1)(a) and (e) are met. |

## A.7.3. Assessment of long-range environmental transportation and impact on environmental compartments

Table A.50 Assessment of LRTAP / POP criteria

| LRTAP / POP criteria | Assessment |
|---|---|
| The active substance or a degradation product is a persistent organic pollutant (POP) listed in Annex I of EC 850/2004 | Ethylene oxide is not listed in Annex I of EC 850/2004 |
| Assessment of long-range transport potential (LRTAP):<br>Vapour pressure <1000 Pa and half-life in air > 2 days or<br>Monitoring data in remote area showing that the substance is found in remote regions or<br>Result of multimedia modelling | Vapour pressure: 146 kPa at 20 °C<br>Estimated half-life in air: 38-382 days |
| The active substance or a degradation product is vP/vB or T | Ethylene oxide is a T substance |
| **Conclusion on LRTAP/POP assessment** | Based on the available data, ethylene oxide has the potential to persist for a long time in the atmosphere. It is furthermore a T substance. However, the high vapour pressure (above the cut-off value) indicates that ethylene oxide is not likely to deposit from the atmosphere to other environmental compartments. |

# B. Exposure assessment and effects of the active substance in the biocidal product(s)

## B.1. General product information

### B.1.1. Identification of the product

Table B.1 Product name(s) and formulation type

| | |
|---|---|
| **Trade name(s) or proposed trade name(s)** | **D495 SS** |
| **Manufacturer's development code and number of the product** | **D495 SS** |
| **Formulation type** | **Compressed gas** |

### B.1.2. Complete qualitative and quantitative composition of the biocidal product

Table B.2 Active substance(s) in the product

| ISO or Trivial name | IUPAC name or other accepted chemical name | EC number | CAS number | Composition / all constituents (upper and lower concentration limit in % (w/w)) | Concentration in the product in % (w/w) |
|---|---|---|---|---|---|
| **Ethylene oxide** | Oxirane | 200-849-9 | 75-21-8 | 99.9% | 99.9% |

The product contains pure active substance, and no co-formulants.

## B.1.3. Physical, chemical and technical properties

Table B.3 Physical, chemical and technical properties

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| Physical state at 25°C and 101.3 kPA | Gas | Not documented | | Merck 2001 |
| Colour at 20°C and 101.3 kPA | Colourless | Not documented | | Merck 2001 |
| Odour at 20°C and 101.3 kPA | Sweet, olefinic | Not documented | | Verschueren 1983 |
| Acidity / alkalinity | | Not documented | EtO does not contain any components that would contribute to dissociation in water and therefore it is not classed as acidic or alkaline (acidic pH < 4, alkaline pH > 10). (See Appendix 2) | - |
| Relative density | 0.8821 g/cm3 at 10 °C | Not documented | | Lide, 2005, CRC handbook |
| **Storage stability, stability and shelf-life** | | | | |
| Accelerated storage | | | For the analysis of EtO in the product (and for the analysis of EtO in manufactured batches), it is not possible to measure EtO directly due to safety concerns as it is extremely flammable and explosive under certain conditions. In addition, pure EtO is toxic and poses a danger to both health of lab personnel when handling the substance and to the environment. Therefore the practical safety concerns and limitations for handling EtO makes it difficult to analyse and generate any study data.<br><br>The storage stability and the need for a storage stability test must be re- | Waiver |
| Long term storage at ambient temperature | | | | |
| Low temperature stability (liquids) | | | | |

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| | | | evaluated during the product approval stage. Please also see the reasoning given for accepting the non-submission of analytical methods for ethylene oxide. | |
| **Effects on content of the active substance** | | | | |
| Light | - | - | - | - |
| Temperature and humidity | - | - | - | - |
| Reactivity towards container material | | | Commercial products are provided in containers made from suitable material such as aluminium canisters, stainless steel drums or carbon-steel cylinders and there are no indications of corrosion or other effects on such containers during storage under recommended conditions. | |
| **Technical characteristics** | | | | |
| Wettability | | | Not applicable for 100% ethylene oxide product | |
| Suspensibility, spontaneity and dispersion stability | | | Not applicable for 100% ethylene oxide product | |
| Wet sieve analysis and dry sieve test | | | Not applicable for 100% ethylene oxide product | |
| Emulsifiability, re-emulsifiability and emulsion stability | | | Not applicable for 100% ethylene oxide product | |
| Disintegration time | | | Not applicable for 100% ethylene oxide product | |
| Particle size distribution, content of dust / fines, attrition, | | | Not applicable for 100% ethylene oxide product | |

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| friability | | | | |
| Persistent foaming | | | Not applicable for 100% ethylene oxide product | |
| Flowability, pourability, dustability | | | Not applicable for 100% ethylene oxide product | |
| Burning rate – smoke generators | | | Not applicable for 100% ethylene oxide product | |
| Burning completeness – smoke generators | | | Not applicable for 100% ethylene oxide product | |
| Composition of smoke – smoke generators | | | Not applicable for 100% ethylene oxide product | |
| Spraying pattern - aerosols | | | Not applicable for 100% ethylene oxide product | |
| Other technical characteristics | - | - | - | - |
| **Physical and chemical compatibility with other products including other biocidal products with which its uses is to be authorised** | | | | |
| Physical compatibility | | | Not applicable – The product will not be combined with any other product | |
| Chemical compatibility | | | Not applicable – The product will not be combined with any other product | |
| Degree of dissolution and dilution stability | | | Ethylene oxide does not contain any dissociation groups | |
| Surface tension | | | Ethylene oxide is too hazardous to be experimentally determined for surface tension. Based on chemical structure, no surface activity is predicted.<br><br>Surface tension will not be used for (eco)toxicological evaluation. The biocidal products containing ethylene oxide is gaseous, hence surface tension | |

| Property | Result | Test method applied or description in case of deviation | Remarks / Discussion / Justification for waiving | References |
|---|---|---|---|---|
| | | | is not relevant. | |
| Viscosity | | | Viscosity is required for liquid substances determined at 20 and 40 °C. Ethylene oxide is a gas at 20 and 40 °C, therefore determination of viscosity by test method OECD 114 is not possible. | |

### B.1.4. Hazard identification for physical and chemical properties

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the physical, chemical hazards for the product are identical to those of the active substance. Please see section A.1.4.

### B.1.5. Analytical methods for detection and identification

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, analytical methods of the product are identical to those of the active substance. Please see section A.1.6 Analytical methods

## B.2. Efficacy

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the efficacy of the product is identical to that of the active substance. Please see section A.2.2.1.

However, it should be noted that the efficacy data described in section A2.2.1. is considered sufficient to conclude on the innate biocidal activity of the active substance. Further information/studies needs to be submitted at the product authorisation stage, which sufficiently covers efficacy towards other claimed target organisms, and compositions of the biocidal product.

### B.2.1. Mode of action

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the mode of action for the product is identical to that of the active substance. Please see section A.2.2.2.

### B.2.2. Resistance

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the description of resistance for the product is identical to that of the active substance. Please see section A.2.2.3.

### B.2.3. Conclusion on efficacy

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the conclusion on efficacy for the product is identical to that of the active substance. Please see section A.2.3.

# B.3. Human exposure assessment

## B.3.1. Identification of main paths of human exposure towards active substance from its use in biocidal product

Table B.4 Summary table: relevant paths of human exposure

| Exposure path | Primary (direct) exposure | | | Secondary (indirect) exposure | | | |
|---|---|---|---|---|---|---|---|
| | Industrial use | Professional use | Non-professional use | Industrial use | Professional use | General public | Via food |
| **Inhalation** | Yes | n.a. | n.a. | n.a. | n.a. | Yes | **n.a.** |
| **Dermal** | Yes | n.a. | n.a. | Yes | n.a. | n.a. | **n.a.** |
| **Oral** | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | **n.a.** |

Ethylene oxide (EtO) is a gas, and its primary biocidal use is the sterilisation of single use medical equipment before these are placed on the market. Once placed on the market, possible release of EtO from the medical device will not be regulated by the BPR, but by the Medical Device Regulation (MDR, (EU) 2017/745).

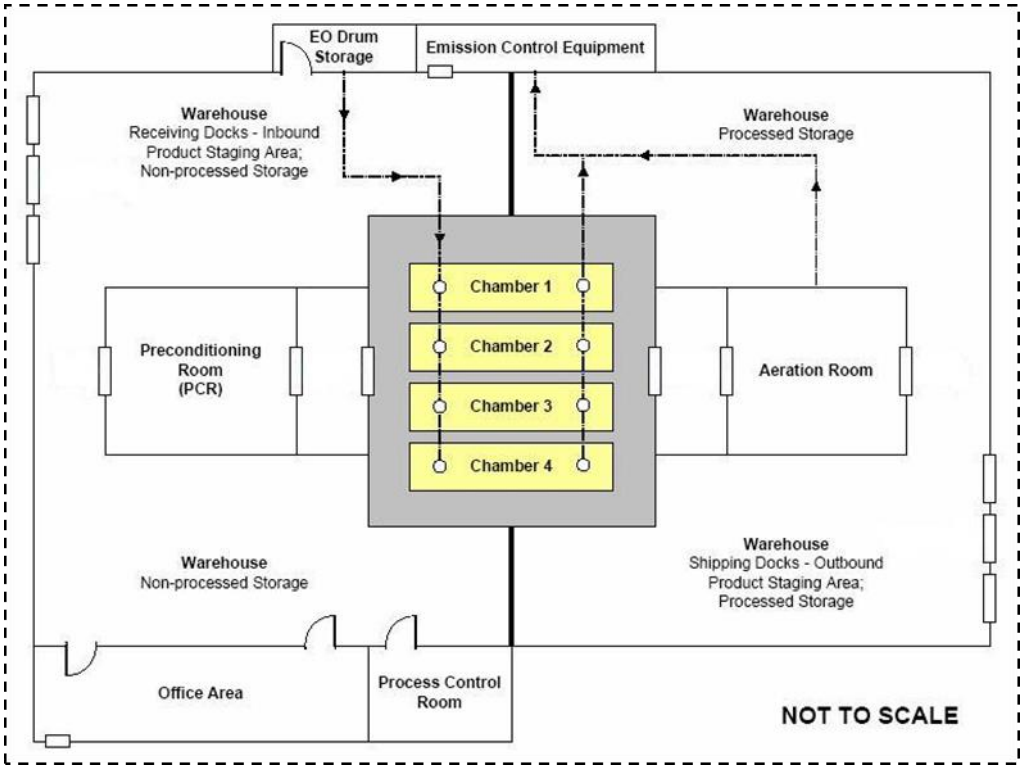
EtO is exclusively used in dedicated, highly specialised industrial sterilisation plants. Exposure to EtO occurs mostly through the inhalation route when small amounts of EtO escapes from the treatment process, or more likely, from post-sterilisation fugitive emissions. Dermal exposure is possible by skin contact with sterilised items with residuals on their surface. The staff are specialised and highly trained, and a high level of operational conditions (OC) and RMM are applied in order to control and reduce the operator exposure as much as possible.

A description of a typical EtO sterilisation process, including the associated tasks and the RMMs involved, prepared by the applicant is presented here (See also: Doc III-B.5.2.; Andrew, D. 2020). These conditions are adapted to the EU harmonised OEL value of 1 ppm already established in Directive (EU) 2017/398. Please be aware that the DMEL derived for professionals is significantly lower than the OEL given in Directive (EU) 2017/2398: 3 ppb versus 1 ppm.

**Ethylene Oxide Sterilisation Process – Overview**

Industrial ethylene oxide (EtO) sterilisation is performed at dedicated plants by specialised and highly trained staff working to clearly defined and rigorously enforced procedures to ensure that the potential for EtO exposure is minimised and within workplace limits. The process involves a high degree of automation with continuous monitoring of airborne EtO concentrations and control of atmospheric emissions. Industrial units typically contain several sterilisation chambers. Chamber sizes vary from those capable of handling between one and 32 pallets (100 $m^3$). Plant designs vary; the design of a typical plant, process description, associated tasks and risk management measures (RMMs) are described in this overview.

Facilities are generally split into two (non-sterilised and sterilised) areas, as indicated below.




**Non-sterilised side↑** **↑ Sterilised side**

Figure 1 Typical industrial EtO sterilisation plant

Effective sterilisation with ethylene oxide is product-specific and dependent on conditions of heat, moisture, gas concentration and time. Each cycle requires validation of sterilisation efficiency using a process challenge device (PCD) containing a biological indicator, placed within the product load at a location where the required sterilisation conditions are most difficult to achieve. Internal PCDs, usually medical products or devices selected by the manufacturer as being difficult to sterilise based on design and composition, are used for validation. External PCDs are placed external to the product load during routine processing to facilitate retrieval after processing.

The process consists of three main stages:

- Pre-conditioning, where product loads are preheated and humidified to predefined levels, ensuring a consistent sterilisation process regardless of pre-processing storage conditions
- Sterilisation, during which the products are exposed to concentrations of ethylene oxide under defined conditions of temperature, moisture and time in order to achieve sterility.
- Aeration, allowing the accelerated off-gassing of sterilised products, facilitating the containment of emissions and ensuring that product residuals meet specified limits.

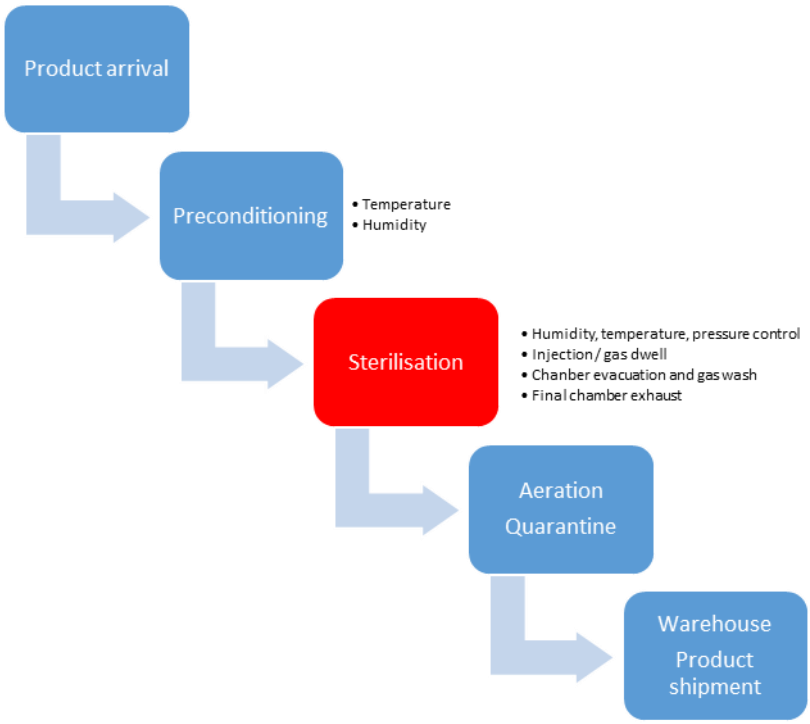



Figure 2 Overview of the ethylene oxide sterilisation process

**Details of the ethylene oxide sterilisation process**

**1. Preconditioning**

The EtO sterilisation processes require preconditioning of the products to be sterilised, a procedure which is performed outside the sterilisation chamber. Preconditioning typically takes between 12-72 hours and is carried out in dedicated preconditioning cells with the same product capacity as the sterilisation chamber, specifically designed to heat and humidify the products to achieve a stable internal temperature (typically 45-55°C) and humidity levels (typically 65% RH) prior to them entering the sterilisation chamber. This process assures that sterilisation is reproducible, regardless of external influences such as climatic conditions.

| | |
|---|---|
| **Associated tasks:** | Loading pallets of product into the preconditioning room (forklift) |
| **Exposure potential:** | There is no potential for exposure to ethylene oxide during preconditioning due to plant design |
| **RMMs:** | No specific RMMs are required |

Once the preconditioning phase is complete, the products are placed in a heated chamber designed to withstand the pressures involved in the sterilisation process (the sterilisation chamber).

| | |
|---|---|
| **Associated tasks:** | Pallets of product are transferred into the sterilisation chamber by hand pallet jack or (more typically in modern plants) by automated loader. The sterilisation chamber is sealed and prepared for sterilisation. |
| **Exposure potential:** | Where there is no conveyor in place, operators enter the sterilisation chamber for loading. In these cases, operators have to follow specific work instructions that define operational conditions, depending on the residual EtO concentration in the sterilisation |

| | |
|---|---|
| | chamber. Routine access to the sterilisation chamber does not occur when a conveyor is in place. |
| **RMMs:** | Where manual loading is required, the use of PPE depends on the levels of EtO in the exposure chamber, which are continually monitored. Levels of EtO are also indicated by warning lights. The use of a self-contained respirator and protective clothing is mandatory where the EtO concentration exceeds 5 ppm (indicated by a red warning light). For concentrations less than 5 ppm (indicated by an orange warning light), operators must use protective clothing and carry a portable detector. There is continuous monitoring of EtO in the vicinity of the sterilisation chamber, linked to an alarm system. |

## 2. Sterilisation

*a) Initial evacuation*

Ethylene oxide sterilisation typically takes 6-15 hours and depends on the removal of at least 97% of the air from the sterilisation chamber by applying a vacuum and performing a series of flushes with nitrogen. The number of inert flushes is dependent on the depth of the vacuum and increment of inert gas used in the flushes.

*i. Deep vacuum process*

The vacuum rate is selected to maintain package integrity by allowing the air trapped inside the package to vent without destroying the seal integrity. This ensures that the sterile barrier properties of the packages are maintained, thereby protecting the sterility of the product once the process is complete.

*ii. Shallow vacuum process*

Some products are not able to withstand a deep vacuum or high pressure; an alternative process is therefore required to avoid damage to the products and/or packaging. For pressure-sensitive products, the shallow vacuum (nitrogen soft cycle) is utilised. In this process, an initial shallow vacuum is followed by an injection of nitrogen (nitrogen wash). This process is repeated several times to ensure the adequate removal of air from the sterilisation vessel. The process is less demanding on packaging seal integrity but adds time to the overall process as additional nitrogen washes are required to remove the EtO.

| | |
|---|---|
| **Associated tasks:** | Initiation, monitoring and control of the sterilisation process (Controlled remotely from a control room using a process computer system programmed for the specific sterilization cycle) |
| **Exposure potential:** | There is no potential for workers to be exposed to ethylene oxide during the sterilisation process due to plant design. The integrity of the chamber is checked prior to gassing. |
| **RMMs:** | Continuous monitoring of EtO levels in the vicinity of the sterilisation chamber, linked to an audible/visible alarm system |

*b) Humidification*

During the previous preconditioning step, heat and moisture were added to the product to predetermined levels. During the initial evacuation phase of the sterilisation process, however, the product may lose a significant amount of moisture which must be replaced prior to introducing the ethylene oxide. This is accomplished by adding humidity in the form of steam injections (while exercising care to avoid overheating), calculated to result in a predetermined relative humidity level. Following the addition of steam, the products are allowed to dwell for the amount of time required to replace the moisture lost during the evacuation phase.

| | |
|---|---|
| **Associated tasks:** | Monitoring and control of the sterilisation process (controlled remotely from a control room using a process computer system programmed for the specific sterilization cycle) |
| **Exposure potential:** | There is no potential for workers to be exposed to ethylene oxide during the sterilisation process due to plant design |
| **RMMs:** | Continuous monitoring of EtO levels in the vicinity of the sterilisation chamber, linked to an audible/visible alarm system |

*c) Gas injection and dwell*

After the humidification phase, liquid ethylene oxide is heated into a gaseous phase and then injected into the sterilisation chamber, using a closed system. The amount of gas required is calculated to attain a concentration () sufficient to achieve product sterility while avoiding high post-sterilisation residual levels. The following application rate, i.e. the concentration of ethylene oxide in the sterilisation chamber, has been substantiated by two ISO 11135:2014 studies validating the efficacy of specific ethylene oxide sterilisation processes: 300 mg/L (based on approx. mean concentration from the two studies). Please note that other application rates may be used, as long as they are sufficiently validated according to ISO 11135:2014. The industry is working towards lowering the treatment concentration of EtO. Quantities are checked prior to injection to ensure the sufficient EtO is available for the sterilisation cycle. Following injection, the product is exposed to predetermined levels of ethylene oxide, heat and humidity for a predetermined amount of time (dwell). Exposure time is determined by careful analysis of the product, load configuration and desired level of sterility. A computerised process control system continually monitors and controls the sterilisation process.

| | |
|---|---|
| **Associated tasks:** | Monitoring and control of the sterilisation process (controlled remotely from a control room using a process computer system programmed for the specific sterilization cycle |
| **Exposure potential:** | There is no potential for workers to be exposed to ethylene oxide during the sterilisation process due to plant design |
| **RMMs:** | Continuous monitoring of EtO levels in the vicinity of the sterilisation chamber, linked to an audible/visible alarm system. Primary controls include chamber pressure drop tests and process control systems supported by LEL and ppm detection for EtO and $O_2$ depletion. |

*d) Post-exposure gas purge and wash phase*

After the exposure phase, ethylene oxide gas is removed from the sterilisation chamber. After initial evacuation of EtO from the chamber, a series of post exposure washes to remove all EtO from the chamber as well as reducing product residuals to defined levels takes place. The number of post exposure washes will be dependent on the depth and increment of inert gas used in each wash, with deep vacuum being more effective in the removal of EtO than shallow vacuum washes. All post exposure evacuations from the chamber are sent to an emission control system prior to venting to the external atmosphere.

| | |
|---|---|
| **Associated tasks:** | Monitoring and control of the sterilisation process (controlled remotely from a control room using a process computer system programmed for the specific sterilization cycle |
| **Exposure potential:** | There is no potential for workers to be exposed to ethylene oxide during the sterilisation process due to plant design |
| **RMMs:** | Continuous monitoring of EtO levels in the vicinity of the sterilisation chamber, linked to an alarm system<br>Obligatory use of e.g. positive pressure self-contained breathing apparatus and protective clothing for manual unloading of the sterilisation chamber. The use of conveyors, workplace transport and automation minimise the handling of products in the post-sterilisation phase.<br>Ethylene oxide is vented from the exposure chamber to the atmosphere via comprehensive treatment / abatement systems (detailed below), to ensure compliance with local emission regulations. |

## 3 Aeration

Following sterilisation, pallets of products are usually placed in a heated room (close to sterilisation temperature) with high volume air changes for approximately 12 hours to allow additional removal of residual gas (primary aeration). The amount of time required for this stage is a factor of the product material composition and the intended use. On completion of the primary aeration phase, products may be transferred to a secondary aeration room, where an optional additional period of storage (quarantine) under increased temperature and ventilation can also be used to further reduce residual ethylene oxide levels, if required. Products typically remain in secondary degassing from 24/48 hours to several days. Unprotected access to these areas is not permitted. After aeration is complete, pallets of product are moved to the warehouse for storage until release.

| | |
|---|---|
| **Associated tasks:** | Pallets of product are transferred out of the sterilisation chamber by hand pallet jack or (more typically in modern plants) by automated loader. The sterilisation chamber is sealed and prepared for sterilisation. |
| **Exposure potential:** | Where there is no conveyor, operators enter the sterilisation chamber for unloading. In these cases, operators have to follow |

| | |
|---|---|
| | specific work instructions that define operational conditions, depending on the EtO concentration in the sterilisation chamber. Routine access to the sterilisation chamber does not occur when a conveyor is in place. |
| **RMMs:** | Where manual unloading is required, PPE is either worn routinely or is triggered by the levels of EtO in the exposure chamber, which are continually monitored. Levels of EtO are also indicated by warning lights. The use of a self-contained respirator and protective clothing is mandatory where the EtO concentration exceeds 5 ppm (indicated by a red light). For concentrations less than 5 ppm (indicated by an orange light), operators must use protective coveralls and carry a portable detector. There is continuous monitoring of EtO in the vicinity of the sterilisation chamber, linked to an alarm system.<br>Ventilation exhaust from the aeration rooms is vented to the external atmosphere via a comprehensive emission abatement system to remove any remaining residual ethylene oxide. |

**Post sterilisation**

The duration of aeration (primary and secondary) is designed to ensure that product EtO residual levels comply with the limits set out in ISO 10993-7. Products are released from aeration once the product EtO residual requirements are met. The product is then stored in the process storage warehouse until the product is picked up for distribution.

| | |
|---|---|
| **Associated tasks:** | Removal of pallets from the aeration chamber / quarantine.<br>Movement of pallets to warehouse & shipment |
| **Exposure potential:** | Potential for exposure when entering the aeration chamber, monitored using a personal detector |
| **RMMs:** | Use of respirator and protective clothing when entering the aeration room |

**Control systems**

*Alarm systems*
EtO levels are continuously monitored and are linked to an alarm system. Sensors are activated when the ethylene oxide concentration exceeds the regulatory limit (typically 1 ppm for more than 15 minutes. All systems are routinely calibrated in line with manufacturer's instructions. Gas detection systems are interlocked to the chamber control systems and initiate shutdown of the sterilisation cycle if the alarm is activated and will put the sterilisers into "safe" condition.

*Emission controls*

Emissions from the sterilisation chamber and the aeration rooms are treated to ensure compliance with local emission limits. The high concentration gas mixture evacuated from the sterilisation chamber after the dwell and gas wash phases is vented to either a catalytic converter or acid scrubber. Catalytic converters are typically used in the EU, while acid

scrubbers are more common in the US. Where sterilisation chamber gases are vented to a catalytic converter, a water balancing tank is used to eliminate peak concentrations. Ethylene oxide dissolves in the water and is then stripped out in a controlled manner and mixed with air containing low EtO concentrations from the aeration rooms. The system is designed and monitored to ensure that EtO concentrations in the mixed air stream remain below the Lower Explosion Limit (LEL) for EtO, before being fed into the catalytic bed and to ensure efficient destruction of the EtO.

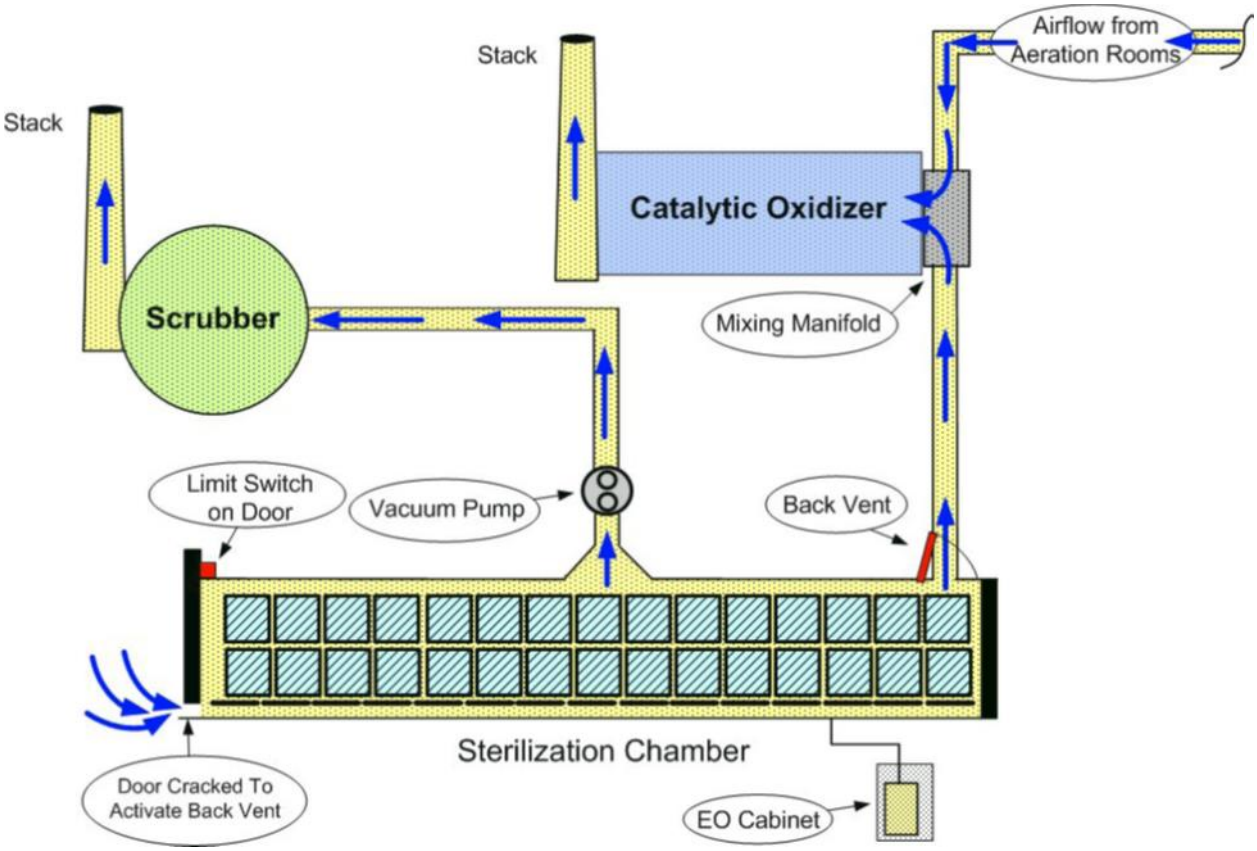



Figure 3 Generic description of an EtO abatement system

**Ancillary tasks**

*Storage, connection / disconnection*

Ethylene oxide is stored in cylinders or drums and is typically housed outside the main sterilisation facility. Drums are handled according to specified safety and quality procedures and are checked for visible damage on arrival at the facility. A leak test is also performed, e.g. by spraying the connections with a leak detection spray. EtO levels in the tank/cylinder area are continuously monitored and are linked to an audible/visible alarm system. The use of a respirator, chemical protective shoes and gloves is specified when connecting / disconnecting drums or cylinders of ethylene oxide.

*Process validation*

Laboratory staff involved in validation activities handle small quantities of treated product; the potential for exposure is therefore limited. The primary laboratory activity is processing the biological indicators/challenge devices. Treated products are removed from the

aeration room with the use of respirator, protective clothing and personal monitor. PCDs are handled in the laboratory under air extraction, laboratory staff carry personal monitors. Laboratory staff are included in the occupational exposure monitoring programme.

*Routine monitoring*

In addition to the constant monitoring of airborne ethylene oxide concentrations in sensitive areas, staff undertaking various tasks associated with a potential for exposure are routinely assessed for exposure using passive (badge) monitors. Furthermore, the use of automated alarm systems in areas of potential exposure ensure the use of positive pressure self-contained breathing apparatus as best practice where EtO levels exceed the workplace limit.

**Monitoring of personnel**

Monitoring of involved personnel is routinely performed in accordance with occupational legislation and should follow the principles given in EN-689:2018.
Some excerpts from information submitted by the applicant is presented below (See Doc. III-B.6.6.). The described routine is specific to one operator and the details in the monitoring programmes may vary among the different operators. The described monitoring routine is, nevertheless, believed to be descriptive for operators in the EU. An example of the internal monitoring routine of one EtO operator is presented in the confidential folder (HS-15.3-SWP-01 Occupational Exposure Monitoring -EtO-CONFIDENTIAL).

Personal sampling and area monitoring play an important part in managing and monitoring occupational exposure. Personal sampling is required to demonstrate compliance with the relevant occupational exposure limit. Area monitoring data supports sampling programmes and is critical in the early identification of ambient EtO levels which may have an impact on employee health.

Monitoring data is reviewed and used to support the assessment of whether existing collective and engineering controls are sufficient and whether any further control measures, other than respiratory protective equipment (RPE) and PPE, are required.

The occupational exposure monitoring programme for ethylene oxide requires both passive and active personal sampling to be carried out at all EtO sterilisation plants and laboratories where biological indicators, EtO residual testing and other analysis of devices sterilised by EtO are carried out. Where local regulations require blood or urine analysis or other health monitoring these requirements shall also be met.

*Passive sampling programme*

Passive sampling using diffusion monitoring badges is performed routinely. Sampling is based on an 8-hour monitoring period with a minimum monitoring duration of 6 hours, covering normal work activities. At the end of each monitoring period, the badges are re-capped and sent for independent laboratory analysis. Full details of the individual and monitoring period are also provided to the laboratory to allow accurate calculation of the corresponding 8-hour TWA. In the event that a monitoring result exceeds the applicable OEL, this exceedance would be reported under internal incident reporting procedures and subsequently investigated. Risk mitigation measures are applied in a strict hierarchy including using other sterilisation technologies where possible, reducing the amount of EtO used per cycle, containment, local extract ventilation, EtO abatement and collective controls including ventilation. Within controlled areas, where EtO levels may exceed safe limits, suitable respiratory protective equipment (RPE) and PPE is used as a last resort.

*Active sampling programme*

While passive sampling is indicative, active sampling provides a more accurate measure of exposure and is therefore an important part of the overall exposure monitoring programme and ongoing analysis of results.

Active personal sampling:

Active sampling uses a flow controlled, rechargeable pump. For personal sampling the pumps are typically attached to a belt with a tube passing to a sampling head attached on the worker's lapel, so it draws sample air in from within the breathing zone. A known volume of air is drawn through a sampling media allowing the time weighted exposure to be analysed

*Active sampling procedure*

Active exposure monitoring sampling must be carried out and supervised by a competent occupational hygiene consultant, who should supply the sampling equipment, monitor the sampling and report the results.

When appointing an occupational hygienist, the facilities should ensure that the consultant can provide sampling to an appropriate standard.

The report issued by the consultant should include their interpretation of the results for each employee.

*Sampling duration*

Occupational exposure is measured as an 8-hour time weighted average (TWA). Therefore, where possible sampling, both passive and active, should be carried out for each individual for 8 hours or full shift. However, an 8-hour TWA may be extrapolated from representative measurements taken over shorter time periods, the minimum acceptable sampling period is 6 hours if that time is representative of the normal working day and includes all the normal activities expected of the employee.

To ensure exposure monitoring results are accurate, it is essential that detailed time records are kept during sampling events. This will include start and end times, as well as times spent in controlled areas.

*Short term exposure level monitoring*

Short-term exposure monitoring, over a period of 15 minutes, may be required where there is a specific concern relating to potential EtO exposure connected with changing connecting and disconnecting EtO cylinders, retrieving BI's, loading and unloading steriliser chambers, and carrying out maintenance tasks. Short-term monitoring should be agreed with the HSSE team.

*Biological Monitoring*

Biological monitoring of employees for EtO exposure, normally by testing blood haemoglobin, is required by some regulatory authorities. Where biological monitoring is provided, this is in addition to and does not replace the requirement to carry out passive and active occupational exposure monitoring as detailed above. Research is ongoing in to reliable and effective biological monitoring for EtO exposure

## B.3.2. List of scenarios

Due to the highly specialised use of Ethylene oxide (EtO) and the high level of RMM applied at the treatment facilities, exposure to industrial workers involved in EtO disinfection is not possible to model in a realistic way through the use of the existing exposure models. Monitoring of exposure to personnel at EtO disinfection plants is mandatory under occupational law (EN 689:2018), the exposure assessment was therefore performed using submitted monitoring data from a number of EtO disinfection plants in several European States.

The identified work tasks involved in the sterilisation process are listed as scenarios but have not been assessed individually.

Table B.5 Summary table: Exposure scenarios

| Scenario number | Scenario (e.g. mixing/ loading) | Primary or secondary exposure Description of scenario | Exposed group (e.g. professionals, non-professionals, bystanders) |
|---|---|---|---|
| **1.** | Pre-treatment | Transfer of products into the pre-treatment area | **Industrial** |
| **2.** | Mixing and loading | Loading of products into the treatment tank | **Industrial** |
| **3.** | Sterilisation | Sterilisation | **Industrial** |
| **4.** | Post treatment | Transfer of the treated products from the treatment tank to the aeration chamber | **Industrial** |
| **5.** | Post treatment | Warehouse | **Industrial** |

## B.3.3. Industrial exposure

The main biocidal use of EtO is sterilisation of heat labile medical equipment in large highly specialised industrial disinfection plants. The EtO treatment units typically contain several EtO chambers which can vary considerably in size.

Engineering controls restrict the exposure to workers who are involved in the process and also any workers in the same or adjacent areas who are not directly involved in the sterilisation process. The process is highly automated, and continuous monitoring of airborne EtO concentrations coupled to audible and visual alarms is performed. Monitoring of worker exposure is mandatory and is regularly performed.

Occupational Exposure Limits:

The current occupational exposure limit (OEL) for EtO is defined in directive (EU) 2017/2398 on *the protection of workers from the risks related to exposure to carcinogens or mutagens at work*, which entered into force 17 January 2020. Directive (EU) 2017/2398 defines a maximum OEL for EtO of 1.8 mg/m$^3$, equivalent to 1 ppm, but more stringent binding OELs can be set nationally by individual Member States.

Prior to this Directive entering into force, the OEL-values for EtO were set nationally. Most

European states operated with an OEL value of 1 ppm. Some States operated with a more stringent OEL-value of 0.5 ppm, while others again had set a higher OEL value of 5 ppm.

Some member states have also defined a 15 minutes short term exposure limit (STEL) which varies between 2 and 5 ppm.
Unless local regulations specify a stricter requirement, sterilisation sites will work to a standard STEL of 5 ppm.

*Exposure to industrial workers*

Exposure to personnel employed at EtO sterilisation plants is not possible to model, due to the highly specialised OC and the employed RMM. The applicant has submitted a data set of exposure data from external audits employing active exposure monitoring to assess the exposure. The measurements originate from four EtO disinfection plants located in different European Member States.

The operator of these plants had agreed to an internal policy to follow a maximum occupational exposure limit of 1 PPM as its regional standard to be followed, unless local regulations require a stricter limit (this was relevant prior to the entry into force of directive (EU) 2017/2398). One of its plants is situated in a Member State with a national OEL of 0.5 ppm, or 0.84 mg/m$^3$. Recent comments provided by the applicant (March 2020) indicate that these facilities operate under a slightly different (and possibly more stringent) operating condition than the general description presented in B.2.1. This also implies that the submitted data must be regarded to represent a realistic best-case scenario. The data do present an example of the exposure situation at plants subject to OELs of both 1 ppm and 0.5 ppm and the operator's ability to comply with these exposure limits.
The complete data set can be found in Doc. III-B.6.6., and some key statistics are tabled below.

Table B.6 Exposure monitoring statistics

| **Substance identity** | |
|---|---|
| CAS no. | 75-21-8 |
| IUPAC name | Oxirane/ Ethylene oxide |
| | |
| **Overall descriptive statistics** | |
| Number of measured values | 49 |
| Arithmetic mean | 0.190 ppm (0.343 mg/m$^3$) |
| Standard deviation | 0.21 ppm (0.38 mg/m$^3$) |
| Max-value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 3** | |
| Number of measured values | 15 |
| National OEL-value | 0.5 ppm (0.84 mg/m$^3$) |
| Arithmetic mean | 0.018 PPM (0.033 mg/m$^3$) |
| Standard deviation | 0.0054 ppm (0.0097 mg/m$^3$) |
| Max. value | 0.0285 ppm (0.051 mg/m$^3$) |

| | |
|---|---|
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 1, 2 and 6** | |
| Number of measured values | 34 |
| OEL-value | 1 ppm (1.8 mg/m$^3$) |
| Arithmetic mean | 0.266 ppm (0.48 mg/m$^3$) |
| Standard deviation | 0.214 ppm (0.38 mg/m$^3$) |
| Max. value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.055 ppm (0.1 mg/m$^3$) |
| | |
| **Overall distribution:** | |
| 10% Percentile | 0.014 ppm (0.025 mg/m$^3$) |
| 50% Percentile | 0.11 ppm (0.21 mg/m$^3$) |
| 75% Percentile | 0.278 ppm (0.5 mg/m$^3$) |
| 90% Percentile | 0.5 ppm (0.91 mg/m$^3$) |
| 95% Percentile | 0.8 ppm (1.45 mg/m$^3$) |

## B.3.4. Professional exposure

Only industrial disinfection with ethylene oxide has been assessed.

### B.3.4.1 Scenario [n]

Not applicable

### B.3.4.2 Combined scenarios

Not applicable

## B.3.5. Non-Professional exposure

Ethylene oxide is not used by non-professionals

### B.3.5.1 Scenario [n]

Not applicable

### B.3.5.2 Combined scenarios

Not applicable

## B.3.6. Secondary exposure of the general public excluding dietary exposure

### Secondary exposure from atmospheric emissions

Ethylene oxide from the treatment chamber and from the aeration rooms is exhausted to the atmosphere via a catalytic converter which converts ethylene oxide to carbon dioxide and water, typically with efficiency greater than 99.9% or, in a relatively small number of cases, via acid scrubbers which convert the ethylene oxide to ethylene glycol, with efficiency typically in the range of 99.5% to 99.9%. Atmospheric emissions of ethylene oxide in Europe are strictly regulated with maximum permissible concentrations in exhaust gas set by individual Member States. A $PEClocal_{air}$ has been calculated to be 0.0001251 $mg/m^3$ (equivalent to 0.068 ppb) as an average air concentration 100 m from the source of emission (chapter 0). It could be discussed whether this value should be adjusted to take into account the long half-life of EtO in air and the potential for local accumulation near a source (B.4.3). This distance is considered to represent the average distance between the emission source and the border of the industrial site.

There are several publications on monitoring of air concentrations of EtO in different areas, mainly from the US (See monitoring reports referred to in A.4.1.4). The measured EtO levels vary significantly, both those reported to be background levels and those reported to be caused by emission from sterilisation plants. One of the publications, Olaguer et al 2020, reports an urban background concentration in Michigan of 0.247 $\mu g/m^3$ (0.14 ppb). The estimated peak 24h exposure to EtO caused by the sterilisation facility in a nearby residential neighbourhood across the street from the sterilisation facility was reported to be 2.08 $\mu g/m^3$ (1.15 ppb) (1.83 $\mu g/m^3$ (1 ppb) when the presumed background level is subtracted).

Halik, T. 2018 reports monitoring data from the Village of Willowbrook, Illinois in the US, where a Sterigenics sterilisation facility is located. EtO levels in air as high as 0.45 ppb inside the Willowbrook police department and 0.25 ppb in a classroom in a nearby middle school is reported. Halik reports the background concentration of EtO upwind from the sterilisation plant to be 0.07 ppb.

Some of the monitoring data exceed the calculated $PEClocal_{air}$ referred to above. Although significant variation is seen in the reported monitoring data, both with regards to the possible atmospheric background level of EtO and to the air concentrations caused by emissions from EtO sterilisation plants, these reports clearly indicate that EtO emissions from treatment plants should be considered, particularly where the treatment plants are located close to residential areas or in urban areas.

The eCA considers that since the currently available monitoring data is from the US, and not from within the EU, and since there could be high local variations in EtO air concentrations, frequent monitoring of the air outside of each EU EtO sterilisation facility would give the most reliable information as to whether the emissions are within an acceptable range or not. No monitoring data (measured air concentrations of EtO) from the surroundings of European sterilisation plants has been submitted.

### Secondary exposure from contact with sterilised products

The primary use of EtO is industrial disinfection of single use medical equipment before these are placed on the market. Once placed on the market, possible release of EtO from the medical device will not be regulated by the BPR, but by the Medical Device Regulation (MDR, (EU) 2017/745).

The accepted residual levels, and thus, secondary exposure of EtO and ethylene chlorohydrin (ECH) under MDR is defined in the ISO-standard ISO 10993-7:2008 (CEN 2008). Secondary exposure to the general public from medical devise was therefore not

further assessed.

The limits defined in ISO 10993-7 and the measures taken by the users to comply with these, are briefly described for information only:

The use of EtO to sterilise medical devices may result in residues being present in the sterilised device. EtO may also react to form ethylene chlorohydrin (ECH) in PVC products, or with water residues to form ethylene glycol (EG). Ethylene oxide, ethylene chlorohydrin and ethylene glycol have known health effects. When determining the suitability of EtO for the sterilisation of medical devices, it is important to ensure that the levels of residual EtO, ECH and EG pose minimal risks to the patient following normal use of the sterilised product.

The potential for the secondary exposure to EtO is managed through the effective aeration of sterile products and management of post-sterilisation logistics and storage. It is important to differentiate between residual levels and post-sterilisation fugitive emissions; the former being strictly controlled by relevant standards and is routinely analysed.

ISO 10993-7:2008 specifies limits for EtO and ECH sterilisation residuals for medical devices. Maximum residue levels are dependent on the type of device defined by the exposure category specified in ISO 10993-1 (limited exposure, prolonged exposure or permanent contact). Other limits for EtO and ECH also apply to specific types of medical device.

No device limits are specified for EG because a risk assessment indicates that the levels likely to occur in a medical device are lower than those potentially causing health effects. While the potential exists for acute haemodynamic and haemolytic effects to occur following rapid intravenous administration of hyperosmolar compounds like EG, the ethylene oxide sterilization of medical devices would not be expected to produce hyperosmolar solutions.

Table B.7 Residual limits for EtO and ECH (ISO 10993-7)

| **Device exposure category*** | | **Exposure time** | **Residual limits** | |
|---|---|---|---|---|
| | | | **EtO** | **ECH** |
| **A** | Limited exposure device | ≤24 h | The average daily dose shall not exceed 4 mg. | The average daily dose shall not exceed 9mg. |
| **B** | Prolonged exposure device | > 24 h - ≤ 30 d | The average daily dose shall not exceed 2 mg/d.<br>The maximum EtO dose shall not exceed:<br>- 4 mg in the first 24 h.<br>- 60 mg in the first 30 days | The average daily dose shall not exceed 2 mg/d.<br>The maximum ECH dose shall not exceed:<br>- 9 mg in the first 24 h.<br>- 60 mg in the first 30 days |
| **C** | Permanent exposure device | > 30 d | The average daily dose shall not exceed 0.1 mg/d.<br>The maximum EtO dose shall not exceed:<br>- 4 mg in the first 24 h.<br>- 60 mg in the first 30 days.<br>- 2.5g lifetime. | The average daily dose shall not exceed 0.4 mg/d.<br>The maximum ECH dose shall not exceed:<br>- 9 mg in the first 24 h.<br>- 60 mg in the first 30 days.<br>- 10 g lifetime. |

*defined in ISO 10993-1

Local effects (e.g. irritation) have also been considered and are incorporated into the tolerable contact limits (TCL) for EtO and ECH for surface-contacting devices and implants. Values are designed to prevent localised irritation due to EtO or ECH released from the device.

Table B.8 Tolerable contact limits for EtO and ECH (ISO 10993-7)

| **Substance** | **Tolerable contact limits (TCL)** (surface of the patient-device interface) | |
|---|---|---|
| **EtO** | ≤ 10µg/cm$^2$ | Or negligible irritation as specified in ISO 10993-10 |
| **ECH** | ≤ 5mg/cm$^2$ | Or negligible irritation as specified in ISO 10993-10 |
| Specific limits are further defined for a number of special devices in ISO 10993-7 | | |

As described above, limits on the residues of EtO and ECH are specific to the type of product. In practice, for most devices, exposure to EtO and ECH is considerably lower than the maximum values specified in ISO 10993-7. Following sterilisation, aeration of the sterilised medical devices ensures that airborne EtO concentrations and residual levels of EtO and ECH are within acceptable levels. Aeration is performed either in the sterilisation chamber and/or in a separate aeration chamber and is controlled to ensure the efficient and reproducible removal of EtO residues until predetermined levels are reached. Facilities sterilising medical devices guarantee compliance with ISO 10993-7 standards. Product batches are not released from the sterilisation facilities until they meet the requirements listed above, based on predetermined post-sterilisation (aeration/quarantine) times and conditions defined by experimental dissipation curves. Products are monitored to ensure continued compliance with residual limits.

### B.3.6.1 Combined scenarios

Not applicable

## B.3.7. Dietary exposure

Not applicable

### B.3.7.1 List of scenarios

Not applicable

### B.3.7.2 Information of non-biocidal use of the active substance

EtO is a substance which is used in high volumes (3.4 mill tonnes in 2011) in polymer industry in Europe. This use is not within the scope of BPR and was therefore not addressed within this CAR as other legislation applies.

### B.3.7.3 Estimating Livestock Exposure to Active Substances used in Biocidal Products

Not applicable

### B.3.7.4 Estimating transfer of biocidal active substances into foods as a result of professional and/or industrial application(s)

Not applicable

### B.3.7.5 Estimating transfer of biocidal active substances into foods as a result of non-professional use

Not applicable

## B.3.8. Exposure associated with production, formulation and disposal of the biocidal product

Manufacture of the active substance, formulation of the product and their packaging are not within the scope of BPR and therefore was addressed within this CAR as other legislation applies.

### B.3.8.1 Combined scenarios

Not applicable

## B.3.9. Combined residential scenarios

Not applicable

## B.4. Environmental exposure assessment

Table B.9 General information on the environmental exposure assessment

| General information | |
|---|---|
| Assessed PT | PT 2 |
| Assessed scenarios | Quantitative exposure assessment of the atmospheric compartment (calculation of $PECloca l_{air}$ in accordance with the Guidance on BPR, vol. IV Part B). Qualitative assessment of possibly indirectly exposed compartments, i.e. discussion of the possible subsequent indirect release surface water and soil, based on physical-chemical properties. |
| ESD(s) used | No ESD has been followed. Ethylene oxide is specifically mentioned in the ESD for PT 2 (van der Poel, 2001). It is stated that sterilisation with ethylene oxide is not covered by an emission scenario, since it is only applied with extreme care and is bound to strict statutory regulations. |
| Approach | Description of the fate and behaviour of ethylene oxide upon entry to the atmosphere, based on the calculated $PECloca l_{air}$ together with available data on the substance's physical chemical properties, environmental degradation and distribution. |
| Distribution in the environment | Investigated based on submitted data and QSAR estimations. |
| Groundwater simulation | No groundwater assessment performed. It is not expected that ethylene oxide will reach groundwater from the intended use covered in this CAR. |
| Lifecycle steps assessed | Production/formulation: No. Ethylene oxide for use as a gaseous sterilising agent is produced on a small scale by specialist manufacturers only. Taking into account the overall limited release of ethylene oxide to the environment from all life cycle steps, the contribution from the production/formulation phase is considered irrelevant for the assessment.<br><br>Use: Yes, qualitatively assessed.<br><br>Service life: No. Releases after the biocidal sterilisation step, i.e. when the single use medical devices are available on the market, are not covered by the BPR. |
| Remarks | The agreements from the early WG discussion of ethylene oxide at WG-I-2018 has been used as guiding principles for the qualitative exposure assessment. |

### General description of the potential for release to the environment

For a description of the intended use of ethylene oxide as a biocide in PT2, as well as potential exposure pathways, please see the heading "Intended use and foreseen exposure pathways" in chapter A.4.

To sum up, the atmosphere is the only compartment which is directly exposed. Although indirect exposure of ethylene oxide to the aquatic and terrestrial environment could potentially happen due deposition from air, this is highly unlikely. The high vapour pressure (146 kPa at 20 °C) makes volatilisation a highly relevant removal pathway. As discussed in chapter A.4, ethylene oxide is highly water soluble and could hence be expected to wash out from the atmosphere via precipitation. However, according to the available information, the rapid volatilisation rate will counteract this potential, leading to a negligible deposition or partitioning to water or soil from the atmosphere. It is furthermore mentioned in the Technical agreements for Biocides (ECHA, 2019) that a risk assessment of subsequent compartments after emission to air is not relevant for highly volatile substances. Even though this entry refers to small scale use, the same physico-chemical principles are likely to be of relevance also for other uses of highly volatile substances.

## B.4.1. Emission estimation

### B.4.1.1 Industrial sterilisation

There is only one emission scenario to address in this risk assessment, and that is the emission from industrial use of ethylene oxide as a gaseous sterilant.

As mentioned in the ESD for PT 2 (van der Poel, 2001), sterilisation with ethylene oxide is only applied to a limited number of objects, and with extreme care following strict statutory regulations. This process is therefore not covered by an emission scenario in the ESD.

Nevertheless, the local emission from a sterilisation unit (daily emission, $Elocal_{air}$) and a $PEClocal_{air}$ at 100 m from the source of emission has been calculated based on information provided by the applicant considered as representative of a realistic worst case sterilisation facility. The potential for indirect exposure to other environmental compartments based on the $PEClocal_{air}$ is also discussed.

**Realistic worst case numbers for the $Elocal_{air}$ calculation**

According to company data for ethylene oxide emission from a high capacity sterilisation plant with emissions to air via a catalytic converter, approximately 400 kg of ethylene oxide per day is exhausted to the catalytic emission control system from the sterilisation units. A further 50 kg/day is exhausted from the aeration rooms. This gives a total of 450 kg of ethylene oxide per day. Assuming the efficiency of the catalytic converter is 99.9%, approximately 0.45 kg of ethylene oxide is released into the atmosphere per day. Atmospheric emissions from smaller units are expected to be considerably less than this.

According to the applicant, 450 kg ethylene oxide per day entering the catalytic converter system is a worst case assumption. Furthermore, it has been communicated from the applicant that for the high capacity facilities, a catalytic converter efficiency of 99.9 % can be assumed, whereas amongst lower capacity facilities (i.e. facilities using less ethylene oxide per day) a lower efficiency could be in place, e.g. 99.5 %.

In some European countries, national restrictions are in place (e.g. with requirements for maximum ethylene oxide concentration in the stack/chimney directly prior to release into the surrounding air). It is not clear to the eCA whether an efficiency of 99.9 % is actually necessary to fulfil these national requirements, or if sufficiently low emissions can be achieved also with lower efficiency of the catalytic converter, depending on other factors at the sterilisation facility.

A reduction from 99.9 % to 99.5 % in the catalytic converter efficiency, will increase the $PEClocal_{air}$ 5-fold. On the other hand, a worst case use of ethylene oxide combined with a worst case efficiency of the catalytic converter, might give a $PEClocal_{air}$ which is purely worst case as opposed to realistic worst case.

Based on the above, it was concluded at WG-III-2020 that an $Elocal_{air}$ of 0.45 kg/day can be used as a realistic worst case emission value. The resulting PEC value was also discussed at WG-III-2020, please see chapter B4.3.

Table B.10 Resulting local emission to relevant environmental compartments

| Compartment | Local emission ($Elocal_{air}$) [kg/d] | Remarks |
|---|---|---|
| Air | 0.45 | - |

## B.4.2. Fate and distribution in exposed environmental compartments

As described above and in section A.4, the only directly receiving compartment from the intended use is the atmosphere. Normally, indirect exposure to soil and water based on deposition from air is a relevant route to consider. However, due to ethylene oxide being a highly volatile substance, it is very unlikely that water or soil will be exposed. In the following table, these two compartments are therefore marked with parentheses. No release to STPs occur from the intended use.

A PEC value has therefore only been calculated for air.

Table B.11 Identification of relevant receiving compartments based on the exposure pathway

| Scenario | Fresh-water | Sedi-ment | Sea-water | Sea-water sedi-ment | STP | Air | Soil | Ground-water |
|---|---|---|---|---|---|---|---|---|
| Industrial use of ethylene oxide as a gaseous sterilant | (-) | - | (-) | - | - | + | (-) | - |

Explanation to the table: a plus sign (+) signifies that there is direct exposure to a compartment, a minus sign indicates no exposure, and a minus sign in brackets indicates potential indirect exposure based on the exposure pathway

Table B.12 Input parameters for calculating the fate and distribution in the environment

| Input | Value | Unit | Remarks |
|---|---|---|---|
| Molecular weight | 44.05 | g/mol | - |
| Log Octanol/water partition coefficient (Log $K_{ow}$) | -0.30 | Log 10 | - |
| Organic carbon/water partition coefficient ($K_{oc}$) | 3.237 | L/kg | QSAR estimation, MCI methodology, (KocWin v. 2.00) |
| Henry's Law Constant | 14-20 | $Pa/m^3/mol$ | at 20ºC |
| Vapour pressure | 146 | kPa | at 20ºC |
| Biodegradability (ready / not ready) | Readily bio-degradable | - | Based on an OECD 301C test (MITI, 1995) |
| $DT_{50}$ for biodegradation in sewage | ≈ 20 days | d | Modified BOD test (Conway *et al.,* 1983), value should be considered purely indicative |
| $DT_{50}$ for hydrolysis in surface water (fresh water) | 34.5-40.2 | d | at 12ºC and pH 7.4 |
| $DT_{50}$ for degradation in air | 38-578 | d | Different sources give different results, based on e.g. OH radical concentrations and assumptions of 12-h or 24-h days. Ethylene oxide has the potential to persist for a long time in the atmosphere. |
| Bioconcentration (BCF), aquatic | 3.16 | L/kg | QSAR estimation (BCFBAF v.3.01) |

## B.4.3. Calculated PEC values

The calculations of the concentration of ethylene oxide in the air are based on equations 43-45 in Guidance on the BPR, vol. IV part B. The $Elocal_{air}$ for ethylene oxide, used as a basis for the PEC calculation, is 0.45 kg/d, as described in chapter A.4.1.4 above.

$Clocal_{air} = Elocal_{air} \times Cstd_{air} = 1.25 \times 10^{-4}$ mg/m$^3$

where
$Clocal_{air}$ = local concentration in air during emission episode
$Cstd_{air}$ (concentration in air at source strength of 1 kg/d) = $2.78 \times 10^{-4}$ mg/m$^3$ (default value)
$Elocal_{air}$ (local direct emission rate to air) = 0.45 kg/d

$Clocal_{air,ann} = Clocal_{air} \cdot Temission / 365 = 1.25 \times 10^{-4}$ mg/m$^3$

where
$Clocal_{air,ann}$ (annual average concentration in air 100 m from point source)
Temission = 365 days

$PEClocal_{air,ann} = Clocal_{air,ann} + PECregional_{air} =$ **$1.25 \times 10^{-4}$ mg/m$^3$**

where
$PEClocal_{air,ann}$ (annual average predicted environmental concentration in air)
$PECregional_{air}$ (regional concentration in air) = 0

Table B.13 Summary table on calculated PEC values

| **Scenario** | **$PEClocal_{air}$ [mg/m$^3$]** |
|---|---|
| Industrial use of ethylene oxide as a gaseous sterilant | $1.25 \times 10^{-4}$ |

**Discussion and concerns regarding the $PEClocal_{air}$**

Some of the US monitoring data described in chapter A.4.1.4 exceed the calculated $PEClocal_{air}$. It could be discussed whether a background concentration (i.e. a $PECregional_{air}$) should be added in the calculation of the $PEClocal_{air}$, or whether the $PEClocal_{air}$ in any other ways should take into account the long half-life of ethylene oxide in air and the potential for local accumulation of ethylene oxide near a source, depending on atmospheric conditions (e.g. weather patterns, wind and convection).

The currently available monitoring data is from the US, and not from within the EU. The eCA asked both the applicant and European Member States whether any monitoring data is available from the surroundings of European ethylene oxide sterilisation plants, in order to have a basis of comparison with the estimated $PEClocal_{air}$ and the US monitoring data. No such monitoring data was obtained. However, information on national restrictions on the release of ethylene oxide was received (compiled responses can be found in the table below).

Since there could be high local variations in ethylene oxide air concentrations, and since no sufficient argumentation has been provided which makes it possible to disregard the US monitoring data (and the fact that these in some cases exceed the $PEClocal_{air}$), the eCA considers that monitoring of the air outside of each EU ethylene oxide sterilisation facility would give the most reliable information both as to whether the $PEClocal_{air}$ is representative and protective for the EU, and as to whether the emissions are acceptable or not with regard to the human health risk assessment. The usefulness of EU monitoring data was supported by the environmental WG (WG-III-2020).

Table B.14 Ethylene oxide emissions and monitoring: responses from MS

| MS | Limits for EtO conc. in surrounding areas / exhaust gas | Additional remarks/information given by the MS |
|---|---|---|
| FI | HTP value (Concentrations Known to be Harmful):<br>1 ppm (1,8 mg/m$^3$)<br>link to more information in Finnish<br>*(*swedish version here*)* | In Finland there is at least two sterilisation plant for pharmaceutical products using EtO, products they are using can be found on our Chemicals register.<br>The plants are claiming that the ethylene oxide is used in strictly controlled conditions during sterilization process and the used ethylene oxide is led to a catalytic abatement system which converts ethylene oxide to carbon dioxide and water. |
| LV | None | In Latvia limits are not set for ethylene oxide emissions and the air monitoring is not being performed. Taking into account the available information in Latvia is only one company who uses ethylene oxide for sterilization (emissions in 2018 were 0.001144 tons per year). |
| LT | Limits for EtO in ambient air:<br>– 0.30 mg/m$^3$ of single (or half an hour) pollution,<br>– 0.03 mg/m$^3$ average of one day | Monitoring for EtO is not required according Integrated pollution prevention and control legislation because determined emissions from EtO sterilisation plants are bellow of the established hazard rate of pollutant. |
| NL | Emission limit for exhaust gas:<br>– 1 mg / Nm$^3$ (normal m$^3$)<br>– Mass flow: 2.5 g/h<br><br>MTR (maximum tolerable risk level): 3 µg/m$^3$<br>VR (negligible risk level): 0.03 µg/m$^3$ | Air quality standards for EtO: https://rvs.rivm.nl/<br>The search system is: https://rvszoeksysteem.rivm.nl/<br>*Explanation search: stofnaam ethylene oxide, select lijst stofklassen voor luchtemissies, select Normen, milieu, select lucht (=air)*<br><br>Emission data from all sources are collected: see http://www.emissieregistratie.nl/erpubliek/bumper.en.aspx<br>*Search: emissions -> make your graph or map -> Dutch Substances of Very High Concern -> Ethylene oxide - > show graph* |
| DK | Air emission limit values for EtO in permit of individual facility:<br>< 20 mg/ normal m$^3$ (10 min value)<br>< 2.5 mg / normal m$^3$ (24 hour value)<br><br>National immission limit (max. contribution of EtO from one facility to surrounding air): 0.005 mg/m$^3$ | The numbers are taken from an environmental permit for a sterilisation facility, given in 2019 by a Danish municipality: https://dma.mst.dk/vis-sag/download/e11b77aa-bcf4-41df-a715-c88312f77d00<br><br>National immission limits (Danish B values) – EtO listed on page 33: https://www2.mst.dk/Udgiv/publikationer/2016/08/978-87-93529-02-1.pdf<br>(From the publication: The purpose of B limits is to protect the general public from pollutants.) |
| SK | Emission limit for exhaust gas:<br>– mass flow of 1.5 g / h, or<br>– concentration of 0.5 mg / m$^3$ | The Slovak Republic has one large medical sterilization facility dedicated to the sterilization of medical materials with ethylene oxide.<br><br>Emission of ethylene oxide is in the range of 0.04 – 0.067 t/year. |
| In addition, the eCA found out that the German TA Luft, which also applies in IE and AT, gives these restrictions for EtO:<br>*The following total mass concentrations or mass flows contained in waste gas may not be exceeded as a minimum requirement, even where several substances of one class occur simultaneously: mass flow: 1.5 g/h, or mass concentration: 0.5 mg/m³* | | |

## B.4.4. Primary and Secondary poisoning

As explained in section A.4.1.3, there is no concern for bioaccumulation of ethylene oxide in the environment. The log Kow is -0.30, the estimated $BCF_{fish}$ is 3.16 L/kg and the estimated $BCF_{earthworm}$ is 0.85 L/kg. No further assessment of secondary exposure via the food chain is therefore considered necessary.

# B.5. Assessment of effects on Human Health for the product

## B.5.1. Product(s)

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the human health hazard properties of the product are identical to those of the active substance. Please see section A.3.

## B.5.2. Dermal absorption

Not relevant. Ethylene oxide is a gas at room temperature; and inhalation exposure is considered the most relevant exposure route.

## B.5.3. Acute toxicity

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the acute toxicity of the product is identical to that of the active substance. Please see section A.2. and A.3.

## B.5.4. Sensitisation

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the sensitisation properties of the product is identical to that of the active substance. Please see section A.3

## B.5.5. Corrosion and irritation

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the corrosion and irritation properties of the product is identical to that of the active substance. Please see section A.2. and A.3.

### Serious eye damage and eye irritation

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the eye damage properties of the product is identical to that of the active substance. Please see section A.2. and A.3.

### Respiratory tract irritation

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the respiratory tract irritation properties of the product is identical to that of the active substance. Please see section A.2. and A.3.

### Overall conclusion on corrosion and irritation

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Please see section A.2. and A.3 for an overall conclusion.

## B.5.6. Other

The product, D495 SS, is consisting of pure active substance, without any co-formulants.

## B.6. Environmental effects assessment for the product

The product, D495 SS, is consisting of pure active substance, without any co-formulants. Hence, the environmental assessment of the product is identical to that of the active substance. Please see section A.4.

# C. Risk characterisation of the biocidal product(s)

## C.1. Risk Characterisation for human health

### C.1.1. Critical endpoints

#### C.1.1.1 Systemic effects

Ethylene oxide is classified as acutely toxic by oral and respiratory route (Acute Cat. 3, H301 and H331), corrosive to skin and eyes and irritating to respiratory tract (Skin Corr. 1B, H314, Eye Dam. 1, H318 and STOT SE 3, H335), causing drowsiness or dizziness (STOT SE 3, H336), causing serious damage to health by prolonged or repeated exposure (STOT RE 1, H372), and damaging fertility and the unborn child (Repr. 1B, H360Fd).

However, ethylene oxide is also classified as a Category 1B Carcinogen (Carc. 1B, H350) and a Category 1B Mutagen (Muta. 1B, H340), and there is strong evidence that the carcinogenicity of ethylene oxide, a direct acting alkylating agent, is caused by a genotoxic mechanism with a non-threshold mode of action. Ethylene oxide has been shown to have genotoxic and mutagenic activity in numerous assays in both somatic and germ cells, and in both prokaryotic and eukaryotic organisms. It is generally assumed that in the absence of data to the contrary, an effect-threshold cannot be identified for genotoxic carcinogens exhibiting direct interaction with DNA, i.e. it is not possible to define a no-effect level for carcinogenicity induced by such agents.

IARC (2012) concluded that there is limited evidence in humans of a causal association of ethylene oxide exposure with lymphatic and haematopoietic cancers (specifically lymphoid tumours, i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia), and breast cancer. There is sufficient evidence in experimental animals for the carcinogenicity of ethylene oxide.

The critical endpoint is therefore the genotoxic carcinogenicity of ethylene oxide with a non-threshold mode of action.

In accordance with the BPR Guidance (Volume III Parts B+C, ECHA, 2017a), normally the AELs for appropriate time frames need to be derived for systemic effects. As ethylene oxide is a genotoxic carcinogen with a non-threshold mode of action, exposure to it must be avoided or minimised as far as technically feasible. The derivation of AELs is considered to be not feasible, as based on its non-threshold mode of action no safe levels of exposure can be established for ethylene oxide. Based on this DMEL needs to be established.

#### C.1.1.2 Local effects

Local effects are observed upon exposure to ethylene oxide; however, as the critical effect of ethylene oxide is the genotoxic carcinogenicity with a non-threshold mode of action, no further risk assessment for local effects is considered warranted.

### C.1.1.3 Absorption

Table C.1 Absorption values for different exposure routes

| Route | Study | Test substance and concentration of a.s. | Value |
|---|---|---|---|
| Oral | n.a. | n.a. | n.a. |
| Dermal | n.a. | n.a. | n.a. |
| Inhalation | Filser and Bolt,1984/ Fennell, 2001<br>Fennell, 2001<br>Brugnone et al. 1985, 1986 | EtO 99.7%, ca. 100-1000 ppm, PBPK modelling<br>PBPK modelling<br>Biological monitoring by analysis of alveolar air and blood in workers employed in a hospital sterilizer unit | Rat:40-60 %<br><br>Mice: 40 %<br>Human: 75-80 % |

Ethylene oxide is a gas, and inhalation is the most relevant route of exposure. A pulmonary uptake of 40-60 % for rat, 40 % for mice and 75-80% for humans has been reported (ref: A.3.1.1). Use of these values in PBPK modelling resulted in simulated blood and tissue levels of EtO which were in good agreement with experimental values (Fennell, 2011).

There seems to be difference between the inhalation absorption of EtO in humans and rodents. However, the underlying data are considered not sufficiently consistent or robust to justify a correction of the DMEL value. An inhalation absorption value of 75-80 % was concluded at HH WG III 2020.

## C.1.2. Reference values

### C.1.2.1 Reference values to be used in Risk Characterisation

Table C.2 Reference values

| Studies selected for reference value derivation | | |
|---|---|---|
| **Reference value** | **Study** | **Rationale for selecting the study** |
| DMEL (inhalation) | NTP TR 326 (1987), Mouse inhalation, Long term duration (Comparable to OECD guideline 451) | Mouse lung adenoma/adenocarcinoma is the most sensitive relevant endpoint for human risk assessment. By using the EFSA BMD Webtool a $BMDL_{10}$ calculation for alveolar/broncheolar adenomas and carcinomas in female mice resulted in a value of 35.5 ppm. |

The following DMEL value for professional could be established:

Table C.3 DMEL for professionals

| **Derivation / Identification of the relevant dose descriptor for carcinogenicity** | |
|---|---|
| Based on the results of an inhalational mice carcinogenicity study (whole body exposure) a $BMDL_{10}$ of 35.5 ppm in female mice is assumed. | |
| | **"Linearised approach"** |
| **Relevant dose descriptor** | 35.5 ppm (female mice, inhalation) |
| **Modification of the relevant dose descriptor** | |
| | **"Linearised approach"** |
| Differences between exposure conditions mice (6h/day, 5 days/week) and human (8h/day, 5 days/week) | 6/8 x 5/5 |
| Difference between activity level: at rest (5.3 $m^3$) versus light activity (10 $m^3$) | 5.3/10 |
| Differences between occupational and lifetime exposure conditions | 52/48 x 75/40 |
| Calculation of corrected dose descriptor | 35.5 x 6/8 x 5/5 x 5.3/10 x 52/48 x 75/40 x |
| **Corrected Dose Descriptor** | 28,65 ppm |
| **Application of assessment factors to get the DMEL** | |
| | **"Linearised approach"** |
| Interspecies extrapolation | Not applied (according to the guidance for non-threshold effects) |
| Intraspecies extrapolation | Not applied (according to the guidance for non-threshold effects) |
| High to low dose extrapolation | 10,000 (linearity 1:100,000) |
| Calculation of DMEL for professionals (corrected BMD divided by overall assessment factor) | 28,7 ppm/10,000 = 0.00287 ppm ≈3 ppb |

The following DMEL value for the general public living in the surrounding area to the disinfection plants could be established:

Table C.4 DMEL for general public living in the surrounding area of the plants

| **Derivation / Identification of the relevant dose descriptor for carcinogenicity** | |
|---|---|
| Based on the results of an inhalational mice carcinogenicity study (whole body exposure) a $BMDL_{10}$ of 35.5 ppm in female mice is assumed. | |
| | **"Linearised approach"** |
| **Relevant dose descriptor** | 35.5 ppm (female mice, inhalation) |
| **Modification of the relevant dose descriptor** | |
| | **"Linearised approach"** |
| Differences between exposure conditions mice (6h/day, 5 days/week) and human (24h/day, 7 days/week) | 6/24 x 5/7 |
| Calculation of corrected dose descriptor | 35.5 x 6/24 x 5/7 |
| **Corrected Dose Descriptor** | 6.3 ppm |
| **Application of assessment factors to get the DMEL** | |
| | **"Linearised approach"** |
| Interspecies extrapolation | Not applied (according to the guidance for non-threshold effects) |
| Intraspecies extrapolation | Not applied (according to the guidance for non-threshold effects) |
| High to low dose extrapolation | 100,000 (linearity 1:1 000,000) |
| Calculation of DMEL for general public living in the surrounding area of the plant (corrected BMD divided by overall assessment factor) | 6.3 ppm/100,000 = 0.000063 ppm ≈0.06 ppb |

## C.1.2.2 Uncertainties and assessment factors

The BPR guidance (Volume III Parts B+C, Section 2.4.1.1, ECHA, 2017a) indicates that for substances with non-threshold mode of action, e.g. for mutagens and/or carcinogens, no reference values such as AEL/AEC can be derived, and a semi-quantitative approach should be followed (e.g. Derived Minimal Effect Level, DMEL) where relevant. Different DMEL values can be calculated, representing different risk levels, e.g. an increase of lifetime cancer risk in 1 per 100.000 exposed individuals ($10^{-5}$) or 1 per 1.000.000 exposed individuals ($10^{-6}$).

The REACH guidance on information requirements and chemical safety assessment (Chapter R8) gives further guidance on how to derive a DMEL (ECHA, 2012).

Since DMELs are used in the assessment of risks to humans, human data can in general be used as an appropriate basis for the derivation of DMELs. However, there are specific uncertainties that deserve attention when using human data. These include the influence of bias, confounding from mixed exposures and other risk factors and accuracy of the exposure information. Therefore, care should be taken when using epidemiological or other human data for setting the limit values (i.e. AELs/DMELs), and the use of relevant animal data should also be considered.

*Human data*
The SCOEL report for EtO (SCOEL, 2012) used the available data on human haemato-poietic/lymphatic cancer as a starting point of published quantitative risk assessments. The

estimated occupational ethylene oxide exposure concentrations corresponding to specified extra risks were calculated for lymphoid cancer mortality as the most appropriate endpoint. The lymphoid cancer mortality was chosen based on sensitivity analyses of other mortality endpoints (i.e. all lymphohaematopoietic tissue cancers, leukaemia). It should however be noted that for the endpoint of lymphoid cancer mortality used by SCOEL, there was no statistically significant exposure-response relationship in the study.

An occupational exposure (40 years of occupational inhalation exposure to ethylene oxide from age 20 to age 60 years) to an inhalation concentration of 2.77 ppm ethylene oxide was estimated to result in an extra risk of lymphoid cancer mortality of 4 in 10 000 (0.0004) in the combined worker population of men and women from the UCC and NIOSH studies based on Cox proportional hazards models (Valdez-Flores *et al,* 2011 in SCOEL, 2012). The occupation exposure concentrations derived by SCOEL are presented in Table C.8.

SCOEL indicated that there is a solid database for biological monitoring, based on N7-(2-hydroxyethyl)valine (HOEtVal)-haemoglobin adduct monitoring. Numerical correlations of human ethylene oxide exposure in ambient workplace air with HOEtVal adduct levels have been established by Angerer et al. (1998 in SCOEL, 2012) and by Boogaard et al. (1999 in SCOEL, 2012), based on field studies. According to these two studies, a constant workplace exposure to 1 ppm ethylene oxide results in a steady-state level of 4.0 and of 6.8 nmol HOEtVal per g globin, respectively (Boogaard 2002 in SCOEL, 2012).

An 8-h time weighted average (TWA) of 0.5 ppm corresponds to a HOEtVal level of 3.2 nmol per g globin. In consequence, a TWA of 0.1 ppm would correspond to a biological haemoglobin adduct value of 0.64 nmol (640 pmol) HOEtVal/g globin. Other extrapolated figures are included in the table below. The values given in the table can still be distinguished from the general background value of about 20 pmol HOEtVal/g globin.

Table C.5 Occupational exposure concentrations (TWA; exposure for working lifetime) and haemoglobin adduct values corresponding to specified extra risks of lymphoid cancer mortality for males and females combined, according to the evaluation of Valdez-Flores et al (2011)

| Extra risk | Corresponding TWA (ppm) | Corresponding Hb adducts (nmol HOEtVal/g globin) |
|---|---|---|
| $4 \times 10^{-3}$ | 21.35 | 136.6 |
| $1 \times 10^{-3}$ | 6.58 | 42.1 |
| $4 \times 10^{-4}$ | 2.77 | 17.7 |
| $1 \times 10^{-4}$ | 0.712 | 4.56 |
| $4 \times 10^{-5}$ | 0.286 | 1.83 |
| $1 \times 10^{-5}$ | 0.072 | 0.46 |

The German Ausschuss für Gefahrstoffe (AGS) also evaluated lifetime risk values for ethylene oxide in 2011 (AGS, 2011). They considered the available human data and the previous (2009) SCOEL evaluation. However, AGS indicated e.g. the following drawbacks regarding the use of human data:

- No evidence for a carcinogenic mode of action of EtO in the epidemiological studies
- Inconsistency between individual studies
- Unclear relevant endpoint for the haematopoietic cancer
- Lack of precision in the diagnosis of hematopoietic disorders
- Exposure estimations too inaccurate; lack of knowledge about the relevant exposure measure

This assessment of AGS was supported by the Dutch Health Council assessment (2014). The Dutch Health Council concluded that, while there are many epidemiological studies on the possible carcinogenic effects of ethylene oxide, only in a few studies associations were

found between exposure to ethylene oxide and cancer, specifically lymphoid/haemato-poietic cancers. Moreover, the diagnosis of these types of cancers has changed over the years, making the studies difficult to compare. The mixed exposure of the workers to other carcinogenic chemicals is also a drawback.

Under the REACH regulation a substance evaluation report for EtO was in 2013 finalised by the Competent Authority Austria. The Austrian authorities decided to use animal data for their $BMD_{10}$ calculation. Austria also decided to use the Benchmark dose model released by EPA in the $BMD_{10}$ calculation and the results were in agreement with results obtained by AGS. However, Austria decided to only use the brain tumors seen in rats and alveolar/bronchiolar adenomas/carcinoma seen in mice as a point of departure for the $BMD_{10}$ calculation. The Austrian calculations confirmed the values set by AGS.

In conclusion, there seems to be insufficient epidemiological evidence to conclude on a causal relation between exposure to ethylene oxide and a cancer risk in humans. Therefore, animal data were considered for the derivation of a DMEL.

*Animal data*

The German Ausschuss für Gefahrstoffe (AGS) considered brain tumors, mononuclear cell (MNC) leukemia and mesothelioma of the peritoneum found in rats in the study of Snelling et al., 1984, and lung tumors reported in mice in the NTP study (1987) for the $BMD_{10}$ calculations (AGS, 2011). AGS considered the peritoneal mesothelioma and the MNC leukemia observed in rats of little relevance to humans. The peritoneal mesothelioma was considered rat-specific without a relevant human counterpart, while MNC leukemia was considered as Fischer rat-specific with a high spontaneous incidence. However, because of the genotoxic mechanism of action of ethylene oxide it could not be ruled out that certain rat-specific tumors could be an indication for human tumors at other sites in the body.

The AGS has used a Multistage Model to derive the $BMD_{10}$ values (benchmark dose for a 10% response) for different types of tumors in rats and mice reported in the two studies, based on the reported incidence values (A.3.9). The results are presented in the table below. The brain tumours are considered to be relevant for humans; however, the AGS calculates a rather high $BMD_{10}$ for this type of tumors (>100 ppm). AGS has also indicated that the human relevance of lung tumors observed in mice is not clear, since these tumors were not found in the epidemiological studies, nor in the rat studies with ethylene oxide. However, the lungs are a relevant entry port also for humans. The $BMD_{10}$ for the lung tumors was calculated by AGS to be 19.44 ppm, which was far lower than the $BMD_{10}$ for brain tumors in rats (>100 ppm) and was within the range of the $BMD_{10}$ for mononuclear cell leukemia in female rats (13.6 ppm). The AGS decided to use the lung tumors in mice as a starting point for the derivation of the cancer risk values.

Table C.6 $BMD_{10}$ values for selected tumours calculated by AGS (AGS, 2011)
(*Please note that the conversion factor 1ppm=2 mg/m³ was used in the ASG publication, while 1ppm=1.8 mg/m³ was used in this CAR.)*

| | **$BMD_{10}$[a] (ppm)** |
|---|---|
| **Rat** | |
| Brain tumours, males | 101.50 |
| Brain tumours, females | 120.89 |
| Spleen MNC leukemia, males | 37.68 |
| Spleen MNC leukemia, females | 13.60 |
| Peritoneal mesothelioma, males | 68.10 |
| **Mice** | |
| Alveolar/broncheolar | |
| - adenomas, males | 64.33 |
| - adenomas, females | 54.27 |
| - carcinomas, males | 53.82 |
| - carcinomas, females | 86.87 |
| - adenomas and carcinomas, males | 19.44 |
| - adenomas and carcinomas, females | 43.11 |

[a] $BMD_{10}$: benchmark dose for a 10% response

The Dutch Health Council (2014) also evaluated the cancer risk values. The Council agreed with AGS that there was too much uncertainty in the epidemiological data to use them as a basis for the risk values. The Council considered the AGS reasons for dismissing the mononuclear cell leukemia in rats as not relevant to humans as doubtful; the lung tumours in mice can be considered equally not relevant to humans, as they often develop spontaneously and biologically strongly differ from the lung tumors observed in humans. The calculations by the Council, using the MNC leukemia as a starting point, would result in slightly lower values than calculated by AGS. Nevertheless, the Dutch Health Council proposed to adhere to the AGS risk values, as these values in their opinion will most probably overestimate the cancer risk in humans, considering the lack of clear evidence of the causal relationship between exposure to ethylene oxide and increased cancer risk in epidemiological studies.
The AGS used the $BMD_{10}$ of 19.44 ppm as the starting point and corrected for exposure duration in the study (6 h) compared to 8 h worker exposure, 48 weeks/year and 40 years worker exposure compared to 52 weeks and 75 years for the general population exposure. No correction was made for inhalative volumes in 8 hours under respective conditions (6.7 $m^3$ for base level, and 10 $m^3$ for light activity, ref REACH guidance (ECHA, 2012)).

$BMD_{10}$ = 19.4 ppm (10% response over background)
$hBMD_{10}$ = 29.6 ppm
(corrected for human exposure situation at workplace: 8h/day, 48 weeks, 40 years)

The following risk values were calculated:

Table C.7 TWA based on the German AGS
(*Please note that the conversion factor 1ppm=2 mg/m³ was used in the ASG publication, while 1ppm=1.8 mg/m³ was used in this CAR.)*

| **Extra risk** | **Corresponding TWA (ppm)** |
|---|---|
| $4 \times 10^{-3}$ | 1.18 |
| $1 \times 10^{-3}$ | 0.296 |
| $4 \times 10^{-4}$ | 0.118 |
| $1 \times 10^{-4}$ | 0.0296 |
| $4 \times 10^{-5}$ | 0.0118 |
| $1 \times 10^{-5}$ | 0.00296 |

*Overall*
The SCOEL used the human data to calculate the cancer risk values, whereas the German AGS, Dutch Health Council and the Austrian Competent Authority responsible for Substance evaluation under REACH used the animal cancer data.

The results of SCOEL and AGS is presented in the table below:

Table C.8 TWA values in ppm and mg/m$^3$ calculated based on human and animal data. (*Please note that the conversion factor 1ppm=2 mg/m$^3$ was used in the ASG publication, while 1ppm=1.8 mg/m$^3$ was used in this CAR.)*

| Extra risk | Corresponding TWA | | | |
|---|---|---|---|---|
| | SCOEL (human data)[1] | | AGS (animal data)[2] | |
| | ppm | mg/m$^3$ | ppm | mg/m$^3$ |
| $4 \times 10^{-3}$ | 21.35 | 39.07 | 1.18 | 2.16 |
| $1 \times 10^{-3}$ | 6.58 | 12.041 | 0.295 | 0.540 |
| $4 \times 10^{-4}$ | 2.77 | 5.069 | 0.118 | 0.216 |
| $1 \times 10^{-4}$ | 0.712 | 1.303 | 0.030 | 0.054 |
| $4 \times 10^{-5}$ | 0.286 | 0.523 | 0.0118 | 0.022 |
| $1 \times 10^{-5}$ | 0.072 | 1.318 | 0.003 | 0.005 |

[1] Ref: SCOEL, 2012
[2] Ref: AGS, 2011; Dutch Health Council, 2014, 2014; REACH RMS Austria, 2013

There are some uncertainties about whether human data or animal data should be used as the point of departure for DMEL setting for EtO. The use of animal data resulted in lower values for the cancer risks in humans compared to the use of human data if the human data are interpreted in line with the SCOELs conclusion. The lowest $BMD_{10}$ calculated by AGS for rat spleen MNC leukemia (females) was in the same range as the $BMD_{10}$ for mice alveolar/bronchiolar adenomas and carcinomas (males); i.e. 13.60 ppm (female rat) vs. 19.44 ppm (male mice).

Based on comments received in the RCOM phase the eCA decided to use the EFSA BMD Webtool for the calculation of $BMDL_{10}$ for relevant findings in the carcinogenicity studies. At HH WG III 2020 it was concluded that animal data should be used in the risk assessment of ethylene oxide. Considering that EtO is a proven genotoxic agent with little or no need for metabolites to create adducts, and that lungs is expected to be the first site of contact, it was considered reasonable to settle with the mouse lung adenoma/adenocarcinoma findings as a point of departure for the DMEL setting (findings was considered the most sensitive, relevant endpoint). The $BMDL_{10}$ calculations performed on males only showed a large confidence interval and should therefore not be used, while the $BMDL_{10}$ calculation performed for females provided reliable results. In conclusion, it was agreed at HH WG III 2020 that the $BMDL_{10}$ of 35.5 ppm for alveolar/broncheolar adenomas and carcinomas in female mice from the long-term mouse inhalation study (NTP TR 326, 1987) should be used as the point of departure in the DMEL setting. Please see Appendix VIII where background BMDL-calculations and further explanations are included for the relevant PoD.

The value is corrected for the exposure duration in the study (6 h) compared to 8 h worker exposure. As exposure to the mice was for 5 days a week, no correction was necessary for weekly occupational exposure. However, a correction is made for activity level (rest versus light activity) in agreement with the guidance (ECHA, 2012). A standard respiratory volume for workers during 8 h working day is assumed (10 m$^3$, based on the default value 1.25 m$^3$/h), reflecting a moderate intensity of activity and not including rest and/or sleep periods (ECHA, 2017b). In lack of a specific default value for rest in the BPR guidance, comparison is made with a value of 5.3 m$^3$/8 hours which is based on the default value for long term exposure of 16 m$^3$/24-hour day (ECHA, 2017b).

Furthermore, a correction was made for occupational exposure conditions; i.e. 48 weeks/year and 40 year compared to 52 weeks/year and 75 years for the general population exposure. No correction was considered necessary for respiratory volumes.

As for the general public living in the surrounding area to the disinfection plants a DMEL was established assuming exposure 24 hours 7 days a week according to the REACH R8 guidance (ECHA,2012).

### C.1.2.3 Maximum residue limits or equivalent

Not relevant. Possible residues levels remaining the finalised sterilised medical devices are regulated through Regulation (EU) No 2017/745, which repeals Directive (EC) No 93/42. In addition, no residues of the active substance are expected in food or feedingstuffs.

### C.1.2.4 Specific reference value for groundwater

Not relevant for EtO.

## C.1.3. Industrial uses

Due to the specialised use of EtO, as described in detail in chapter B.3.3, the exposure assessment was performed on the basis of submitted monitoring data of workers at several EtO disinfection plants in Europe. The monitoring data are the result of actual measurements from the active sampling program at the plants and are performed by external occupational hygiene consultants. Please see the description presented in chapter B.3.3. for further detail on the monitoring programs applied at EtO disinfection plants.

Prior to the recent entry into force of directive (EU) 2017/2389 *on the protection of workers from the risks related to exposure to carcinogens or mutagens at work* (entered into force 17 January 2020), the OEL-value for EtO was set nationally in the individual States. Most States operated with an OEL of 1 ppm, some states had decided a more stringent OEL-value of 0.5 ppm, while others again operated with an OEL-value of 5ppm.

The exposure data was submitted by an EtO user who had defined an internal policy to follow an OEL of 1 ppm, unless local regulations required a stricter limit (only relevant prior to the entry into force of directive (EU) 2017/2389). The submitted data includes monitoring data from a plant located in a State with a national OEL of 0.5 ppm (0.84 $mg/m^3$). Recent comments provided by the applicant (March 2020) indicate that these facilities operate under a slightly different (and possibly more stringent) operating condition than the general description presented in chapter B.2.1. As such, the submitted monitoring data represent a best-case scenario, as other operators on the market also may have operated with the higher OEL of 5 ppm applicable in e.g. UK and Ireland. The data does, however, give valuable information about the actual operator exposure levels at EtO plants subject to OELs of both 0.5 ppm and 1 ppm and the operator's ability to comply with these exposure limits.

The duties and working practices of individuals monitored during the sampling surveys are argued by the applicant to reflect normal working activities and, consequently, also reflect normal potential exposure. They also state that the nature and duration of the EtO sterilisation process is highly standardised and consistent across all products; there is therefore very little variation in potential exposure risks from day to day and between different sites.

Key figures and statistics are presented in the table below. The complete data set can be found in Doc III-B.6.6.

Table C.9 Exposure monitoring statistics

| Exposure monitoring statistics | |
|---|---|
| **Substance identity** | |
| CAS no. | 75-21-8 |
| IUPAC name | Oxirane |
| | |
| | |
| **Overall descriptive statistics** | |
| Number of measured values | 49 |
| Arithmetic mean | 0.190 ppm (0.343 mg/m$^3$) |
| Standard deviation | 0.21 ppm (0.38 mg/m$^3$) |
| Max-value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 3** | |
| Number of measured values | 15 |
| National OEL-value | 0.5 ppm (0.84 mg/m$^3$) |
| Arithmetic mean | 0.018 PPM (0.033 mg/m$^3$) |
| Standard deviation | 0.0054 ppm (0.0097 mg/m$^3$) |
| Max. value | 0.0285 ppm (0.051 mg/m$^3$) |
| Min. value | 0.012 ppm (0.0127 mg/m$^3$) |
| | |
| **Statistics country 1, 2 and 6** | |
| Number of measured values | 34 |
| OEL-value | 1 ppm (1.8 mg/m$^3$) |
| Arithmetic mean | 0.21 ppm (0.38 mg/m$^3$) |
| Standard deviation | 0.214 ppm (0.38 mg/m$^3$) |
| Max. value | 0.888 ppm (1.6 mg/m$^3$) |
| Min. value | 0.055 ppm (0.1 mg/m$^3$) |
| | |
| **Overall distribution:** | |
| 10% Percentile | 0.014 ppm (0.025 mg/m$^3$) |
| 50% Percentile | 0.11 ppm (0.21 mg/m$^3$) |
| 75% Percentile | 0.278 ppm (0.5 mg/m$^3$) |
| 90% Percentile | 0.5 ppm (0.91 mg/m$^3$) |
| 95% Percentile | 0.8 ppm (1.45 mg/m$^3$) |

### C.1.3.1 Systemic effects

In order to perform a risk characterisation of the exposure to personnel involved in EtO sterilisation, the exposure values from the submitted monitoring data set was compared

directly to the DMEL-value.

Table C.10 Conclusion of risk characterisation for industrial use

| Task/ Scenario | Tier/ PPE | DMEL (ppb) | Measured exposure (8-hour TWA) (ppb) | Measured exposure/ DMEL (%) | Acceptable (yes/no) |
|---|---|---|---|---|---|
| Overall max. value | | 3 | 888 | 29600% | No |
| Overall average | | 3 | 190 | 6333% | No |
| Max value country 9 | | 3 | 888 | 29600 | No |
| Average country 9 | | 3 | 324 | 10792 | No |
| Min. value country 9 | | 3 | 56 | 1850 | No |
| Max value country 3 | | 3 | 28.5 | 950% | No |
| Average country 3 | | 3 | 18 | 600% | No |
| Min value country 3 | | 3 | 12 | 400% | No |

### C.1.3.2 Local effects

Not applicable

### C.1.3.3 Conclusion

Monitoring data from 4 EtO disinfection plants in Europe were compared directly with the DMEL-value. Recent comments (March 2020) provided by the applicant indicate that these facilities operate under a slightly different (and possibly more stringent) operating condition than the general description presented in chapter B.2.1. Even the minimum value from the data set results in an exceedance of the DMEL-value of 400%. Thus, no acceptable risk for industrial workers involved in EtO disinfection could be demonstrated, based on an elevated lifetime cancer risk of $1 \times 10^{-5}$.

It is evident from the submitted monitoring data that the operator exposure at the plant subject to the lower national OEL-limit of 0.5 ppm (country 3) is significantly lower than the operator exposure at the other plants. The reasons behind this difference is not known, and details on the individual plants have not been submitted by the applicant for confidentiality reasons. One could only speculate if this individual plant may be of a newer date and better constructed. It may be more automated than the others, or it may be caused by its size or the specific type of products being sterilised there.

In order to be able to comply with a DMEL of 3 ppb, it is obvious that significant changes in the procedures applied at the EtO disinfection plants need to be implemented, both with regard to engineering measures, automatization, to the use of PPE and with regard to exposure monitoring and analysis methodology. It is an obvious challenge that the detection limit for EtO in air is given to be 9.72 ppb (17.5 µg/m3) (See chapter A.16.).

The corresponding estimated elevated lifetime cancer risk for exposure levels exceeding the DMEL-value are tabled below.

Table C.11 Estimated elevated lifetime cancer risk to different exposure levels

| **Estimated elevated lifetime cancer risk to exposure levels exceeding the DMEL-value** | |
|---|---|
| **Exposure (8-hour TWA)** | **Lifetime cancer risk** |
| 1 ppm | $3.3 \times 10^{-3}$ |
| 0.1 ppm | $3.3 \times 10^{-4}$ |
| 10 ppb | $3.3 \times 10^{-5}$ |
| 3 ppb | $1 \times 10^{-5}$ |

## C.1.4. Professional uses

Not applicable. Only industrial use of EtO has been assessed.

### C.1.4.1 Systemic effects

Not applicable

### C.1.4.2 Local effects

Not applicable

### C.1.4.3 Conclusion

Not applicable

## C.1.5. Non-professional users

Not applicable. EtO is not used by non-professionals.

### C.1.5.1 Systemic effects

Not applicable

### C.1.5.2 Local effects

Not applicable

### C.1.5.3 Conclusion

Not applicable

## C.1.6. Secondary (indirect) exposure as a result of use

**Secondary exposure from contact with medical device**

The primary biocidal use of EtO is the industrial disinfection of single use medical equipment before these are placed on the market. Once placed on the market, these items are no longer regulated by the BPR, but by the Medical Device Regulation ((EU) 2017/745; MDR). The accepted residual levels, and thus, secondary exposure of EtO and ethylene chlorohydrin (ECH) under MDR is defined in the ISO-standard ISO 10993-7:2008. Secondary exposure to the general public from medical device was therefore not further assessed.

**Secondary exposure to atmospheric emissions**

Ethylene oxide from the sterilisation chamber and the aeration room is exhausted to the atmosphere via a catalytic converter which converts ethylene oxide to carbon dioxide and water, typically with efficiency greater than 99.9% or, in a relatively small number of cases, via acid scrubbers which convert the ethylene oxide to ethylene glycol, with efficiency typically in the range of 99.5 to 99.9%. Atmospheric emissions of ethylene oxide in Europe are strictly regulated with maximum permissible concentrations in exhaust gas set by individual Member States. A $PEClocal_{air}$ has been calculated to be 0.0001251 $mg/m^3$ (equivalent to 0.068 ppb) as an average air concentration 100 m from the source of emission (chapter B.3.3). This distance is considered to represent the average distance between the emission source and the border of the industrial site.

Published reports on monitoring data of EtO levels in air, mainly from the US, indicate that the calculated $PEClocal_{air}$ cannot be regarded as a very conservative estimate, but rather is well within, and even lower, than the reported monitoring data (Please see chapter A.4.1.4 and B.3.6.).

Although significant variation is seen in the reported monitoring data, both with regards to the presumed atmospheric background level of EtO and to the air concentrations caused by emissions from EtO sterilisation plants, these reports clearly indicate that EtO emissions from treatment plants should be considered, particularly where the treatment plants are located close to residential or urban areas. No monitoring data (measured air concentrations of EtO) from the surroundings of European sterilisation plants has been submitted.

The eCA considers that since the currently available monitoring data is from the US, and not from within the EU, and since there could be high local variations in ethylene oxide air concentrations, frequent monitoring of the air outside of each EU ethylene oxide sterilisation facility would give the most reliable information as to whether the emissions are within an acceptable range or not.

## C.1.6.1 Systemic effects

In order to assess the risk level for non-professionals/the general public subject to secondary exposure to atmospheric emissions of EtO from treatment plants, an increased lifetime cancer risk level of $1 \times 10^{-6}$ was regarded as a tolerable risk level. An exposure of 24 hours per day, 7 days per week was assumed and was compared to the calculated $PEClocal_{air,}$ representing the air concentration of EtO 100m away from the point of emission.

Based on these calculations, secondary exposure to atmospheric emissions of EtO results in an unacceptable risk to the general public.

Table C.12 Conclusion of risk characterisation for general public secondary exposure

| Scenario | Tier | DMEL | Estimated exposure | Estimated exposure/ DMEL (%) | Acceptable (yes/no) |
|---|---|---|---|---|---|
| General public, secondary exposure | | 0.06 ppb | 0.07 ppb | 116.7% | No |

### C.1.6.2 Local effects

Not applicable

### C.1.6.3 Conclusion

Secondary exposure to the general public from atmospheric emissions of EtO, assuming an average air concentration of 0.07 ppb 100m from the source of emission, was deemed to be non-acceptable based on an increased lifetime cancer risk level of $1 \times 10^{-6}$.

## C.1.7. Indirect exposure via food

Not applicable

## C.1.8. Production / formulation of active substance

Manufacture of the active substance, formulation of the product and their packaging are not within the scope of BPR and was therefore not addressed within this CAR as other legislation applies.

### C.1.8.1 Systemic effects

Not applicable

### C.1.8.2 Local effects

Not applicable

### C.1.8.3 Conclusion

Not applicable

## C.1.9. Aggregated exposure

Not applicable

## C.2. Risk characterisation for the environment

A qualitative assessment has been conducted, due to the limited exposure of ethylene oxide from the intended use. The only direct emission to the environment will be to the atmosphere. A $PEClocal_{air}$ has been calculated, at $1.25 \times 10^{-4}$ mg/m$^3$. In the human health assessment, the $PEClocal_{air}$ is compared with the DMEL for the general public living in the surrounding areas of European sterilisation plants. The $PEClocal_{air}$ exceeds the DMEL, and hence unacceptable risk is identified. Risks to terrestrial vertebrates with regard to inhalation effects and exposure from nearby sterilising facilities can therefore not be excluded. This aspect is however considered covered by the human health risk assessment. The eCA is of the opinion that requesting additional data specifically for the terrestrial risk assessment will not add anything to the overall outcome of the risk assessment.

It is not likely that the levels of ethylene oxide in the atmosphere will give rise to concern for organisms in other environmental compartments. It is expected that ethylene oxide will be deposited to water or soil from the atmosphere only to an insignificant extent, due to the high vapour pressure and hence rapid volatilisation. It is in other words not likely that the ethylene oxide emitted to the atmosphere from the intended use will impact organisms in other environmental compartments. Based on the effects assessment, any limited amounts of ethylene oxide potentially reaching water or soil from the atmospheric release would be expected to give concentrations which are far from harmful to aquatic organisms and there is no reason for concern regarding bioaccumulation in the aquatic or terrestrial compartment.

According to the applicant, any potential liquid waste containing ethylene oxide is not released to STPs and subsequently to surface waters, but handled specifically as toxic waste and disposed of without emissions to the environment. This has been a prerequisite for the environmental risk assessment, and should be a requirement for the sites where ethylene oxide is used as intended by the applicant.

## C.3. Risk characterisation for the physico-chemical properties

Ethylene oxide is a flammable and explosive gas, normally stored under pressure. A harmonised classification of ethylene oxide exists, please see section 2. Ethylene oxide is classified as a flammable gas, and pressurised gas (pictograms GHS02 and GHS04). Some additional data has also been provided under section A.1.5.

Ethylene oxide may catch fire or cause an explosion in concentrations greater than 3 % in air. Below 10 °C it forms an extremely flammable liquid which may be ignited by heat, sparks, or flames, vapours may travel to a source of ignition and flash back. The auto-ignition temperature is 457 °C. Furthermore, ethylene oxide reacts with water, strong acids, alkalis, and oxidizers and chlorides of iron, tin, and aluminium or oxides of iron and aluminium.

Based on this, ethylene oxide is regarded as an active substance of high hazard, that is a great risk to the user. Therefore, special care must be taken when handling ethylene oxide. This includes, special training of personnel, specialised equipment for storing and use, special equipment for transportation of ethylene oxide.

## C.4. Measures to protect man, animals and the environment

Engineering controls are required, and equipment must be compliant with ATEX (Appareils destinés à être utilisés en Atmosphères Explosibles) Directives. Equipment location must be in compliance with national guidance if appropriate.

The applicant has submitted recommendations regarding measures to protect man, animals and the environment, which are presented in Doc III B8. The recommendations must be re-evaluated prior to the product authorisation phase in order to ensure that they comply with the hazards of the active substance and the risk assessment of the product.

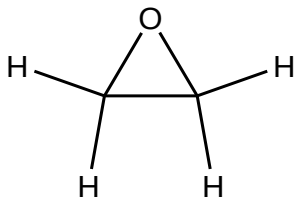
# D. Appendices

## APPENDIX I: LIST OF ENDPOINTS

### Chapter 1: Identity, Physical and Chemical Properties, Classification and Labelling

| | |
|---|---|
| Active substance (ISO Name) | Ethylene oxide |
| Product-type | 2 |

**Identity**

| | |
|---|---|
| Chemical name (IUPAC) | Oxirane / Ethylene oxide |
| Chemical name (CA) | Oxirane |
| CAS No | 75-21-8 |
| EC No | 200-849-9 |
| Other substance No. | None |
| Minimum purity of the active substance as manufactured (g/kg or g/l) | 99.9% |
| Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg) | None |
| Molecular formula | $C_2H_4O$ |
| Molecular mass | 44.05 g/mol |
| Structural formula |  |



**Physical and chemical properties**

| | |
|---|---|
| Melting point | -112.5 °C |
| Boiling point | 11 °C |
| Thermal stability / Temperature of decomposition | Not relevant. Please see waiver in Section A.1.3. |
| Appearance | Colourless gas at room temperature |
| Relative density | 0.8821 g/cm$^3$ at 10 °C |
| Surface tension (state temperature and concentration of the test solution) | Please see waiver in section A.1.3. |
| Vapour pressure (in Pa, state temperature) | 66 kPa at 0 ºC<br>100 kPa at 10 ºC<br>146 kPa at 20 ºC<br>208 kPa at 30 ºC |
| Henry's law constant (Pa m$^3$ mol$^{-1}$) | 14 Pa m3 mol$^{-1}$<br><br>19.86 Pa m3 mol$^{-1}$ |
| Solubility in water (g/l or mg/l, state temperature) | 1000 g/L at 25 ºC |
| | |
| | |
| Solubility in organic solvents (in g/l or mg/l, state temperature) | Soluble in alcohol and ether |

| | |
|---|---|
| Stability in organic solvents used in biocidal products including relevant breakdown products | Not relevant |
| Partition coefficient (log $P_{OW}$) | Log $P_{OW}$: -0.30 |
| | |
| | |
| Dissociation constant | Not relevant. Please see waiver in Section A.1.3. |
| UV/VIS absorption (max.) (if absorption > 290 nm state $\varepsilon$ at wavelength) | Absorbance maxima at 159 nm, 171 nm and 174 nm. (NIST 2009a) |

**Physical hazards**

| | |
|---|---|
| Explosives | Not available. Please see waiver in Section A.1.4 |
| Flammable gases | Yes. Flammable limits of 3 to 100% in ambient air<br>Auto ignition temperature 457 ºC |
| Flammable aerosols | Not relevant. |
| Oxidising gases | Not available. Please see waiver in Section A.1.4 |
| Gases under pressure | Yes. According to the harmonized classification under CLP, please also see the Note U[1]). |
| Flammable liquids | Not applicable to gasses. |
| Flammable solids | Not applicable to gasses. |
| Self-reactive substances and mixtures | Not applicable to gasses. |
| Pyrophoric liquids | Not applicable to gasses. |
| Pyrophoric solids | Not applicable to gasses. |
| Self-heating substances and mixtures | Not applicable to gasses. |
| Substances and mixtures which in contact with water emit flammable gases | Not applicable to gasses. |
| Oxidising liquids | Not applicable to gasses. |
| Oxidising solids | Not applicable to gasses. |
| Organic peroxides | Not relevant. |
| Corrosive to metals | Not available. Please see waiver in Section A.1.4 |
| Auto-ignition temperature(liquids and gases) | Not available. Please see waiver in Section A.1.4 |
| Relative self-ignition temperature for solids | Not applicable to gasses. |
| Dust explosion hazard | Not applicable to gasses. |

1) Note U (of CLP as amended by Commission Delegated Regulation (EU) 2016/918 of 19 May 2016): When put on the market gases have to be classified as 'Gases under pressure', in one of the groups compressed gas, liquefied gas, refrigerated liquefied gas or dissolved gas. The group depends on the physical state in which the gas is packaged and therefore has to be assigned case by case. The following codes are assigned: Press. Gas (Comp.), Press. Gas (Liq.), Press. Gas (Ref. Liq.), Press. Gas (Diss.). Aerosols shall not be classified as gases under pressure (See Annex I, Part 2, Section 2.3.2.1, Note 2).

**Classification and proposed labelling**

| | |
|---|---|
| with regard to physical hazards | Flam. Gas 1; H220<br>Press. Gas |

| | |
|---|---|
| with regard to human health hazards | Carc. 1B; H350<br>Muta. 1B; H340<br>Repr. 1B; H360Fd<br>Acute Tox. 3; H301<br>oral: ATE = 100 mg/kg bw'<br>Acute Tox. 3; H331<br>inhalation: ATE = 700ppm (gases)<br>STOT SE 3; H335<br>STOT SE 3; H336<br>STOT RE 1; H372 (nervous system)<br>Skin Corr. 1; H314<br>Eye Dam. 1; H318 |
| with regard to environmental hazards | None. |

## Chapter 2: Methods of Analysis

**Analytical methods for the active substance**

| | |
|---|---|
| Technical active substance (principle of method) | Waiver |
| Impurities in technical active substance (principle of method) | Please see confidential appendix. |

**Analytical methods for residues**

| | |
|---|---|
| Soil (principle of method and LOQ) | Waiver (limited exposure) |
| Air (principle of method and LOQ) | Gas chromatography with Electron capture detector (ECD),<br>LOQ: 17.5 µg/m$^3$ (9.72 ppb) |
| Water (principle of method and LOQ) | Waiver (limited exposure) |
| Body fluids and tissues (principle of method and LOQ) | Gas chromatography with Flame ionisation detector (FID),<br>LOQ in blood: 4 µg/L<br>LOQ in tissue: 0.10 µg/L |
| Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes) | Not relevant |
| Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes) | Not relevant |

## Chapter 3: Impact on Human Health

**Absorption, distribution, metabolism and excretion in mammals**

| | |
|---|---|
| Rate and extent of oral absorption: | Not established, inhalation is the most relevant route of exposure. |
| Rate and extent of dermal absorption*: | Not established, inhalation is the most relevant route of exposure. |
| Rate and extent of inhalation absorption | A pulmonary uptake of 75-80% for humans has been reported. |
| Distribution: | EtO is rapidly and fully distributed through the body. |

| | |
|---|---|
| Potential for accumulation: | The half-life in human blood has been calculated to be 48 minutes; thus no accumulation of ethylene oxide in humans is expected. |
| Rate and extent of excretion: | At a steady state in humans 20-25 % of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound. The major amount of ethylene oxide is metabolized by hydrolysis, while ca. 20 % are converted to glutathione conjugates. |
| Toxicologically significant metabolite(s) | Two major pathways of ethylene oxide metabolism have been established: conjugation with glutathione by glutathione transferase and hydrolysis to ethylene glycol (probably chemical rather than via epoxide hydrolase). |

* The dermal absorption value is applicable for the active substance and might not be usable in product authorization

**Acute toxicity**

| | |
|---|---|
| Rat LD50 oral | LD50 is between 50 mg/kg bw and 300 mg/kg bw.<br>[Oral ATE = 100 mg/kg bw' from 14$^{th}$ ATP to CLP] |
| Rat LD50 dermal | No information available. |
| Rat LC50 inhalation | LC50 (4-hr) = 660 (95% CI = 509-856 ppm) (mouse, female).<br>[Inhalation: ATE = 700ppm (gases) from 14 ATP to CLP] |

| | |
|---|---|
| Skin corrosion/irritation | EtO is corrosive to skin. |

| | |
|---|---|
| Eye irritation | As ethylene oxide is considered to cause serious eye damage as it is classified as corrosive to skin. |

| | |
|---|---|
| Respiratory tract irritation | EtO is classified as an active substance that causes respiratory irritation. |

| | |
|---|---|
| Skin sensitisation (test method used and result) | There is a lack of evidence for a potential to cause skin sensitisation. Hence, no classification is warranted. |

| | |
|---|---|
| Respiratory sensitisation (test method used and result) | The available data on asthmatic symptoms do not present evidence that justifies classification for specific respiratory sensitisation to ethylene oxide. |

**Repeated dose toxicity**

**Short term**

| | |
|---|---|
| Species / target / critical effect | Only limited information on short-term inhalation toxicity of ethylene oxide is available. Available information is not considered sufficient for a reliable NOAEL derivation. As ethylene oxide is a genotoxic |

| | |
|---|---|
| | carcinogen with a non-threshold mode of action, AELs normally derived for acute, subchronic and chronic effects will not be relevant for this active. A DMEL (by inhalation) is the only relevant threshold to be used in the risk assessment for EtO. |
| Relevant oral NOAEL / LOAEL | - |
| Relevant dermal NOAEL / LOAEL | - |
| Relevant inhalation NOAEL / LOAEL | - |

**Sub-chronic**

| | |
|---|---|
| Species/ target / critical effect | Ethylene oxide is classified as a genotoxic carcinogen, hence a subchronic NOAEL is not considered relevant for the human risk assessment. Despite the lack of full OECD guideline studies in 2 species, the available studies by inhalation indicate that ethylene oxide affects different species (rat, mice, monkey, dog, rabbit), at concentrations in the same dose range as low as 50 ppm. |
| Relevant oral NOAEL / LOAEL | - |
| Relevant dermal NOAEL / LOAEL | - |
| Relevant inhalation NOAEL / LOAEL | - |

**Long term**

| | |
|---|---|
| Species/ target / critical effect | Ethylene oxide is classified as a genotoxic carcinogen, hence a long term NOAEL is not considered relevant for the human risk assessment. |
| Relevant oral NOAEL / LOAEL | - |
| Relevant dermal NOAEL / LOAEL | - |
| Relevant inhalation NOAEL / LOAEL | - |

**Genotoxicity**

| |
|---|
| Ethylene oxide has been shown to be genotoxic in virtually all systems studied, these include tests in yeast and fungi as well as in bacterial and mammalian systems and also in transgenic mice. Human data confirm the genotoxic properties of ethylene oxide. Ethylene oxide is a direct alkylating agent that reacts with nucleophiles without the need for metabolic transformation. |

**Carcinogenicity**

| | |
|---|---|
| Carcinogenicity | The studies provide conclusive evidence that ethylene oxide was carcinogenic in two species (rat and mice).<br>Despite limited evidence in humans for the carcinogenicity of ethylene oxide, IARC consider ethylene oxide to be carcinogenic to humans (Group 1). |

| | |
|---|---|
| Species/type of tumour | Rat:<br>Increase in the incidence of mononuclear cell leukemia at 50 ppm (male) and brain glioma and peritoneal mesothelioma at 100 ppm (male). Treatment related increase in peritoneal mesotheliomas in males and brain tumours in both sexes at 33 and 100 ppm and subcutis fibroma in males at 100 ppm. Dose-related increased prevalence of mononuclear cell leukemia at all exposure concentrations; incidence statistically significantly increased only in females at 100 ppm. 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. Also, statistically significant increase in the number of rats with multiple neoplasms was observed starting from 10 ppm.<br><br>Mice:<br>Mice were exposed to 0, 50 or 100 ppm EtO. In both male and female mice a dose related increase in the incidence of benign or malignant neoplasms in the lung and benign neoplasms in the Harderian gland was noted. Ethylene oxide also caused an increase in malignant tumours of the uterus, mammary gland and lymphomas in female mice.<br><br>Human:<br>In epidemiological data and case reports on carcinogenicity of ethylene oxide in humans have serval findings been observed. IARC have concluded that there is limited evidence in humans for a causal association of ethylene oxide with lymphatic and haematopoietic cancers (specifically lymphoid tumours, i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia), and breast cancer. |
| Relevant $BMDL_{10}$ | 35.5 ppm (alveolar/broncheolar adenomas and carcinomas in female mice). |

**Reproductive toxicity**
**Developmental toxicity**

| | |
|---|---|
| Species/ Developmental target / critical effect | There are indications of the developmental effects of ethylene oxide.<br>Rat: At lower dose levels decreased foetal weights were observed which at least in one study were reported to occur in the absence of maternal toxicity. In addition, one study reported increased incidence of skeletal variations, but at the dose levels at which maternal toxicity was also observed. At higher dose levels variations and malformations were observed. Due to limited reporting it could not be concluded that these malformations would have been in all cases secondary to maternal toxicity.<br>Rabbit: No effect seen at 150 ppm in the study provided, indicating that that the rabbit is less sensitive than the rat. |
| Relevant maternal NOAEC | 100 ppm (highest dose tested in a rat study)<br>[LOEAC of 150 ppm in another study, also rat] |
| Relevant developmental NOAEC | 33 ppm<br>[LOEAC of 100 or 150 ppm, two different studies in rat] |

**Fertility**

| | |
|---|---|
| Species/critical effect | Ethylene oxide causes adverse effects on fertility.<br>In the one-generation study in rats, decreased number of implantations and born foetuses per implantation site (indicating post-implantation losses) was observed without any signs of parental toxicity at 100 ppm. These findings are supported by other studies in mice and rats, showing increased incidences of resorptions and/or decreased incidences of implantations. Additional support for the fertility effects comes from the studies reporting specific effects on spermatogenesis and sperm morphology in rats, mice, guinea pigs and monkey. |
| Relevant parental NOAEC | 100 ppm |
| Relevant offspring NOAEC | 33 ppm |
| Relevant fertility NOAEL | 33 ppm |

**Neurotoxicity**

| | |
|---|---|
| Species/ target/critical effect | Human evidence together with data from experimental animals provides sufficient evidence to support the classification of ethylene oxide as STOT RE 1 for neurotoxicity [STOT RE 1, H372; Causes damage to nervous system through prolonged or repeated exposure]. |

**Developmental Neurotoxicity**

| | |
|---|---|
| Species/ target/critical effect | No study provided. |

**Immunotoxicity**

| | |
|---|---|
| Species/ target/critical effect | No studies on immunotoxicity of ethylene oxide were submitted. |

**Developmental Immunotoxicity**

| | |
|---|---|
| Species/ target/critical effect | No studies on developmental immunotoxicity were provided. |

Other toxicological studies

Medical data

**Summary**

| | Value | Study | Safety factor |
|---|---|---|---|
| DMEL prof | 3 ppb | NTP TR 326, Mouse inhalation, Long term duration (Comparable to OECD guideline 451)<br><br>$BMDL_{10}$ for alveolar/broncheolar adenomas and carcinomas in female mice: 35.5 ppm.<br><br>Corrected dose descriptor: 28.7 ppm (Differences between exposure conditions mice (6h/day, 5 days/week) and human (8h/day, 5 days/week)<br><br>Difference between activity level: at rest (5.3 $m^3$) versus light activity (10 $m^3$)<br><br>Differences between occupational (48 weeks/year for 40 years) and lifetime (52 weeks/year for 75 year) exposure conditions) | High to low extrapolation: 10,000<br><br>(linearised approach 1:100,000; basis for dose descriptor was $BMDL_{10}$) |
| DMEL priv | 0.06 ppb | NTP TR 326, Mouse inhalation, Long term duration (Comparable to OECD guideline 451)<br><br>$BMDL_{10}$ for alveolar/broncheolar adenomas and carcinomas in female mice: 35.54 ppm.<br><br>Corrected dose descriptor: 6.3 ppm. (Differences between exposure conditions mice (6h/day, 5 days/week) and human (24h/day, 7 days/week)] | High to low extrapolation: 100,000<br><br>(linearised approach 1:1 000,000; basis for dose descriptor was $BMDL_{10}$) |

**MRLs**

| | |
|---|---|
| Relevant commodities | Not relevant. |

**Reference value for groundwater**

| | |
|---|---|
| According to BPR Annex VI, point 68 | Not relevant. |

**Dermal absorption**

| | |
|---|---|
| Study (in vitro/vivo), species tested | No study provided. Inhalation is the most relevant route of exposure. |
| Formulation (formulation type and including concentration(s) tested, vehicle) | - |
| Dermal absorption values used in risk assessment | - |

## Chapter 4: Fate and Behaviour in the Environment

**Route and rate of degradation in water**

| | |
|---|---|
| Hydrolysis of active substance and relevant metabolites/ degradants ($DT_{50}$) (state pH and temperature) | Ethylene oxide, $DT_{50}$:<br>– Freshwater, pH 7.4, 12 °C: 34.5-40.2 d<br>– Saltwater, pH 7.0, 9 °C: 33.4-39.6 d<br><br>Ethylene glycol (relevant hydrolysis degradant): hydrolytically stable<br><br>Ethylene chlorohydrin: not known |
| Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites/ degradants | Ethylene oxide is not expected to undergo photolysis, since it does not absorb solar radiation in wavelengths > 290 nm.<br><br>The same is true for ethylene glycol and ethylene chlorohydrin. |
| Readily biodegradable (yes/no) | Yes |
| Inherent biodegradable (yes/no) | Not determined |
| Biodegradation in freshwater | Not determined |
| Biodegradation in seawater | Not determined |
| Non-extractable residues | Not determined |
| Distribution in water / sediment systems (active substance) | Not determined, but substance is unlikely cause exposure to water/sediment from the intended use. |
| Distribution in water / sediment systems (metabolites/ degradants) | Not determined. Ethylene oxide hydrolyses to ethylene glycol, but ethylene oxide will to a very low extent end up in the aquatic compartment from the intended use. |

**Route and rate of degradation in soil**

| | |
|---|---|
| Mineralization (aerobic) | Not determined |
| Laboratory studies (range or median, with number of measurements, with regression coefficient) | Not determined |
| $DT_{50}$ lab (20°C, aerobic): | Not determined |
| $DT_{90}$ lab (20°C, aerobic): | Not determined |
| $DT_{50}$ lab (10°C, aerobic): | Not determined |
| $DT_{50}$ lab (20°C, anaerobic): | Not determined |
| degradation in the saturated zone: | Not determined |

| | |
|---|---|
| Field studies (state location, range or median with number of measurements) | Not determined |
| $DT_{50}$ f: | Not determined |
| $DT_{90}$ f: | Not determined |
| Anaerobic degradation | Not determined |
| Soil photolysis | Not determined |
| Non-extractable residues | Not determined |
| Relevant metabolites - name and/or code, % of applied a.s. (range and maximum) | Not determined |
| Soil accumulation and plateau concentration | Not determined. The estimated Koc for ethylene oxide is 3.24 L/kg. It is hence not strongly adsorbed to soil and therefore unlikely to accumulate in this compartment. |

**Adsorption/desorption**

| | |
|---|---|
| Ka , Kd<br>Kaoc , Kdoc<br>pH dependence (yes / no) (if yes type of dependence) | Koc (estimated): 3.24 L/kg |

**Fate and behaviour in air**

| | |
|---|---|
| Direct photolysis in air | Not relevant, no absorption at relevant wavelengths. |
| Quantum yield of direct photolysis | Not relevant |
| Photo-oxidative degradation in air | Latitude: ..... Season: ..... $DT_{50}$ ..... |
| Volatilization | |

**Reference value for groundwater**

| | |
|---|---|
| According to BPR Annex VI, point 68 | Not relevant for ethylene oxide. |

**Monitoring data, if available**

| | |
|---|---|
| Soil (indicate location and type of study) | |
| Surface water (indicate location and type of study) | |
| Groundwater (indicate location and type of study) | |
| Air (indicate location and type of study) | Please see table directly below. |

| Reference | Ethylene oxide concentration in air | Remarks |
|---|---|---|
| Halik, T. (2018), Ethylene oxide air monitoring report. GHD Services Inc., Report No. 01 | 0.58 µg/m$^3$ = 0.32 ppb | Highest measured concentration in air outside Sterigenics facility |
| Holmes, J.R. (1993), Persistence of ethylene oxide in the atmosphere, California EPA, Research Note 93-6 | 0.03 µg/m$^3$ = 0.02 ppb | Remote coastal locations (according to the author believed to represent hemispheric background concentrations for ethylene oxide) |
| | 7.20 µg/m$^3$ = 4.00 ppb | Downwind of an ethylene oxide fumigation plant |
| | 0.05 µg/m$^3$ = 0.03 ppb | Los Angeles area, suburbs |
| | 1.44 µg/m$^3$ = 0.8 ppb | Los Angeles area, downtown |
| Olaguer, E. *et al.* (2019), Ethylene oxide exposure attribution and emissions quantification based on ambient air measurements near a sterilization facility. Int. J. Environ. Res. Public Health 2020, 17, 42 | 0.25 µg/m$^3$ = 0.14 ppb | Urban background concentration near facility |
| | 2.08 µg/m$^3$ = 1.16 ppb | Highest 24 h concentration occurred in a parking lot / residential neighbourhood immediately across the street from the sterilization facility and directly downwind from the site |
| | 0.30 µg/m$^3$ = 0.17 ppb | Background concentration which was by the author not thought to be contributed to by the facility. |
| WHO (2003), Concise International Chemical Assessment Document 54, Ethylene oxide | 0.04-956 µg/m$^3$ = 0.02-553 ppb | 24-h ambient air concentrations in Los Angeles, reported by Havlicek et. al (1992). |
| | 0.03-0.40 µg/m$^3$ = 0.02-0.22 ppb | Northern California |
| | 0.03-0.36 µg/m$^3$ = 0.02-0.20 ppb | Remote coastal regions, California |

## Chapter 5: Effects on Non-target Species

| Toxicity data for aquatic species (most sensitive species of each group) | | | | |
|---|---|---|---|---|
| **Substance** | **Species** | **Time-scale** | **Endpoint** | **Toxicity** |
| | **Fish** | | | |
| Ethylene oxide | Fathead minnow (*Pimephales promelas*) | 96 h | Mortality | $LC_{50}$ = 84 mg/L |
| Ethylene glycol | Goldfish (*Carassius auratus*) | 24 h | Mortality | $LC_{50}$ > 5 000 mg/L |
| Ethylene chlorohydrin | Fathead minnow (*Pimephales promelas*) | 96 h | Mortality | $LC_{50}$ = 67 mg/L |
| | **Invertebrates** | | | |
| Ethylene oxide | *Daphnia magna* | 48 h | Mortality | $EC_{50}$ = 212 mg/L |
| Ethylene glycol | *Daphnia magna* | 48 h | Mortality | $EC_{50}$ > 10 000 mg/L |
| Ethylene chlorohydrin | *Daphnia magna* | 48 h | Mortality | $EC_{50}$ = 100 mg/L |
| | **Algae[1)]** | | | |
| Ethylene oxide | *Selenastrum capricornutum* | 96 h | Growth rate | $EC_{50}$ = 240 mg/L [2)] |
| | **Microorganisms** | | | |
| Ethylene | Activated sludge | 16 h | Growth | $IC_{50}$ = 10-100 mg/L |

| | | | | |
|---|---|---|---|---|
| oxide | (sewage) microorganisms | | inhibition | |
| Ethylene glycol | Activated sludge (sewage) microorganisms | 16 h | Growth inhibition | $IC_{50}$ = 10 000 mg/L |
| Ethylene oxide | Activated sludge (sewage) microorganisms | 3 h | Respiration inhibition | $EC_{50}$ > 713 mg/L |

1) Experimental algae data are not available for ethylene glycol (ECOSAR green algae 96 h $EC_{50}$ = 3 500 mg/L) or ethylene chlorohydrin.

2) The algae data are for propylene oxide, but are considered indicative for ethylene oxide.

**Effects on earthworms or other soil non-target organisms**

| | |
|---|---|
| Acute toxicity to …….. | Not determined |
| Reproductive toxicity to …… | Not determined |

**Effects on soil micro-organisms**

| | |
|---|---|
| Nitrogen mineralization | Not determined |
| Carbon mineralization | Not determined |

**Effects on terrestrial vertebrates**

| | |
|---|---|
| Acute toxicity to mammals | Not determined |
| Acute toxicity to birds | Not determined |
| Dietary toxicity to birds | Not determined |
| Reproductive toxicity to birds | Not determined |

**Effects on honeybees**

| | |
|---|---|
| Acute oral toxicity | Not determined |
| Acute contact toxicity | Not determined |

**Effects on other beneficial arthropods**

| | |
|---|---|
| Acute oral toxicity | Not determined |
| Acute contact toxicity | Not determined |
| Acute toxicity to …….. | Not determined |

**Bioconcentration**

| | |
|---|---|
| Bioconcentration factor (BCF) | $BCF_{fish}$ (calc. from log Kow): 3.16 L/kg<br>$BCF_{earthworm}$ (calc. from log Kow): 0.85 L/kg |
| Depuration time ($DT_{50}$) | Not determined |
| Depuration time ($DT_{90}$) | Not determined |
| Level of metabolites (%) in organisms accounting for > 10 % of residues | Not determined |

## Chapter 6: Other End Points

# APPENDIX II: HUMAN EXPOSURE CALCULATIONS

No additional appendixes on human exposure calculations are available, as all the necessary calculations are shown in the CAR.

# APPENDIX III: ENVIRONMENTAL EMISSION (AND EXPOSURE) CALCULATIONS

No additional appendixes on human exposure calculations are available, as all the necessary calculations are shown in the CAR.

# APPENDIX IV: LIST OF TERMS AND ABBREVIATIONS

| Standard term/abbreviation | Explanation |
|---|---|
| AEL | Acceptable exposure level |
| AEC | Acceptable exposure concentration |
| AF | Assessment factor |
| AGS | German Ausschuss für Gefahrstoffe |
| a.i. | Active ingredient |
| ANOVA | Analysis of varianse |
| APCI | Atmospheric pressure chemical ionization |
| APF | Applied protection factor |
| a.s. | Active substance |
| BCF | Bioconcentration factor |
| BMD | Benchmark dose |
| BOD | Biological oxygen demand |
| BPR | Biocidal Product Regulation (EU 528/2012) |
| bw | Body weight |
| CAR | Competent Authority Report |
| CI | Confidence interval |
| CLP | Classification, Packaging and Labelling of Substances and Mixtures |
| COD | Chemical oxygen demand |
| DOC | Dissolved organic carbon |
| $DT_{50}$ | Degradation half-life (period required for 50 % dissipation) |
| DMEL | Derived minimal effect level |
| dw | Dry weight |
| $EC_{50}$ | Median effective concentration |
| $ED_{50}$ | Median effective dose |
| eCA | Evaluating competent authority |
| ECHA | European Chemicals Agency |
| $E_rC_{50}$ | Median effective concentration growth rate |
| ESD | Emission scenario document |
| EtO | Ethylene oxide |
| EU | European Union |
| EUSES | European Union System for the Evaluation of Substances |
| g | gram(s) |
| GC | Gas chromatography |
| GHS | Globally Harmonized System of Classification and Labelling of Chemicals |
| GLP | Good laboratory practice |
| h | hour(s) |
| HPLC | High performance liquid chromatography |
| $IC_{50}$ | Median inhibitory concentration |
| kg | kilogram(s) |
| $K_{oc}$ | Partition coefficient octanol-carbon |

| Standard term/abbreviation | Explanation |
|---|---|
| $K_{ow}$ | Partition coefficient octanol-water |
| $LC_0$ | Highest concentration tested with no mortality |
| $LC_{50}$ | Median lethal concentration |
| $LD_0$ | Highest dose tested with no mortality |
| LC/MS | Liquid chromatography/Mass selective detector |
| $LD_{50}$ | Median lethal dose |
| LEL | Lower Explosion Limit |
| LEV | Local exhaust ventilation |
| LO(A)EL | Lowest observable (adverse) effect level |
| LO(A)EC | Lowest observable (adverse) effect concentration |
| LOEC | Lowest observable effective concentration |
| m | metre(s) |
| MATC | Maximum tolerable concentration |
| MDR | Medical Device Regulation (EU) 2017/745 |
| MEL | Minimal effect level |
| M factor | Multiplication factor |
| MIC | Minimum inhibitory concentration |
| min | minute(s) |
| MOE | Margin of exposure |
| MOS | Margin of safety |
| MQL | Maximum quantification limit |
| MRL | Maximum residue level |
| MS | Mass spectrometry |
| MW | Molecular weight |
| n | number of observations |
| NA or N/A | Not applicable |
| NO(A)EL | No observable (adverse) effect level |
| NO(A)EC | No observable (adverse) effect concentration |
| NOEC | No observed effect concentration |
| $NOE_bC$ | No observed effect concentration, based on biomass |
| $NOE_rC$ | No observed effect concentration, based on growth rate |
| OC | Organic carbon |
| OC | Operational Conditions |
| ODP | Ozone-depleting potential |
| OECD | Organisation for Economic Cooperation and Development |
| OEL | Occupational exposure limit |
| Pa | Pascal |
| PBT | Persistent, bioaccumulative, toxic |
| PBPK | Physiologically based pharmacokinetics |
| PEC | Predicted environmental concentration |
| PCD | Process Challenge Device |
| PNEC | Predicted no effect concentration |
| POP | Persistant organic pollutant |
| PPE | Personal protective equipment |
| ppm | Parts per million |
| ppb | Parts per billion |
| (Q)SAR | (Quantitative) structure activity relationships |
| RCR | Risk characterization ratio |
| RfD | Reference dose |
| RH | Relative humidity |
| RHO | Density |
| RMM | Risk mitigation measure |
| RPE | Respiratory protective equipment |
| SCL | Specific concentration limit |
| SD | Standard deviation |
| SED | Solvent Emissions Directive |
| SOP | Standard operating procedures |
| STEL | Short Term Exposure Limit |

| Standard term/abbreviation | Explanation |
|---|---|
| STP | Sewage treatment plant |
| TLC | Thin layer chromatography |
| ThOD | Theoretical oxygen demand |
| TOC | Total organic carbon |
| TWA | Time weighted average |
| USEPA | United States Environmental Protection Agency |
| UV | Ultraviolet |

# APPENDIX V: OVERALL REFERENCE LIST (INCLUDING DATA OWNER AND CONFIDENTIALITY CLAIM)

| Author(s) | Year | Reference to Doc III | Title. Source (where different from company) Company, Report No. GLP (where relevant) / (Un)Published | Data Protec-tion Claim-ed (Yes/ No) | Owner |
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| [redacted] | 2018 b | IIIB5.10/07 | Renewed functional assessment and renewed performance assessment according to EN 11135 of the program 2 bar / 60 °C / 140 min / 5 h of the ethylene oxide sterilizer | Yes | [redacted] |
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| Author(s) | Year | Reference to Doc III | Title.<br>Source (where different from company)<br>Company, Report No.<br>GLP (where relevant) / (Un)Published | Data Protec-tion Claim-ed (Yes/ No) | Owner |
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| Author(s) | Year | Reference to Doc III | Title.<br>Source (where different from company)<br>Company, Report No.<br>GLP (where relevant) / (Un)Published | Data Protec-tion Claim-ed (Yes/ No) | Owner |
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# APPENDIX VI: CONFIDENTIAL INFORMATION

Confidential appendices:

Appendix VI_Conf_Ethylene oxide_PT02_ref_spec_agreed
Appendix VI_Conf_table_A10
Appendix VI_Conf_reference_list
Appendix VI_Conf_annex_DocIIIA
Appendix VI_Occupational Exp Monitoring_EtO_HS-15.3_SWP-01_conf

# APPENDIX VII: STUDY SUMMARIES

Study summaries provided separately:

Doc IIIA_3_Physchem_EtO_final CAR
Doc IIIA_4_Analytical methods_EtO_final CAR
Doc IIIA_5_Efficacy_EtO_final CAR
Doc IIIA_6_Toxicology_EtO_final CAR
Doc IIIA_7_Ecotox_EtO_final CAR
Doc IIIA_7_Fate_EtO_final CAR
Doc IIIA_8_Protection_measures_EtO_final CAR
Doc IIIA_9_Classification_EtO_final CAR

Doc IIIB_3_Pyschem_EtO_final CAR
Doc IIIB_4_Analytical_methods_EtO_final CAR
Doc IIIB_5_Efficacy_EtO_final CAR
Doc IIIB_5.2-01_Elsmore_2009_appendix_EtO_final CAR
Doc IIIB_5.2-09_Andrew_2019_appendix_EtO_final CAR
Doc IIIB_6_Toxicology_EtO_final CAR
Doc IIIB_7_Environment_EtO_final CAR
Doc IIIB_8_Measures_EtO_final CAR

# APPENDIX VIII: EFSA BMDL10 CALCULATIONS

Documents provided separately:

Appendix VIII_EtO_LungCarc_ Females_BMDcalc
Appendix VIII_EtO_LungCarc_both sexes_BMDcalc
Appendix VIII_EtO_LungCarc_Males_BMDcalc