Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

ethylene oxide; oxirane

EC Number: 200-849-9
CAS Number: 75-21-8

CLH-O-0000001412-86-164/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

22 September 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Ethylene oxide, oxirane

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

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

Index Number: **603-023-00-X**

Contact details for dossier submitter:

Environment Agency Austria, Spittelauer Lände 5, A-1090 Vienna

on behalf of the Austrian Competent Authority (Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management, Stubenring 1, 1010 Vienna, Austria)

Version number: **03** **Date:** **August 2016**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Ethylene oxide, oxirane
EC number:	200-849-9
CAS number:	75-21-8
Annex VI Index number:	603-023-00-X
Degree of purity:	Typical concentration: ≤100.0 % (w/w) Concentration range: ≥99.9 - ≤100.0 % (w/w)
Impurities:	unknown impurities (concentration range: ≥0.0 - <0.1 % (w/w))

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Press. Gas (*Note U*) Flam. Gas 1, H220 Skin Irrit. 2, H315 Eye Irrit. 2, H319 Acute Tox. 3 *, H331 STOT SE 3, H335 Carc. 1B, H350 Muta. 1B, H340
Current proposal for consideration by RAC	Skin Sens 1, H317 Acute Tox.3, H301 Acute Tox. 3, H331 (removal of asterisk) Skin Corr 1B, H314 (Causes severe skin burns and eye damage)

	Eye Dam 1, H318 STOT RE1 (H372: Causes damage to nervous system through prolonged or repeated exposure) Repr. 2, H361fd
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Press. Gas (*Note U*) Flam. Gas 1, H220 Eye Dam 1, H318 Skin Corr 1B, H314 Skin Sens 1, H317 Acute Tox.3, H301 Acute Tox. 3, H331 STOT SE 3, H335 STOT RE1, H372 Carc. 1B, H350 Muta. 1B, H340 Repr. 2, H361fd

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Not assessed in this dossier.
2.2.	Flammable gases	Flam. Gas 1, H220	-	Flam. Gas 1, H220	Not assessed in this dossier
2.3.	Flammable aerosols	-	-	-	Not assessed in this dossier.
2.4.	Oxidising gases	-	-	-	Not assessed in this dossier.
2.5.	Gases under pressure	Press. Gas	-	Press. Gas	Not assessed in this dossier
2.6.	Flammable liquids	-	-	-	Not assessed in this dossier.
2.7.	Flammable solids	-	-	-	Not assessed in this dossier.
2.8.	Self-reactive substances and mixtures	-	-	-	Not assessed in this dossier.
2.9.	Pyrophoric liquids	-	-	-	Not assessed in this dossier.
2.10.	Pyrophoric solids	-	-	-	Not assessed in this dossier.
2.11.	Self-heating substances and mixtures	-	-	-	Not assessed in this dossier.
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Not assessed in this dossier.
2.13.	Oxidising liquids	-	-	-	Not assessed in this dossier.
2.14.	Oxidising solids	-	-	-	Not assessed in this dossier.
2.15.	Organic peroxides	-	--	-	Not assessed in this dossier.
2.16.	Substance and mixtures corrosive to metals	-	-	-	Not assessed in this dossier.
3.1.	Acute toxicity - oral	Acute Tox. 3, H301	-	-	
	Acute toxicity - dermal	-	-	-	No data available
	Acute toxicity - inhalation	Acute Tox. 3, H331	-	Acute Tox. 3 *, H331	
3.2.	Skin corrosion / irritation	Skin Corr 1B, H314	-	Skin Irrit. 2, H315 -	

3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318	-	Eye Irrit. 2, H319	
3.4.	Respiratory sensitisation	-	-	-	Conclusive but not sufficient for classification
	Skin sensitisation	Skin Sens 1, H317	-	-	
3.5.	Germ cell mutagenicity	Muta. 1B, H340	-	Muta. 1B, H340	Not assessed in this dossier
3.6.	Carcinogenicity	Carc. 1B, H350	-	Carc. 1B, H350	Not assessed in this dossier
3.7.	Reproductive toxicity	Repr.2, H361fd	-	-	
3.8.	Specific target organ toxicity –single exposure	STOT SE 3, H335		STOT SE 3, H335	Respiratory tract irritation not assessed in this dossier.
3.9.	Specific target organ toxicity – repeated exposure	STOT RE1, H372	-	-	
3.10.	Aspiration hazard	-	-	-	Not assessed in this dossier.
4.1.	Hazardous to the aquatic environment	-	-	-	Not assessed in this dossier.
5.1.	Hazardous to the ozone layer	-	-	-	Not assessed in this dossier.

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Hazard statements: H301, H331, H314, H317, H335, H340, H350, H361fd, H372,
Precautionary statements: No statement codes are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

Note U: 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.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Classification of ethylene oxide has been included into Annex I of Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances by Commission Directive 91/325/EEC of 1 March 1991 adapting to technical progress for the twelfth time.

In 1999 a classification for environment has been discussed at the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances, Pesticides (ECBI/43/99 Rev. 2). The Group agreed not to classify ethylene oxide for the environment.

Classification of ethylene oxide has been revised by Commission Directive 2009/2/EC adapting Council Directive 67/548/EEC to technical progress for the 31st time (inclusion of risk phrase R6).

Ethylene oxide is now covered by index number 603-023-00-X in Annex VI, part 3, Table 3.1 (list of harmonized classification and labelling of hazardous substances) of Reg. (EC) No 1272/2008 (CLP regulation).

2.2 Short summary of the scientific justification for the CLH proposal

The Competent Authority of Austria has initiated substance evaluation for ethylene oxide according to Article 45(4) of the REACH Regulation. In the course of the evaluation, the evaluating MSCA noted that the current harmonised classification entry is incomplete. Based on an in-depth evaluation of the hazard data it is proposed that the current harmonised classification entry for human health should further include classification for STOT RE, due to neurological effects (primarily sensorimotor polyneuropathy) seen in humans and animal studies. As reproductive toxicity has been seen in various species including humans a classification as Repr. 2 is proposed. In addition ethylene oxide has to be classified as sensitizer (Skin Sens 1). Allergies of the immediate type are documented and case reports describing contact dermatitis after ethylene oxide contact or allergic reactions after parenteral administration are available. Moreover a classification for acute oral toxicity (Acute Tox 3, H301) and Skin corrosion (Skin Corr 1B) is warranted based on available animal data. The asterisk (*) indicating minimum CLP classification for acute inhalation toxicity (Acute Tox 3, H331) is no longer necessary since the data confirm the current classification.

Based on thorough evaluation of available data a revision and an extension of the current harmonised classification entry are deemed necessary and an adaption is proposed.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Table 4: Current Annex VI Table 3.1 – Harmonised classification and labelling of hazardous substances

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling	
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)
603-023-00-X	ethylene oxide; oxirane	200-849-9	75-21-8	Press. Gas ([1]) Flam. Gas 1 Carc. 1B Muta. 1B Acute Tox. 3 * Eye Irrit. 2 STOT SE 3 Skin Irrit. 2	H220 H350 H340 H331 H319 H335 H315	 GHS02 GHS04 GHS06 GHS08 Dgr	H220 H350 H340 H331 H319 H335 H315

([1]) Note U (*) Minimum Classification

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Self-classification notifications for ethylene oxide by industry are summarized in the C&L inventory (http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database). 38 aggregated notifications are presented in the inventory; the total number of notifiers is 2405 (n=2405) (accessed on 09th of December 2015).

In addition to the harmonized classification given above the registrants classify ethylene oxide for the following properties:

- Acute Tox. 4 (H302: Harmful if swallowed) (n=1743)
- STOT RE 1 (H372: Causes damage to nervous system through prolonged or repeated exposure (n=1637)
- Eye Irrit 2A (H319: Causes serious eye irritation) (n=52)

- Acute Tox 3 (H301: Toxic if swallowed.) (n=28)
- Skin Sens 1 (H317: May cause an allergic skin reaction) (n=28)
- Acute Tox 2 (H330: Fatal if inhaled.) (n=47)
- Aquatic Chronic 3 (H412: Harmful to aquatic life with long lasting effects) (n=28)

RAC general comment
During the public consultation (PC) one member state competent authority (MSCA) noted that after adaptation to the technical and scientific progress (cf. 4. ATP to the CLP Regulation) the hazard class "Flammable gases (including chemically unstable gases)" in section 2.2 of Annex I to CLP Regulation has been amended and therefore, ethylene oxide has to be classified as Flam. Gas 1; H220, Chem. Unst. Gas A; H230. However, since physico-chemical hazard classes were not proposed for classification by the dossier submitter (DS) and were not open for PC, an evaluation by RAC of this hazard class was not possible. Ethylene oxide is a colourless gas at room temperature. It has a boiling point of 10.7°C. During storage and transport, ethylene oxide is kept as a liquid under moderate pressure. In those situations exposure to liquid ethylene oxide may occur. Throughout this document, ethylene oxide has also been abbreviated as ETO.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

According to Article 36(3) of the CLP Regulation for a substance that fulfils the criteria for other hazard classes or differentiations than those of CMR or respiratory sensitisation (Cat. 1) a harmonised classification and labelling proposal can be submitted if a justification is provided demonstrating the need for such action at Community level.

According to Article 45(4) of the REACH Regulation the Competent Authority of Austria has initiated substance evaluation for ethylene oxide. In the course of the evaluation, the evaluating MSCA noted that the current harmonised classification entry is incomplete. Therefore, the current harmonised classification of ethylene oxide needs to be revised.

Ethylene oxide is a very important industrial chemical and commonly used in the sterilization of heat sensitive materials. Beside its effects on the nervous system ethylene oxide is a well-known and well documented sensitizer. Therefore an update of the Annex VI ethylene oxide entry of Regulation (EC) No. 1272/2008 is warranted to include those considerable additional endpoints to subsequently ensure a high level of protection of human health according to CLP regulation Article 1(1) and to raise awareness amongst workers.
The toxicological data provided in the registration dossier by the lead registrant and open literature indicated that ethylene oxide should be additionally classified as Acute Tox 3, H301; Skin Corr 1B, H314; Eye Dam 1, H318; STOT RE1, H372; Skin Sens 1, H317; Repr. 2, H361fd.

The current classification for ethylene oxide has been introduced by Commission Directive 91/325/EEC (12th ATP). This harmonised classification has been translated into harmonised CLP classification but the DSD criteria sometimes did not fully correspond to a classification according to the CLP criteria. A minimum classification for acute inhalation toxicity category 3 (Acute Tox 3*) was introduced. To minimize further uncertainty in classification of ethylene oxide this endpoint has been evaluated as well and revised in this proposal.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

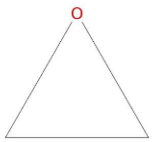
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	200-849-9
EC name:	ethylene oxide
CAS number (EC inventory):	75-21-8
CAS number:	75-21-8
CAS name:	oxirane
IUPAC name:	oxirane
CLP Annex VI Index number:	603-023-00-X
Molecular formula:	C2H4O
Molecular weight range:	44.0526

Structural formula:



1.2 Composition of the substance

Table 6: Constituents

Constituent	Typical concentration	Concentration range	Remarks
Ethylene oxide EC no 200-849-9	≤100.0 % (w/w)	≥99.9 - ≤100.0 % (w/w)	

Table 7: Impurities

Impurity	Typical concentration	Concentration range	Remarks
Unknown impurities	0.05 % (w/w)	≥0.0 - <0.1 % (w/w)	

1.2.1 Composition of test material

This information is given in the study descriptions in the relevant chapters if available.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties[1]

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	colorless gas of sweetish ethereal odour	REACH registration /CSR	-
Melting/freezing point	-111 °C	REACH registration /CSR	-
Boiling point	10.7 °C at 1013 hPa	REACH registration /CSR	-
Relative density	0.89 g/cm^3 at 10 °C (liquid density at boiling point) 2.9 kg/m3 at 20 °C (gas density)	REACH registration /CSR	-
Vapour pressure	1456 hPa at 20 °C	REACH registration /CSR	-
Surface tension	not surface active	Data waived in REACH registration	Based on chemical structure, no surface activity is predicted.
Water solubility	miscible in all proportions	REACH registration /CSR	-
Partition coefficient n-octanol/water	-0.3 at 25 °C	REACH registration /CSR	-
Flash point	not relevant	Data waived in REACH registration	Regardless of the substance being a gas at room temperature, and the flash point consequently being of no relevance under REACH. Flash points of -57 to -17 °C are reported in the technical literature.
Flammability	extremely flammable gas	Data waived in REACH registration	The substance is not pyrophorice, and yields no flammable gases on contact with water. Given the flammability limits in air of 2.6 - 100 vol%, however, the substance is extremely flammable. Aqueous solutions of ethylene oxide are flammable to highly flammable liquids, depending on the concentration.
Explosive properties	explosive under the influence of a flame	REACH registration /CSR	The substance is stable at room temperature, however tends to polymerize violently in the presence of impurities. The substance is not sensitive against shock or friction,

[1] Based on registration data, updated Nov 2014

			however explodes under influence of a flame. Explosiveness depends on pressure, temperature, concentration, the type, form, and energy of the ignition source, and the type of container. Decomposition temperature: 571 °C (calculated).
Self-ignition temperature	429 °C	REACH registration /CSR	-
Oxidising properties	no oxidising properties	Data waived in REACH registration	The Substance is incapable of reacting exothermically with combustible materials on the basis of the chemical structure. The substance is extremly flammable.
Granulometry	not applicable	Data waived in REACH registration	Substance is marketed or used in a non solid or granular form.
Stability in organic solvents and identity of relevant degradation products	not applicable	Data waived in REACH registration	The stability of the substance is not considered as critical
Dissociation constant	not applicable	Data waived in REACH registration	The substance does not contain any ionic structure.
Viscosity	not applicable	Data waived in REACH registration	Substance is a gas. Values of 0.00945 mPa_s at 20 °C (gas phase) and 0.254 mPa_s at 10 °C (liquid phase) are reported.

2 MANUFACTURE AND USES

2.1 Manufacture

Ethylene oxide has been fully registered as a joint submission in a tonnage band of 1,000,000 + tonnes per annum and individual submissions for intermediate use only have been submitted (ECHA dissemination website, accessed May 2015).

2.2 Identified uses

Ethylene oxide is registered for manufacture, formulation, industrial use and use by professional workers. Ethylene oxide is mainly used for polymer production, as an intermediate and as laboratory agent. But also the use of ethylene oxide in coatings and sealings or in plant protection products is registered.

Detailed information on registered uses is given in table 9-13 (according to REACH dissemination website; accessed May 2015).

Table 9: Manufacture

Manufacture and distribution of ethylene oxide	
Process category	PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)
Environmental release category	ERC 1: Manufacture of substances
Use as a laboratory agent	
Process category	PROC 15: Use as laboratory reagent
Environmental release category	ERC 1: Manufacture of substances

Table 10: Formulation

Polymer	
Process category	PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation)
Chemical product category	PC 32: Polymer preparations and compounds
Environmental release category	ERC 2: Formulation of preparations
Use in production of rocket motors.	
Process category	PROC 1: Use in closed process, no likelihood of exposure
Chemical product category	PC 13: Fuels PC 32: Polymer preparations and compounds
Environmental release category	ERC 3: Formulation in materials

Table 11: Uses at industrial sites

Polymer production	
Process category	PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small

	containers (dedicated filling line, including weighing)
Environmental release category	ERC 6c: Industrial use of monomers for manufacture of thermoplastics
Sector of end use	SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals
Use as an intermediate	
Process category	PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)
Environmental release category	ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates)
Sector of end use	SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals
Use of Polymer	
Process category	PROC 21: Low energy manipulation of substances bound in materials and/or articles PROC 26: Handling of solid inorganic substances at ambient temperature
Environmental release category	ERC 5: Industrial use resulting in inclusion into or onto a matrix
Sector of end use	U 14: Manufacture of basic metals, including alloys SU 15: Manufacture of fabricated metal products, except machinery and equipment
Industrial use of EO cartridge as auxiliary to specific Medical Device	
Process category	PROC 3: Use in closed batch process (synthesis or formulation)
Environmental release category	ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles
Coatings and sealants	
Process category	PROC 10: Roller application or brushing
Environmental release category	ERC 8c: Wide dispersive indoor use resulting in inclusion into or onto a matrix
Sector of end use	SU 19: Building and construction work

Table 12: Uses by professional workers

Use as a laboratory agent	
Process category	PROC 15: Use as laboratory reagent
Environmental release category	ERC 1: Manufacture of substances
Polymer to be used in a Plant Protection Product	
Process category	PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 11: Non industrial spraying
Environmental release category	ERC 8d: Wide dispersive outdoor use of processing aids in open systems
Sector of end use	SU 1: Agriculture, forestry and fishing
Professional use of EO cartridge as auxiliary to specific Medical Device	
Process category	PROC 3: Use in closed batch process (synthesis or formulation)
Environmental release category	ERC 8a: Wide dispersive indoor use of processing aids in open systems
Coatings and sealants	
Process category	PROC 10: Roller application or brushing
Environmental release category	ERC 8f: Wide dispersive outdoor use resulting in inclusion into or onto a matrix

Table 13: Article Service life

Used in the manufacture of polymers	
Environmental release category	ERC 1: Manufacture of substances ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates) ERC 6c: Industrial use of monomers for manufacture of thermoplastics
Use in production of rocket motors.	
Environmental release category	ERC 3: Formulation in materials

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Ethylene oxide is classified as Press. Gas; Flam. Gas 1, H220. No further evaluation done.

4 HUMAN HEALTH HAZARD ASSESSMENT

This CLH proposal is based on the information from REACH registration, public available literature and the information given by industry in the course of a substance evaluation done by the MSCA[2].

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination) (SCOEL, 2012; Fennell, 2001)

Toxicokinetic models for ethylene oxide have been developed and gradually improved (Fennell, 2001; Csanady, 2000). Ethylene oxide is readily taken up by the lungs. In humans at steady state 20–25 % of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound, and 75–80 % is taken up by the body (pulmonary uptake) and metabolised. For mice and rats the uptake was set at 40% and 43% respectively. Ethylene oxide is relatively stable in aqueous solution ($t_{1/2}$ ~76h) facilitating its ability to distribute readily throughout the body. The half-life of ethylene oxide in human blood has been calculated to be 48min (Fennell, 2001). Accordingly, no accumulation of ethylene oxide in humans over a working week is to be expected.

In rats treated with 100ppm ^{14}C-labelled ethylene oxide for 6 hours, 18 hours after the end of exposure, 60 % of the recovered radioactivity was found in the urine, about 6 % in the faeces, and about 9 % as CO_2 and 1 % as unchanged ethylene oxide in the exhaled air. In the internal organs, the highest level of radioactivity was found in the liver, followed by the red blood cells, kidneys and adrenals.

An overview on the metabolic pathways of ethylene oxide is given in Figure 1. 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 (100ppm and above) and non-linearity in metabolic elimination of ethylene oxide. According to available experimental data humans lacking the glutathione transferase human (h)GSTT1 gene are more susceptible towards the sister-chromatid-exchange inducing effect of ethylene oxide than were carriers of the hGSTT1 gene. Genetic factors are therefore jointly responsible for differences in susceptibility of humans to effects of ethylene oxide.

Due to its intrinsic chemical reactivity ethylene oxide alkylates a variety of different sites of biological macromolecules (i.a. proteins at the electron-rich functional groups of the amino acids cysteine, histidine and valine) without requiring prior metabolic activation. In the blood 2-hydroxyethyl adducts with hemoglobin are reported for ethylene oxide exposed humans. For an

[2] http://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/substance-rev/3082/term

occupational exposure (8 h/day, 5 days/week) to 1 ppm EO an hemoglobin adduct level of 2.4 nmol/g Hb is expected at steady state (Casanady, 2000).

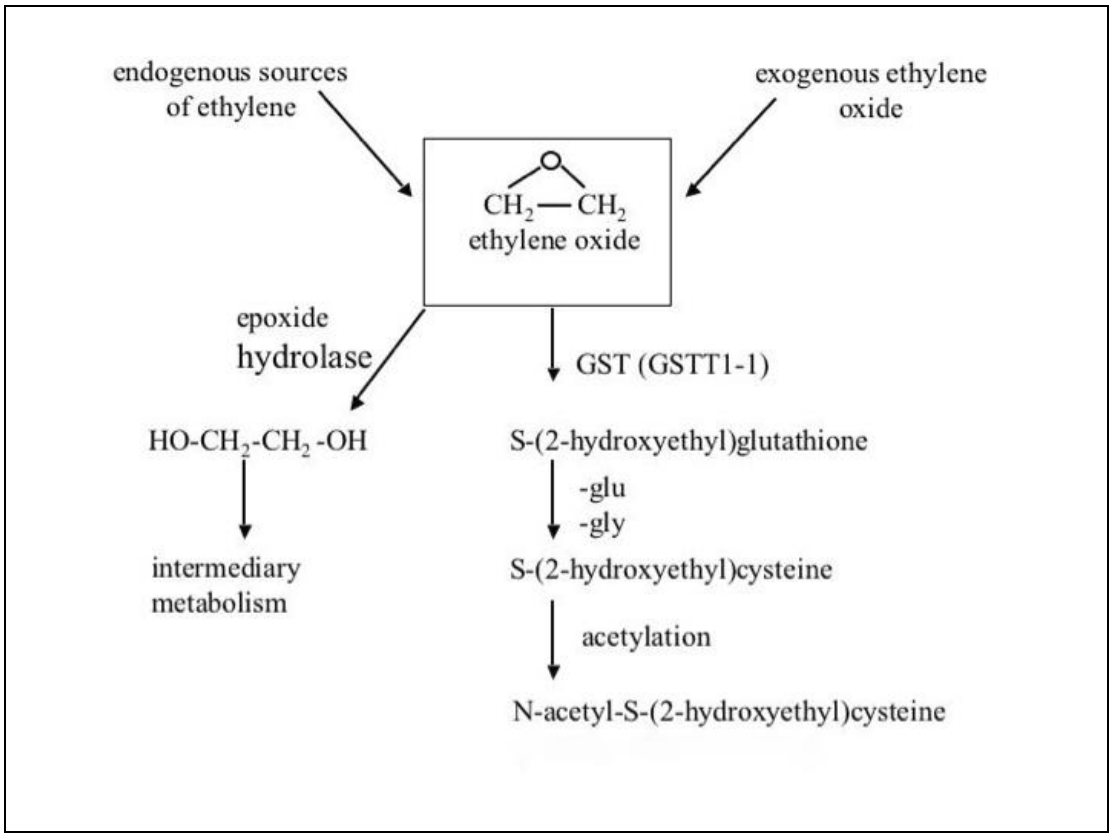


Figure 1: Metabolic pathways of ethylene oxide (SCOEL, 2012).

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Ethylene oxide is not classified for acute oral toxicity so far. The relevant studies for this endpoint are given in Table 14.

Table 14: Summary table of acute oral toxicity studies.

Method	Results	Remarks	Reference
rat (Wistar) male oral: gavage ~10 animals/group Acute Tox Class method	Rat: LD_{50}= 330 mg/kg bw (m) Guinea pig LD_{50} = 270mg/kg bw (m/f)	2 (reliable with restrictions) Key study weight of evidence no data on exact	Smyth H.F. (1941)

Observation for 14d		number of animals and doses administered available. Test material: ethylene oxide	
rat oral: feed equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity)	LD_{50}: 330 mg/kg bw	2 (reliable with restrictions) Supporting study Test material: ethylene oxide	Bruhin H. (1961) (*)
mice, guinea pig oral	LD_{50}: 280 and 365mg/kg bw (f/m mice) LD_{50}: 270mg/kg bw (guinea-pig)	Supporting study	Woodard G. (1971). *as cited in WHO, 2003, Study not available*

(*) this study is mentioned in the aggregated CSR, provided by ECHA during substance evaluation. No further evaluation possible as it is not publically available.

Only a few data on the oral route of exposure are available but they all show LD_{50} values in the same order of magnitude.

Smyth (1941) investigated acute oral toxicity of 60 glycol and glycol derviates in male Wistar rats and guinea pigs (m/f). Test substance was administered by gavage. The diluent was always water (except for insufficient soluble material which then was handled as temporary dispersions in 1% aqueous sodium sulphate of heptadecanol). No information on concentrations or number of animals per dose is given. All deaths within 14 days after exposure were considered for LD_{50} calculation. For rats an LD_{50} of 330mg/kg bw (95% C.I. 290-360mg/kg bw) and for guinea pigs an LD_{50} of 270mg/kg bw (95% C.I. 190-380mg/kg bw) was calculated.

Bruhin (1961) confirms an LD_{50} of 330mg/kg bw for rats while Woodard (1971) investigated mice (LD_{50}=280mg/kg (f) and LD_{50}=365mg/kg (m)) and guinea pigs (LD_{50}=270mg/kg). No further details on these studies are available.

Due to the physico-chemical properties of ethylene oxide the physical state at room temperature is gaseous. In the available literature describing the oral acute toxicity tests no information is given on how the oral application was performed. Ethylene oxide is liquid at 10°C and completely miscible with water (high water solubility) therefore administration by oral route is possible. Some of the applied EO might have been lost by evaporation during handling/administration so the actual LD_{50} values might even be lower than reported.

4.2.1.2 Acute toxicity: inhalation

Ethylene oxide is classified for this endpoint as Acute Tox. 3*, H331 (Toxic if inhaled). The relevant studies are given in Table 15.

Table 15: Summary table of relevant acute inhalation toxicity studies

Method	Results	Remarks	Reference
rat (Sprague-Dawley) male (882-2298ppm) (10 rats per dose) Mice (533-1365ppm) (10 mice per dose) Dogs (327-2830ppm) (3 dogs per dose) inhalation: gas 4h chamber exposure 14day observation	LC_{50} (4 h): 1460 ppm (rat) LC_{50} (4 h): 835ppm (mice) LC_{50} (4 h): 960ppm (dogs)	2 (reliable with restrictions) supporting study Test material: ethylene oxide	Jacobson, K.H. (1956)
rat (Sprague-Dawley) male, female (5 rats per dose) ethylene oxide vapour 850-2182ppm 4h chamber exposure 14day observation	LC_{50} (4h): 1972ppm (male), 1537ppm (female), 1741ppm (combined sexes)	2 (reliable with restrictions) Test material: ethylene oxide	Nachreiner D.J. (1991) and Snellings W.M (2011)
rat (Sprague-Dawley) male (5 rats per dose) Conc: 3609-6161ppm 1h chamber exposure 14day observation	LC_{50} (1h): 5748ppm (male), 4439ppm (female), 5029ppm (combined sexes)	2 (reliable with restrictions) Test material: ethylene oxide	Nachreiner D.J. (1992) and Snellings W.M (2011)
Mice B6C3F1 (5 animals per sex and dose) 4h chamber exposure 100-1600ppm	LC_{50} (4h): 660ppm (female) No LC_{50} calculated for males	2 (reliable with restrictions) key study GLP Test material: ethylene oxide	NTP (1987)

Jacobson, 1956 investigated the effects of acute exposure to ethylene oxide in male white rats, female white mice and male beagle dogs. Animals were exposed for 4h with exposure concentration varying for rats from 882-2298ppm, for mice from 533-1365ppm and for dogs from 327-2830ppm. Mortality is shown in Table 16. The LC_{50} for rats was estimated to be 1460ppm, for mice 835ppm and for dogs 960ppm. Signs of toxicity in rats were nasal discharge, lacrimation, diarrhea, gasping and salivation.

Table 16: Mortality after exposure to ethylene oxide vapour for 4 hours (Jacobson, 1956).

rats		mice		dogs	
ppm	mortality	ppm	mortality	Ppm	mortality
2298	10/10	1365	10/10	2830	3/3
1992	10/10	1343	10/10	1393	3/3
1843	9/10	960	7/10	710	0/3
1648	4/10	882	3/10	327	0/3
1343	2/10	860	6/10		
882	2/10	533	1/10		

In another 4h acute inhalation study, groups of five male and five female Sprague-Dawley rats were exposed to ethylene oxide (99.9%) vapor at 850, 1443 or 1021ppm. Groups of five males also were exposed to 2026 or 2182ppm and five females were exposed to 1637ppm (Table 17). The animals were exposed in a 1300-L glass and stainless steel dynamic chamber. Surviving animals were observed for 14 days after exposure. The LC_{50} was 1972ppm (C.I. = 1887 to 2061) for male rats, 1537ppm (C.I. = 1391 to 1698ppm) for female rats, and 1741ppm (C.I. = 1655 to 1831ppm) for the combined sexes. During exposure, signs of eye, nasal and oral irritation (blepharospasm; wetness and encrustation around the eyes, nose, and mouth; swollen eye tissue), hypoactivity, and signs of respiratory distress (audible respiration, mouth breathing, increased or shallow respiration, and gasping) were noted. Clinical signs immediately after exposure included tremors and an absence of tail and toe pinch reflex in some groups. Clinical signs indicative of eye and respiratory tract irritation and neurologic effects were observed during the first 3 or 4 days after exposure. No clinical signs were observed after the day of exposure in the 1021ppm group or after day 4 in the other exposure groups (Nachreiner, 1991 as cited in National Research Council, 2010; published in Snellings, 2011).).

In a 1h acute inhalation study groups of five male rats were exposed to measured concentrations of 6161, 5546 or 4827ppm and groups of five female rats to concentrations of 4827, 4202, 4064, 3966 and 3609ppm. No deaths occurred in the male group exposed to 4827ppm or in the female group exposed to 3609ppm (Table 17). The LC_{50} was 5748ppm (95% C.I. = 5276 to 6262ppm,) for males, 4439ppm (C.I. = 4034 to 4884ppm) for females, and 5029ppm (95% C.I. = 4634 to 5459ppm) for the combined sexes. Because of extreme variations in the analytic concentrations (3584 to 4432ppm), which probably explain the unusual mortality rate, the 4064ppm female group was not included in the calculation for the LC_{50}. Clinical signs of toxicity were observed in all groups during and after the 1-h exposure up to day 3 or 4 postexposure. Restlessness was observed in all groups during the first 10 min of exposure. In all groups of males and in the 4827ppm female group, only lacrimation was observed on the day of exposure; periocular wetness was observed in the remaining female groups. These findings suggest that ethylene oxide was irritating to the eyes and the respiratory tract and toxic to the nervous system. Gross examination showed effects in the nose,

lungs, and kidneys. Lung weights were elevated in animals that died before the study ended compared with the lungs of animals that survived until study termination, particularly in the male groups (Nachreiner, 1992 as cited in National Research Council, 2010; published in Snellings, 2011). An extrapolation of the results to 4 hour testing exposure (by dividing by a factor of 2) results in an LD_{50} (4h) = 2220ppm for the most sensitive female rats.

Table 17: Mortality rate in mice after 4h and 1h exposure to ethylene oxide (Nachreiner, 1991 and 1992)

4h exposure (Nachreiner, 1991)			1h exposure (Nachreiner, 1992)		
ppm	male	females	ppm	male	females
2182	4/5		6161	4/5	
2026	4/5		5546	1/5	
1850	0/5	5/5	4827	0/5	5/5
1637		4/5	4202		1/5
1443	0/5	1/5	4064		5/5[(1)]
1021	0/5	0/5	3966		2/5
			3609		0/5

[(1)] *Not included in LC_{50} calculation*

In an inhalation study by NTP (1987) groups of five male and female mice were exposed to ethylene oxide concentrations of 100, 200, 400, 800, 1600ppm for 4h. No animals died after exposure to 100, 200 and 400ppm. All males exposed to 800ppm died 2 to 6 days after exposure and four females exposed to 800ppm died 1 to 3 days after exposure. All male and female mice exposed to 1,600 ppm died within 4 h after exposure (see Table 18). Lacrimation and dyspnea were observed at 800ppm; severe dyspnea, incoordination, semiconsciousness, and diarrhea were observed in animals exposed to 1600ppm. No clinical signs were described for the 100- and 400ppm groups. An LC_{50} value of 660ppm (95% C.I. = 509 to 856ppm) (female mice) was calculated by the Spearman-Karber method.

Table 18: Mortality of mice after exposure to ethylene oxide vapour for 4 hours (NTP, 1987).

$B6C3F_1$ mice		
ppm	mortality males	mortality females
100	0/5	0/5
200	0/5	0/5
400	0/5	0/5
800	5/5	4/5
1600	5/5	5/5

4.2.1.3 Acute toxicity: dermal

No acute dermal toxicity studies are available. No classification for this endpoint.

4.2.2 Human information

Casuistic reports of human intoxications showed symptoms like 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, cardiac arrhythmia. The symptoms of systemic intoxication (e.g. headaches, vomiting) often appear before the local effects (irritation). 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. Permanent health impairment as a result of acute ethylene oxide intoxication has not been described (DFG, 1993).

4.2.3 Summary and discussion of acute toxicity

Oral:

The available data on this route of exposure is limited. For oral exposure an LD_{50} = 330 mg/kg bw for male rats can be derived (Smyth, 1941; Bruhin, 1961). Mice and Guinea pigs were the most sensitive species with an LD_{50} of 280mg/kg bw and 270mg/kg bw respectively. LD_{50} values are all in the same order of magnitude.

Inhalation:

Acute toxicity after inhalation has been investigated in 3 species showing toxic effects like dyspnea, diarrhea, lacrimation, incoordination, semiconsciousness, tremor, etc. These effects are the result of irritation of the eyes and the respiratory tract as well as toxicity to the nervous system. For inhalatory exposure (4h) of rats an LC_{50} = 1460ppm (Jacobson, 1956) or LC_{50} = 1741ppm (Nachreiner 1991) can be derived. For mice an LC_{50} = 660ppm (NTP, 1987) can be derived. Generally the lowest valid value would be the basis for classification. Currently ethylene oxide is classified according to Regulation (EC) No. 1272/2008 as acute toxic (Acute Tox 3*) via the inhalation route.

4.2.4 Comparison with criteria

Oral:

According to the CLP criteria, classification as Acute Toxicity 3 (oral) needs to be assigned if the acute toxicity value expressed as LD_{50} value or as acute toxicity estimates is between 50 and 300 mg/kg bw.

The LD_{50} deduced from the existing studies is 270mg/kg bw (guinea pig).

Inhalation:

According to Table 3.1.1 of Regulation (EC) No. 1272/2008 LC_{50} values between 500 and 2500ppm (4h exposure) needs to be classified as Acute Tox 3 (inhal).

The results of the available studies are well between these limits. The most sensitive species (mice) showed an LD_{50}=660ppm for 4h exposure (NTP. 1987).

4.2.5 Conclusions on classification and labelling

Based on the criteria for the oral route of exposure ethylene oxide has to be classified as Acute Tox 3, H301

According to the criteria ethylene oxide shall be classified as Acute Tox 3, H331. Currently ethylene oxide is harmonised classified as Acute Tox 3* (H331) for the inhalatory route of exposure. A removal of the asterisk (*) is proposed. The asterisk indicates a minimum CLP classification which is no longer necessary since the data confirm the classification.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

The DS summarised three studies (all predating GLP and OECD test Guidelines) on oral toxicity of ethylene oxide. Smyth *et al.* (1941) presented LD_{50} values of 60 glycols and glycol derivates, one of them being ethylene oxide, obtained from studies with male Wistar rats and male/female guinea pigs exposed by gavage. No details on test material concentrations were reported and in most cases ten animals per dose group were used. The LD_{50} for ethylene oxide in rats was reported as 330 mg/kg bw and for guinea pigs as 270 mg/kg bw. All deaths occurring within 14 days after exposure were considered when calculating the LD_{50} values.

The LD_{50} of 330 mg/kg bw was later confirmed in a study equivalent or similar to OECD Test Guideline (TG) 401, where rats were exposed to ethylene oxide via the feed (Bruhin *et al.,* 1961). No further details of the study were available.

LD_{50} values of 280 mg/kg bw and 365 mg/kg bw were obtained for female and male mice, respectively, in an oral study (Woodard and Woodard, 1971; cited in WHO, 2003). The same study showed an LD_{50} of 270 mg/kg bw for the guinea pig. No further details of the study were available.

The DS pointed out that ethylene oxide is gaseous at room temperature. No details were available on how, and under which conditions, the oral application was performed in the acute oral toxicity studies. The DS concluded that a proportion of the ethylene oxide might have evaporated during handling/administration, meaning that the actual exposure doses might have been lower than reported.

The DS proposed classification as Acute Tox. 3; H301.

Acute toxicity: inhalation

Acute inhalation studies have been performed with ethylene gas.

An NTP study (1987) performed with male and female B6C3F1 mice was identified by the DS as the key study for acute inhalation toxicity of ethylene oxide. 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 LC_{50} value of 660 ppm (95% CI 509-856 ppm) was calculated (female mice) based on the results.

The effects of acute inhalation exposure to ethylene oxide were studied in groups of five male Sprague-Dawley rats exposed for 4 h at concentrations of 1021, 1443, 1850, 2026 or 2182 ppm and in groups of five females exposed at concentrations of 1021, 1443, 1637, 1850 ppm (Nachreiner, 1991; described in Snellings, 2011). Surviving animals were observed for 14 days after exposure. Clinical signs indicative of eye, nasal and oral irritation (blepharospasm; periocular/perinasal/perioral wetness, swollen eye tissue), hypoactivity and signs of respiratory distress (audible respiration, gasping), as well as absence of tail/toe pinch reflex were observed during or immediately after the exposure. Similar clinical signs indicating eye and respiratory tract irritation and neurological effects were observed during the next 3-4 days after exposure. No indications of clinical effects were observed after day 4. The numbers of dead male animals were as follows: 1850 ppm: 0/5; 2026 ppm: 4/5; 2182 ppm: 4/5. An LC_{50} value of 1972 ppm (95% confidence interval (CI) 1887-2061 ppm) was calculated for male rats. For female animals the following deaths occurred: 1443 ppm: 1/5; 1637 ppm 4/5; 1850 ppm: 5/5, leading to LC_{50} = 1537 ppm (95% CI 1391-1831 ppm). The LC_{50} value for the combined sexes was 1741 ppm (95% CI 1655-1831 ppm).

Exposure of groups of Sprague-Dawley rats to ethylene oxide for 1 h by inhalation resulted in LC_{50} values of 5748 ppm (95% CI 5276-6262 ppm) for males, 4439 ppm (95% CI 4034-4884 ppm) for females, and 5029 ppm (95% CI 4634-5459 ppm; combined group) (Nachreiner, 1992; described in Snellings, 2011). The exposure concentrations for groups of male rats (n=5) were 4827, 5543 and 6161 ppm, and for female rats (n=5) 3609, 3966, 4064, 4202 and 4827 ppm. When adjusted to 4 h of exposure, the corresponding LC_{50} values were 1437 ppm (males) and 1110 ppm (females).

In an inhalation study (Jacobson and Hackley, 1956), the acute effects of ethylene oxide were studied in male Sprague-Dawley rats, female white mice and beagle dogs. The animals were exposed for 4 h at concentrations of 882-2298 ppm (rats), 533-1365 ppm (mice) and 327-2830 ppm (dogs). Based on the mortality observed in the study, the LC_{50} values were calculated as follows: rats 1460 ppm, mice 835 ppm and dogs 960 ppm. Clinical signs of toxicity included nasal discharge, lacrimation, diarrhoea, gasping and salivation.

The DS proposed classification as Acute Tox. 3; H331.

Acute toxicity: dermal

No data were available and no classification was proposed.

Comments received during public consultation

Comments on acute oral toxicity were received from one MSCA and one national authority who supported the proposal for classification as Acute Tox. 3; H301. Two industrial/trade association stakeholders commented that it is questionable whether it is relevant to classify ethylene oxide for acute oral toxicity as it is gaseous at room temperature.

Two MSCAs and one national authority supported classification as Acute Tox. 3; H331. No other comments were received on acute toxicity by inhalation.

Assessment and comparison with the classification criteria

Acute toxicity: oral

The lowest LD_{50} value, 270 mg/kg bw, was obtained in two studies with guinea pigs. An LD_{50} value of 280 mg/kg bw was obtained in a study with female mice. According to the CLP criteria, classification is required where the LD_{50} is ≤ 2000 mg/kg bw. Furthermore, if the acute toxicity value expressed as LD_{50} is between 50 mg/kg bw and 300 mg/kg bw, the resulting classification is Acute Tox. 3; H301. Thus, RAC agrees with the proposal of the DS to classify ethylene oxide for oral acute toxicity as **Acute Tox. 3; H301 (Toxic if swallowed)**. Ethylene oxide is a colourless gas at room temperature. It has a boiling point of 10.7°C. During storage and transport, ethylene oxide is kept as a liquid under moderate pressure. In those situations exposure to liquid ethylene oxide may occur.

Acute toxicity: inhalation

In the inhalation studies, the LC_{50} values, calculated for 4-h exposure, varied between 660 ppm (female mice) and 1972 ppm (male rats). According to the CLP criteria, classification is required where the LC_{50} is ≤ 20000 ppm. For classification as Acute Tox. 3; H331, the LC_{50} needs to be between 500 and 2500 ppm. All LC_{50} derived from the different studies were well between these limits. RAC agrees with the proposal of the DS to classify ethylene oxide for acute toxicity by inhalation as **Acute Tox. 3; H331 (Toxic if inhaled)**.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Ethylene oxide is classified as irritant to the respiratory tract (STOT SE 3). In addition effects on the nervous system after single exposure are described in literature.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

4.3.1.1 Human information

Human evidence and effects seen in a study with rats give some concern about neurotoxicity after single exposure to ethylene oxide.

Table 19: Human evidence for neurotoxicity after single exposure.

Method	Results	Remarks	Reference
Case report (43-year-old female nurse) Accidental release of ethylene oxide vapour (estimated 500ppm)	Nausea, stomach spasms, paleness, light. headedness, short periods of unconsciousness, convulsive movements of arms and legs, periods of apnea, muscle twitching, nausea	Supporting study	Salinas E. (1981) (cited in US EPA, 2010)

Method	Results	Remarks	Reference
Duration: 2-3min	Inability to perform minor motor tasks continued for up to 1 week after exposure		
Case report (n=5) Accidental release ; >260ppm Duration: 30min	Irrit. of upper resp.tract, headache, intense generalized pruritus muscular weakness in one worker	Supporting study Coexposure ethylene oxide and carbon dioxide	Deleixhe P.A. (1986) (cited in US EPA, 2010)
Survey (n=165) 11-23.5ppm Duration per cycle: 2.77-11.75min	Headaches, skin and eye irritation, dry mouth, sore throat, skin rash, loss of sense of smell, shortness of breath, nausea, numbness in fingers, drowsiness		Bryant H.E. (1989) (cited in US EPA, 2010)

After short term/single exposure due to accidents effects on the central nervous system have been observed. Salinas (1981) described the case of a 43-year old nurse. Exposure to ethylene oxide during disposal of a dropped ampule (2-3min, 500ppm) resulted in nausea, stomach spasms, paleness, light. headedness, short periods of unconsciousness, convulsive movements of arms and legs, periods of apnea, muscle twitching and nausea. Malaise continued for 24h after exposure. Malaise and an inability to perform minor tasks continued for up to 1 week after exposure. The patient was asymptomatic 2 months after exposure (US EPA, 2010).

Five hospital workers are described by Deleixhe (1986), who were exposed for 30min to ethylene oxide vapors emitted from a leaky sterilized (>260ppm). The sterilizing gas was a mixture of ethylene oxide and carbon dioxide. Two workers experienced only headache and diarrhea which disappeared within 70h after exposure. The other three showed more serious signs of toxicity like irritation of the upper respiratory tract, dry mouth and thirst, conjuctival irritation, severe headache, intense generalized pruritus; muscular weakness in one worker and dizziness in another (US EPA, 2010).

Bryant (1989) made a survey on sterilizer workers in 27 hospitals; 165 workers were identified by a questionnaire to have short term-exposure to ethylene oxide. The exposure duration per cycle ranged from peaks of 11ppm to 23.5ppm. The total exposure concentration per sterilizer cycle ranged from undetectable to 10.7 ppm with exposure durations per cycle ranging from 166 s (2.77 min) to 705 s (11.75 min). The mean concentration per cycle was 3.4ppm. The detection of the ethylene oxide odor suggests that the concentrations exceeded 260 ppm, at least briefly. The most prevent 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. No distinction between short-term effects and effects due to repeated exposure is possible (US EPA, 2010).

4.3.1.2 Non-human information

Effects on the nervous system of animals have been observed in an acute exposure study by Snellings (2011). Groups of five Sprague-Dawley rats/sex were exposed for 1h or 4h to ethylene oxide concentrations ranging from 1443ppm up to 6161ppm, and clinical signs and mortality were recorded. For details see chapter 4.2. In both the 1-hour and 4-hour studies, clinical signs of ataxia, tremors, absence of the startle reflex, absence of the tail/toe pinch reflex, and decreased respiration rate were noted, and all of these could have a neurologic effect from EO exposure as a component. For all groups no clinical signs were observed in survivors after postexposure (day 5 after 4h exposure, day 2/3 after 1h exposure).

US EPA (2010) describes an acute neurotoxicity study (Mandella, 1997a) where groups of 10 male and 10 female Sprague-Dawley rats were exposed by whole body inhalation to ethylene oxide at 0, 100, 300, or 500 ppm for 6 h and observed for 14 days after exposure. Neurobehavioral assessments that included the standard functional observational battery (FOB) and motor activity tests were performed on all animals on day 1 and on days 8 and 15 after exposure. The results of the FOB assessment showing exposure-related effects (slightly impaired locomotion, drooping half-closed eyelids, no reaction to approach, low arousal). No clear exposure related effects were observed on day 8 and 15 (reversibility of effects).

Table 20: Neurotoxicity in animals after single exposure

Method	Results	Remarks	Reference
Acute neurotoxicity study Sprague-Dawley rats (10m and 10f each group) Concentration: 1,100, 300, 500ppm Exposure: inhalation 6h	NOAEC=100ppm FOB (day 1): The incidences of low arousal and no response to approach were significantly increased in male rats and both sexes combined at 300 and 500 ppm, and the incidence of droopy, half-closed eyelids was significantly increased in both sexes at 500 ppm. Motor activity was decreased in both sexes at 500 ppm and in males at 300 ppm and was correlated with the decrease in normal exploratory activity. No clear exposure-related effects were observed on day 8 or 15.	Supporting study Study not available	Mandella R.C. (1997a) (cited in US EPA. 2010)
Acute inhalation toxicity study Sprague-Dawley rats (5f+5m per group)	4h: absence of tail/toe pinch reflex, tremor No signs after postexposure day 5	Supporting study GLP	Snellings W.M. (2011)

Method	Results	Remarks	Reference
Exposure: 1h, 4h Concentration: 4h: 2182-1443ppm 1h: 6161-3966ppm	1h: absent startle reflex ataxia, tremors No signs after postexposure day 2 (m), 3 (f)		

4.3.2 Comparison with criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure have to be classified in Category 1. Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of:

(a) reliable and good quality evidence from human cases or epidemiological studies; or
(b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.

Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance value (inhalation) for classification as STOT SE Category 1 is C≤2500ppmV/4h and for STOT SE Category 2 20000≥C≤2500ppmV/4h.

Human evidence after exposure to ethylene oxide vapor is available. Effects on the nervous system have been seen after two accidental exposures at estimated concentrations of 500ppm (n=1) or >260ppm (n=1). The effects seen in the survey cannot clearly be attributed to a short term exposure. First effects in rats were seen at concentrations of 300ppm (NOEAC=100ppm).

4.3.3 Conclusions on classification and labelling

There is some evidence from two case reports that ethylene oxide affects the nervous system after single exposure. Animal studies also show impairment of the nervous system but these effects were reversible in rats.
Based on the minor severity of effects and the reversibility no classification for STOT SE is proposed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Ethylene oxide has an existing classification as STOT SE 3; H335 for respiratory irritation.

These effects were not included in the evaluation of the DS which focused on neurological effects relevant for classification for STOT SE.

Information on humans

Three studies indicating human neurological effects after single exposure were included in the dossier.

In a case report on a 43-year old nurse, effects occurring after exposure to ethylene oxide (estimated concentration 500 ppm) for 2-3 minutes during disposal of a dropped ampule were reported (Salinas, 1981; cited in US EPA 2010). The symptoms included nausea, stomach spasms, paleness, light-headedness, short periods of unconsciousness, convulsive movements of arms and legs, periods of apnoea, muscle twitching and nausea. Malaise and an inability to perform minor tasks persisted for up to one week after exposure. Two months after the exposure, the patient was asymptomatic.

Another case report (Deleixhe, 1986; cited in US EPA 2010) described the exposure of five hospital workers, due to a leakage of sterilising gas consisting of ethylene oxide and carbon dioxide. Based on the odour, it was estimated that the concentration of ethylene oxide was ≥ 260 ppm. Two of the workers suffered from headache and diarrhoea, which disappeared within 70 h after exposure. The other three exposed workers had more severe symptoms, including severe headache, upper respiratory tract irritation, conjunctival irritation, intense generalised pruritus, dry mouth and thirst. Furthermore, one of the workers suffered from muscular weakness and another one from dizziness.

In a survey distributed to hospitals, 165 steriliser workers were identified as having short-term exposure to ethylene oxide (Bryant 1989, cited in US EPA 2010). The exposure levels ranged from undetectable to 10.7 ppm per steriliser cycle, the mean concentration being 3.4 ppm (duration 166 – 705 s). Based on the odour it was estimated that the peak concentrations were > 260 ppm, at least during short times. The most prevalently reported symptoms included headache, skin and eye irritation, dry mouth and sore throat. Other symptoms were skin rash, runny nose, loss of sense of smell, shortness of breath, nausea, numbness in fingers and drowsiness.

Non-human information

In an acute neurotoxicity study, groups of 10 male or 10 female Sprague-Dawley rats were exposed for 6 h to concentrations of 0, 100, 300 or 500 ppm ethylene oxide by inhalation (Mandella, 1997; cited in US EPA 2010). The animals were observed for 14 days after exposure. On days 1, 6 and 15, neurobehavioral assessments, including a standard functional battery and motor activity tests, were carried out. In the groups of male rats exposed to ethylene oxide at 300 ppm and 500 ppm, the incidences of low arousal and no response to approach were significantly increased. The same result was obtained when combining the outcome among males and females at these concentrations. The incidence of droopy, half-closed eyelids was significantly increased both for males and females. Decreased motor activity was observed among both sexes at 500 pm and among males at 300 ppm. No clear exposure-related effects were observed at follow-up on days 8 and 15.

Acute toxicity studies with Sprague-Dawley rats reported signs of neurological effects after inhalation exposure for 4 h (exposures: males 1021-2182 ppm; females 1021-1850 ppm) or 1 h (exposures: males 3609-6161 ppm; females 3609-4827 ppm) (Snellings, 2011). The findings included ataxia, tremors, absence of the startle reflex, absence of the tail/toe pinch reflex and decreased respiration rates were observed in rats exposed to ethylene oxide for 1 h or 4 h. The 1-h study resulted in LC_{50} values of 5748 ppm in males and 4439 ppm in

females (see Acute toxicity). The effects were reversible and on day 5 after the 4-h exposure and day 2/3 after the 1 h exposure, no clinical signs were observed.

The DS compared the available data on effects observed upon single exposure with the classification criteria for STOT SE Category 1 or 2 and proposed no classification for STOT SE, on the basis of the minor severity of effects and the reversibility of the findings. STOT SE 3 (H336) was not considered by the DS.

Comments received during public consultation

One MSCA commented that the appropriateness of the current classification as STOT SE 3; H335 should have been evaluated. Another MSCA commented that a classification for narcotic effects (STOT SE 3; H336) should have been discussed, as such effects were reported in humans and in animals in acute inhalation toxicity studies.

Assessment and comparison with the classification criteria

There are two case reports indicating acute neurological effects in humans at high, accidental exposures of up to 500 ppm. In one of those cases, there was co-exposure with carbon dioxide. In the third human study presented by the DS, symptoms of mild central nervous system (CNS) effects were reported among steriliser workers. These reports, although limited, suggest that ethylene oxide may have at least transient effects on the CNS. An acute animal neurotoxicity study showed reversible CNS depression, with significantly increased responses including low arousal, decreased motor activity and partly closed eyelids in both sexes, at 300-500 ppm. Acute animal inhalation studies have shown clinical signs including ataxia, tremors, absence of the startle reflex, absence of the tail/toe pinch reflex, low arousal, and no response to approach. Since the dose levels were close to LC_{50}, some of these effects may also have been related to the general toxicity. These effects have been summarised in the tables below.

Table. Clinical findings in male and female Sprague-Dawley rats exposed to ethylene oxide vapour for 4 h. The LC_{50} values calculated from the study were 1972 ppm for males and 1537 ppm for females. Source: Nachreiner 1991, as reported by National Research Council (2010).

	Males			Females			
	Concentration (ppm)			Concentration (ppm)			
Effects	2182	2026	1850	1850	1637	1443	1021
During exposure							
Bleopharospasm	+	+	+	+	+	+	+
Wetness around eyes and nose	+	+	+	+	+	+	+
Hyperactivity	+	+	+	+	+	+	+
Mouth breathing	+			+			
After exposure							
Unkempt fur	+	+	+	+	+		
Wetness or encrustation around eyes, nose and mouth	+	+	+	+	+	+	+

Swollen tissue around eyes							+
Mouth breathing	+	+	+	+	+	+	
Audible respiration	+	+	+	+	+	+	
Gasping	+	+	+	+			
Decreased, increased or shallow respiration	+	+	+ *	+	+		+ *
Absence of tail and toe pinch reflex		+			+		
Hypoactivity	+	+	+	+	+	+	
Tremors		+				+	

* Increased respiration rate and shallow respiration only

Table. Clinical findings in male and female Sprague-Dawley rats exposed to ethylene oxide vapour for 1 h. The LC_{50} values calculated from the study were 5748 ppm for males and 4439 ppm for females. Source: Nachreiner 1992, as reported by National Research Council (2010).

	Males			Females				
	Concentration (ppm)			Concentration (ppm)				
Effects	6161	5546	4827	4827	4202	4064	3966	3609
During exposure								
Restlessness	+	+	+	+	+	+	+	+
Wetness around eyes	+	+	+	+	+	+	+	+
Lacrimation	+	+	+	+				
Mouth breathing	+							
Hypoactivity	+	+	+	+	+	+	+	+
No acoustic startle reflex	+	+	+	+				
After exposure								
Unkempt fur	+	+		+	+	+	+	+
Wetness or encrustation around eyes, nose and mouth			+	+	+			+
Decreased respiration	+	+		+	+	+	+	
Hypoactivity	+	+		+		+	+	+
Ataxia	+				+	+	+	+
Tremors	+	+			+	+	+	

Because of the limited data on the severe effects in humans and symptoms, which are more attributable to transient CNS depression in animals, RAC considers that classification for STOT SE 1 is not applicable for ethylene oxide. Neither were animal data identified which would have fulfilled the criteria for classification as STOT SE 2. According to the criteria, transient CNS effects observed in animals or humans should be classified as STOT SE 3 rather than STOT SE 1 or 2. RAC considers that classification of ethylene oxide as STOT SE 2 is not justified.

A classification as STOT SE 3; H336 can be assigned if exposure to a chemical causes CNS depression, including narcotic effects (drowsiness, narcosis, reduced alertness, loss of

reflexes, lack of coordination, and vertigo) in humans. According to the CLP guidance, the effects can be manifested as severe headache or nausea. Clinical signs may include reduced judgement, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reduced reaction time, or sleepiness. The classification may also be appropriate if studies with experimental animals show transient effects, including lethargy, lack of coordination, loss of righting reflex, and ataxia.

Two human case reports on acute, accidental exposure to ethylene oxide present symptoms (e.g. headache, light-headedness, dizziness, unconsciousness) indicating transient narcotic effects. In one of the case reports the exposure involved a mixture of ethylene oxide and carbon dioxide. The outcome of a survey among hospital workers exposed to ethylene oxide indicated the occurrence of symptoms including headache and dizziness. Reports on neurological effects in humans upon repeated exposure also presented symptoms, which are often more related to acute exposure than repeated exposure, like dizziness or nausea.

Acute animal inhalation toxicity studies showed significantly increased clinical signs, including ataxia, tremors, absence of the startle reflex, absence of the tail/toe pinch reflex, low arousal, and no response to approach, which may be related to CNS effects of ethylene oxide.

Taking into account the observations from one acute neurotoxicity study and one acute inhalation toxicity with rats, and the reported symptoms related to accidental ethylene oxide exposure in sterilising units, RAC concludes that the criteria for classification for specific target organ toxicity, based on transient, narcotic effects, are fulfilled and classification as **STOT SE 3; H336 (May cause drowsiness and dizziness)** is warranted.

Ethylene oxide is currently classified as STOT SE 3; H335 based on its potential to cause respiratory irritation. An evaluation of this classification was not considered by the DS, and no data were included in the dossier. RAC did not therefore evaluate the classification for respiratory irritation.

4.4 Irritation

Ethylene oxide has a harmonised classification according to Regulation (EC) No. 1272/2008 as skin and eye irritant (Skin Irrit. 2, Eye Irrit. 2) and STOT SE3, H335 (May cause respiratory irritation).

4.4.1 Skin irritation

The data on skin irritation testing are presented in Chapter 4.5 as they indicate corrosivity of ethylene oxide.

4.4.2 Eye irritation

It should be noted that if a substance or mixture is classified as Skin corrosive Category 1 then serious damage to eyes is implicit and there is no need to proceed with classification for eye effects (CLP guidance, chapter 3.3[3]). Such substances are automatically considered to be severely

[3] Guidance on the application of CLP criteria. Version 4.1 June 2015

damaging to the eye and are classified but not labelled for serious eye damage in addition to skin corrosion.

For completeness the available test data (McDonald, 1977) are presented below.

Non-human information

Table 21: Summary table of available studies on eye irritation

Method	Results	Remarks	Reference
rabbit (New Zealand White) Vehicle: dilution with a balanced salt solution 1%, 0.1% and 0.01% ethylene oxide in physiologic salt solution methods of Draize (1955) and Baldwin et al. (1973)	irritant Severity of corneal cloudness see table below	2 (reliable with restrictions) Supporting study Test material (EC name): ethylene oxide	McDonald T. O. (1977)

McDonald (1977) examined the toxicity of a 0.1% and 1% dilution of ethylene oxide on normal (n=6) and irritated eyes (n=6) (0.1ml of a commercial available shampoo was diluted and used to produce mild, transient ocular irritation). No information on the stability of the test preparations is given. Control groups included six normal untreated eyes, six eyes that were irritated when receiving the vehicle, six normal eyes which received the vehicle and six irritated eyes with no vehicle treatment. Physiologic salt served as vehicle control. All treated eyes received topical ocular instillation of 0.05ml/dose at 10min intervals for 6hours. Ocular changes were graded at the end of 6h and at 24h (and 48h for one concentration) according to the method of Draize and Baldwin. With increasing concentration of ethylene oxide various ocular pathologic changes were observed (congestion, swelling, discharge, infrequent incidence of flare, iritis, and evidence of corneal cloudiness associated with loss of epithelia cells). A dose response relationship for these changes was observed.

Table 22: Severity of corneal cloudiness after 6h topical ocular instillation of ethylene oxide in rabbit eyes (McDonald, 1977).

Ethylene oxide conc.	eye	Severitiy (mean ocular score* and number of animals)			
		0h	6h	24h	48h
1%	irritated§	0.9 (11/12)	1.1 (10/12)	1.0 (9/12)	0.5 (4/12)
	normal	-	1.2 (11/12)	1.9 (12/12)	0.8 (7/12)
0.1%	irritated§	1.0 (6/6)	0.8 (5/6)	0.0 (0/6)	-
	normal	-	0.0 (0/6)	0.0 (0/6)	-
0.01%	irritated§	0.8 (4/6)	0.7 (4/6)	0.0 (0/6)	-
	normal	-	0.0 (0/6)	0.0 (0/6)	-

Physiol. salt solution	irritated§	1.1 (21/24)	0.9 (18/24)	0.2 (6/24)	0.0 (0/24)
	normal	-	0.0 (0/24)	0.0 (0/24)	0.0 (0/24)
Untreated controls	irritated§	1.0 (21/24)	1.1 (18/24)	0.2 (5/24)	0.0 (0/24)
	normal	-	0.0 (0/24)	0.0 (0/24)	0.0 (0/12)

* Maximum score = 4, § pretreated with diluted shampoo

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

As ethylene oxide was proposed to be classified as skin corrosive, the DS concluded that serious damage to the eyes is implicit, and the substance is automatically considered to be severely damaging to the eye. However, for completeness, the DS also presented the available data on eye irritation.

One eye irritation study was available (McDonald, 1977). NZW rabbits were used for the examination of the toxicity of 0.1% or 1% dilutions of ethylene oxide in normal (n=6) and irritated eyes (n=6, irritation performed using a diluted shampoo). No information on the stability of the test solutions was presented. The treatment was performed by topical ocular application of 0.05 mL of the dilution at 10 min intervals for 6 h. At the end of the exposures (6 h), ocular changes were evaluated and scored for severity (0-4) according to the method of Draize and Baldwin. Follow-ups were performed for all animals at 24 h, and for the 1% dose at 48 h. A dose-response relationship for ocular pathologic changes, including congestion, swelling, discharge, infrequent incidence of flare, iritis, and evidence of corneal cloudiness associated with loss of epithelia cells, was observed. The highest score (1.9) was assigned at the 24 h examination of the group exposed to 1% ethylene oxide in normal eyes (see table below).

Table: Severity of corneal cloudiness after 6h topical ocular instillation of ethylene oxide in rabbit eyes (McDonald, 1977).

Ethylene oxide conc.	eye	Severity (mean ocular score* and number of animals)			
		0 h	6 h	24 h	48 h
1%	irritated§	0.9 (11/12)	1.1 (10/12)	1.0 (9/12)	0.5 (4/12)
	normal	-	1.2 (11/12)	1.9 (12/12)	0.8 (7/12)
0.1%	irritated§	1.0 (6/6)	0.8 (5/6)	0.0 (0/6)	-
	normal	-	0.0 (0/6)	0.0 (0/6)	-
0.01%	irritated§	0.8 (4/6)	0.7 (4/6)	0.0 (0/6)	-
	normal	-	0.0 (0/6)	0.0 (0/6)	-
Physiol. salt solution	irritated§	1.1 (21/24)	0.9 (18/24)	0.2 (6/24)	0.0 (0/24)
	normal	-	0.0 (0/24)	0.0 (0/24)	0.0 (0/24)

Untreated controls	irritated§	1.0 (21/24)	1.1 (18/24)	0.2 (5/24)	0.0 (0/24)
	normal	-	0.0 (0/24)	0.0 (0/24)	0.0 (0/12)

* Maximum score = 4,
§ pre-treated with diluted shampoo

Comments received during public consultation

One MSCA and one national authority supported the classification as Eye Dam. 1; H318. In a comment form industry, it was suggested that further *in vitro* skin irritation/corrosion tests need to be performed in order to justify the classification for skin irritation/corrosion, and only after that can a decision be made on eye damage/irritation.

Assessment and comparison with the classification criteria

According to CLP, skin corrosive substances shall be considered as leading to serious eye damage as well. The hazard statement for skin corrosion addresses also serious eye damage (H314: Causes severe skin burns and eye damage). Thus, there is no need for comparison of the available data from one eye irritation study with the classification criteria for eye damage/irritation.

As ethylene oxide is proposed to be classified as Skin Corr. 1, RAC agrees that it shall also be classified as Eye Dam. 1; H318. According to CLP, the hazard statement H318 (Causes serious eye damage) is in these situations not included on the label because of redundancy.

4.4.3 Respiratory tract irritation

No further evaluation done.

4.4.4 Summary and discussion of irritation

Information on skin irritation/corrosion is presented and discussed in Chapter 4.5.

One study on eye irritation (McDonald, 1977) is available. The ethylene oxide concentration tested is very low (max 1%) and no information in the stability of the test preparation is given, therefore the value of this study is limited. However ethylene oxide is a corrosive substance and such substances are automatically considered to be severely damaging to the eyes.

4.4.5 Comparison with criteria

A substance will be classified as Eye Irrit 1 when it produces (1) at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (2) at least in 2 of 3 tested animals, a positive response of corneal opacity (≥ 3) and/or iritis (> 1,5) calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

A substance will be classified as Eye Irrit 2 when it produces at least in 2 of 3 tested animals, a positive response of corneal opacity (≥ 1) and/or iritis (≥ 1), and/or conjunctival redness (≥ 2) and/or conjunctival oedema (chemosis) (≥ 2) calculated as the mean scores following grading at 24,

48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days.

Skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1) (Regulation EC No 1272/2008).

Ethylene oxide is corrosive (proposed classification as Skin Corr 1B) therefore serious damage to eyes (Category 1) is considered implicit.

4.4.6 Conclusions on classification and labelling

The available studies on skin irritation show a corrosive potential of ethylene oxide (resulting in a classification as Skin Corr 1B), therefore no classification as skin irritant is warranted (see Chapter 4.5).

Ethylene oxide currently is harmonized classified as Eye Irrit 2, H319. The evidence from the available study on eye irritation is limited due to low test concentration. However for skin corrosive substance (like ethylene oxide) serious damage to eyes is considered implicit (GLP guidance). This is already indicated in the hazard statement for skin corrosion (H 314: Causes severe skin burns and eye damage). Thus, in this case both classifications - Skin Corr. 1B, H314 and Eye Dam. 1, H318 are required for ethylene oxide. The hazard statement H318 ‘Causes serious eye damage’ is not indicated on the label because of redundancy (CLP Article 27) (CLP guidance).

4.5 Corrosivity

4.5.1 Non-human information

For evaluation of the corrosive potential of ethylene oxide two non-GLP studies are available. Study details are given in the table below.

Table 23: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
skin rabbit (New Zealand White) n=6 patch test (abraded) – 4h exposure 0.5ml undiluted liquid DOT method	corrosive subdermal hemorrhages and chemical burns at any time of examination	2 (reliable with restrictions) Non GLP supporting study experimental result Test material: ethylene oxide (undiluted)	Celanese Chemical Co., Inc. (1972)
skin rabbit Aqueous solution of ethylene	hyperemia and edema resulted when the duration of skin contact was 6 minutes or longer. The severer exposures	2 (reliable with restrictions) key study experimental	Hollingsworth, R.L. et al. (1956)

oxide (10% and 50%) Coverage: occlusive (shaved) Exposure 1-60min	resulted in scar formation. The intensity of response was roughly proportional to the length of exposure time and concentration	result Test material: ethylene oxide (10-50%)	

Celanese (1972) conducted a skin irritation test on New Zealand white rabbits. The test protocol was modelled after DOT (Department of Transportation, Hazardous Materials Regulation), non-GLP. 0.5ml of undiluted liquid ethylene oxide was applied on intact and abraded skin (four epidermal incisions) of six rabbits. Test sites were immediately covered with gauze patches, secured with masking tape and wrapped with plastic sheeting. At the end of four hours the plastic wrappings and patches were removed and the test sides were examined and scored for erythema and edema on a graded scale of 0 to 4. After 24 and 72 hours the sites were again scored (see Table 24:). Ethylene oxide showed irritation scores of 4 with subdermal hemorrhages and chemical burns at any time of examination.

Table 24: Results of the skin irritation test in Albino rats (Celanese, 1972).

	Scores for abraded skin						Scores for intact skin					
No	4h		24h		72h		4h		24h		72h	
	Eryth	Edema	Eryth	Edema	Eryth	Edema	Eryth	Edema	Eryth	Edema	Eryth	Edema
1	4	4*	4	4*§	4	4*§	4	4*	4	4*§	4	4*§
2	4	4§	4	4*§	4	4*§	4	4§	4	4*§	4	4*§
3	4	4*	4	4*§	4	4*§	4	4*	4	4*§	4	4*§
4	4	4*	4	4*§	4	4*§	4	4*	4	4*§	4	4*§
5	2	1*	4	4*§	4	4*§	2	3*	4	4*§	4	4*§
6	4	4*	4	4*§	4	4*§	4	4*	4	4*§	4	4*§
mean	3.7	3.5	4	4	4	4	3.7	3.8	4	4	4	4

(*) subdermal hemorrhages, (§) chemical burns

Hollingsworth (1956) reported a test on intact shaved abdominal skin of immobilized rabbits under plastic cover for periods of time ranging from 1 to 60 minutes. The animals were observed for six to seven days following exposure. 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. No further information (e.g. scoring, number of animals) is available in the original literature.

4.5.2 Human information

Exposure of large skin areas to a 1% aqueous solution of ethylene oxide for about 2h resulted in a severe blistering in human individuals after 12-14h (Sexton, 1949).

Series of aqueous solutions with ethylene oxide concentrations between 1 and 90% were tested on human skin (Sexton, 1950). The 50% solution produced the most severe skin reaction, which was

attributed to the rapid evaporation of the more concentrated solutions, which prevented more prolonged skin contact (reviewed in ATSDR, 1990).

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.

4.5.3 Summary and discussion of corrosivity

Liquid ethylene oxide causes severe skin lesions (chemicals burns, hemorrhages, scar formation) in in vivo animal testing. Case reports also demonstrate corrosive potential of the substance.

Ethylene oxide or solutions of ethylene oxide are highly reactive alkylating agents that react with many constituents of tissue resulting in cellular and tissue dysfunction and destruction. Ethylene oxide is strongly corrosive with rapid evaporation from skin.

4.5.4 Comparison with criteria

A corrosive substance is a substance that produces destruction of skin tissue (visible necrosis through the epidermis and into the dermis). Three subcategories for classification as corrosive are provided: subcategory 1A, where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B, where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C, where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

For ethylene oxide one positive study with exposure duration of 4h (showing chemicals burns, hemorrhages) and one positive study with exposure durations from 1-60 minutes (showing hyperemia, edema at 6min or longer) is available. Positive evidence from humans is available.

4.5.5 Conclusions on classification and labelling

Due to the available human and animal data and the knowledge about the reactivity of ethylene oxide a classification as Skin Corr 1B is recommended.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Human information

One study (Sexton, 1950; cited in ATSDR, 1990) examining effects occurring after application of ethylene oxide as an aqueous solution at concentrations of 1-90% on human skin has been reported. The 50% aqueous solution presented the most severe reactions. At higher concentrations, the evaporation of ethylene oxide increased and the skin contact time was thus

shorter. No further details available.

Exposure of large skin areas to 1% aqueous solution of ethylene oxide for 2 h was reported to cause a severe blistering after 12-14 h (Sexton, 1949).

A number of cases of patients in hospitals are reported showing skin reactions (erythema, blister formation, scaling, crusted ulcerations and severe/second degree burns) after contact to e.g., gauze, gowns, drapes or breast implants that had been sterilised with ethylene oxide (Alomar, 1981; Hanifin, 1971; cited in ATSDR, 1990).

Non-human data

In a non-GLP study performed with New Zealand White (NZW) rabbits, intact (n=6) and abraded (n=6) skin was exposed to 0.5 mL of undiluted ethylene oxide for 4 h under occlusive conditions (Celanese Chemical Co., Inc., 1972). At the end of the exposure time, the plastic wrapping was removed and the test sites were scored for erythema and oedema on a graded scale from 0 to 4. The sites were re-examined and scored again after 24 and 72 h. The exposure resulted in clear signs of irritation: subdermal haemorrhages and chemical burns were observed immediately after the exposure as well as during re-examination.

Another report (Hollingsworth *et al.,* 1956) presented results from skin exposure of rabbits using 10% and 50% aqueous solutions of ethylene oxide. The solution was applied on shaved skin and covered with plastic for 1-60 min. In animals exposed for six minutes or longer, hyperaemia and oedema were observed. Scar formation was observed upon longer exposure. The severity of the effects was roughly proportional to the exposure duration and concentration of the test solution. No further details on the study conditions, scoring, or reversibility of effects, were presented.

The DS concluded that liquid ethylene oxide can cause severe skin lesions, as has been documented in animal studies and human case reports. It was pointed out that ethylene oxide or its solutions are highly reactive alkylating agents which can react with many constituents of tissue, resulting in cellular and tissue dysfunction and destruction. The DS proposed to classify ethylene oxide as Skin Corr. 1B.

Comments received during public consultation

Two MSCAs and one national authority supported the proposed classification as Skin Corr. 1B. One industrial stakeholder commented that classification as Skin Corr. 1 or Skin Corr. 1C should be considered instead of Skin Corr. 1B, and that *in vitro* guideline tests would be needed in order to get data applicable for the decision on subcategorization. The justification for this was that ethylene oxide is volatile and evaporates rapidly. As the animal tests were performed under occlusive conditions, instead of semi occlusive conditions, they represent a worst-case situation.

Assessment and comparison with the classification criteria

The reports on skin irritation/corrosion include a number of human case reports, describing the corrosive potential of ethylene oxide. Two animal studies were found. One described clearly the corrosive potential of ethylene oxide after exposure to undiluted liquid for 4 h. The effects were observed on intact, as well as abraded skin. The other study reported hyperaemia and oedema already after 6 min of exposure to 10% or 50% aqueous solution of ethylene oxide. The severity of effects increased with prolonged exposure time. No details on the test

conditions, or scoring when evaluating the skin irritation, were reported. Both animal studies included exposure under occlusive conditions. Current standard *in vivo* tests for skin irritation/corrosion include exposure under semi occlusive patches. In the CLP guidance, it is stated that "Especially in borderline cases of classification the method of application should be accounted for in the evaluation of effects".

RAC considers that the available data provides evidence for the corrosive potential of ethylene oxide. The 4-h animal study describes outcomes that justify a subcategorization as Skin Corr. 1C (criteria: responses occur after exposures between 1 and 4 h and observations up to 14 days). The study protocol of the other *in vivo* study, with an exposure time of up to 1 h, is not reported in detail. It was indicated that effects were observed already after 6 min of exposure, but due to the lack of details, RAC considers that this study cannot be used as a key study to justify a classification as Skin Corr. 1B (criteria: responses occur following exposure between 3 min and 1 h). RAC also considers, that the fact that the studies were performed using occlusive patches makes a detailed interpretation of the study results complicated.

In the CLP Guidance it is stated that "Where the substance is classified as a skin corrosive but the data used for classification does not allow differentiation between the skin corrosion subcategories 1A/1B/1C, then the substance should be assigned skin corrosive Category 1".

On the basis of the arguments presented above, RAC concludes that ethylene oxide should be classified as **Skin Corr. 1; H314 (Causes severe skin burns and eye damage)** without sub-categorisation.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 25: Summary table of relevant sensitisation studies (non-human and human)

Method	Results	Remarks	Reference
Non-human information			
guinea pig (Hartley) male no further details given	not sensitising	4 (not assignable) Test material: ethylene oxide	G. Woodard and M. Woodard (1971) *As cited in CSR,* *Reviewed in ATSDR, 1990* *study not available*
(1) Immunization	Immunization of mice (ip application) with either	2 (reliable with	Chapman J. et al. (1986)

CAF_1 and $B_6D_2F_1$ mice (Lewis Brown Norway) rat No information on number of animals used (2) Passive cutaneous anaphylaxis (PCA) assay in Sprague Dawley rat	ethylene oxide - ovalbumin or ethylene oxide - keyhole limpet hemocyanin resulted in the production of ethylene oxide specific antibodies. LBN rats failed to respond to the ethylene oxide proteins.	restrictions) supporting study Test material: ethylene oxide	
Evidence from humans			
Patch test (with sterilized materials like rubber, PVC)	Skin reaction (irritation) directly correlated to the total dose of EO received One subject developed sensitivity to ethylene oxide (100ppm in PVC) - mild delayed reaction	Supporting study	Shupack J.L. et al. (1981)
Case report (nurse, n=1)	Urticaria, rhinitis, asthma Prick-test with ethylene oxide sterilized Latex-gloves was positive (negative with gamma ray sterilized latex gloves)	Abstract only (French)	Jacson F. (1991)
Case report (nurse, 30years old, wearing sterilized gown) (n=1) Comparative test with material sterilized/not sterilized	Eczema on forearms Allergic delayed-type hypersensitivity reaction No IgE to ethylene oxide detectable	Supporting study	Caroli U.M. et al. (2005)
Case report (nurse, 35years old, wearing sterilized gown) (n=1) Comparative test with material sterilized/not sterilized	Eczema on forearms Allergic contact dermatitis (delayed-type)	Supporting study	Kerre S. et al. (2009)
Clinical surveillance Detection of ethylene oxide specific cytophilic antibodies	Antibodies found in 35 of 83 dialyse patients (42%), 22 of them had anaphylactoid reactions during dialysis	Weight of evidence	Bommer J. et al. (1985) (reviewed in SCOEL, 2012)

Case descriptions	Anaphylactic reactions in dialysis patients	Weight of evidence	Röckel A. et al. (1988) (reviewed in SCOEL, 2012)
Clinical surveillance RAST	High RAST values were associated with anaphylactoid reactions during dialysis and with chronic asthma	Weight of evidence	Rumpf K.W. et al. (1985) (reviewed in SCOEL, 2012
Clinical surveillance IgE against HSA - ethylene oxide	6/7 hemodialysis patients with immediate-type allergic reactions positive (85%) 0/6 hemodialysis patients without reaction positive	Weight of evidence	Grammer L. C. et al. (1984)
Clinical surveillance Total antibody and IgE against HSA – ethylene oxide	16 of 24 patients with reaction during dialysis had detectable levels of IgE (66%) 3 of 41 patients without reaction had detectable levels of IgE (7%)	Weight of evidence	Grammer L.C. et al. (1985)
Clinical surveillance (1) Skin prick test with ethylene oxide-human serum albumin conjugate (2) RAST	(1) Skin prick test: patients receiving chronic hemodialysis: 5/56 (9%) pos. patients receiving peritoneal dialysis: 0/30 pos. (2) RAST patients receiving chronic hemodialysis: 13/107 (12%) pos. patients receiving peritoneal dialysis: no pos. results	Weight of evidence	Marshall C. et al. (1984)
Clinical surveillance	ethylene oxide specific IgE antibodies in: 22/25 patients with acute allergic reactions (88%) 5/37 patients without allergic reaction (13%)	Weight of evidence	Marshall C.P. et al. (1985)

	Normal control were negative		
Clinical surveillance Allergosorbent test (IgE antibodies for ethylene oxide)	7 of 9 (78%) patients who experienced severe hypersensitivity reaction during dialyse had high titers of IgE Patients with mild hypersensitivity show IgE in the normal range (30/37)	Weight of evidence	Lemke H.D. (1987)
Case report (n=1)	Hemodialysis patient, severe allergic reactions after exposure to sterilized articles positive RAST to HSA-ethylene oxide positive skin test and in vitro histamine release	Weight of evidence	Dolovich J. et al. (1978)
(1) Clinical surveillance in patients with allergic reactions (2) Survey of current chronic hemodialysis population in the hospital	(1) 27 patients with acute allergic-type reactions during hemodialysis; Positive RAST 22/27 (81%) (2) 9% positive allergy skin test; 12% positive RAST; sensitized patients had no symptoms	Weight of evidence	Dolovich J. et al. (1984)
Case reports (n=4)	Patients with dialyzer-hypersensitivity syndrom (anaphylactoid reaction) High incidence of positive RAST to HSA-ethylene oxide conjugate	Weight of evidence	Caruana R.J. et al. (1985)
Clinical surveillance RAST (radioallergosorbent test) - IgE against ethylene oxide and HSA-ethylene oxide	138 patients with hypersensitivity during dialyse: 63% positive 78 patients without reaction (control): 11% positive	Weight of evidence	Pearson F. et al. (1987)
Clinical surveillance - Skin-prick test	Hypersensitivity in 6/600 plateletpheresis donors Skin-prick-test: 4/6 positive donors and 0/40 controls	Weight of evidence	Leitman S.F. et al. (1986)

- RAST - Histamine release	Positive RAST: 4/6 positive donors and 1/145 controls Histamin release: 6/6 positive donors and 0/4 controls		
Case study (n=1)	Developed ethylene oxide allergy during dialysis Positive RAST	Weight of evidence	Monbaliu D. et al (2010)
Case study (n=1)	Hypersensitivity reactions during dialysis Serum samples covering a 7-year period of clinical treatment were analysed: Changes in titers of IgE and IgG antibodies correlate to the time of ethylene oxide exposure as well as clinical symptoms	Weight of evidence	Wass U. et al (1988)

4.6.1.1 Non-human information

Chapman (1986) developed an animal model for ethylene oxide-specific IgE mediated hypersensitivity reactions. The hypothesis was that residual ethylene oxide in a medical device can react with serum proteins during medical procedures to form ethylene oxide-protein conjugates that can result in patient sensitisation and elicitation of anaphylactic reactions. Mice and rats were immunized by intraperitonial application of ethylene oxide protein conjugates (ethylene oxide - ovalbumin, ethylene oxide – keyhole limpet hemocyanin) on day 0 (with adjuvant aluminium hydroxide and/or *Bordetella pertussis*), day 9 and day 30. No information on number of animals used is given. To boost IgE response cyclophosphamide was given (ip) on day 28. Blood was taken on day 14, 20 and 40. To evaluate the mice and rat serum for ethylene oxide-specific IgE antibodies the passive cutaneous anaphylaxis (PCA) assay was used (passive cutaneous anaphylaxis reactions are manifested by the appearance of blue spots at the site of intradermal injection of diluted serum from treated animals in Sprague Dawley rats). Immunization of both strains of mice with either ethylene oxide - ovalbumin or ethylene oxide - keyhole limpet hemocyanin resulted in the production of ethylene oxide specific antibodies. LBN rats failed to respond to the ethylene oxide proteins. For detailed results see Table 26 (Chapman, 1986).

Table 26: Effect of Immunization protocol – ethylene oxide specific IgE responses (Chapman, 1986).

Immunogen (µg/injection)	Adjuvant	Ethylene oxide specific IgE titer *		
		Bleed 1	Bleed 2	Bleed 3

		(day 14)	(day 20)	(day40)
CAF_1 mice				
EO-OA (1)	Alum, BP	<4	<4	4
EO-OA (10)	Alum, BP	4	4	20
EO-OA (1)	Alum	4	4	80
EO-OA (10)	Alum	40	4	320
EO-KLH (1)	Alum, BP	<4	<4	80
EO- KLH (10)	Alum, BP	20	4	4
EO- KLH (1)	Alum	20	20	80
EO- KLH (10)	Alum	20	4	4
$B_6D_2F_1$ mice				
EO-OA (1)	Alum, BP	<4	4	40
EO-OA (10)	Alum, BP	Alum	<4	<4
EO-KLH (1)	Alum, BP	<4	4	640
EO-KLH (10)	Alum, BP	20	4	160
EO-KLH (10)	Alum	80	4	160
LBN rats				
EO-OA (1)	Alum	<4	<4	<4
EO-OA (10)	Alum	<4	<4	<4
EO-KLH (1)	Alum	<4	20	<4
EO-KLH (10)	Alum	<4	<4	<4

Alum = aluminium hydroxide; BP = heat-killed *B. perussis;* EO = ethylene oxide; OA = ovalbumin; KLH = keyhole limpet hemocyanin

* The ethylene oxide specific IgE titer is expressed as the reciprocal of the greatest dilution of a serum which, in a PCA assay, produced a blueing of the skin greater than 5mm in diameter.

A skin sensitization study in guinea pigs was negative, but the validity is insufficient (Woodard, 1971; study not available).

4.6.1.2 Human information

Dermal application studies using human volunteers by Sexton (1950) (no further information available) and Shupack (1981) (patch tests, one of 12 volunteers showed a recurrent reaction) have provided some evidence that ethylene oxide is a skin sensitizer.
Ethylene oxide is corrosive and differentiation between an irritant and an allergic contact dermatitis is difficult. However allergic contact dermatitis (delayed-type) after wearing ethylene oxide sterilized gowns is described by Caroli (2005) and Kerre (2009). Urticaria is described by Jacson (1991) (see case studies Table 25).

Ethylene oxide is used for sterilization of heat-sensitive medical devices[4]. Anaphylactic reactions in dialysis patients (parenteral route of exposure) with attacks of sneezing, retrosternal burning pains, larynx oedema, bronchial obstruction and hypersecretion, flushing and pruritus and sometimes even anaphylactic shock have been described by several authors (Bommer, 1985, Röckel, 1989, Rumpf, 1985). There exist different potential causes for the observed effects; however, various authors came independently to the conclusion that by far the main factor in the provocation of such reactions is allergy of immediate type to ethylene oxide. In these cases the presence of conjugates of ethylene oxide with human serum albumin (HSA) could be demonstrated by RAST (radioallergosorbent test) (Bommer, 1985; Grammer, 1984; Röckel, 1989; Rumpf. 1985; Lemke, 1987; Caruana, 1985; Pearson, 1987; Dolovich, 1984; Dolovich, 1978; Leitman, 1986).

In a study of 83 dialysis patients, 16 dialysis unit personnel and 44 healthy control persons ethylene oxide-HSA specific IgEs occurred more frequently in dialysis patients compared to the control group. Patients with increased IgE levels had allergic complications more frequently than patients without antibodies. IgE levels decreased when other sterilisation methods were applied instead of ethylene oxide and clinical symptoms had suddenly improved. Re-exposure to ethylene oxide sterilised materials resulted in reappearance of the clinical symptoms (Bommer, 1985) (reviewed in SCOEL 2012).

Pearson (1987) examined 138 patients who experienced hypersensitivity reactions during dialysis (reactors). 78 patients without reactions were also evaluated (control). Elevated serum RAST values were more common in reactors (63%) than in controls (11%) demonstrating the role of ethylene oxide in dialysis associated hypersensitivity reactions.

Lemke (1987) concluded that ethylene oxide causes most severe hypersensitivity reactions by an IgE-mediated mechanism after he demonstrated high titers of IgE antibodies against ethylene oxide in seven of nine patients who had experienced severe hypersensitivity reaction. In most patients with mild hypersensitivity reaction during dialysis (n=37) plasma levels of IgE specific for ethylene oxide were in the normal range (30/37).

In a study by Grammer (1985) 16 of 24 patients with anaphylaxic reactions during hemodialysis had detectable levels of IgE to ethylene oxide-HSA, whereas only 3 of 41 nonreacting patients had detectable levels. An association between the presence of antibodies and immediate anaphylactic reactions was demonstrated.

Skin prick test and RAST was used by Marshall (1984) to demonstrate ethylene oxide related sensitisation. Skin prick test with a conjugate of human serum albumin (HSA) and EO was positive in five of 56 (8.9%) hemodialysis patients and 0 of 30 peritoneal dialysis patients. In the ethylene oxide-HSA radioallergosorbent test (RAST) the sera of 13 of 107 (12.1%) hemodialysis patients including sera from 5 patients with negative skin tests were positive. Sensitized patients in this population did not experience allergic-type reactions during hemodialysis. There were no positive ethylene oxide-HSA RAST results which could be ascribed to peritoneal dialysis patients. The lower sensitivity of the skin prick test in comparison to RAST was explained by a significant reduced cutaneous responsiveness of renal failure patients compared with normal adult subjects.

Marshall (1985) also examined patients receiving long-term hemodialysis. Serum was obtained from 25 patients who experienced acute allergic reactions during hemodialysis and 37 unselected patients receiving hemodialysis. Sera from 22 of 25 (88%) of the allergic reaction group and from

[4] The residues that may be found after sterilization of medical devices with ethylene oxide are beside ethylene oxide itself ethylene chlorhydrin (CAS 107-07-3; Acute Tox 2*, H300; Acute Tox 1, H310; Acute Tox 2*, H330) and ethylene glycol (CAS 107-21-1; Acute Tox 4*, H302).

five of 35 (13%) of the unselected group were demonstrated to contain IgE antibodies with specificity for EO. Corresponding IgG antibodies were also present. No such antibodies were detected in serum from normal controls or ragweed-allergic patients.

Dolovich (1978) describes a patient who developed severe allergic reactions after hemodialysis. He showed positive skin test, in vitro histamine release and RAST. In a later study by the same author (Dolovich, 1984) 27 patients with acute allergic-type reactions during hemodialysis were tested. RAST for antibodies to ethylene oxide was positive for 22/27. In a survey of the current chronic hemodialysis population for ethylene oxide related antibodies 9% had a positive allergy skin test and 12 % had a positive RAST. The sensitized individuals of this survey had no distinctive symptoms.

Leitman (1986) observed immediate-type hypersensitivity reactions in 6 of 600 donors (prevalence of 1%) who underwent automated plateletpheresis procedure (ethylene oxide gas was used for sterilization). Positive skin-prick test (using ethylene oxide-HSA reagent) was seen in 4/6 donors who had hypersensitivity reactions and in 0/40 controls. RAST showed that serum from 4/6 donors with reactions and 1/145 controls contained IgE antibodies to ethylene oxide-HSA. 6/6 donors with positive reactions and 0/4 controls had specific ethylene oxide induced basophil histamine release.

Also case studies are reported in literature. Monbaliu (2010) describes a patient who developed ethylene oxide allergy during hemodialysis (positive RAST) and Wass (1988) monitored a patient with hypersensitivity reactions during dialysis for a period of 7 years. He concluded that the changes in titers of IgE and IgG antibodies correlated to the time of ethylene oxide exposure as well as to clinical symptoms of hemodialysis patients.

4.6.1.3 Summary and discussion of skin sensitisation

Ethylene oxide is a direct and potent alkylating agent and reacts with hydroxyl, sulfhydryl, amino and carboxyl groups in human macromolecules. As a hapten it becomes an active allergen after binding to human proteins (e.g. HSA-ethylene oxide conjugates). There are only few animal data: one negative guinea pig test of poor quality and insufficient reporting of the applied protocol and one positive passive cutaneous anaphylaxis (PCA) assay. In addition a considerable amount of human data is available. For ethylene oxide especially allergies of the immediate type are documented and case reports describing contact dermatitis after dermal exposure are reported (SCOEL, 2012).

Besides sensitizing effects via the dermal route of exposure also effects after parenteral exposure have been described several times for ethylene oxide. The development of a sensitization is always a systemic process but allergic reactions can occur at localized sites (exposed skin areas) or systemic (anaphylaxis after parenteral exposure). In principle the systemic availability of sensitized immune cells circulating throughout the body always has to be kept in mind as they can respond when challenge occurs at sites other than the original site of sensitization (WHO, 2012). For ethylene oxide positive skin tests after parenteral exposure are described (Leitman, 1986; Dolovich 1978/84; Marshall, 1984).

4.6.1.4 Comparison with criteria

Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) if there are positive results from an appropriate animal test.

Subcategory 1A may be appropriate for substances showing a high frequency of occurrence in humans and/or a high potency in animals. They can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered. For low to moderate frequency and/or potency subcategory 1B may be appropriate.

For ethylene oxide there is evidence in humans that the substance can lead to sensitisation by skin contact after induction via the dermal but also via the parenteral route of exposure. Ethylene oxide is a direct and potent alkylating agent. As a hapten it becomes an active allergen after binding to human proteins

4.6.1.5 Conclusions on classification and labelling

Ethylene oxide is a sensitizing agent. Type I (anaphylaxis) and Type IV (contact dermatitis) hypersensitivity reactions have been observed in individuals exposed to ethylene oxide (WHO, 2003). No animal studies according to standard test protocols are available. Classification for this endpoint is based on human data (dermal and parenteral route of exposure) and the known mechanism (alkylating agent). Ethylene oxide should be classified as skin sensitizer (Category 1), H317 (May cause an allergic skin reaction).

According to CLP guidance document[5] classification into sub-categories can only carried out if data are sufficient. The available human data (clinical surveillance and case reports) do not provide information on the size of the exposed population, or on the extent (no information on release of ethylene oxide from sterilized material) and the frequency of exposure. Based on the available data no classification into a subcategory is proposed.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Non-human data

No animal studies performed according to OECD guidelines were found. One guinea pig study has been mentioned in literature. Ethylene oxide was reported to be not sensitising, but no details on the study were available (Woodard and Woodard, 1971; cited in ATSDR, 1990).

In a study on CAF1 and B6D2F1 mice, intraperitoneal immunisation with ethylene oxide – ovalbumin or ethylene oxide – keyhole limpet haemocyanin resulted in the production of ethylene oxide specific IgE antibodies. In the same study, no immune response was observed in Lewis Brown Norway rats (Chapman *et al.,* 1986).

Human data

Patch tests performed with sterilised materials such as rubber and PVC on 12 healthy volunteers showed a clear correlation between skin irritation and the ethylene oxide dose. One

[5] Guidance on the application of CLP criteria. Version 4.1 June 2015

of the individuals showed a mild delayed sensitivity reaction (Shupack *et al.,* 1981).

One abstract presented information on a nurse suffering from urticaria, rhinitis and asthma. Prick-tests with ethylene oxide sterilised latex gloves were positive. No further details were available.

Two reports described cases of nurses with eczema on their forearms when wearing a sterilised gown. In the first case (Caroli *et al.,* 2005), the reaction was diagnosed as delayed-type hypersensitivity reaction and in the second case (Kerre *et al.,* 2009), as delayed-type allergic contact dermatitis. Patch tests were performed, showing positive reactions in both cases. No IgE to ethylene oxide was detectable.

Ethylene oxide has frequently been used for sterilisation of heat-sensitive medical devices. Several reports on anaphylactic reactions in dialysis patients have been published. In all cases, the exposure occurred by the parenteral route. These reports are summarised in the table below.

Table: Reports on anaphylactic reactions due to the exposure to ethylene oxide via medical devices.

Method	**Results**	**Reference**
Clinical surveillance Detection of ethylene oxide specific cytophilic antibodies	Antibodies found in 35 of 83 dialyse patients (42%), 22 of them had anaphylactoid reactions during dialysis	Bommer *et al.,* 1985 (reviewed in SCOEL, 2012)
Case descriptions	Anaphylactic reactions in dialysis patients	Röckel *et al.,* 1988 (reviewed in SCOEL, 2012)
Clinical surveillance RAST	High RAST values were associated with anaphylactoid reactions during dialysis and with chronic asthma	Rumpf *et al.*, 1985 (reviewed in SCOEL, 2012
Clinical surveillance IgE against HSA - ethylene oxide	6/7 haemodialysis patients with immediate-type allergic reactions positive (85%) 0/6 haemodialysis patients without reaction positive	Grammer *et al.*, 1984
Clinical surveillance Total antibody and IgE against HSA – ethylene oxide	16 of 24 patients with reaction during dialysis had detectable levels of IgE (66%) 3 of 41 patients without reaction had detectable levels of IgE (7%)	Grammer *et al.*, 1985
Clinical surveillance (3) Skin prick test with ethylene oxide-human serum albumin conjugate (4) RAST	(1) Skin prick test: patients receiving chronic haemodialysis: 5/56 (9%) positive patients receiving peritoneal dialysis: 0/30 positive (2) RAST: patients receiving chronic haemodialysis: 13/107 (12%) positive patients receiving peritoneal dialysis: no positive results	Marshall *et al.*, 1984
Clinical surveillance	ethylene oxide specific IgE antibodies in: 22/25 patients with acute allergic reactions (88%) 5/37 patients without allergic reaction (13%) Normal control were negative	Marshall *et al.*, 1985

Clinical surveillance Allergosorbent test (IgE antibodies for ethylene oxide)	7 of 9 (78%) patients who experienced severe hypersensitivity reaction during dialyse had high titrs of IgE Patients with mild hypersensitivity show IgE in the normal range (30/37)	Lemke, 1987
Case report (n=1)	Haemodialysis patient, severe allergic reactions after exposure to sterilised articles positive RAST to HSA-ethylene oxide positive skin test and *in vitro* histamine release	Dolovich *et al.*, 1978
(3) Clinical surveillance in patients with allergic reactions (4) Survey of current chronic haemodialysis population in the hospital	(1) 27 patients with acute allergic-type reactions during haemodialysis; Positive RAST 22/27 (81%) (2) 9% positive allergy skin test; 12% positive RAST; sensitised patients had no symptoms	Dolovich *et al.,* 1984
Case reports (n=4)	Patients with dialyzer-hypersensitivity syndrome (anaphylactoid reaction) High incidence of positive RAST to HSA-ethylene oxide conjugate	Caruana *et al.*, 1985
Clinical surveillance RAST (radioallergosorbent test) - IgE against ethylene oxide and HSA-ethylene oxide	138 patients with hypersensitivity during dialyse: 63% positive 78 patients without reaction (control): 11% positive	Pearson *et al.,* 1987
Clinical surveillance - Skin-prick test - RAST - Histamine release	Hypersensitivity in 6/600 plateletpheresis donors Skin-prick-test: 4/6 positive donors and 0/40 controls Positive RAST: 4/6 positive donors and 1/145 controls Histamine release: 6/6 positive donors and 0/4 controls	Leitman *et al.,* 1986
Case study (n=1)	Developed ethylene oxide allergy during dialysis Positive RAST	Monbaliu *et al*., 2010
Case study (n=1)	Hypersensitivity reactions during dialysis Serum samples covering a 7-year period of clinical treatment were analysed: Changes in titres of IgE and IgG antibodies correlate to the time of ethylene oxide exposure as well as clinical symptoms	Wass *et al.,* 1988

The DS concluded that type I (anaphylaxis) and type IV (contact dermatitis) hypersensitivity reactions have been observed in humans exposed to ethylene oxide. Furthermore, the DS pointed out that ethylene oxide is an alkylating agent that reacts with hydroxyl, sulfhydryl, amino and carboxyl groups in human macromolecules. As a hapten, it becomes an active allergen after binding to human proteins. Based on the reactions following upon dermal and parenteral exposure the DS concluded that ethylene oxide should be classified as Skin Sens. 1; H317. No subcategorization was proposed.

Comments received during public consultation

One MSCA and one national authority supported the classification as Skin Sens. 1; H317, based essentially on data from haemodialysis patients.

One comment received from industry did not support the classification for skin sensitisation. It was stressed that the reports related to parenteral exposure of dialysis patients cannot be used for classification purposes for this hazard class, as they do not investigate dermal exposure. The three case reports on dermal exposure as well as one study on healthy volunteers were considered as not showing convincing evidence of skin sensitisation following exposure to ethylene oxide.

Assessment and comparison with the classification criteria

With regard to human data, the CLP criteria (a) require evidence on sensitisation by skin contact in a substantial number of individuals. The data presented in the dossier contains only a few case reports, each presenting one individual with skin reactions after exposure. Taking into consideration that ethylene oxide has been extensively used for sterilisation purposes for decades, the number of case reports is considered very low. The case reports do not clearly identify the observed reactions as outcomes of ethylene oxide sensitisation. As the substance causes skin irritation/corrosivity, it is possible that the reported eczema may also have occurred due to irritation.

Severe allergic-type reactions and ethylene oxide IgE antibodies among dialysis patients have been reported in several clinical surveillance studies and case reports. All of these reports focused on situations in which individuals were exposed to ethylene oxide parenterally (sterilised medical equipment). As these reports do not include information on sensitisation following skin contact, RAC does not consider them relevant for the evaluation of classification for skin sensitisation.

No appropriate animal tests have been performed.

Based on the reactions following upon dermal and parenteral exposure, the DS concluded that ethylene oxide should be classified as Skin Sens. 1; H317.

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.

4.6.2 Respiratory sensitisation

Table 27: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
Case report accidental exposure 4h/day for 4d	Symptoms after 4d of 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)
Case report	Increase in airway reactivity after challenge with ethylene oxide	*Article not available*	Dugue, P. et al., (1991) (cited in Hayes F.G. (1994))

4.6.2.1 Non-human information

No information available

4.6.2.2 Human information

Deschamps (1992) described a case of persistent nonimmunologic asthma and slight peripheral neuropathy that developed in a worker exposed to ethylene oxide 4 h/day for 4 days. The worker noticed an odor, suggesting that the concentration was ≥700ppm. Signs and symptoms after the 4-day exposure included coughing, shortness of breath, and wheezing. Respiratory symptoms persisted and 1 year after the accident, pulmonary function tests showed bronchial obstruction and bronchial hyperreactivity. The forced vital capacity was 93% of the predicted value, forced expiratory volume in 1 s (FEV1) was 74% of the predicted value. The respiratory effects persisted for at least 3 years after exposure. Immunologic tests showed no formation of immunoglobulin E antibodies to ethylene oxide. None of the other five exposed workers had respiratory complaints. The rapid onset of symptoms, the high atmospheric concentration, the persistence of symptoms after removal from exposure and the absence of ethylene oxide IgE antibodies argue for a persistent asthma after high irritant exposure (reactive airway dysfunction syndrome-RADS).

A nurse involved in the cold sterilisation of dialysis equipment showed work related asthmatic symptoms. Increase airway reactivity occurred after challenge with ethylene oxide (Dugue, 1991 as cited in Hayes, 1994). Another case of occupational asthma is mentioned by Verraes (1995) (cited in PSL assessment report, 2001) but no further information is available.

4.6.2.3 Summary and discussion of respiratory sensitisation

The case reports documented in the literature show asthmatic symptoms (coughing, shortness of breath, and wheezing) and bronchial hyperreactivity. Due to the inherent properties of ethylene oxide an irritant induced asthma cannot be excluded.

4.6.2.4 Comparison with criteria

A substance shall be classified as respiratory sensitiser if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity (asthma, rhinitis/conjunctivitis, alveolitis) and/or if there are positive results from animal tests. Immunological mechanisms do not have to be demonstrated.

For ethylene oxide no animal tests are available. Three case reports describe respiratory symptoms after ethylene oxide exposure. Baur (2012) lists ethylene oxide as substance causing irritant-induced occupational asthma.

4.6.2.5 Conclusions on classification and labelling

It is evident that inhalation of ethylene oxide causes respiratory symptoms and that the substance is a direct and potent alkylating agent. The substance is classified for respiratory tract irritation (STOT SE 3). Based on the available data respiratory sensitisation cannot be finally evaluated.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS included three case reports showing asthmatic symptoms.

Accidental exposure of a worker to ethylene oxide 4 h/d for four days resulted in coughing, shortness of breath, and wheezing (Deschamps, 1992). The worker noticed an odour, and based on that the ethylene oxide concentration was most likely ≥ 700 ppm. One year after the exposure, pulmonary function tests showed bronchial obstruction and bronchial hyper-reactivity. The respiratory effects persisted for at least three years. No IgE antibodies to ethylene oxide were detected in immunological tests. It was concluded that this may be a case of reactive airway dysfunction syndrome (RADS). Five other workers exposed at the same time did not suffer from any respiratory symptoms.

A nurse working with sterilisation of dialysis equipment showed work related asthmatic symptoms. Challenge with ethylene oxide resulted in increased airway reactivity. No further details were available (Dugue, 1991; cited in Hayes, 1994).

A third case of occupational asthma has been mentioned in literature, but no further details were available (Verraes, 1995; cited in PSL assessment report, 2001).

The DS concluded that based on the available data, respiratory sensitisation cannot be evaluated. Due to the inherent properties of ethylene oxide it is not possible to exclude an irritant induced asthma. No classification was proposed.

Comments received during public consultation

One MSCA commented on this hazard class, asking whether the DS had considered the use of QSAR analysis to predict the potential of ethylene oxide to cause respiratory sensitisation.

Assessment and comparison with the classification criteria

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 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 supports the proposal of the DS for **no classification** for respiratory sensitisation.

4.7 Repeated dose toxicity

Repeated dose studies relevant for STOT RE classification are described under 4.8.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Neurological effects have been reported frequently after exposure to ethylene oxide. Human as well as animal data are available documenting peripheral neuropathy.

Hematotoxicity was described after exposure to ethylene oxide sterilized medical devices. Ethylene oxide has effects on RBC, Hb, Ht and reticulocytes in animal studies and in humans. A clear mechanism could not be established so far.

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.1.1 Neurotoxicity

Human information

Neurological effects (primarily sensorimotor polyneuropathy) have been observed in workers exposed to ethylene oxide for decades. Early observations are documented by Blackwood (1938), von Oettingen (1939) and Sexton (1949) with symptoms like headache, nausea and vomiting. No information on exposure levels is available (cited from ATSDR, 1990).

Neurologic effects after long-term occupational inhalation exposure to ethylene oxide are described by Gross (1979). Four sterilizer operators exposed to ethylene oxide for up to two months on an intermittent basis at levels of approximately 700 ppm (estimated by the authors based on the fact that the exposed workers could smell the vapours emitted from a leaking apparatus) reported headaches, nausea, vomiting, clumsiness, blunting of the senses, lethargy, numbness and weakness in the extremities, and, in the case of one operator, recurrent major motor seizures at 20- to 30-minute intervals near the end of the work shift. Nerve conduction studies indicated sensorimotor neuropathy. These conditions were reversed in the case of one of these operators who was returned to a position without ethylene oxide exposure, but the results of nerve conduction studies remained abnormal in the cases of two of the three workers who were returned to positions of lower ethylene oxide exposure (50 ppm or less) (ATSDR, 1990).

SCOEL (2012) cited a study by Garry (1979) where neurological symptoms were seen in 12 persons who had been exposed occupationally to ethylene oxide for about 6 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. This value was, however, not considered to be representative of the whole exposure period. In addition, in four persons with local and neurological symptoms the level of sister chromatid exchange in cultured peripheral lymphocytes was increased relative to the control values and showed no tendency to decrease even 18 weeks after the end of exposure.

Kuzuhara (1983) reports 2 cases (out of several employees without symptoms) showing sensorimotor polyneuropathy. All worked at ethylene oxide sterilization for 8h/day. Workers smelled gas for several minutes when the door of the sterilizer was open, indicating exposure greater than 700ppm. Patient one noted paresthesia and weakness in the distal limbs three months after first exposure. Examination in hospital showed distal limb weakness and cutaneous sensory loss. Symptoms subsided spontaneously in a few weeks. Few weeks after return to work again paresthesia and weakness appeared. Symptoms again subsided gradually within 2 months. Patient 2 noticed paresthesia six months after exposure started. Examination showed weakness in distal limb muscles, mildly decreased sensations (touch, pain, warm/cold). Symptoms cleared one month later. Nerve biopsie implied axonal degeneration and regeneration. Muscle changes suggested denervation and reinnervation.

Another case is reported by Schröder (1985). A worker in a sterilization factory showed weakness in the lower extremities and a progressive gait unsteadiness after 5 months of exposure (up to 500ppm 2-3 times daily). Nerve conduction studies were markedly abnormal (decreased sensory and motor conduction velocity) indicating moderate to severe polyneuropathy. Reexamination 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.

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 CPT (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.

Klees (1990) describes a group of 25 hospital workers chronically exposed to ethylene oxide (8h TWA of 4.7ppm) 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.

12 operating-room nurses/technicians developed symptoms like headache, hand numbness, memory loss and rash on wrists where they had contact with ethylene oxide sterilized gowns (Brashear, 1996). Neurologic evaluation revealed neuropathy in 9/12 patients, elevated vibration threshold in 4/9, abnormal pressure threshold in 10/11 and neuropathy on conduction studies in 4/10. Neuropsychological testing demonstrated mild cognitive impairment in four of six patients. Sural nerve biopsy in the most severely affected patient showed findings of axonal injury. Several patients in this group displayed signs of peripheral and CNS dysfunction following exposure to EO. 7/12 had persistent hand symptoms for at least a year despite removal from EO-treated products. The ethylene oxide level in the gown cuff 18 days poststerilization (stored in sealed packs) was

298ppm and ethylene chlorohydrin[6] about 373ppm. Peak levels of exposure may have been even higher. Patients may be exposed via the dermal and the inhalatory route.

Patch (2001) concluded in his study that chronic ethylene oxide exposure also has impact in the area of intellectual functioning (IQ) and anxiety. In a neuropsychological examination psychological effects of 22 individuals suffering from ethylene oxide exposure (working in a medical setting for 24 to 108 months) were compared to those of 64 victims of traumatic brain injury (TBI) (time from date of injury 1 to 73 months). Individuals of both groups underwent cognitive examinations (Wechsler IQ, Fingertapping test, Reaction time test, MMPI, MAACL[7]). Intelligent test scores resulted in low average to average range for the TBI group and borderline intellectual functioning for the ethylene oxide group compared to the established mean for the general public. The reaction and movement time scores (finger tapping, reaction time test) indicate impairment for each group.
The MMPI test indicated preoccupation with bodily concerns, anxiety, depression and tendency to channel stressful feelings into physical symptoms and feeling of alienation, isolation and social disconnectedness for both groups. In the MAACL all scores (anxiety, depression, hostility) were elevated for both groups compared to nonbrain injured reference groups with the ethylene oxide exposed individuals exhibiting more feelings of anxiety, fear, edginess and loss of control. In the absence of data from control groups it is difficult to judge these results.

Several studies report peripheral neuropathy, impaired hand-eye coordination, and memory loss after single or long-term exposure of workers to ethylene oxide (Crystal, 1988; Estrin, 1987/1990; Finelli, 1983; Kuzuhara, 1983; Salinas, 1981; Schroeder, 1985; Zampollo, 1984; De Freitas, 1991). In some studies sural nerve biopsies showed axonal degeneration and regeneration (Kuzuhara, 1983; Schroeder, 1985; De Freitas, 1991; Brashear, 1996). Symptoms improved after exposure to ethylene oxide terminated (Crystal, 1988, Kuzuhara, 1983; Zampollo, 1984, Gross, 1979, Fukushima, 1986). Relevant information (affected workers, symptoms and exposure concentration) for these studies is compiled in Table 28.

Effects on the nervous system after short term/single exposure are described in Chapter 4.3.

Table 28: Human evidence for neurotoxicity after repeated exposure.

Method	**Results**	**Remarks**	**Reference**
Case reports (n=4) Operators exposed to ethylene oxide due to a leaking sterilizer up to 2 months Case 1: 3 weeks Case 2: 3 weeks	Peripheral neuropathy Case1: headache, nausea, vomiting, lethargy, motor seizures at 20-30min intervals; patient was fully recovered 2 months later Case 2: headache, limb weakness, fatigability,	Supporting study No information on exposure concentrations available. [700ppm are estimated by the	Gross J.A (1979)

[6] Ethylene Chlorohydrin (ECH) is a residue after sterilization that may be formed when ethylene oxide comes into contact with free chloride ions ((CAS 107-07-3; Acute Tox 2*, H300; Acute Tox 1, H310; Acute Tox 2*, H330).

[7] Instruments sensitive to neurobehavioral status: Minnesota Multiphasic Personality Inventory (MMPI) and Multiple Affect Adjective Checklist (MAACL)

Method	Results	Remarks	Reference
Case 3: 2 weeks Case 4: 2 months	wide based unsteady gait; shift to work without exposure resulted in significant improvement Case 3: headache, altered memory and thinking, fatigability, cramps; further work under condition of lower exposure (50ppm) resulted in no improvement of nerve conduction studies Case 4: asymptomatic but nerve conduction studies showed sensorimotor polyneuropathy ; further work under condition of lower exposure (50ppm) resulted in no improvement of nerve conduction studies	authors as the workers could smell the chemical] Possibility of short time exposure to high levels of ethylene oxide no assessed	
Clinical study Occupational exposure during ethylene oxide gas sterilization	headaches , nausea, speech disorders and impairment of short-term memory, vertigo and incoordination	Supporting study	Garry V.F. (1979) (cited in SCOEL, 2012)
Case report (43-year-old female nurse) Accidental release of ethylene oxide vapour (estimated 500ppm) Duration: 2-3min	Nausea, stomach spasms, paleness, light. headedness, short periods of unconsciousness, convulsive movements of arms and legs, periods of apnea, muscle twitching, nausea Inability to perform minor motor tasks continued for up to 1 week after exposure	Supporting study	Salinas E. (1981) (cited in US EPA, 2010)
Case report (n=5) Accidental release ; >260ppm Duration: 30min	Irrit. of upper resp.tract, headache, intense generalized pruritus muscular weakness in one worker	Supporting study Coexposure ethylene oxide and carbon dioxide	Deleixhe P.A. (1986) (cited in US EPA, 2010)
Survey (n=165)	Headaches, skin and eye irritation, dry mouth, sore throat, skin rash, loss of		Bryant H.E. (1989)

Method	Results	Remarks	Reference
11-23.5ppm Duration per cycle: 2.77-11.75min	sense of smell, shortness of breath, nausea, numbness in fingers, drowsiness		(cited in US EPA, 2010)
Case report (n=3)	Polyneuropathy (bilateral foot drop, denervation potential on electromyography)	Supporting study	Finelli P.F. (1983) (cited in DFG, 1993)
Case report (n=2) Occupational exposure during ethylene oxide sterilization Several months of exposure, about 1,5h/day Concentration: estimated peak exposure ~ 700ppm (smelling) when opening the sterilizer	Sensorimotor neuropathy (axonal sural nerve degeneration) Symptoms improved after termination of exposure	Supporting study	Kuzuhara S. (1983)
Case report (n=2) among 12 female workers Two years of exposure (ethylene oxide sterilizer)	Peripheral neuropathy Cease of exposure resulted in swift remission of symptoms and complete normalisation of the electromyography record	Supporting study Exposure fluctuating between 10 and 400ppm	Zampollo A. (1984)
Case report (n=1) 5 months of exposure Concentration: up to 500ppm, 2-3 times daily	Polyneuropathy (distal weakness of lower extremities and transitory reduced nerve conduction velocity, nerve fibre degeneration) Improvement in re-examination 1 year after exposure	Supporting study	Schroeder J.M. (1985)
Case report (n=4) Exposure 8-10 times/day while transporting sterilized products and once daily while exchanging	Polyneuropathy (impairment of lower limbs and titubation) All patients show motoneuron disease, dorsal cord disorder, cranial and	Supporting study	Fukushima T. (1986) (as cited in NEDO, 2004)

Method	Results	Remarks	Reference
containers	autonomic disorders Reversible		
Case report (n=1) 10 years of exposure (adjacent to an ethylene oxide chemical sterilizer)	After 7 years symptoms like impaired memory, increased irritability, clumsiness, falling Symptoms markedly improved few months after exposure ceased Symptoms 1 year after exposure ceased: emotional lability, impaired concentration, cognitive slowing, impaired recent and remote memory	Supporting study 4,2ppm (when the sterilizer was closed)	Crystal H.A. (1988)
Clinical study (measurement of nervous system function) 8 hospital workers and 8 control persons Exposure 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	Test substance: sterilizer using 12% ethylene oxide + 88% chlorodifluoromethan or 100% ethylene oxide Estimated average exposure: less than 1ppm Second measurement: up to 250ppm	Estrin W.J (1987) and Estrin W.J (1990)
Cross-sectional study 25 exposed workers (for 1-11years) – 4 excluded after review of questionaire 24 unexposed control workers Evaluation with a self-administered questionnaire and a neuropsychological screening	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	Low dose exposure (8h TWA 4.7ppm) but peak exposure possible Bias: awareness of exposure among subjects may result in an exaggerated	Klees J.E. (1990)

Method	Results	Remarks	Reference
battery		self-reporting on the questionnaire	
Case report (n=1) Seven years of exposure	mild sensorimotor polyneuropathy (axonal degenerative type) sural nerve biopsy: mild loss of myelinated fibres, fibres with axonal degeneration	Supporting study No detailed information available	De Freitas M.R. (1991)
Clinical survey (n=12) 12 operating-room nurses/technicians Inhalative and dermal exposure (vapour in package and residue retained in surgical gowns)	Rash on arm and wrist, dysesthesia, headache Neuropathy in 9/12 further symptoms: memory loss, mild cognitive impairment, elevated vibration threshold, abnormal pressure threshold sural nerve biopsy: axonal injury persistent hand symptoms for at least 1 year after removal	Supporting study Exposure to ethylene oxide and ethylene chlorohydrin measurement in gown cuff: Ethylene oxide =298ppm Ethylene chlorohydrin = 373ppm Peak level exposure unknown	Brashear A. (1996)
Neuropsychological examination of exposed workers (n=22) Exposure: 24-108 months	IQs of ethylene oxide exposed individuals lower than for general population Feelings of anxiety, fear, edginess and loss of control	Supporting study Examination of sequelae of ethylene oxide exposure No information on exposure concentrations available. No information on effects like eg. neuropathy in exposed workers is given.	Patch P.C. (2001)

Non-human Information

Available animal data for the evaluation of neurotoxic effects of ethylene oxide is compiled in Table 29.

Table 29: Animal studies relevant for evaluation of neurotoxicity

Method	Results	Remarks	Reference
Chronic neurotoxicity studies			
Cat Subacute inhalation study Concentration: about 100 or 200ppm Exposure: 1-several h/day, up to 22d No information on number of animals or available control group	lethal for the animals after about 22 days Paralysis of hind limbs, unsteady gait Autopsy: generalized hyperaemia of the internal organs and the brain, perivascular haemorrhage and liver and kidney damage.	Study not available Testing material : ethylene oxide Actual concentrations were probably much higher than stated	Koelsch F (1930) (as cited in DFG, 1993)
Rats, mice, guinea pigs, rabbits, monkeys (for details on numbers of animals see Table 30) Concentration: 841, 357, 204, 113, 49ppm, control Exposure: 7h/day, the duration of exposure was varying from 10d to 357days (dependent on concentration and animal used)	841ppm (10days of exposure): all mice died, no effects on neurotoxicity in other animals 357ppm (48-357d): paralysis, impairment of nervous system, muscular atrophy, poor reflexes; guinea pigs showed no effect on nervous system 204ppm (176-226d): monkeys showed partial paralysis and muscle atrophy rabbits show slight/marked paralysis in rear legs 113ppm (176-226d): no effects 49ppm (180-184d): no effects	Supporting evidence Small number of animals in each exposure group Test material: ethylene oxide vapour	Hollingsworth R.L. (1956)
Cynomolgus monkeys (12 animals per group)	No clinical evidence of neurotoxicity or effect on peripheral nerve	Study not available Small number of	Sprinz H (1982)

Method	Results	Remarks	Reference
Concentration: 50, 100ppm, control Exposure: 6h/day, 5d/week, 24 months	conduction Light microscopy: - no difference between ulnar and sciatic nerves - Demyelination in fasciculus gracilis in 1 of 2 monkeys each dose group	monkeys examined Test material: ethylene oxide	(as cited in ECETOC, 1984)
Monkeys - Macaca fascicularis, male, obtained from the wild, unknown age) (1 animals per group) - Cynomolgus monkeys (12 animals per group) chronic (inhalation chamber exposure) Concentration: 50 and 100 ppm Ethylene oxide (nominal conc.), control (13 animals each) Exposure: 24 months (7 h/day, 5 d/weeks)	MCV: there was no significant difference between oxide exposure groups and controls (two animals in the 100ppm group showed a large reduction in MCV between 12 months and the termination of exposure) EEG: no detectable differences between exposed and control groups Neuropathology (2 animals from each exposure group): Axonal bodies in the nucleus gracilis at 50ppm and 100ppm, demyelination in the extreme distal portion of the fasciculus gracilis in one monkeys at 50ppm and one at 100ppm exposure Under the condition of this study no significant neurophysiological effects were found.	2 (reliable with restrictions) Test material: ethylene oxide Difficulties of the study: Small number of monkeys examined and possibility of age-related effects	Setzer, J.V. (1996)
Fischer 344 (rats), CD1 and CF1 (mice) male/female subchronic (inhalation: vapour) (whole body) Concentration : 450, 150, 100, and 50 ppm (nominal conc.), control Vehicle: no data Exposure: 7 - 8 weeks (6 h/d, 5	no NOAEC identified 450ppm (rats, mice): high mortality by the end of week2 (mice) and week 3 (rat); tremor, convulsion, paresis of hindquarters was seen before death (week 2-3) neuro-muscular function examination at 150ppm	Study report not available, evaluation based on IUCLID dataset GLP 2 (reliable with restrictions) Test material: ethylene oxide	Snellings, W.M. (1982)

Method	Results	Remarks	Reference
d/w) Animals per group: 35m rats, 35 f rats, 15 m CD1 mice, 15 f CD1 mice, 15 m CF1 mice, 15 f CF1 mice	(rats only): no appreciable differences were noted between the EO-exposed and control groups	This study was conducted according to the protocol and amendments prepared by the Chemical Hygiene Fellowship.	
F344 rats, male (80 per group) Concentration: 0, 50, 100ppm Exposure: 7h/d, 5d/w for 2 years	Multifocal areas of atrophy, degeneration of skeletal muscle fibres at 100ppm Changes were not accompanied by any changes in nerves (detectable in light microscopy)	2 (reliable with restrictions) supporting study Test material: ethylene oxide	Lynch, D.W., (1984)
Cynomolgus monkey rats Concentration: 0, 50, 100 ppm Exposure: 2 years (7 h/d, 5 d/w)	Rats 100ppm: increased incidence of skeletal muscle myopathy, multifocal areas of atrophy and degeneration of skeletal muscle fibers. Monkey 100ppm: slight demyelination of the brains, no changes in routine electrocardiograms Rats 50ppm: brain lesions in rats.	Study not available supporting study experimental result Test material: ethylene oxide No information on number of animals used	Lynch, D.W., (1984a) (cited in ATSDR, 1990)
mouse ($B6C3F_1$) male/female subchronic inhalation study (vapour) Concentration : 250, 100, 50 or 10 ppm The actual mean chamber concentration levels 236, 104, 48, and 10 ppm, respectively, were close to the target concentrations.	NOAEC: 10 ppm (male/female) (overall effects) Neuromuscular screening test: Dose-related trend of response for reduced locomotor function and abnormal posture (250-100-50ppm) No histologic alterations in	2 (reliable with restrictions) weight of evidence (small sample size) experimental result Test material ethylene oxide	Snellings, W.M. (1984)

Method	Results	Remarks	Reference
(nominal conc.) Number of animals: 30m, 30f Exposure: 6 hours/day, 5 days/week for 10 weeks (males) or 11 weeks (females)	muscle or nervous tissue.		
Wistar rats, male (5 exposed, 5 control) Concentration 500ppm Exposure: 6h/days, 3days/week for 13 weeks	Axonal degeneration of myelinated fibres in the fasciculus gracilis and the hindleg nerve Electron microscopic findings: fasciculus gracilis – decrease of myelinated fibre densitiy. decrease in the median diameter of myel. fibres hindleg nerv - myelinated fibres with multifocal breakdown of the myelin sheath	2 (reliable with restrictions) Test substance ethylene oxide	Ohnishi A. (1985)
Wistar rats Concentration 250ppm Exposure: 6h/days, 5days/week for 9 months	No definite abnormality of the gait or posture in control and test rats Histolog. examination: Distal axonal degeneration of myelinated fibres in sural nerves and fasciculus gracilis extent of distribution and severity of degenerative findings was variable.	Abstract only No further information available	Ohnishi A. (1986)
Wistar rats, male and female Concentration 250ppm Exposure: 6h/days, 5days/week for 17 weeks	Both (m and f) showed paresis of hindlegs, but sexual difference did not affect the degree Axonal degeneration of the myelinated fibres in the peroneal nerv, the nerv to	Abstract only No further information available	Mori K. (1990)

Method	Results	Remarks	Reference
	the soleus muscle and in the fasciculus gracilis Sexual differences played no part in the severity of degenerations		
Range finding study Sprague-Dawley rats (5m and 5f each group) Concentration: 1,100, 300, 400, 500ppm Exposure: inhalation, 4 weeks	NOAEL= 100ppm One female rat in the 500-ppm group was found dead on day 18. Clinical signs observed at 500 ppm included irregular gait, decreased fecal volume, lethargy, prostration, emaciation, yellow anogenital staining, moist rales, labored breathing, paleness, black and brown stains on the snout Body weight: decreased 12-42% in m and f at 330, 400, 500ppm Neurologic. Assessment: hindlimb grip strength decreased 22% to 36% in both sexes at 300, 400, and 500 ppm; Landing foot splay decreased 29% to 42% in both sexes at week 3 or 4 at 400 and 500 ppm Postmortem examination: decreased absolute brain weight in males with 500-ppm exposure. No exposure-related gross lesions were observed, and only minimal to slight vacuolation of the white matter of the thalamus and medulla oblongata was observed in both sexes at 500 ppm.	Study not available Testing material : ethylene oxide	Mandella R.C. (1997b) (cited in US EPA, 2010)
Subchronic neurotoxicity study Sprague-Dawley rats (15m and	NOAEL= 100ppm No exposure-related effects at 100 ppm and	Study not available	Mandella R.C. (1997c) (cited in US EPA. 2010)

Method	Results	Remarks	Reference
15f each group) Concentration: 0, 25, 50, 100, 200ppm Exposure: inhalation, 14 weeks	no exposure-related effects observed for clinical signs, mortality, or cholinesterase activity at any concentration. Body weight gain decreased 16% to 17% during exposure to 200ppm Neurobehavioral assessment: no exposure-related effect except for a 25% decrease in hindlimb grip strength in females exposed to 200 ppm. The level of motor activity did not differ between exposed and control rats. Postmortem examination: no exposure-related gross or microscopic lesions in nervous system tissue	Testing material : ethylene oxide	

In an early study with cats (Koelsch, 1930), exposure for one to several hours daily to ethylene oxide concentrations which were presumably much higher than stated (100 or 200 ppm) was lethal for the animals after about 22 days. Reduced food consumption and also apathy, paralysis of the hind limbs and an unsteady gait were the most conspicuous symptoms; autopsy revealed generalized hyperaemia of the internal organs and the brain, perivascular haemorrhage and liver and kidney damage (cited in DFG, 1993). DFG, 1993 also cites a subacute study by Jacobson (1956) where dogs were exposed to 290ppm ethylene oxide (6h/d, 6 weeks). 2 of 3 dogs showed tremor, vomiting and weakness in the hind legs (atrophy of the hind leg muscles).

In a repeated dose study by Hollingsworth (1956) rats, mice, guinea pigs, rabbits and monkeys were exposed to various concentrations (841, 357, 204, 113, 49ppm) of ethylene oxide. At 357ppm (exposure period varying from 48 to 357days) the growth of all species was markedly subnormal. Several animals died during exposure period. Rats, rabbits and monkeys showed impaired function of the nervous system (lumbar and sacral region), paralysis and subsequent atrophy of the muscles of the hind limbs. Recovery appeared to be complete in all species surviving the testing period. Guinea pigs showed no effect on the nervous system. In addition to the symptoms above monkey showed poor or non-existent knee jerk reflexes, poor pain perception in the hind quarters and about the genitalia. The cremasteric reflex was elicited. The extensor reflex of the palms of the hind feet was non-existent. During exposure to 204ppm ethylene oxide for up to 226 days monkeys developed less active knee jerk reflexes and the Babinski reflex was positive. Partial paralysis and

some evidence of muscular atrophy of the rear extremities were noted. After 196 days rabbits also exhibited slight to marked paralysis in the rear legs. No adverse effects were seen at 113 and 49ppm. Table 30 gives an overview on exposed animals and effects observed. No detailed information on the number of affected animals is given in the study.

Table 30: Study results of repeated 7h/days, 5d/week vapour exposure (Hollingsworth, 1956).

Test concentration (ppm)	Animal (number)	duration	effect
841	Rats (10) Guinea pigs (8) Rabbits (2) Mice (5) Monkey (1)	8 exposures in 10 days	Irritation of the respiratory tract All animals died
357 (pilot study)	Rats m (10) Rats f (10) Mice f (10)	7 exposures in 9 days	2 rats and 4 mice died Moderate loss of body weight Severe lung injury in rats and mice
357	rats m (10) Rats f (10) Rabbits (2) Mice f (10) Monkey f (1) Guinea pigs m+f (8) Monkeys m+f (2)	33-59 exposures in 48-85 days 123 exposures in 176-357 days	All mice died after 33 exposures * 1 rabbit (m) died after 48 exposures All rats (except two males) died after 38 exposures *. These 2 rats were given 42 exposures and then allowed to recover * Secondary infection was the primary cause of death in rats and mice. Towards the end of exposure duration rats, rabbits and monkey showed impaired function of the nervous system at the level of lumbar and sacral region. Paralysis and atrophy of the muscles of the hind

	Monkeys m (2)	38-41 exposures in 60 days 94 exposures in 140 days	limbs. All these delayed effects were reversible in the recovery periode up to 132 days. Guinea pigs showed no effects on the nervous system. Monkeys showed impairment of function of the nervous system, paralysis, muscular atrophy of the hind limbs, poor knee jerk reflex, poor pain perception, no extensor reflex of the palms
204	Rats (20) Mice f (10) Guinea pigs (8) Rabbits (4) Monkeys f (2)	122-157 exposures in 176-226 days	Some rats and mice died due to secondary respiratory infection Rats: Depressed growth in rats, marked increase in lung weights Monkeys: less active knee jerk reflexes, pos. Babinski-reflex, partial paralysis, evidence of muscular atrophy of the rear extremities Rabbits: slight to marked paralysis in the rear legs
113	Rats (20) Guinea pigs (8) Rabbits m (2) f (2) Monkeys f (2)	122-157 exposures in 176-226 days	Growth depression in male rats Moderate increase in lung weight in male rats
49	Rats (20)	127-131 exposures in	No adverse effects

	Guinea pigs (8) Rabbits m (2) f (2) Mice (10)	180-184 days	
Control (air exposed)	Well matched with the experimental animals in respect to number, age, sex and body weight		

Snellings (1982) reported a subchronic inhalation study with male and female rats and mice (GLP compliant). The animals were exposed to vapour concentrations of 450, 150, 100 and 50ppm (in inhalation chambers) for 6 hours per day and 5 days per week over a study period of 7 - 8 weeks. Within 2 to 3 weeks, high numbers of mortalities and other significant treatment-related effects for both rats and mice occurred at the 450ppm exposure level. Before death occurred in rats and mice of this exposure group, observations of tremors, convulsions, and paresis of the hindquarters were observed in several animals. Although there was no clear pathogenesis, the most probable cause of death for the rats was vascular damage or nasal cavity obstruction. Histologic changes noted for the rats of the 450ppm concentration group, which were sacrificed after 2 or 3 weeks of exposure, were various lesions in the nasal cavity mucosa, lymphoid tissue atrophy, and testicular degeneration. Neuromuscular Function Tests were performed on male and female rats of the 150 ppm and control groups on a Friday (i.e. following 5 completed exposure days) and on a Monday (i.e. following subsequent exposure after 2 days of no exposure). It was found that there were no major differences noted in the observations made on these two days for the male and female rats in the 150 ppm exposure group. During the 5, 6 and 7th exposure weeks, the same detailed evaluations were performed on the 150 ppm and control groups of rats. Because of time limitations, only a small sample size was used during these evaluations. During this time period, no appreciable differences were noted between the EO-exposed and control groups. At the evaluation prior to the final sacrifice, the number of animals evaluated was larger. As before, no significant differences were noted. Only a few significant findings in organ weight determinations and clinical pathology values were noted in the rats of the 150ppm exposure level; however, none of these were supported by histopathologic alterations. White blood cell counts for 150, 100 and 50ppm female rats exposure groups at week 8 were statistically lower than control (but no dose response). No overall NOAEC could be established as adverse effects were seen at concentrations ≥ 50ppm. For neurotoxicity a NOAEC of 150ppm can be derived.

Snellings (1984) conducted a vapour inhalation GLP-study in mice for a duration of 10 weeks for males and 11 weeks for females. Male and female mice were given concentrations of 0, 10, 50, 100 and 250ppm ethylene oxide in an inhalation chamber for 6 hours per day and 5 days per week. There was no appreciable difference in mortality for the exposed groups and the controls. No common cause of death was obvious for those animals that died or that were sacrificed in a moribund condition. The gain in body weight for the animals of the highest exposure group was statistically significantly lower than that of the control for only the last exposure week. The statistically significant pathologic findings that could be indicative of a toxic response were observed in the 250 ppm exposure group only. They included minimal changes in certain erythroid parameters, increased liver weight, decreased testicular weight, and decreased spleen weight (noted also in the 100ppm group. However, of the tissues examined grossly or microscopically, there were no histopathologic findings to support any of these apparent treatment-related effects. Results of a

neuromuscular function test indicated that certain reflex responses and locomotor activities were affected in the ethylene oxide-exposed animals. A dose-related trend of response in the 250, 100, and 50ppm exposure groups was noted in the evaluation of locomotor functions (abnormal posture, reduced locomotor activity); at 250ppm a statistical difference for abnormal reflexes of righting, toe pinch, tails pinch was observed. However, because of the small sample size (5 mice were selected for neuromuscular screening testing), determination of what concentrations were effect or no-effect levels is difficult. There were no accompanying histopathologic alterations in muscle and central or peripheral nervous tissue. The NOAEC was found to be 10ppm for male and female mice.

A two-year study in rats (Lynch, 1984) showed a skeletal muscle myopathy in 100ppm exposed rats. These changes were not accompanied by any changes in the nerves.

Lynch (1984a) reported a chronic inhalation study with monkeys and rats (number of animals unknown). The animals were exposed to vapour concentrations of 50 and 100ppm for 7 hours per day and 5 days per week over a study period of 2 years. The 100ppm group had a statistically significant reduced mean body weight compared to the control group beginning at week 19 and continuing through week 104. Five monkeys died during the 2-year exposures, one each in the ethylene oxide 50ppm and ethylene oxide 100ppm groups. These deaths did not appear to be related to ethylene oxide exposure. The study reported an increased incidence of skeletal muscle myopathy in rats exposed to ethylene oxide at 100 ppm. Lesions consisted of multifocal areas of atrophy and Degeneration of skeletal muscle fibers. Chronic exposures to ethylene oxide at 100 ppm resulted in slight demyelination of the brains of monkeys and exposure to 50 ppm resulted in brain lesions in rats. No treatment related changes were observed in routine electrocardiograms taken from monkeys throughout the study. Evidence of neurotoxicitiy and demyelination was found at both doses. Exposure to 100ppm decreased nerve conduction velocities. No haematological effects were seen in monkeys. In the discussion the author point out that intraspecies variation might be considerable and some individuals more susceptible than others (reviewed in ATSDR, 1990).

ECETOC (1984) cites a study by Sprinz (1982) where Cynomolgus monkeys (12 animals per group) were exposed to 50 and 100ppm of ethylene oxide. They showed neither clinical evidence of neurotoxicity nor any effect on peripheral nerve conduction. Light-microscopic investigations were performed with pairs of animals from each group. These revealed no differences between the ulnar and sciatic nerves from exposed and control monkeys. However, demyelination was observed in the distal portion of the fasciculus gracilis in 1 of 2 monkeys of both exposure groups. An axonal dystrophy was also noted in the nucleus gracilis. There were no clinical findings which could account for the histological changes. The pathogenesis and biological significance of these findings in this very small population of exposed primates is uncertain.

In a study by Setzer (1996) (evaluated by US EPA, 2010) groups of 12 Cynomolgus monkeys + 1 *Macaca fasc*. were exposed whole body to ethylene oxide (99.7%) in 3.5m^3 stainless-steel and glass chambers at concentrations of 0, 50, or 100ppm for 7 h/day, 5 days/week for 24 months. The monkeys obtained from the wild were of unknown age. Two animals from each group were sacrificed at the end of the exposure period for neuropathologic examination. The remaining animals were maintained for additional 7 years without ethylene oxide exposure, at which time two additional animals per group were subjected to neuropathologic examination. Mean body weight of monkeys exposed to 50ppm was similar to that of controls, but the 100ppm group weighed significantly less than controls from week 25 to the termination of exposure. The maximum nerve conduction velocity (MCV) of the monkeys exposed to ethylene oxide did not differ significantly from that of controls at any time during exposure, but it was consistently lower in the 100ppm group than in controls from 12 months to the termination of exposure. The investigators noted that MCV of two animals in the 100ppm group showed a large decline between 12 months and the termination of exposure. The MCV was not significantly affected in animals exposed to ethylene

oxide at the end of the 7-year recovery period. No significant effect was observed on EEG measurements. Neuropathologic examination of two monkeys per group after exposure for 2 years showed lesions indicating axonal dystrophy in the medulla oblongata, restricted to the nucleus gracilis at 50 and 100ppm. The lesions were negative/trace or negative in the two controls, slight or severe in the two 50ppm monkeys, and negative or slight in the two 100ppm monkeys. Demyelination in the extreme distal portion of the fasciculus gracilis was seen in one monkey in each group; the lesion was severe at 100ppm. Neuropathologic examination of two monkeys per group maintained for the additional 7 years showed slight or moderate axonal dystrophy in the two monkeys in each group, including controls. The authors concluded that no statistically significant group differences in neurophysiological or neuropathological endpoints were found but they also underline the symptoms seen in the 100ppm group where the two examined monkeys showed substantial decrease in MCV over the first 12 months of exposure. One of these also showed severe demyelination of the fasciculus gracilis. No final conclusion on neurotoxicity of ethylene oxide can be drawn as the study results are limited by the small number of monkeys examined (2 animals from each group) and the possibility of age-related effects.

In a 4-week range-finding study (Mandella, 1997b; cited in US-EPA, 2010) groups of five male and five female Sprague-Dawley rats were exposed by whole-body inhalation to ethylene oxide vapor at concentrations of 0, 100, 300, 400, or 500ppm. No exposure- related effects were observed at 100 ppm. One female rat in the 500-ppm group was found dead on day 18. Clinical signs observed at 500 ppm included irregular gait, decreased fecal volume, lethargy, prostration, emaciation, yellow anogenital staining, moist rales, labored breathing, paleness, and black and brown stains on the snout. Body weights of males and females exposed to 300, 400, or 500ppm decreased by 12% to 42% at study termination and food consumption decreased by 15% and 18% in females and males, respectively, during the first week. The neurologic assessment at weeks 3 and 4 showed that hindlimb grip strength decreased 22% to 36% in both sexes at 300, 400, and 500 ppm; this effect was more severe at 400 and 500ppm. Landing foot splay decreased 29% to 42% in both sexes at week 3 or 4 at 400 and 500ppm; this effect was more severe at 500 ppm. The postmortem examination showed decreased absolute brain weight in males with 500-ppm exposure. No exposure-related gross lesions were observed and only minimal to slight vacuolisation of the white matter of the thalamus and medulla oblongata was observed in both sexes at 500ppm. The NOAEL for the 4-week inhalation study was 100 ppm.

Mandella (1997c) (cited in US-EPA, 2010) describes a subchronic neurotoxicity study where groups of 15 male and 15 female Sprague-Dawley rats were exposed by whole body inhalation to ethylene oxide vapour at concentrations of 0, 25, 50, 100, or 200 ppm for 14 weeks. Neurobehavioral assessments (functional observational battery) were conducted on 10 rats of each sex after exposure for 5, 9, and 14 weeks and after a 13-week recovery period. Five rats of each sex were assessed for gross and microscopic lesions after exposure for 14 weeks and after the 13-week recovery period. No exposure-related effects were observed at 100 ppm and no exposure-related effects were observed for clinical signs, mortality, or cholinesterase activity at any concentration. Body weight gain decreased 16% to 17% during exposure to 200 ppm with a concomitant decrease in food consumption. The neurobehavioral assessment showed no exposure-related effect except for a 25% decrease in hindlimb grip strength in females exposed to 200 ppm. The level of motor activity did not differ between exposed and control rats. Postmortem examination showed no exposure-related gross or microscopic lesions in nervous system tissue. The NOAEL for this study was 100 ppm.

Ohnishi (1985) studied the effect of inhaled ethylene oxide vapour on neuropathy in rats. Five male Wistar rats were exposed to ethylene oxide at a concentration of 500 ppm, 6 h/day, 3 days/week for

13 weeks. Five pair-fed animals exposed to ambient air served as controls. Clinical signs in the exposed rats included an awkward gait at weeks 5 to 8 and slight to moderate hindlimb ataxia starting at week 9 or 10. Light and electron microscopic examination of peripheral nerves showed axonal degeneration of myelinated fibers in the fasciculus gracilis and hindlimb nerves. The degenerative changes accounted for the ataxia observed in these animals (US EPA, 2011).

In another study by Ohnishi (1986) an exposure of Wistar rats for 6h/week, 5 days/week for 9 months to 250ppm ethylene oxide also showed axonal degeneration of myelinated fibres in sural nerves and fasciculus gracile. Sexual differences in Wistar rats play no part in the severitiy of degenerations (Mori, 1990).

Effects on the nervous system have been observed frequently in laboratory animals exposed to ethylene oxide. Acute studies (Mandella, 1997a; Snellings, 2011) (Chapter 4.3) show slight impaired locomotion, tremor, absence of reflexes; these effects were not persistent in rats. In subchronic or chronic studies in rats, mice, rabbits and monkeys there was a range of neurological effects, including awkward or ataxic gait, paralysis, and atrophy of the muscles of the hindlimbs, accompanied in some cases by pathological evidence of axonal degeneration of myelinated fibres in nerves of the hind legs. Effects on various reflexes (righting, tail pinch, toe pinch) were also noted. Demyelination has been seen in primates at 50ppm (Sprinz, 1982; Setzer, 1996) (but small number of animals investigated), similar to effects seen in humans. ATSDR derived a LOAEL=50ppm and a NOAEL=10ppm for neurotoxicity based on the study of Snellings (1984). More recent studies in rats show a NOAEL=100ppm (Mandella, 1997b, c).

4.8.1.2 Hematotoxicity

General introduction:

Ethylene oxide reacts with amino acids (cysteine, N-terminal valine, histidine) in haemoglobin. The main difference between species is the 12 and 170 times higher reactivity of cysteine in mouse and rat Hb, respectively, than in human Hb (Segerbäck, 1990). According to Osterman-Golkar (1976) these alkylations do not alter the life span of erythrocytes. This effect has been used for monitoring of exposure to ethylene oxide. Due to the stability and lack of repair of haemoglobin adducts the level measured reflects the integrated exposure over a period of 4 months – the lifespan of the erythrocytes (Yong, 2001).

Non human information

Table 31: Animal studies relevant for evaluation of hematotoxicity

Method	Results	Remarks	References
In vitro hemolysis test (Autian J Authorized toxicity testing protocols, Vol I) New Zealand rabbit	Dose response relationships between hemolysis and ethylene oxide conc or duration of exposure at concentrations	Supporting study Testing material : ethylene oxide	Anand V.P.(2003)

blood	>500µg/ml, 30% hemolysis at 1250 µg/ml		
Mice C57BL/6J male inhalation study 6h/d, 5d/week (for 1d,2d, 4d, 8d, 14d, 4wk, 6wk, 8wk, 10wk) 255ppm 4 animals/group	Hb↓, Ht↓, RBC↓, Decrease in the bone marrow cell density, and the number of lymphocytes Bone marrow populations and leukocytes in the peripheral blood perturbed Alterations in the cell cycle data of the bone marrow	2 (reliable with restrictions) Supporting study Testing material : ethylene oxide	Popp D.M. (1986)
B6C3F1 mice Inhalation study 10 mice of each sex per group 0, 50, 100, 200, 400, 600ppm 6h/d, 5d/week for 14 weeks	Mice exposed at 400 and 600ppm died in week 1-4, necropsy done Thymic lymphocytic necrosis in males (10/10) and females (6/10) at 600 ppm; Lymphocytic necrosis of the spleen in males (5/10) at 600ppm	2 (reliable with restrictions) According to NTP standard Testing material : ethylene oxide	NTP (1987)
Subchronic inhalation study B6C3F1 mice (10 mice per sex and dose group) Conc: 0, 10, 50, 100, 250ppm 6h/d, 5d/week, 10(m)/11(f) weeks	250ppm: RBC↓, PCV↓, Hb↓ No effects at lower conc. Histology of spleen, liver. testis and brain normal Organs weights at 250ppm: liver (f) ↑, spleen (m, f) ↓ No information on	2 (reliable with restrictions) This study was conducted according to the protocol and amendments prepared by the Chemical Hygiene Fellowship. Testing material : ethylene oxide	Snellings W.M. (1984)

	secondary effects.		
Wistar rats male Inhalation study, chronic 500ppm	Coproporphyrin excretion ↑ (250%) Urinary coproporphyrin/mg creatinine ↑ (141%)	Abstract only, report not available (Japanese)	Fujishiro K. (1989)
Wistar rats, male Inhalation study Pair-fed Control n=28 500ppm, n=28 6hr/d; 3d/week for 2, 6 and 13 weeks	Macrocytic, normochromic anemia (Hb↓, Ht↓, RBC↓, MCV↑, reticulocytes↑) Glutathion reductase activity ↓ No information on secondary effects. No histopathology	Key study 2 (reliable with restrictions) Testing material : ethylene oxide	Mori K.(1990)
Wistar rats, male Inhalation study Pair-fed 500ppm 6hr/d; 3d/week (for 2, 6 and 13 weeks) 8 animals per group	Normocytic and normochromic anemia (Hb↓, Ht↓, RBC↓, reticulocytes↑) ALA synthase ↑, ferrochelatase ↓, uroporphyrin ↑, coproporphyrin excretion ↑, hepatic cytochrome P-450 ↓ No information on secondary effects.	Key study 2 (reliable with restrictions) Testing material : ethylene oxide	Fujishiro K. (1990)
Wistar rat, male 500ppm 3d/week, 3 months	Normocytic and normochronic anemia (no further details) hepatic cytochrome P-450 ↓ (28%) hepatic hemeoxygenase ↑ liver and renal functions normal	Abstract only, report not available (Japanese)	Matsuoka M. (1988)
Zew Zealand rabbits Chamber exposure	Haematological and GSH measurement did not differ between	Abstract only	Yager JW.(1982)

Conc: 0, 10, 50, 250ppm 6h/d, 5d/week, 12weeks	control and exposed groups		
Dog (Beagle) (n=3) 100ppm for 6 months 6h/d, 5d/week; --- 290ppm for 6 weeks	In 2/3 dogs: RBCs↓, Hb↓ --- In 2/3 dogs: RBC↓, haemoglobin↓, haematocrit↓ - mild normochromic anemia	reliable with restriction no measured data available Testing material : ethylene oxide	Jacobson KH. (1956)
Dog Subcutaneous injection 6, 18, 54 mg/kg bw The highes dose was reduced to 36,mg/kg bw on day 7 of exposure	reduced haemoglobin and haematocrit values at all dosage levels, extramedullary haematopoiesis sever local tissue injury at the injection sites	Original literature not available	Woodard (1971) cited in FDA, 1978

Anand (2003) published an in vitro hemolysis test (New Zealand white rabbit blood) where concentrations up to 500µg ethylene oxide/ml for 4h did not result in haemolytic index[8] exceeding 5% (NOAEL=500µg/ml). At doses > 500µg/ml a dose and time dependent increase of hemolysis could be observed. Evidence of significant hemolysis was first observed at a concentration of 1250µg/ml after 4 h of exposure (haemolytic index 30.3%). For details see Table 32. This study simulates acute exposure to ethylene oxide.

Table 32: Hemolysis (%) of rabbit blood after incubation with ethylene oxide (average of three replicates) (Anand, 2003).

	EO concentration (µg/ml)								
Incubation (h)	25	50	100	250	500	1,250	2,500	5,000	10,000

[8] Hemolytic index (%) = [(av. abs. value of test article – av. absorb. of negative control) /(av. abs. value of pos ctrl. – av. abs.. of negative control)] x 100

Average absorbance was determined at 545nm against a saline blank

1	1.03 [0.5]	1.37 [0.4]	0.73 [0.4]	0.79 [0.2]	0.41 [0.3]	-0.13 [0.3]	8.45 [1.0]	94.76 [0.9]	84.23§ [2.2]
2	-0.12 [1,4]	1.74 [0.3]	0.99 [1.0]	1.05 [0.4]	0.92 [1.9]	1.38 [1.3]	28.63 [4.0]	75.52§ [19.9]	81.82§ [3.2]
4	2.23 [2.2]	0.06 [0.4]	1.01 [1.7]	0.08 [0.6]	0.75 [0.5]	30.27 [3.0]	52.91 [13.6]	79.45§ [4.5]	47.4§ [0.7]

[standard deviation], underlined numbers indicate >5% hemolysis, § formation of dark green reaction product interfering in assay

In an in vivo study (Popp, 1986) blood and bone marrow from mice exposed to 255ppm ethylene oxide for 6h/d for 5d/week was analysed after 1, 2, 8, 14 days and 4, 6, 8, and 10 weeks. Decrease in the number of erythrocytes (RBC), the quantity of hemoglobin (Hb), the haematocrit value (Ht), the bone marrow cell density, and the number of lymphocytes was observed. Details are presented in Table 33. Corresponding information on % alteration compared to control are presented in Table 35. 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 (see Table 34) 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 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 G_0 stem-cell pool.

Table 33: Hematology and stem cell analysis of mice exposed to 255ppm ethylene oxide, mean±SD (Popp, 1986).

Exp.	No of mice	WBC/mm³ ($x10^3$)	HCT (%)	RBC/mm³ ($x10^6$)	Hb (g/dl)	MCV (fl)	MCHb (pg)	BM ($x10^6$)	CFU-S/M
C	#	6.1±0.5	45.5±0.3	10.8±0.2	15.4±0.3	42.1±0.6	14.3±0.3	75.2±1.8	13635±534
1d	8	5.9±0.6	46.8±0.5*	11.1±0.4	15.2±0.1	42.4±1.1	15.2±0.1	67.1±2.5*	10625±293*
2d	8	3.2±0.6*	46.9±0.9	11.1±0.3	17.7±0.4*	42.3±0.9	15.4±0.5	66.6±6.5	10091±1091*
4d	4	7.0±0.1	43.8±0.5*	9.4±0.3*	15.0±0.1	46.9±1.9*	16.0±0.6*	65.0±6.3	9818±469*
8d	4	4.8±0.9	41.5±0.7*	9.7±0.2*	14.7±0.3	42.8±1.0	15.1±0.4	72.0±6.8	13539±1164
14d	4	6.7±0.8	45.3±0.8	9.8±0.7	15.2±0.2	46.7±3.0*	15.6±1.0	69.0±5.5	12288±1397
4wk	4	4.2±0.3*	44.8±0.5	11.7±0.4*	15.4±0.1	38.5±1.5*	13.3±0.5	61.8±2.5*	10879±699*

6wk	4	5.7±0.4	43.5±0.5*	10.1±0.5	14.6±0.2	43.1±0.8	14.4±0.5	85.31.7*	9376±1587*
8wk	4	2.1±0.3*	44.0±0.9	11.2±0.4	13.7±0.3*	39.5±0.9*	12.3±0.3*	78.8±6.1	12800±1270
10wk	4	4.6±0.6	43.0±0.7*	9.8±0.2*	14.0±0.3*	43.9±1.7	14.3±0.6	64.7±4.9*	9741±147*

number of mice varying for each endpoint between 8 and 13 animals
* $p<0.05$
MCV=(HCT/RBC)x10
MCHb=(Hb/RBC)x10

Table 34: White blood cell differential and number and percent of Normal for Lymphocytes (L), Granulocytes (G), Monocytes (M) and Eosinophils (E) (Popp, 1986).

Time	Differential (%)				No of cells/mm³				% normal				
	L	G	M	E	L	G	M	E	L	G	M	E	Total WBC
C	75	17	5	3	4408	1013	301	149	100	100	100	100	100
1d	50	43	6	2	3325	1828	336	181	75	181	112	122	97
2d	31	62	7	0.4	1048	2065	238	18	24	204	79	12	56
4d	63	25	9	4	4379	1738	626	278	99	208	195	187	118
8d	67	28	4	1	3199	1337	191	48	73	132	63	32	81
14d	73	16	8	3	4911	1076	538	202	111	106	179	136	115
4wk	65	25	7	4	2714	1044	293	167	62	103	97	112	71
6wk	69	25	6	3	3970	1430	345	173	90	141	115	116	98
8wk	43	54	3	1	897	1127	63	21	20	111	21	14	36
10wk	58	27	4	4	2674	1245	184	184	61	123	61	123	79

Table 35: % Alteration of blood parameters after exposure to 255ppm ethylene oxide (Popp, 1986).

	WBC			Ht			RBC			Hb			BM			CFU-S/M		
		% from c	diff		% from c	diff		% from c	diff		% from c	diff		% from c	diff		% from c	diff
Control	6,10	100,00	0,00	45,50	100,00	0,00	10,80	100,00	0,00	15,40	100,00	0,00	75,20	100,00	0,00	13635	100,00	0,00
1d	5,90	96,72	-3,28	46,80	102,86	2,86	11,10	102,78	2,78	15,2	98,70	-1,30	67,1	89,23	**-10,77**	10625	77,92	**-22,08**
2d	3,20	52,46	**-47,54**	46,90	103,08	3,08	11,10	102,78	2,78	17,7	114,94	**14,94**	66,6	88,56	**-11,44**	10091	74,01	**-25,99**
4d	7,00	114,75	**14,75**	43,80	96,26	-3,74	9,40	87,04	**-12,96**	15	97,40	-2,60	65	86,44	**-13,56**	9818	72,01	**-27,99**
8d	4,80	78,69	**-21,31**	41,50	91,21	-8,79	9,70	89,81	**-10,19**	14,7	95,45	-4,55	72	95,74	-4,26	13539	99,30	-0,70
14d	6,70	109,84	9,84	45,30	99,56	-0,44	9,80	90,74	-9,26	15,2	98,70	-1,30	69	91,76	-8,24	12288	90,12	-9,88
4wk	4,20	68,85	**-31,15**	44,80	98,46	-1,54	11,70	108,33	8,33	15,4	100,00	0,00	61,8	82,18	**-17,82**	10879	79,79	**-20,21**
6wk	5,70	93,44	-6,56	43,50	95,60	-4,40	10,10	93,52	-6,48	14,6	94,81	-5,19	85,3	113,43	**13,43**	9376	68,76	**-31,24**
8wk	2,10	34,43	**-65,57**	44,00	96,70	-3,30	11,20	103,70	3,70	13,7	88,96	**-11,04**	78,8	104,79	4,79	12800	93,88	-6,12
10wk	4,60	75,41	**-24,59**	43,00	94,51	-5,49	9,80	90,74	-9,26	14	90,91	-9,09	64,7	86,04	**-13,96**	9741	71,44	**-28,56**

Bold numbers indicate difference of ≥10% from control

In a 14 week study (NTP, 1987) B6C3F1 mice were exposed to concentrations up to 600ppm. All mice exposed at 400 and 600ppm died before end of the study (600ppm: all died in week 1, 400ppm: animals died in week 1-4). A necropsy was performed on all animals, including those found dead. Thymic lymphocytic necrosis was observed in males (10/10) and females (6/10) at 600ppm. Lymphocytic necrosis of the spleen was found in males (5/10) at 600ppm. Renal tubular necrosis was seen in male (8/10) and female (5/10) mice at 600ppm. No examination of hematology has been done (NTP, 1987).

B6C3F1 mice exposed to 250ppm for 10/11 weeks showed slightly depressed RBC and Hb in males and significant reduced RBC, PCV and Hb in females at 250ppm (see Table 36 and Table 37). As demonstrated in Table 38 (for males) and Table 39 (for females) the effects did not reach a reduction of 10% from control. There were no changes in mean corpuscular volume and no evidence of bone marrow hyperplasia or of nucleated red blood cells in the peripheral blood smears. Statistically significant differences in spleen, liver and testis weights were recorded at 250ppm (see Table 40) however there were no histopathological findings (Snellings, 1984).

Table 36: Haematological values for male mice after ethylene oxide exposure for 10 weeks (Snellings, 1984).

Concentration (ppm)	RBC[a] ($x10^6/mm^3$)	PCV[a] (%)	Hb[a] (g/dl)	MCV[a] (μm^3)	MCH[a] (pg)	MCHC[a] (%)	WBC/mm^{3a}
250	8.922* (0.332)	45.7 (1.9)	14.48* (0.60)	52.6 (0.8)	16.1 (0.3)	31.6 (0.7)	3760 (2626)
100	8.534 (1.908)	44.2 (8.7)	14.02 (2.58)	54.1 (4.3)	16.7 (1.9)	31.8 (1.3)	4400 (2149)
50	9.174 (0.316)	47.0 (2.4)	14.81 (0.56)	52.7 (1.0)	16.1 (0.3)	31.4 (1.0)	3900 (1986)
10	9.335 (0.432)	47.4 (1.9)	15.04 (0.58)	52.4 (1.2)	16.0 (0.0)	31.8 (0.9)	3050 (2020)
0	9.346 (0.530)	47.8 (2.8)	15.16 (0.75)	52.7 (1.1)	16.1 (0.3)	31.9 (0.7)	4250 (1844)

[a] mean value and SD in brackets
* 0.05>p>0 01 in comparison to control

Table 37: Haematological values for female mice exposed to ethylene oxide for 11 weeks (Snellings, 1984).

Concentration (ppm)	RBC[a] ($x10^6/mm^3$)	PCV[a] (5)	Hb[a] (g/dl)	MCV[a] (μm^3)	MCH[a] (pg)	MCHC[a] (%)	WBC/mm^{3a}
250	8.694** (0.451)	44.7** (4.0)	14.76** (0.64)	52.7 (0.7)	17.0** (0.8)	33.0 (1.2)	2430 (682)
100	9.228 (0.510)	47.0 (2.9)	15.35 (0.51)	52.4 (1.4)	16.5 (0.7)	32.6 (0.7)	2630* (753)
50	9.429 (0.201)	47.6 (2.3)	15.40 (0.65)	51.9 (0.8)	16.2 (0.4)	32.4 (0.5)	2089 (247)
10	9.514 (0.316)	47.9 (2.1)	15.53 (0.35)	51.6 (0.5)	16.2 (0.4)	32.4 (1.0)	2340 (645)
0	9.539 (0.365)	48.2 (3.5)	15.54 (0.47)	52.1 (0.8)	16.2 (0.4)	32.1 (0.7)	1980 (432)

[a] mean value and SD in brackets
* 0.05>p>0 01 in comparison to control
** p<0 01 in comparison to control

Table 38: Snellings (1984) – blood parameters after exposure to ethylene oxide up to 250ppm, % difference from control - MALES

Concentration (ppm)	RBC ($x10^6/mm^3$)		PCV (%)		Hb (g/dl)		MCV (μm^3)		MCH (pg)		MCHC (%)		WBC/mm^3	
		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)
250	8.922	-4,54	45,7	-4,39	14,48	-4,49	52,6	-0,19	16,1	0,00	31,6	-0,94	3760	-11,53
100	8.534	-8,69	44,2	-7,53	14,02	-7,52	54,1	2,66	16,7	3,73	31,8	-0,31	4400	3,53
50	9.174	-1,84	47	-1,67	14,81	-2,31	52,7	0,00	16,1	0,00	31,4	-1,57	3900	-8,24
10	9.335	-0,12	47,4	-0,84	15,04	-0,79	52,4	-0,57	16	-0,62	31,8	-0,31	3050	-28,24
0	9.346	0,00	47,8	0,00	15,16	0,00	52,7	0	16,1	0	31,9	0	4250	0

Table 39: Snellings (1984) - blood parameters after exposure to ethylene oxide up to 250ppm, % difference from control - FEMALES

Concentration (ppm)	RBC ($x10^6/mm^3$)		PCV (%)		Hb (g/dl)		MCV (μm^3)		MCH (pg)		MCHC (%)		WBC/mm^3	
		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)		Diff (%)
250	8.694	-8,86	44,7	-7,26	14,76	-5,02	52,7	1,15	17	4,94	33	2,80	2430	22,73
100	9.228	-3,26	47	-2,49	15,35	-1,22	52,4	0,58	16,5	1,85	32,6	1,56	2630	32,83
50	9.429	-1,15	47,6	-1,24	15,4	-0,90	51,9	-0,38	16,2	0,00	32,4	0,93	2089	5,51
10	9.514	-0,26	47,9	-0,62	15,53	-0,06	51,6	-0,96	16,2	0,00	32,4	0,93	2340	18,18
0 (c)	9.539	0,00	48,2	0,00	15,54	0,00	52,1	0	16,2	0	32,1	0	1980	0

Table 40: Mean values of organ weights (Snellings, 1984)

	Liver (absolute weight (g) / % of body weight)		Spleen (absolute weight (g) / % of body weight)		Testes (absolute weight, (g))
	male	female	male	female	male
250ppm	1.428 / 5.180	1.372 / 5.645*	0.052* / 0.188*	0.060* / 0.246*	0.106*
control	1.604 / 5.161	1.306 / 5.269	0.078 / 0.252	0.083 / 0.335	0.116

* $p<0.05$ in comparison to control

Mori (1990) examined effects of ethylene oxide on some factors which contribute to hemolysis. When male wistar rats were exposed to 500ppm ethylene oxide for 2, 6 and 13 weeks after 2 weeks only the Hb of exposed rats decreased when compared with pair-fed control. At 6 weeks the anemia progressed and was accompanied by an increase of reticulocytes by 100%. At 13 weeks the RBC had slightly recovered, but the Hb and Ht further decreased and reticulocytes were increased by 70%. After 6 and 13 weeks the MCV had increased but the MCH or the MCHC had not changed (Table 41 and Table 42). The body weight gain was not significantly affected; histopathological effects on spleen, kidney or liver were not examined. From these results the authors concluded that chronic or subchronic exposure to ethylene oxide induces macrocytic, normochromic anemia with high reticulocyte count. To clarify further if alterations of the metabolism in erythrocytes are the cause of hemolysis ATP and GSH content in erythrocytes were investigated. The inhibition of glutathione reductase (GR) after 13 weeks of exposure might be related to the anemia as GR is a key enzyme in the regulation of metabolism in erythrocytes (hexose monophosphate cycle). This decrease of GR activity by ethylene oxide might be due to alkylation of the GR molecules. As ATP content in erythrocytes was not affected the Embden-Meyerhof pathway and the Lapoport-Leubering cycle were thought to be intact. Membrane fragility as a second possible mechanism of hemolysis was not affected. However the mechanism of ethylene oxide induced hemolysis could not be clarified. No signs of haemorrhaging were observed during the study (Mori, 1990).

Table 41: Haematological findings in rats after exposure to 500ppm ethylene oxide (Mori, 1990) (mean±SD of 12 animals)

blood parameter	2 weeks		6 weeks		13 weeks	
	.Control	Exp.	Control	Exp.	Control	Exp.
RBC ($x10^6/mm^3$)	8.83±0.48	8.44±0.91	9.14±0.27	7.16±0.54*	9.15±0.22	7.85±0.38*
Hb (g/dl)	17.15±0.05	16.23±0.66*	16.43±0.64	15.12±1.57*	16.28±0.48	14.67±0.59*
Ht (%)	52.57±2.47	50.51±3.20	49.77±1.69	46.91±3.75	49.82±1.60	45.58±1.94*
MCV (μ^3)	59.45±1.63	60.25±2.63	54.08±1.00	65.50±2.02*	54.42±1.00	58.09±1.45*
MCH (µ µg)	19.45±1.02	19.35±1.36	19.16±0.25	19.83±0.90	18.39±0.30	18.72±0.54

MCHC (%)	32.65±1.34	32.18±1.14	33.23±0.27	32.23±1.49	32.68±0.53	32.18±0.56
Reticulocyte (%)	2.14±0.71	2.84±0.96	2.22±0.91	4.42±0.97*	1.82±1.24	3.09±1.26*

*significantly different from pair-fed control, $p<0.05$

Table 42: Haematological effects in % of control after exposure to 500ppm ethylene oxide (Mori, 1990)

	2 weeks				6 weeks				13 weeks			
	Control	500 ppm	% from control	Diff.	Control	500 ppm	% from control	Diff.	Control	500 ppm	% from control	Diff.
RBC	8,83	8,44	95,6	-4,4	9,14	7,16	78,3	**-21,7**	9,15	7,85	85,8	**-14,2**
Hb	17,15	16,23	94,6	-5,4	16,43	15,12	92,0	-8,0	16,28	14,67	90,1	-9,9
Ht	52,57	50,51	96,1	-3,9	49,44	46,91	94,9	-5,1	49,82	45,58	91,5	-8,5
MCV	59,45	60,25	101,3	1,3	54,08	65,5	121,1	**21,1**	54,42	58,09	106,7	6,7
MCH	19,45	19,35	99,5	-0,5	19,16	19,83	103,5	3,5	18,39	18,72	101,8	1,8
MCHC	32,65	32,18	98,6	-1,4	33,23	32,23	97,0	-3,0	32,68	32,18	98,5	-1,5
Reticulocyte	2,14	2,84	132,7	**32,7**	2,22	4,42	199,1	**99,1**	1,82	3,09	169,8	**69,8**

Bold numbers indicate difference of ≥10% from control

In another study in male Wistar rats the same exposure pattern as by Mori (1990) was applied: 6h/d, 3d/week for 2, 6 or 13 weeks (Fujishiro, 1990). After 13 weeks of exposure there were no significant changes in body or liver weight between control and exposed rats. Haematological effects after 13 weeks of ethylene oxide exposure are given in Table 43 and Table 44. Hb, Ht and RBC significantly decreased and the number of reticulocytes doubled. There were no changes in the light microscopy appearance of erythrocytes. The authors concluded that these results indicate a normocytic and normochromic anemia caused by ethylene oxide. No data are given for exposure duration of 2 and 6 weeks. Furthermore the study tried to clarify the mechanism of heme depletion by ethylene oxide. Analysis of enzymes of the porphyrin-heme metabolism show that hepatic mitochondrial ferrochelatase was significantly inhibited 13 weeks after exposure while ALA synthase was significantly increased (see Table 45). In addition a decrease in hepatic microsomal cytochrome P-450 (control: 0.61nmol/mg prot ± 0.08, ethylene oxide: 0.48nmol/mg prot ± 0.09*; $p<0.01$) could be seen.

Table 43: Haematological effects after 13 weeks of exposure to 500ppm ethylene oxide (Fujishiro, 1990).

	Hb (g/dl)	Ht (%)	RBC (x10^4µl)	MCV (fl)	MCHC (%)	Reticulocyte (%)
Control	15.6 ± 0.7	45.4 ± 2.8	869 ± 38	52.2 ± 2.3	34.4 ± 0.8	10.9 ± 3.4
500ppm	14.0 ± 0.7***	40.3 ± 2.3**	763 ± 36***	52.8 ± 1.8	34.6 ± 0.4	20.1 ± 9.3*

Results are expressed as mean±SD of 8 samples
Significantly different from control: *p<0.05, **p<0.01, ***p<0.001 (Student´s *t*-test)

Table 44: Haematological effects in % of control after 13 week exposure to 500ppm ethylene oxide (Fujishiro, 1990).

	13 weeks			
	Control	500 ppm	% from control	Diff.
RBC	869	763	87,8	**-12,2**
Hb	15,6	14	89,7	**-10,3**
Ht	45,4	40,3	88,8	**-11,2**
MCV	52,2	52,8	101,1	1,1
MCHC	34,4	34,6	100,6	0,6
Reticulocyte	10,9	20,1	184,4	**84,4**

Bold numbers indicate difference of ≥10% from control

Table 45: Effects of 500ppm ethylene oxide (13 weeks) on enzymes of the porphyrin-heme metabolism (Fujishiro, 1990).

	ALA synthase[a]	ALA dehydratase[b]		Ferrochelatase[c]
	Liver	Erythrocyte	Liver	Liver
control	20.5 ± 1.5	2.62 ± 0.54	140.8 ± 18.1	1.20 ± 0.18
500ppm	27.3 ± 3.4**	2.41 ± 0.92	132.4 ± 17.7	0.90 ± 0.16*

Results are expressed as mean±SD of 8 samples
Significantly different from control: *p<0.01, **p<0.001 (Student´s *t*-test)
[a] ALA-synthase activity is expressed as nmol of ALA formed/h per g wet liver
[b] ALA-dehydratase activity is expressed as µmol of ALA utilized/min per 1 RBC or mg protein
[c] Ferrochelatase activity is expresses as nmol of heme formed/min per mg protein

In rats exposed to 500ppm for three months (pair-fed) haematological examination revealed normocytic and normochromic anemia. Hepatic cytochrome P-450 and protoheme decreased by 28% and 19% respectively. The activity of hepatic heme oxygenase showed a 2-fold increase (Matsuoka, 1988 – abstract only).

Alterations of the porphyrin-heme metabolism by ethylene oxide including results from Fujishiro, 1990 and Matsuoka, 1988 are shown in Figure 2.
Integrating the results from Matsuoka (1988) Fujishiro (1990) tried to clarify the mechanism behind heme depletion by ethylene oxide. Possible mechanisms are

(1) inhibition of mitochondrial ferrochelatase,
(2) induction of hemeoxygenase and/or
(3) microsomal P-450 destruction

In the study mitochondrial ferrochelatase was significantely inhibited after 13 weeks of exposure. The inability to convert rapidly forming protoporphyrin into heme may result in protoporphyrin accumulation (see Figure 2). But in this study protoporphyrin tended to accumulate in the liver and erythrocyte but did not change significantly (Table 46).
The induction of hemeoxygenase (shown by Matsuoka, 1988) may also play a role in heme depletion in the liver of exposed rats.
It is also possible that ethylene oxide destroys cytochrome P-450 by prosthetic heme alkylation because it is a strong alkylating agent. A significant decrease in hepatic microsomal cytochrome P-450 was seen in this study, but no green pigment, considered to be alkylated heme, was observed in exposed rat livers.

Table 46: Effects chronic of ethylene oxide exposure (500ppm) on porphyrins and their precursors (Fujishiro, 1990)

	Urine		Liver			Erythrocyte
	Coproporph. (µg/mg creatinine)	ALA (µg/mg creatinine)	Uroporph. (ng/g)	Coproporph (ng/g)	Protoporph (ng/g)	Protoporph (µg/dl RBC)
Control,	1.14±0.53	62.6±23.1	89.0±22.5	19.9±14.0	88.7±25.9	33.2±9.6
Ethylene oxide 500ppm	6.92±1.86	114.1±38.0	122.1±27.2	33.5±16.7	100.4±14.0	39.8±9.2

Fujishiro (1989, abstract only) reports that ethylene oxide induces porphyria as after chronic exposure to 500ppm ethylene oxide (3d/week, several weeks) the daily coproporphyrin excretion and urinary coproporphyrin per mg creatinine increased by 250% and 141%, respectively. Daily excretion of delta-aminolevulinic acid in the urine tended to increase but did not increase significantly by creatinine correction. Daily urinary volume was increased by 200-300% from the first week to the fifth week of exposure.

Figure 2: Investigated enzymes of the heme metabolism and their response to chronic ethylene oxide exposure (Fujishiro, 1990).

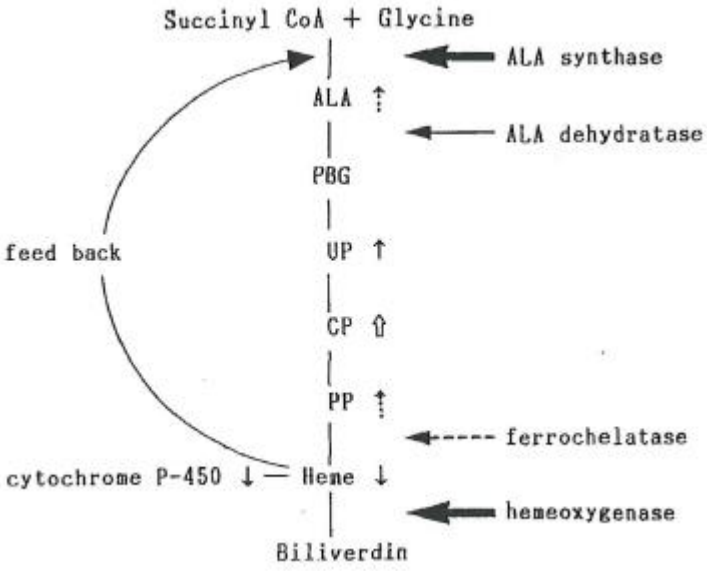


Fig. 4. Simplified scheme of the chronic effects of ethylene oxide inhalation on hepatic porphyrin-heme metabolism. ⇐ induction, ← normal, ←-- inhibition, ⇧ greatly increased, ↑ increased, ⇡ tended to increase, ↓ decreased, ALA: δ-aminolevulinic acid, PBG: porphobilinogen, UP: uroporphyrin, CP: coproporphyrin, PP: protoporphyrin.

New Zealand rabbits exposed to 0, 10, 50 and 250 ppm ethylene oxide in an inhalation chamber (6h/day, 5d/week) for 12 weeks showed no changes in haematological parameters (red or white blood cell counts, haematocrit, haemoglobin or white cell differential) during exposure and during recovery periode. GSH in liver and blood measured at the end of week 12 was not affected compared with unexposed controls. Sister chromatide exchange (SCE) rate was increased at 50 and 250ppm (Yager, 1982 – abstract only).

Dogs exposed to 290ppm for 6 weeks showed significant haematological changes. In 2/3 there was a significant decrease in RBC, haemoglobin and haematocrit. In the third there was a decrease (probably significant) in haemoglobin, with no changes in RBC and haematocrit (no details available). Thus two dogs developed a mild normochromic anemia. As the result of exposure to 100ppm for 6 months, 2 of 3 dogs showed a few significant haematological changes. In one dog there were significant decreases in RBC and haemoglobin. In another there were slight decreases (probably not significant) in RBC, haemoglobin and haematocrit. There were no changes in the third dog. One dog had a normochromic anemia, and similar anemia was suggested but not established in another dog. No detailed data available (Jacobson, 1956).

Woodard (1971, cited in FDA, 1978) exposed dogs to concentration of 6, 18 and 54mg/kg bw by daily subcutaneous injection for 30 days. The highest dosage was reduced after 7days of exposure to 36mg/kg bw. Dogs on the highest dose level showed extensive and sometimes inflammatory changes, whereas dogs at lower dose levels showed marked local inflammatory changes. The study showed increased mortality at the high level dosage and reduced haemoglobin and haematocrit values at all dosage levels. Haematological changes of dose related severity attributed to sever local tissue injury at the injection sites were reported. Hepatic changes such as increased liver weights at each dose and cholestasis at the high dose in each dog and at the mid dose in 1 of 4 dogs were observed. Increased ectopic hematopoesis was observed in 2/4 dogs at all dosage levels. No further

details available. But no effect was observed in the experiment using the same amount administered for 21 days by subcutaneous administration (Bolaz, 1976 as cited in NEDO, 2004).

Human information

The acute hemolytic potential of ethylene oxide in humans is described in literature in connection with ethylene oxide residues in medical devices after sterilization processes. But there is little agreement about the NOAEL/LOAEL of ethylene oxide in medical devices as the extraction of ethylene oxide varies with type of material. E.g. the threshold for ethylene oxide induced hemolysis was >4000µg/g for PVC, but <400µg/g for Cuprophane (cellulosic membrane) (cited in Anand, 2003, original literature not available). According to literature ethylene oxide concentration of ≥ 80µg/ml in circulating blood resulted in hemolysis. This implies that a device could deliver up to 400mg total residual ethylene oxide to the patient, before hemolysis could be expected (assuming distribution in an average total blood volume of 5000ml) (cited in Anand, 2003, original literature not available). Jones (1979) investigated the haemolytic potential of ethylene oxide in solution in three test systems, diluted whole blood in isotonic saline, erythrocytes washed and resuspended in isotonic saline, and erythrocytes washed and resuspended in isotonic phosphate buffer. Concentrations of 2000 µg/ml were necessary before cell lysis was observed in either of the isotonic saline systems. This value increased to 10,000 µg/ml in the isotonic buffer system. The hemolysis results in isotonic phosphate buffer are relevant to in vivo blood exposure because blood has similar buffering capacity and osmolarity.

First investigations to clarify the hematotoxicity of chronic exposure to ethylene oxide in humans were done in the sixties. Hemolysis of blood due to exposure to ethylene oxide sterilized plastic tubing was published by Hirose, 1963. When 31 persons who had been exposed to ethylene oxide for several years were examined and compared with a control group, the following pathological findings were obtained: lymphocytosis, reduced Hb level, 1 case of leukaemia and 3 cases of anisocytosis (Ehrenberg, 1967). On the other hand, the clinical and clinical chemical examination of 37 workers in the chemical industry who had been exposed for more than 10 years to 5–10ppm ethylene oxide and of a control group revealed no evidence of substance-related impairment of health (Joyner, 1964) (cited in DFG, 1993; studies not available).

Table 47: Human information in relation to hematotoxicity (chronic exposure).

Method	Results	Remarks	Reference
Canine or human blood Exposure for up to 24h to various types of plastic tubing, sterilized with steam or ethylene oxide	Ethylene oxide sterilization exaggerate haemolytic effect in all types of tubing	Abstract only No further details available	Hirose T (1963) cited in DFG, 1993
31 persons Ethylene oxide exposure for several years	lymphocytosis, reduced Hb level, 1 case of leukaemia and 3 cases of anisocytosis	No further details available	Ehrenberg L (1967) cited in DFG, 1993

Method	Results	Remarks	Reference
clinical examination of 37 workers in the chemical industry exposure for more than 10 years to 5-10 ml/m^3 ethylene oxide	no evidence of substance-related impairment of health	No further details available	Joyner R.E. (1964) cited in DFG, 1993
Cross-sectional study Women workers (US and Mexican), n=68 Exposure: none (0). low (>0-32ppm/h, high (> 32ppm/h)	US workers: Ht ↓, Hb↓ Lymphocyte percentage ↑ Neutrophil percentage ↓ No statistically significant results for Mexican workers	Supporting study	Schulte PA. (1995)
Cross sectional study n=84 yearly average TWA: - of loading operating technicians: 1977 <1ppm, 1980 = 1.7ppm -other jobs: <1ppm Peak exposure most below 20ppm	Haematological changes (Hb, Ht, RBC, WBC, %Lymphocytes) did not reach statistical significance	one for one matching of potentially exposed with unexposed individuals possibly also exposed to ethylene glycol, ethylene dichloride, biphenyl, biphenyl oxide,	Currier M.F. (1984)
Cross-sectional study workers exposed n=36 control n=35 exposure periode: 1-14 years concentration: below detection limit (personnel air sampler,	No statistically significant difference between workers and control in any immunolog. and haematology. Parameter Chromosome analysis gave no statistically significant difference for any type of abberations in lymphocytes.	Apart from ethylene oxide various other chemicals are used but they are not believed to be associated with the effects.	Van Sitter N.J. (1985)

Method	Results	Remarks	Reference
detection limit 0.05ppm), occasionally up to 8ppm Additional indirect measurement of ethylene oxide exposure (N-(2´-hydroxyethyl)-L-histidine) - no statistically significant differences between workers and control			
Medical surveillance n=36 (Caucasians) on-site control n=15 off-site control n=12 8h TWA = 0.07ppm (personal monitoring, since 1987)	Cross-sectional comparison of the complete blood cell data from exposed and non-exposed hospital workers showed no significant difference		LaMontagne A.D. (1993)
Cross-sectional study n=47 control n=88 concentration: 0.01-0.06ppm	Absolute mean number of monocytes ↑, Absolute mean number of eosinophils ↑, Absolute mean number of lymphocytes↓, Haematocrit↑ Absolute mean number of red blood cells ↑ Absolute mean number of platelets↓,	Supporting study For each exposed worker to unexposed were matched by sex, age and smoking habits Detailed questionnaire for included sujects	Shaham J. (2000)
Case report (n=1) 35 year old man Exposure: 6years	Symptoms: exhaustion, fatigue, petechial bleeding decreased platelet count reversible	Supporting study	Aydin G. (2010)

Haematological effects were observed among a group of 68 women exposed to ethylene oxide released from sterilizers while employed at nine hospitals in the USA and one in Mexico (Schulte, 1995). Exposure was classified as none, low, or high, based on mean 4- month cumulative exposure categories of 0, >0–32ppm, or >32 ppm/h, respectively. Monitoring data revealed mean 8-h TWA exposures in the US hospitals of 0.08ppm and 0.17ppm for the low and high exposure categories, respectively; the corresponding measurements in the Mexican hospital were 0.04 mg/m^3 and 0.99 mg/m^3 (range = 0.5–2.5 mg/m^3), respectively. Among the US workers, haematocrit and haemoglobin levels were reduced in the high-exposure group (see Table 48). Compared with unexposed controls, US workers in the high-exposure subgroup exhibited a statistically significant ($p = 0.04$) increase in the percentage of lymphocytes and a reduction ($p = 0.03$) in the percentage of neutrophils in the blood (see also Table 49). Among the Mexican workers, there were no statistically significant relationships between exposure to ethylene oxide and changes in haematocrit or haemoglobin levels (there was only one worker in the unexposed group), although an exposure-related increase (not statistically significant) in the percentage of neutrophils in the blood was observed. This study, investigating effects of low levels of exposure, gives some supporting evidence although the results are not conclusive (failure to see the same pattern in Mexicans, one-time biological sampling, high level of imprecision as count were performed on 100 cells only).

Table 48: Hematologic effects of ethylene oxide on female workers (Schulte, 1995) (mean adjusted and standard error).

Exp. ppm-hr	Red Blood cells (10^6/mm^3)		Hemoglobin (gm/dl)		Hematocrit (vol/dl)		White blood cells (10^6/mm^3)		Lymphocytes (% total leukocytes)		Neutrophils (% total leukocytes)	
	US	Mexico	US	Mexico	US	Mexico	US	Mexico	US	Mexico	US	Mexico
(1) 0 [a]	4.48 (0.15)	-	13.32 (0.40)	-	40.44 (1.27)	-	6.88 (1.24)	-	31.37 (3.64)	-	63.82 (3.41)	-
(2) >0-32	4.56 (0.09)	5.09 (0.14)	13.70 (0.21)	13.80 (0.45)	41.98 (0.68)	43.60 (1.41)	7.84 (0.65)	4.89 (0.58)	37.23 (1.86)	42.15 (1.64)	57.81 (1.75)	52.68 (1.84)
(3) >32	4.27 (0.15)	4.99 (0.12)	12.76 (0.36)	14.46 (0.39)	38.82 (1.15)	44.24 (1.22)	5.42 (1.09)	6.37 (0.51)	41.46 (3.08)	39.51 (1.42)	54.07 (2.09)	57.29 (1.59)
Signif. p value	-	-	0.03 (2) vs (3)	-	0.02 (2) vs (3)	-	-	-	0.04 (1) vs (3)	-	0.03 (1) vs (3)	-

[a] only one person represents Mexican values in this category

Table 49: Hematologic effects in % of control for US female workers (Schulte, 1995).

	RBC		HB		Ht		WBC		Lymphocytes		Neutrophils	
	10^6/mm^3	% diff from c	gm/dl	% diff from c	vol/dl	% diff from c	10^3/mm^3	% diff from c	% total leuco	% diff from c	% total leuco	% diff from c
control	4,48	0	13,34	0,0	40,44	0,0	6,88	0,0	31,37	0,0	63,82	0,0
US: 0-32ppm	4,56	1,8	13,7	2,7	41,98	3,8	7,84	**14,0**	37,23	**18,7**	57,81	-9,4
US: >32ppm	4,27	-4,7	12,76	-4,3	38,82	-4,0	5,42	**-21,2**	41,46	**32,2**	54,07	**-15,3**

Bold numbers indicate difference of ≥10% from control

Shaham (2000) reported a cross sectional study comparing 88 non-occupationally exposed controls (matched for age, sex, and smoking habits), among 46 hospital workers exposed (at three hospitals in Israel with a mean period of employment of 6.6 years) to 0.01-0.06ppm ethylene oxide (area air samples). There were statistically significant ($p < 0.01$) increases in the mean absolute numbers of red blood cells (+5.6% from c), increases in the percentage of haematocrit (+3.6% from c), and reduction in mean absolute number of platelets (-8,6% from c) in the exposed group compared with control group (see Table 50). No significant differences in the absolute mean number of the total WBCs were found. In the WBC differentials the absolute mean numbers of monocytes (+17.5% from c) and eosinophils (+29.4% from c) were significantly ($p < 0.01$) elevated; the absolute mean number of lymphocytes was significantly lower in exposed workers (-13% from c).

Table 50: Absolute numbers of CBC and WBC differentials in exposed (n=46) and non-exposed (n=88) hospital workers (Shaham, 2000). (mean±SD[§])

Parameter	Control	Exposure
CBC differential		
WBC (k/µL)	7.52±0.22	6.91±0.29
RBC (k/µL)	4.63±0.05	4.89±0.07*
HGB (g/dL)	13.97±0.12	14.14±0.15
HCT (%)	40.83±0.35	42.29±0.46*
PLT (k/µL)	247.33±6.60	225.98±8.58*
WBC differential (k/µL)		
NEUT	4.30±0.17	3.93±0.23
LYMP	2.46±0.07	2.14±0.09*
MONO	0.40±0.02	0.47±0.02*
EOS	0.17±0.01	0.22±0.02*
BASO	0.06±0.01	0.06±0.01

*p<0.01

[§]Least squares means from fitted model

A 35 year old man, working in an ethylene oxide sterilization room for 6 years, developed symptoms like exhaustion, fatigue and petechial bleeding (Aydin, 2010). Laboratory tests showed decreased platelet count of 125000/mm^3. It then decreased gradually to 100000, 98000 and 85000/mm^3. Before working in ethylene oxide sterilization his platelet count was 158000/mm^3. Exposure concentrations are not given in the report but there was no specific aeration inside the sterilizer, no safety closing measure for the sterilizer door and protective devices were used rarely. After removal from this working position platelet counts began to rise gradually.

LaMontagne (1993) described an apparent relative lymphocytosis which persisted over 3-4 years in sterilization workers with documented TWA exposure averaging 0.07ppm.
A comparison with control groups showed that this effect could not be associated with ethylene oxide exposure. He also describes three workers who had a history of acutely toxic overexposure to ethylene oxide (irritation, central nervous effects). Only one showed a relative lymphocyte count above 35% and one showed a high absolute white blood cell count (further examination showed alternately high and within normal range values over time without ethylene oxide exposure). RBC, Hg, Ht were normal in exposed group over various surveillance sessions.

Van Sitter (1985) published a study of 36 exposed and 35 non exposed workers in a plant manufacturing ethylene oxide. Exposure concentration was generally below the detection limit (using personnel air samplers) but transient concentrations up to 8ppm were occasionally recorded. Indirect measurement of ethylene oxide exposure (amount of N-(2´-hydroxyethyl)-L-histidine in haemoglobin) resulted in no statistically significant differences between workers and control (mean value was only slightly higher in workers). There was no statistically significant difference between the frequency of any types of aberrations noted in the lymphocytes of plant workers compared with control. In addition there was no statistically significant difference between plant workers and control in any of the immunological and haematological parameters examined (white blood cell count, T- and B-cells, monocytes, neutrophiles, serum concentrations of IgA, IgG, IgM). Because of technical difficulties not all plant workers and control subjects were examined by immunological and haematological analysis.

Also no haematological changes (Hb, Ht, RBC, WBC, %Lymphocytes) were observed in a group of 84 male workers involved in the manufacture of ethylene oxide who were exposed to estimated concentrations of <1ppm (Currier, 1984).

In general an overall examination of the available human data is difficult as cofounders like smoking habits, infections, etc. are not always assessed properly and the exposure to ethylene oxide is not always documented.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

According to the CLP regulation substances are classified for target organ toxicity STOT RE 1 if they have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.
Substances are classified on the basis of:

- reliable and good quality evidence from human cases or epidemiological studies; or
- observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

For classification based on the results obtained from studies conducted in experimental animals guidance values are given. Thus classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose inhalation study conducted in experimental animals (rat) are seen to occur at or below 50ppm. This guidance value is not intended as strict demarcation value.

Substances are classified in category 2 for target organ toxicity (repeat exposure) (STOT RE2) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure

concentrations. On the basis of evidence from studies in experimental animals it can be presumed that the substance has the potential to be harmful to human health following repeated exposure. According to the guidance given in 1272/2008/EC classification in Category 2 is warranted if toxic effects occur in a range from 50 to 250ppm (90d inhal. exposure, rat). But they are not intended as strict demarcation values.

Effects considered to support classification for specific target organ toxicity following repeated exposure (according to CLP regulation EC 1272/2008 Chapter 3.9.2.7) amongst others are:

- significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);
- any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

Small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects (when such changes or effects are of doubtful or minimal toxicological importance) are considered not to support classification for specific target organ toxicity following repeated exposure.

CLP guidance (ECHA, 2015) gives some additional guidance on the evaluation of haemolytic anemia: In the former legislation (67/548/EEC) the major criterion for haemolytic anemia was "any consistent changes in haematology which indicate severe organ dysfunction" (see also Muller, 2006). In the CLP regulation this criterion has been changes to "any consistent and significant adverse changes in haematology" - this indicates that less adverse effects are considered for classification according to CLP. Therefore a reduction in Hb at ≥20% fulfils the criterion of a consistent and significant adverse effect (ECHA, 2015; Chapter 3.9.2.5.2).

For ethylene oxide target organ toxicity after repeated exposure (STOT RE) for the nervous system and the hematopoietic system was evaluated:

Neurotoxicity:

For ethylene oxide several clinical studies with sterilization operators and 18 human cases are described in literature. Although information on exact exposure concentrations is limited adverse effects are well described as sensorimotor polyneuropathy after repeated exposure. Some reports indicate reversibility of effects several months after end of exposure. Animal studies in different species (monkeys, rats, mice, rabbits) show effects of neurotoxicity (evidence of demyelination, reduced locomotor function, abnormal posture) at 50ppm (Lynch, 1984a; Snellings, 1984) or above. Ethylene oxide often was tested only at higher concentrations. A compilation of relevant study results in comparison to corrected cut-off values for classification according to CLP-regulation is shown in Table 51.

Table 51: Compilation of most relevant chronic animal studies (neurotoxicity) and their corresponding cut-off values for classification as STOT RE.

Dose/effect	Study duration	guidance value (accord. CLP regulation) (ppm)		Reference
		STOT RE 1 (90d: C ≤50ppm)	STOT RE 2 (90d: 50<C≤250)	

204ppm - Monkeys: less active knee jerk reflexes, pos. Babinski-reflex, partial paralysis, evidence of muscular atrophy of the rear extremities - Rabbits: slight to marked paralysis in the rear legs	Up to 226 days	20ppm	100ppm	Hollingsworth (1956)
100ppm, rat: Multifocal areas of atrophy, degeneration of skeletal muscle fibres	2 years	6.5ppm	32ppm	Lynch (1984)
100ppm: - rats: skeletal muscle myopathy, atrophy, degeneration of mucle fibres; - monkey: demyelination 50ppm: brain lesions in rats	2 years	6.5ppm	32ppm	Lynch (1984a)
50ppm, mice: reduced locomotor function	11 weeks	59ppm	293ppm	Snellings (1984)
250ppm, rat: distal axonal degeneration of myelinated fibres No lower conc tested	9 months	17ppm	83ppm	Ohnishi (1986)
250ppm, rat: axonal degeneration of the myelinated fibres No lower conc tested	17 weeks	38ppm	190ppm	Mori (1990)
300ppm, rat: Hind limb grip strength decreased 500ppm, rat: decreased absolute brain weight, minimal to slight vacuolisation of the white matter	4 weeks	150ppm	750ppm	Mandella (1997b)
200ppm, rat: 25% decrease in hindlimb grip strength in female	14 weeks	46ppm	230ppm	Mandella (1997c)

Hematotoxicity:

In a vitro study ethylene oxide induces hemolysis with a NOAEL of 500µg/ml; 30% haemolysis at 1250µg/ml and 4h incubation (Anand 2003). In mice and rats a decrease in RBC, Hb (Snellings 1984) and Ht was observed (Popp, 1986; Mori, 1990; Fujishiro, 1990) at concentrations of 250 and 500ppm respectively indicative of anaemia. An increase of reticulocytes was observed by Mori (1990) and Fujishiro (1990) at 500ppm indicating a bone marrow erythropoietic response (compensatory effect). In rabbits no effects could be seen at 250ppm (Yager, 1982).
For a better comparison of effects seen with the CLP criteria for STOT RE a short compilation of effects in animals (shown as % deriv. from control) is given in the following table (for fluctuations in dose-response see study descriptions in Chapter 4.8.1.2.).

Table 52: Effects seen in animals after exposure to ethylene oxide.

Author	Animal	Conc.	Exp time	RBC (% dev. from control)	Hb (% dev. from control)	Ht (% dev. from control)	Additional comment
Snellings, 1984	Mice male	100	10wk	-8.7%	-7.5%	not examined	Differences in spleen-, liver- and testis weights without histo. findings
Snellings, 1984	Mice female	250	10wk	-8.9%	-5%	not examined	
Popp, 1986	Mice	255	10wk	-9.3%	-9.1%	-5.5%	-
NTP, 1987	Mice	600 ppm	14wk	not examined	not examined	not examined	thymic lymphocytic necrosis, lymphocytic necrosis of the spleen
Mori, 1990	Rat	500ppm	13wk	-14.2%	-9.9%	-8.5%	Reticulocytes +69.8% Inhib. of Glutathione reductase
Fujishiro, 1990	Rat	500ppm	13wk	-12.2%	-10.3%	-11.2%	Reticulocytes +84.4% Hep. mitoch. ferrochelatase ↓, ALA synth ↑, hepatic micros. Cyt P-450 ↓
Yager, 1982	Rabbit	250ppm	12wk	No effects	No effects	No effects	-
Jacobson, 1956	Dog	290ppm	6wk	↓	↓	↓	-
Woodard, 1971	Dog	54/36mg/kg bw	30d	-	↓	↓	Increased liver weight, ectopic

							hematopoesis
Bolaz, 1976	Dog	54/36mg/kg bw	21d	No effects	No effects	No effects	-

The mechanism of ethylene oxide induced hemolysis could not be clarified but inhibition of glutathione reductase (Mori, 1990), inhibition of ferrochelatase, increase of ALA synthase (Fujishiro, 1990) and increase of heme oxygenase (Matsuoka, 1988) could be shown indicating interference of ethylene oxide with the heme metabolism.

Human data are more variable which may be explained by the low exposure levels. Some studies show no effect of ethylene oxide on hematology (Currier, 1984; VanSitter, 1985; LaMontagne, 1993) while others show reduced haematocrit and haemoglobin levels (Schulte, 1995) or increased haematocrit and red blood cell count (Shaham, 2000). Increased proportion of lymphocytes could also be seen (Schulte, 1995). For a rough overview see Table below.

Table 53: Overview on findings in humans after chronic ethylene oxide exposure.

Author	Exposure	RBC (% dev. from control)	Hb (% dev. from control)	Ht (% dev. from control)	Additional comment
Hirose, 1963	-	-	-	-	Haemolytic effect seen
Ehrenberg, 1967	-	-	↓	-	
Joyner, 1964	5-10ppm	No effects seen			
Schulte, 1995	32ppm	-4.7%	-4.3%	-4.0%	WBC -21.2%
Currier, 1984	-	No significant changes			
Van Sitter, 1985	up to 8ppm	No significant changes			
LaMontagne, 1993	0.07ppm	No significant changes			
Shaham, 2000	0.01-0.06ppm	+5.6&	Not examined	+3.6%	Number of platelets: -8.6%
Aydin, 2010	-	-	-	-	Case, report, decreased platelet count

Haemolytic anaemia is a toxicological significant adverse effect in itself. Indicators of anemia are reduced Hb, RBC and Ht. Animals are comparable to humans for these parameters. According to CLP guidance (ECHA, 2015) a reduction in Hb at ≥20% fulfils the criterion of a consistent and significant adverse effect. Ethylene oxide exposure results in a reduction of Hb of less than 20% in animal studies and human studies show a diffuse picture. Secondary effects of anemia have only been investigated in a few studies showing exhaustion, fatigue (Aydin, 2010), extramedullary hematopoiesis (Woodard, 1971) or affected organ weights (Snelling, 1984).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Neurotoxicity:

Based on the available human data showing clear neurotoxic effects and evidence from animal studies ethylene oxide should be classified according to CLP-Regulation as STOT RE1 (H372: Causes damage to nervous system through prolonged or repeated exposure).

Hematotoxicity:

Based on available chronic animal and human data no classification of ethylene oxide for this endpoint is proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS provided both human and animal data on the repeated dose effects of ethylene oxide.

In humans, there were 18 cases describing peripheral neuropathy, impaired coordination and memory loss after inhalation exposure to ethylene oxide. There were also four clinical studies suggesting effects on CNS and peripheral nervous system (PNS) functions in exposed workers. Animal studies in different species (monkeys, rats, mice, rabbits) showed neurotoxicity, including evidence of demyelination, reduced locomotor function and abnormal posture after inhalation exposure of 50 ppm or higher.

Ethylene oxide has shown haemolytic effects *in vitro*. *In vivo*, after inhalation of ethylene oxide, in mice and rats a decrease of 5-14% in RBC, Hb and/or Ht has been observed in different studies at concentrations of 250 and 500 ppm. Also, an increase in reticulocytes up to 70-85% has been observed in two studies at 500 ppm, indicating a bone marrow erythropoietic response (compensatory effect).

Human studies have shown variable results which may be explained by the low exposure levels: while some studies have not shown any effects of ethylene oxide on haematology, in others slightly (4-5%) reduced haematocrit and haemoglobin levels or increased haematocrit and red blood cell count have been reported. An increased proportion of lymphocytes was reported in one study.

On the basis of the available human data showing clear neurotoxic effects and evidence from animal studies, the DS proposed to classify ethylene oxide according to CLP Regulation as STOT RE 1 (H372: Causes damage to nervous system through prolonged or repeated exposure). Regarding haematotoxic effects, according to the CLP guidance a reduction in Hb ≥ 20% fulfils the criterion of a consistent and significant adverse effect. Since ethylene oxide exposure resulted in a reduction of Hb of less than 20% in animal studies and human studies showed a diffuse picture, the DS did not propose classification based on haematotoxic effects.

Comments received during public consultation

Two MSCAs and one national authority supported classification for STOT RE 1; H372.

Assessment and comparison with the classification criteria

Both human and animal data are available on the repeated dose effects of ethylene oxide. The main effects of concern are neurotoxicity and haematotoxicity, which have been evaluated in several studies.

Neurotoxicity in humans

The majority of the data on the repeated dose neurotoxicity in humans comes from case reports. These reports describe neurological effects, mainly characterised by peripheral neuropathy, impaired hand-eye coordination and memory loss in steriliser workers after 2 weeks to 10 years of exposure to ethylene oxide. In many cases, these effects were accompanied with symptoms of more acute in nature; e.g. headache, nausea, fatigue, drowsiness and irritation suggesting that exposures may have been rather high. Peripheral neuropathy has been shown in many cases by measuring nerve conduction velocities or by nerve biopsies. In many cases, the effects have been at least partly reversible after the cessation of exposure. For example, in four operators exposed to ethylene oxide (ETO), nerve conduction velocities indicated sensorimotor neuropathy, which was reversible during the follow-up in 2 out of 4 workers (Gross, 1979). In two cases reported by Kazuhara (1983), axonal degeneration and regeneration was observed in nerve biopsies of workers exposed to ETO and showing sensorimotor neuropathy. Also, Brashner (1996) reported impaired nerve conduction velocities in 4 out of 10 workers showing symptoms related to ETO exposure. Sural axonal injury was observed in nerve biopsy of the most severely affected person. Similar polyneuropathy findings were observed in the reports by Finelli (1983), Zampollo (1984), Schoeder (1985), Fukushima (1986) and DeFreitas (1991). CNS effects, including impaired memory have been suggested in case reports by Crystal (1988), Garry (1979) and Braeshner (1996). Further information on these case reports are given in the table below. Case reports related to single accidental exposures have been excluded from the table.

Table: Case reports on the neurological effects of ethylene oxide in humans.

Subjects and exposure	Results	Remarks	Reference
Case reports (n=4) Operators exposed to ethylene oxide due to a leaking steriliser up to 2 months Case 1: 3 weeks Case 2: 3 weeks Case 3: 2 weeks	Peripheral neuropathy Case 1: headache, nausea, vomiting, lethargy, motor seizures at 20-30 min intervals; patient was fully recovered 2 months later Case 2: headache, limb weakness, fatigability, wide based unsteady gait; shift to work without exposure resulted in significant improvement Case 3: headache, altered memory and thinking, fatigability,	Supporting study No information on exposure concentrations available. 700 ppm, as estimated by the authors as the workers could smell the chemical	Gross, 1979

Case 4: 2 months	cramps; further work under condition of lower exposure (50 ppm) resulted in no improvement of nerve conduction studies 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	Possibility of short time exposure to high levels of ethylene oxide no assessed	
Clinical study: Occupational exposure during ethylene oxide gas sterilisation	Headaches, nausea, speech disorders and impairment of short-term memory, vertigo and incoordination	Supporting study	Garry, 1979 (cited in SCOEL, 2012)
Survey (n=165) 11-23.5ppm Duration per cycle: 2.77-11.75 min	Headaches, skin and eye irritation, dry mouth, sore throat, skin rash, loss of sense of smell, shortness of breath, nausea, numbness in fingers, drowsiness		Bryant, 1989 (cited in US EPA, 2010)
Case report (n=3)	Polyneuropathy (bilateral foot drop, denervation potential on electromyography)	Supporting study	Finelli, 1983 (cited in DFG, 1993)
Case report (n=2), Occupational exposure during ethylene oxide sterilisation Several months of exposure, about 1.5 h/d. Concentration: estimated peak exposure ~ 700 ppm (smelling) when opening the steriliser	Sensorimotor neuropathy (axonal sural nerve degeneration) Symptoms improved after termination of exposure	Supporting study	Kuzuhara, 1983
Case report (n=2) among 12 female workers Two years of exposure (ethylene oxide steriliser)	Peripheral neuropathy Cease of exposure resulted in swift remission of symptoms and complete normalisation of the electromyography record	Supporting study Exposure fluctuating between 10 and 400 ppm	Zampollo, 1984
Case report (n=1) 5 months of exposure Concentration: up to 500 ppm, 2-3 times daily	Polyneuropathy (distal weakness of lower extremities and transitory reduced nerve conduction velocity, nerve fibre degeneration) Improvement in re-examination 1 year after exposure	Supporting study	Schroeder, 1985

Case report (n=4) Exposure 8-10 times/d while transporting sterilised products and once daily while exchanging containers	Polyneuropathy (impairment of lower limbs and titubation) All patients show motoneuron disease, dorsal cord disorder, cranial and autonomic disorders Reversible	Supporting study	Fukushima, 1986 (as cited in NEDO, 2004)
Case report (n=1) 10 years of exposure (adjacent to an ethylene oxide chemical steriliser)	After 7 years symptoms like impaired memory, increased irritability, clumsiness, falling Symptoms markedly improved few months after exposure ceased Symptoms 1 year after exposure ceased: emotional lability, impaired concentration, cognitive slowing, impaired recent and remote memory	Supporting study 4.2 ppm (when the steriliser was closed)	Crystal, 1988
Case report (n=1) Seven years of exposure	Mild sensorimotor polyneuropathy (axonal degenerative type) sural nerve biopsy: mild loss of myelinated fibres, fibres with axonal degeneration	Supporting study No detailed information available	De Freitas, 1991
Case report reporting a cluster of 12 (n=12) operating-room nurses/technicians with neurologic symptoms and findings Inhalative and dermal exposure (vapour in package and residue retained in surgical gowns)	Rash on arm and wrist, dysesthesia, headache Neuropathy in 9/12 Further symptoms: memory loss, mild cognitive impairment, elevated vibration threshold, abnormal pressure threshold Sural nerve biopsy: axonal injury Persistent hand symptoms for at least 1 year after removal	Supporting study Exposure to ethylene oxide and ethylene chlorohydrin Measurement in gown cuff: Ethylene oxide = 298 ppm Ethylene chlorohydrin = 373 ppm. Peak level exposure unknown	Brashear, 1996

There were only three small controlled studies available on the neuropsychological effects of ethylene oxide.

Estrin *et al.* (1987) compared the performance of 8 workers chronically exposed via inhalation to ethylene oxide (+ chlorodifluoromethane) in a computerised psychometric test battery, nerve conduction studies, P-300 event-related potential and EEG spectral analysis to the performance of 8 age and sex matched control persons. The exposed group performed more poorly (not statistically significant) in the psychometric test battery (cognition, memory, attention and coordination (Hand-Eye Coordination Test)). A relationship between years of exposure and decreasing performance on the continuous performance test and reduction in sural velocity was observed. P-300 and EEG spectral analysis showed no significant results.

Klees *et al*. (1990) compared the neuropsychological performance of 22 hospital workers chronically exposed to ethylene oxide (8 h TWA of 4.7 ppm) via inhalation to that of 24 unexposed workers. 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) suggesting some CNS dysfunction and cognitive impairment related to chronic ethylene oxide exposure.

Patch *et al.* (2001) studied the neuropsychological performance of 22 workers exposed to ethylene oxide in medical settings for 24-108 months and compared it to the performance of 64 victims of traumatic brain injury (TBI) (time from date of injury 1 to 73 months). Intelligence test were lower in ETO exposed workers, compared to TBI patients and both groups showed lower scores for reaction and movement times (finger tapping, reaction time test) when compared to the means of the general population. Both groups also showed preoccupation with bodily concerns, anxiety, depression and tendency to channel stressful feelings into physical symptoms and feelings of alienation, isolation and social disconnectedness for both groups, but ethylene oxide exposed individuals exhibited more feelings of anxiety, fear, edginess and loss of control than TBI patients. Due to the lack of a properly matched control group, it was difficult to draw conclusions from this study.

Overall, there were several human case reports showing neurological effects, especially peripheral neuropathy in workers after repeated or long term occupational exposure to high levels of ETO. Controlled studies in ETO exposed workers were limited in size and/or methodology but in principle supported the signs and symptoms reported in case reports.

Neurotoxicity in animals

There were several studies in different animal species on the repeated dose neurotoxicity of ethylene oxide. Most of these describe signs and symptoms of neurotoxicity, including paralysis of hind limbs, degeneration of muscle fibres, demyelinisation/degeneration of nerve fibres, and e.g. impairment in locomotor function. However, some of these studies have used only high exposure levels (250 ppm or higher). Critical studies in animals included the studies by Mandella *et al*. (1997b and c), Lynch *et al*. (1984) and Snellings *et al.* (1984).

In a 4-week range-finding study (Mandella *et al*., 1997b), groups of five male and five female Sprague-Dawley rats were exposed by whole-body inhalation to ethylene oxide vapour at concentrations of 0, 100, 300, 400 or 500 ppm. Clinical signs observed at 500 ppm included irregular gait, decreased faecal volume, lethargy, prostration, emaciation, yellow anogenital staining, moist rales, laboured breathing, paleness, and black and brown stains on the snout. One female rat in the 500 ppm group was found dead on day 18. Body weights of males and females exposed to 300, 400 or 500 ppm decreased by 12% to 42% at study termination and food consumption decreased by 15% and 18% in females and males, respectively, during the first week. The neurologic assessment at weeks 3 and 4 showed that hindlimb grip strength decreased 22% to 36% in both sexes at 300, 400 and 500 ppm; this effect was more severe at 400 and 500 ppm. Landing foot splay decreased 29% to 42% in both sexes at week 3 or 4 at 400 and 500 ppm; this effect was more severe at 500 ppm. At 500 ppm, the post-mortem examination showed decreased absolute brain weight in males and minimal to slight vacuolisation of the white matter of the thalamus and medulla oblongata in both sexes. No exposure-related effects were observed at 100 ppm (NOAEL).

In a follow-up subchronic neurotoxicity study, groups of 15 male and 15 female Sprague-Dawley rats were exposed by whole body inhalation to ethylene oxide vapour at concentrations of 0, 25, 50, 100 or 200 ppm for 14 weeks (Mandella *et al.*, 1997c). Neurobehavioral assessments (functional observational battery) were conducted in 10 rats of each sex after exposure for 5, 9, and 14 weeks and after the 13-week recovery period. Body weight gain decreased 16% to 17% during exposure to 200 ppm with a concomitant decrease in food

consumption. The neurobehavioral assessment showed a 25% decrease in hindlimb grip strength in females exposed to 200 ppm. No exposure-related effects were observed at 100 ppm (NOAEL) and no exposure-related effects were observed for clinical signs (including motor activity), mortality, cholinesterase activity or in macroscopic or microscopic examination of nervous system tissue at any concentration.

In the GLP-compliant study by Snellings *et al.* (1984), male and female mice were given concentrations of 0, 10, 50, 100 and 250 ppm of ethylene oxide in an inhalation chamber for 6 h per day and 5 days per week. No increased mortality was observed among the exposed groups. During the last week of exposure lower body weight gain was observed in the highest exposure group. Minimal changes in certain erythroid parameters, increased liver weight, decreased testicular weight and decreased spleen weight were observed at 250 ppm; decreased spleen weight was noted also in the 100 ppm group. However, there were no histopathological findings to support these weight changes. A dose-related trend of response in the 250, 100 and 50 ppm exposure groups was noted in the evaluation of locomotor functions (abnormal posture, reduced locomotor activity); at 250 ppm a statistically significant difference for abnormal righting reflex, toe pinch and tail pinch was observed. The small sample size (5 mice were selected for neuromuscular screening testing) resulted in uncertainties in the determination of no-effect levels. No accompanying histopathologic alterations in muscle and central or peripheral nervous tissue were observed. The NOAEC was reported as 10 ppm for male and female mice.

Two year inhalation exposure of rats to concentrations of 50 and 100 ppm (for 7 h/d and 5 d/wk) resulted in an increased incidence of skeletal muscle myopathy in the absence of any sciatic nerve neuropathology (Lynch *et al.*, 1984). Brain lesions were observed in histopathology already at 50 ppm. A statistically significant increase in mortality was observed in all groups of exposed rats compared to controls. In a similar 2-year study in monkeys, slight demyelination of the brains of monkeys was reported at 100 ppm (Lynch *et al.*, 1984, cited in ATSDR 1990). No original study report on this study was available.

Other studies have reported effects either at higher dose levels, or have employed a limited number of animals. For example, in the study by Setzer (1996), neuropathological changes were reported in 1 out of 2 examined monkeys/dose group after 24 months exposure to 50 or 100 ppm per day (7 h/d, 5 d/wk). Similarly, demyelination in *fasciculus gracilis* was reported in 1 of 2 monkeys/dose group after inhalation exposure to 50 and 100 ppm (6 h/d, 5 d/wk, for 24 months) in the study by Sprinz *et al.* (1982). Several studies reported clear neurotoxic effects in rodents, rabbits or monkeys at doses of 200 ppm or higher (Hollingsworth *et al.*, 1956; Snellings *et al.*, 1982; Ohnishi *et al.*, 1985/1986; Mori *et al.*, 1990) after exposure periods ranging from 7 weeks to 24 months. Generally, the findings were very similar across the studies and supported the specific peripheral neurotoxicity of ethylene oxide.

Haematotoxicity in experimental systems and in animals

In vitro, ethylene oxide has been shown to cause haemolysis at doses > 500 µg/L. The mechanism of ethylene oxide induced haemolysis is uncertain but inhibition of glutathione reductase (Mori *et al.*, 1990), inhibition of ferrochelatase, increase of ALA synthase (Fujishiro *et al.*, 1990) and increase of haeme oxygenase (Matsuoka, 1988) could play a role in the interference of ethylene oxide with the haeme metabolism. Ethylene oxide has caused decreased red blood cell, haemoglobin and haematocrit levels in animals after sub-chronic inhalation exposure to 250-500 ppm. The main studies and their main haematological findings are discussed below.

In the study by Popp *et al*. (1986), mice exposed to 255 ppm ethylene oxide for 6 h/d for 5

d/wk were studied after 1, 2, 8 and 14 days, and 4, 6, 8 and 10 wk. There were up to 11% decreases in the number of erythrocytes (RBC), the quantity of haemoglobin (Hb) and the haematocrit value (Ht), but the levels varied between different time points. Transient increases were interpreted by the authors as transient compensatory bursts (see table below). In addition, changes in white blood cell counts were seen, but also in this case the results at different time points were variable. In WBC differential analysis, there was a shift towards increased granulocytes numbers while lymphocytes were lost from the circulation. Similarly, in the bone marrow, a trend towards depressed bone marrow cellularity or stem cell numbers were seen, although the exact cell numbers varied.

Table: Alterations of blood parameters after inhalation exposure to 255 ppm ethylene oxide (Popp *et al*., 1986).

	WBC			Ht			RBC			Hb			BM			CFU-S/M		
		% from c	diff		% from c	diff		% from c	diff		% from c	diff		% from c	diff		% from c	diff
Control	6.10	100.00	0.00	45.50	100.00	0.00	10.80	100.00	0.00	15.40	100.00	0.00	75.20	100.00	0.00	13635	100.00	0.00
1 d	5.90	96.72	-3.28	46.80	102.86	2.86	11.10	102.78	2.78	15.2	98.70	-1.30	67.1	89.23	**-10.77**	10625	77.92	**-22.08**
2 d	3.20	52.46	**-47.54**	46.90	103.08	3.08	11.10	102.78	2.78	17.7	114.94	**14.94**	66.6	88.56	**-11.44**	10091	74.01	**-25.99**
4 d	7.00	114.75	**14.75**	43.80	96.26	-3.74	9.40	87.04	**-12.96**	15	97.40	-2.60	65	86.44	**-13.56**	9818	72.01	**-27.99**
8 d	4.80	78.69	**-21.31**	41.50	91.21	-8.79	9.70	89.81	**-10.19**	14.7	95.45	-4.55	72	95.74	-4.26	13539	99.30	-0.70
14 d	6.70	109.84	9.84	45.30	99.56	-0.44	9.80	90.74	-9.26	15.2	98.70	-1.30	69	91.76	-8.24	12288	90.12	-9.88
4 wk	4.20	68.85	**-31.15**	44.80	98.46	-1.54	11.70	108.33	8.33	15.4	100.00	0.00	61.8	82.18	**-17.82**	10879	79.79	**-20.21**
6 wk	5.70	93.44	-6.56	43.50	95.60	-4.40	10.10	93.52	-6.48	14.6	94.81	-5.19	85.3	113.43	**13.43**	9376	68.76	**-31.24**
8 wk	2.10	34.43	**-65.57**	44.00	96.70	-3.30	11.20	103.70	3.70	13.7	88.96	**-11.04**	78.8	104.79	4.79	12800	93.88	-6.12
10 wk	4.60	75.41	**-24.59**	43.00	94.51	-5.49	9.80	90.74	-9.26	14	90.91	-9.09	64.7	86.04	**-13.96**	9741	71.44	**-28.56**

Bold numbers indicate difference of ≥10% from control

In the study by Snellings *et al.* (1984), B6C3F1 mice exposed via inhalation to 10-250 ppm ethylene oxide for 10/11 weeks showed slight reductions in RBC, PVC and Hb levels in males and females. In males the effects were more pronounced at 100 ppm than at 250 ppm. In females, Hb levels were decreased 5%, and PCV and RBC levels were decreased 7-9% at 250 ppm, whereas levels at 100 ppm were only 1-3% lower than in controls. In males, 7-9% decreases in the parameters were observed at 100 ppm, but at 250 ppm the decreases were only ~4.5%. There were no changes in mean corpuscular volume and no evidence of bone marrow hyperplasia or nucleated red blood cells in peripheral blood.

Other inhalation studies, like Mori *et al*. (1990) and Fujishiro *et al*. (1990) described similar, up to 11% decreases in haemoglobin and haematocrit levels effects at the doses of 500 ppm. RBC counts were also depressed (12-14% after 13 weeks of exposure). Both studies described also significant compensatory increase in reticulocyte levels. In the old study in dogs (Jacobson *et al*., 1956), exposure to 290 ppm for 6 weeks resulted in a significant decrease in RBC, haemoglobin and haematocrit levels in 2 out of 3 dogs. At 100 ppm, 1 out of 3 dogs showed significant decreases in RBC and Hb levels.

Overall, these studies show that at high exposure levels, ethylene oxide is able to affect blood parameters in experimental animals. However, the effects even at these high levels (500 ppm)

were rather moderate with changes in blood parameters only up to 10-14%. It should be noted that at these high levels (levels exceeding 200 ppm) rodents are likely to be more sensitive than humans for the adverse effects of ethylene oxide, since in rodents elimination of ETO occurs via glutathione (GSH) conjugation and at these levels GSH is depleted resulting in lower elimination. In humans, ETO is primarily eliminated via hydrolysis.

Haematotoxicity in humans

The acute haemolytic potential of ethylene oxide in humans has been described in literature in connection with ethylene oxide residues in medical devices after sterilisation processes. There is, however, little agreement about the NOAELs/LOAELs of the haemolytic effects of ethylene oxide when exposed via the medical devices as the extraction of ethylene oxide varies with type of material.

An early study by Ehrenberg *et al.* (1967) described lymphocytosis, reduced Hb level, one case of leukaemia and three cases of anisocytosis among 31 persons who had been exposed to ethylene oxide for several years. Schulte *et al.* (1991) studied the haematological effects of ETO among a group of women employed at nine hospitals in the USA and one in Mexico (Schulte *et al.*, 1995). Exposure was classified as none, low, or high, based on mean 4-month cumulative exposure categories of 0, >0–32 ppm-h, or >32 ppm-h, respectively. Mean 8-h TWA exposures in the US hospitals were 0.08 ppm and 0.17 ppm for the low and high exposure categories, respectively; the corresponding measurements in the Mexican hospital were 0.02 ppm and 0.55 ppm (range = 0.3–1.4 ppm), respectively. Among the US workers, haematocrit and haemoglobin levels were reduced about 4%, and neutrophil levels were reduced 15% in the high-exposure group when compared to unexposed controls. Percentage of lymphocytes was increased by about 32% in the high dose group. The absolute number of lymphocytes, however, showed no relationship with exposure. Among the Mexican workers, there were no statistically significant relationships between exposure to ethylene oxide and changes in haematocrit or haemoglobin levels, although estimated exposure was higher than in US workers. Not statistically significant exposure-related increase in the percentage of neutrophils was, however, observed. Uncertainties related to this study include the failure to see the same pattern in Mexicans, only single biological sampling and the high level of imprecision as the counts were performed on only 100 cells.

Another study in humans (Shaham *et al.*, 2000) reported statistically significant ($p < 0.01$) increases in the mean absolute numbers of red blood cells (+5.6% from control), increases in the percentage of haematocrit (+3.6% from control), and reduction in mean absolute number of platelets (-8.6% from control) in the group of 46 ETO exposed hospital workers when compared to the controls matched with age, sex, and smoking habits. In the WBC differential counts, the absolute mean numbers of monocytes (+17.5% from control) and eosinophils (+29.4% from control) were significantly ($p < 0.01$) elevated whereas the absolute mean number of lymphocytes was significantly lower in exposed workers (-13% from control). Measured ETO levels at these hospitals were rather low (0.01-0.06 ppm).

In the study by Van Sitter (1985), no statistically significant effects were seen in ETO manufacturing plant workers in any of the immunological and haematological parameters examined (white blood cell count, T- and B- cells, monocytes, neutrophils, serum concentrations of IgA, IgG, IgM). The inhalation exposure concentration was generally below the detection limit (using personnel air samplers), but transient concentrations up to 8 ppm were occasionally recorded. Also, no haematological changes (Hb, Ht, RBC, WBC, %Lymphocytes) were observed in a group of 84 male workers involved in the manufacture of ethylene oxide and exposed to estimated concentrations < 1 ppm (Currier, 1984). Neither did

ethylene oxide exposures averaging 0.07 ppm result in changes in blood parameters in a cross-sectional survey of 36 hospital workers by LaMontagne *et al.* (1993). One case report (Aydin *et al.*, 2010) described lowered platelet counts in a male working in an ethylene oxide sterilisation room for 6 years and a rise in platelet counts following removal from this work setting.

Overall, human data on the possible haematological effects of ETO is limited and contradictory and cannot provide supportive evidence on these effects in humans.

Comparison with the criteria

In the case of ethylene oxide, there are animal data showing clear effects on peripheral nervous system. In most of the cases, effects have been observed in animals in long term studies (1 to 2 years) at exposures of 200 ppm or higher. Mandella (1997b) reported decreased hindlimb grip strengths at 300 ppm already after 4 weeks of exposure. In the long term study (14 weeks), Mandella *et al.* (1997c) reported similar findings at 200 ppm, but not at 100 ppm. Lynch (1984a) reported myopathy in rats after exposure to 100 ppm for 2 years, and Snellings *et al.* (1984) a dose-related reduction in locomotor function and abnormal posture starting from 50 ppm in a subchronic (10-11 weeks) study. If only animal data on the neurotoxicity of ethylene oxide is taken into account, the weight of evidence supports STOT RE 2 classification for neurotoxicity. However, the similar effects on the PNS has been reported also in several human case reports. These reports describe not only neurotoxic symptoms in humans, but also measured effects on nerve conduction velocities indicative of sensorimotor neuropathy, and axonal degeneration observed in nerve biopsies of exposed workers. Controlled studies in ETO inhalation exposed workers are limited in size and/or methodology but supported the signs and symptoms reported in case reports. RAC is in the opinion that the human evidence together with data from experimental animals provides sufficient evidence to support the classification of ethylene oxide as STOT RE 1 for neurotoxicity.

Regarding haematotoxicity, in animals ethylene oxide caused effects on blood parameters at subchronic (13 weeks) exposures to 255-500 ppm. However, the effects even at 500 ppm were rather moderate and decreases were seen only up to 10-14% in haemoglobin, RBC or haematocrit levels. The data from humans was limited and variable. According to the CLP criteria, the substance can be classified for STOT RE if there are any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis. In the case of blood parameters, reduction in Hb ≥ 20% is considered as a significant adverse effect. When taking into account that in the case of ethylene oxide the reductions in haemoglobin levels stayed well below 20% even at doses exceeding the guidance range of 50-250 ppm for STOT RE 2, no classification on the basis of haematological effects is proposed.

RAC agrees to classify ethylene oxide as **STOT RE 1; H372** for effects on the **nervous system**.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated; already harmonized classification as Muta. 1B, H340.

4.10 Carcinogenicity

Not evaluated; already harmonized classification as Carc. 1B, H350.

4.11 Toxicity for reproduction

Table 54: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
rat (Fischer 344) male/female One-Generation study (similar to OECD 415) Route of exposure: inhalation - vapour (whole body chamber exposure) Conc: 10; 33; 100 ppm Exposure: Prior to mating: 12w; 6 h/d, 5 d/w) During cohabitation: 1-2w (6h/d, 7d/w) Gestation: 19days (6h/d, 7d/w) Parturition + 5d: no exposure Post parturition: 16d (6h/d, 7d/w)	NOAEC (F_0): 33ppm (male/female) (overall effects) NOAEC (F_1): 33ppm (male/female) (overall effects) 100ppm: fertility indices↓, gestation periode↑, number of born pups↓, implantation sites↓	key study (fertility) 2 (reliable with restrictions) experimental result Test material: ethylene oxide	Snellings, W.M. (1982c)
Hybrid mice, female Route of exposure: inhalation 0 or 1200 ppm 1.5 h/day for 4 consecutive days before mating 300 ppm 6 h/day for 10 exposures over a 14-day premating period	300ppm: number of implants ↓, percentage of resorptions↑ 1200ppm: percentage of resorptions↑ induced loss of conceptuses was 15.7% at 1200 ppm and 58.2% at 300 ppm LOAEC (fertility, f)= 300ppm	Study not available experimental result Test material ethylene oxide	Generoso W.M. (1987) (Cited in US EPA, 2010)

Method	Results	Remarks	Reference
Sprague-Dawley rats, f New Zealand white rabbits, f Rats n=32-45 Rabbits n=23-30 Route of exposure: inhalation - chamber exposure Conc: 150ppm 7h/d Exposure • Group 1 (rat, rabbit): no exposure • Group 2 (rat, rabbit): GD 7-16 or 7-19 • Group 3 (rat, rabbit): GD 1-16 or 1-19 • Group 4 (rat only): three weeks before + GD 1-16	Rat: Resorptions ↑ in group 3, fetal body weight↓ in all groups, crown-rump length↓ in all groups, reduced skeletal ossification in all groups Maternal toxicity: absolute and relative kidney and spleen weights ↑in all groups, body weight ↓ in group 4 LOAEC rat (dev.) = 150ppm ——————— Rabbit: no effects NOAEC (maternal toxicity): 150ppm (overall effects) NOAEC (teratogenicity): 150ppm (overall effects)	2 (reliable with restrictions) weight of evidence experimental result Test material ethylene oxide	Hardin B.D. (1983) (publishing data from Hackett P.L., 1982)
two-generation study route of exposure: inhalation (1) guinea pig (n=8) Conc: 357ppm (640mg/m^3) – 176days, 7h/d (2) rat (n=20), guinea pigs (n=8) Conc: 204ppm (370mg/m^3) similar to OECD 416	(1)guinea pigs: 357ppm: moderate growth depression, appreciable degeneration of the tubules of the testes (2)rats and guinea pigs: 204ppm: slight decrease in testes weights, not statistically significant Rats, 204ppm: testes appeared small, microscopically there was evidence of slight degeneration of a few	2 (reliable with restrictions) experimental result Test material ethylene oxide	Hollingsworth R.L. (1956)

Method	Results	Remarks	Reference
	tubules LOEAC (fertility,m)= 204ppm		
Wistar rat, males Route of exposure: inhalation, chamber exposure 50, 100, 250ppm 6 rats per group 6h/d, 5d/w for 13 weeks	50ppm: abnormal sperm heads, teratic type 100ppm: abnormal sperm heads, teratic type 250ppm: total abnormal sperm heads↑, testicular degeneration, epididymal weight ↓ LOAEC (fertility, m) = 50ppm	2 (reliable with restrictions) Test material ethylene oxide Food intake of control and low dose groups was restricted according to the intake of the high dose group	Mori K.(1991)
Wistar rat, males Route of exposure: inhalation, chamber exposure Conc: 0, 500ppm 6 h/d, 3d/w for 2, 4, 6, or 13 w n=6-8 per group	mild degeneration of germ cells at 2 weeks, conspicuous degeneration at 4 weeks exfoliation of germ cells at 6 weeks marked reduction in germ cells in about 50% of seminiferous tubules, which contained only Sertoli cells, at 13 weeks GST activity↑ (4w, 6w, 13w) LOAEC (fertility, m) = 500ppm	2 (reliable with restrictions) Test material ethylene oxide Pair-feed to minimize differences due to food-intake	Mori K.(1989)
B6C3F1 mice Route of exposure: inhalation, chamber exposure	No effects on survival or body weight 250ppm: absolute testicular weights	2 (reliable with restrictions) Test material ethylene oxide	Snellings W.M.(1984)

Method	Results	Remarks	Reference
Conc: 0, 10, 50, 100, 250ppm n=30m+30f per group 10weeks (males) 11 weeks (females) 6h/d, 5d/wk	depressed, normal histology NOAEC (fertility, m) = 100ppm NOAEC (fertility, f) = 250ppm		
Mice, Swiss Webster, male Route of exposure: inhalation, chamber exposure Conc: 0, 200, 400ppm 6h/d, 5d/week for 1, 3, 5 weeks mouse-sperm-morphology-test	Statistically significant increase ($p<0.01$) in the percentage of abnormal sperms heads LOAEC (fertility, m) = 200ppm	2 (reliable with restrictions) Test material ethylene oxide ip-injection of cylcophosphamide was used as positive control	Ribeiro L.R. (1987)
Cynomolgus monkey Route of exposure: inhalation, Conc: 0,50, 100ppm 7h/d, 5d/w 24 months	significant reduction in sperm counts and motility 100ppm: decreased bw	Study not available (abstract only)	Lynch D.W., 1984a (as cited in NEDO, 2004)
rat (Fischer 344) route of exposure: inhalation, vapour (whole body) Conc: 10; 33; 100 ppm Exposure: gestation day 6 - 15 6 h/day equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	No treatment related effects on litter size and resorption sites 100ppm:significant depression of body weight in fetuses NOAEC (maternal toxicity): 100ppm (overall effects) NOAEC (dev): 33ppm	2 (reliable with restrictions) weight of evidence experimental result Test material: ethylene oxide	Snellings, W.M. (1982b)

Method	Results	Remarks	Reference
	(overall effects)		
Rat, Sprague-Dawley, f Route of exposure: inhalation, chamber exposure (1) Conc: 0, 400, 800, 1200ppm 0.5 h/d, GD 6-15 (2) Conc: 0, 200, 400, 800,1200 ppm 0.5 h, 3 times per day, GD 6-15	(1) 1200ppm dilated renal pelvis and ureter (2) 800ppm: fetal body weights ↓ 1200ppm: Reduced maternal weight gain, fetal body weights ↓ NOAEC (dev) short time = 400ppm	2 (reliable with restrictions) Test material ethylene oxide	Saillenfait A.M. (1996)
Mice, C57BL/6J, f Route of exposure: inhalation, chamber exposure Conc: 2100ppm-h (1400ppm x 1,5h or 700ppm x 3h or 350ppm x 6h) 2700ppm-h (1800ppm x 1.5h or 1543ppm x 1,75h or 1350ppm x 2h, 900ppm x 3h or 450ppm x 6h for 1.5, 3 or 6h Single exposure on GD 7	Maternal effects: body weight ↓in all groups, death in high exposure groups Fetal effects: fetal death↑, fetal weights ↓, eye malformations↑, crown-rump length↓	2 (reliable with restrictions) Test material ethylene oxide	Weller E. (1999)
CD rat, f Route of exposure: inhalation. chamber exposure Conc: 0, 50, 125, 225 ppm, n=25 per group	Maternal toxicity: 225ppm: body weight gain↓, food consumption ↓, relative liver weight↑ 125ppm: body weight gain↓, relative liver weight↑ Fetal toxicity:	Abstract only Test material ethylene oxide	Neeper-Bradley T.L. (1993)

Method	Results	Remarks	Reference
6 h/d, GD 6-15	Fetal body weight ↓ in all groups, skeletal variations at 225ppm (n=12)and 125ppm (n=3) LOAEC (dev) = 50ppm		
Mice Route of exposure: inhalation (1) Conc: 0, 1200ppm 1.5h single exposure 1,6, 9, 25h after mating (2) Conc: 0,1800ppm 1.5h single exposure 6h after mating	Exposure 1h after mating: number of live fetuses↓, abnormal foetuses ↑ Exposure 6h after mating: number of live fetuses↓, abnormal foetuses ↑	2 (reliable with restrictions) Abstract only	Rutledge J.C. (1989) (cited in US EPA, 2010)
CD-1 mice, f Route of exposure: Intravenously admin. Conc: 0, 75, 150mg/kg bw At 4 periods during gestation (I) day 4-6 (II) day 6-8 (III) day 8-10 (IV) day 10-12 n= approx. 10 per dose 4 replicates in a period of 6 months	150mg/kg: maternal toxicity Fetal body weight ↓ Malformed fetuses in group II	2 (reliable with restrictions) GLP Test material ethylene oxide (high volatility was considered during preparation and application)	LaBorde J.B. (1980)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Effects on fertility have been investigated by the inhalatory route of exposure only.

In the study by Snellings (1982c) Fischer344 rats were randomly assigned to one of five groups. Each group consisted of 30 males and 30 females. Three groups were exposed to ethylene oxide vapour at approximately 100, 33, or 10 ppm, and two control groups similarly maintained were exposed only to room air. The premating exposure period for males and females was 12 weeks. 5 days after parturition the dams were separated from their pups and exposed again to ethylene oxide vapour through day 21 post partum. There were no treatment-related effects on body weight gain throughout the 12 weeks of exposure for males or females of any exposure group. No statistically significant adverse effects were observed in the 100ppm exposure group when the body weights of the pups per litter were determined at day 4, 14, or 21 post partum. The results of the analyses of the fertility indices (percentages of females pregnant and percentages of males proven fertile) show that those for the 100-ppm exposure group were lower (but not statistically significant) than one or both air-control groups (see Table 55). Sperm parameters were not investigated. There were statistically significantly more females in the 100ppm exposure group whose gestation period was greater than 22 days (7/14) than in either air-control group (4 females 23d, 2 females 25/26, 1 female undeterminable). According to the author the biological significance of this effect is unknown as the gestation length for the laboratory rat is reported to be 21 to 23 days. The major treatment-related adverse effect observed after mating was that the median number of pups born on day 0 post partum per litter for the 100ppm exposure group was significantly ($p < 0.001$) lower than the medians for both air-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 100ppm exposure group. At parturition, no pups were found dead in the 100ppm exposure group or in either air-control group, and there was no evidence of cannibalization. The median number of implantation sites per pregnant female in the 100ppm exposure group was 6, which is significantly lower than the median of 10 or 11 for the air-control groups. The median for the 33- and 10-ppm exposure group was 11. The ratio of the number of fetuses born to the number of implantation sites per female was determined for each litter. The median value of this ratio for the 100ppm exposure group was statistically significantly lower (57) than the value of either air-control group (92 or 100) (see Table 55). There were no statistically significant effects on the survival rate of the F1a generation.

Snellings (1982b) showed that there are no effects on litter size and resorptions when rats are exposed to ethylene oxide (10, 33, 100ppm) on days 6 through 15 (6h/d) of the gestation period. For further study details see Chapter 4.11.2 (Develpomental toxicity).

In a study to assess the effect of inhaled ethylene oxide on preovulatory oocytes Generoso (1987) exposed female mice to ethylene oxide at 0 or 1200 ppm (2160 mg/m^3) for 1.5 h/day for 4 consecutive days before mating or to 300 ppm (540 mg/m^3) for 6 h/day for 10 exposures over a 14-day premating period. Chamber and analytic procedures were not described. The dams were killed on GD 17 to assess the effect on resorptions, midgestational deaths, and late fetal deaths. The number of implants per female was significantly reduced at 300 ppm but not at 1200 ppm. However, the percentage of resorptions in both groups of females exposed before mating was significantly elevated by 10.8% (3.0% in controls) and 41.1% (6.4% in controls) at 1200 and 300 ppm, respectively. Midgestational deaths and late fetal deaths were slightly elevated but not statistically significantly; the induced loss of conceptuses was 15.7% at 1200 ppm and 58.2% at 300

ppm, showing that exposure to the lower concentration for a longer time was more effective than the high concentration for a short time (cited in US EPA, 2010).

Table 55: Reproductive parameters for rats exposed to ethylene oxide via inhalation (Snellings, 1982c)

Parameter	Exposure (ppm)				
	100	33	10	0 (control 1)[1]	0 (control 2)[1]
Number of females pregnant[a]	17/27 (63%)	25/28 (89%)	25/30 (83%)	24/29 (83%)	19/28 (68%)
Number of males proven fertile[b]	15/22 (68%)	20/23 (87%)	19/23 (83%)	17/21 (81%)	12/20 (60%)
Litters totally resorbed	2	0	0	0	0
Numbers of pups at day 0 postpartum	64	212	237	222	174
Numbers of pups born dead	0	1	3	0	0
Median number of stained implantation sites per pregnant rat	6.0*	11.0	11.0	11.0	10.0
Median number of foetuses born per number of implantation sites (x100)	57*(1)	90	92	92	100

[a] ratio of number of pregnant rats to number mated less number of non-pregnant rats mated for only one mating.
[b] ratio of number of males proven fertile to number mated less number mated for only one of the two matings
* $p<0.001$ for comparison to control 1 and $0.001>p>0.001$ for comparison to control 2
* (1) $p<0.001$ in comparison to either control group
[1] two control groups were used so that normal variability between similarly treated concurrent control groups could be evaluated

Exposure of female rats and rabbits to 150ppm ethylene oxide (Hardin, 1983; publication of study report Hackett, 1982) before mating and/or during gestation caused no mortality but maternal rats showed increased kidney and spleen weights and decreased body weights at necropsy. Rats starting exposure three weeks before mating up to gestation day 16 showed a statistical significant increase in the incidence of resorptions; for detailed information (Hackett, 1982) see Table 61. Rats exposed only during gestation showed no significant effects on the incidence of resorptions. Rabbits appeared to be unaffected only exposed during gestation.

Effects of ethylene oxide exposure on male fertility was examined in rats, mice, guniea pigs and monkeys.

In an early study Hollingsworth (1956) examines the toxicity of ethylene oxide on various animals via different routes and durations of exposure. Effects on fertility were seen in male guinea pigs after administration of 357ppm ethylene oxide via inhalation for a period of 176days (7h/day). Histopathological findings showed degeneration of the tubules of the testes with replacement fibrosis in the males. This effect was accompanied by a moderate growth depression in males. Except a slight increase in lung weight of male animals no significant organ weight changes were found. Exposure of rats to 204ppm for 176-226 days revealed small testes in rats with microscopical evidence of slight degeneration of a few tubules combined with a depressed growth.

In a study by Mori (1991) male Wistar rats were exposed to 50, 100 or 250ppm ethylene oxide (6h/d, 5d/w) for 13 weeks. The same volume of food was given to all groups to minimise the effects on sperm heads due to reduced food intake. At 250 ppm a significant decrease in epididymal weights (Table 56), slight degenerations in the seminiferous tubules, significant decreased sperm counts in tail and body of epididymis, and significant increased numbers of abnormal sperm heads in the tail of the epididymis ($p<0.01$) were found; these were not seen at lower doses. When abnormal sperm heads were classified into immature and teratic types, the rate of teratic types increased in all treated groups ($p<0.05$) but not in relation to the concentration of EO. The rate of immature types of sperm heads increased only in the 250 ppm group ($p<0.01$).

Table 56: Effects of ethylene oxide on body, testicular and epididymal weights (Mori, 1991).

Ethylene oxide concentration (ppm)	Body weight (g, mean, SD)	testicular weight (g, mean, SD)	epididymal weight (g, mean, SD)
0 (n=12)	462.7 (33.3)	3.73 (0.27)	1.32 (0.11)
50 (n=6)	489.7 (20.5)	3.33 (0.26)	1.35 (0.09)
100 (n=6)	467.2 (31.9)	3.40 (0.23)	1.26 (0.08)
250 (n=6)	443.3 (37.3)	3.60 (0.39)	1.06 (0.11)*

*Significantly different from control, $p<0.01$

In an earlier study by Mori (1989) male Wistar rats were exposed to ethylene oxide at a concentration of 500 ppm, 6 h/d, 3d/w for 2, 4, 6, or 13 weeks. Six to eight animals were used for each group. Testicular toxicity and changes in glutathione metabolism in the testis were investigated. The relative weights of the testes and the epididymes of the exposed group decreased in a time dependent manner while body weight gain of the exposed group was not different from control (see Table 57). Light microscopic examination revealed degeneration and exfoliation of germ cells. At 2 weeks, disorder of the arrangement and mild degeneration were observed. At 4 weeks, the degeneration of mature spermatids became conspicuous and the nuclear vacuolization 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. However, the other tubules contained more type B spermatogonia and spermatocytes than those at 6 weeks and some of them had almost normal maturation phase spermatids. Mild proliferation of the Leydig cells occasionally was observed only at 13 weeks. Although the severity of damage became apparent over the course of exposure, some seminiferous tubules showed germ cell recovery at 13 weeks compared with 6 weeks. Plasma testosterone concentration was not affected. In spite of some alterations in the glutathione redox cycle (inhibition of the activity of Glutathione reductase at all endpoints,

alterations of glutathione peroxidase) GSH concentration in the testes was not affected. Glutathione-S-transferase (GST) activity, the major enzyme detoxifying ethylene oxide in the testis, increased during the course of exposure. GST activity was measured with two compounds, CDNB and 1,2-epoxy- 3-(p-nitrophenoxy)propane, as substrates. Activity with CDNB increased by 63.0% at 6 weeks and by 72.8% at 13 weeks, and activity with 1,2-epoxy-3-(p-nitrophenoxy)propane increased by 13.1% at 4 weeks and further increased by 54.6% at 6 weeks and by 81.9% at 13 weeks.

Table 57: Effects on relative testicular eights and epididymal weights (mean±SD) (Mori, 1989).

Exposure period (wk)	Rel. testicular weight (%)		Rel. epididymal weight (%)	
	control	500ppm EO	control	500ppm EO
2	1.248±0.101 (6)	1.302±0.183 (6)	0.308±0.056 (6)	0.314±0.062 (6)
4	1.129±0.087 (6)	0.924±0.060 (6)*	0.344±0.004 (6)	0.297±0.016 (6)**
6	1.117±0.049 (8)	0.602±0.059 (8)**	0.347±0.018 (8)	0.248±0.035 (8)**
13	1.006±0.066 (8)	0.466±0.113(8)**	0.344±0.042 (8)	0.204±0.029 (8)**

* p< 0.01; ** p<0.001

Snellings (1984) exposed mice to concentrations up to 250ppm. Effects were only seen at 250ppm. Significant effects on RBC and Hb at 250ppm are presented in Table 36. Testicular weights (absolute) were statistically significant depressed without clinical or histopathological findings to suggest a pathologic effect (Table 58).

Table 58: Absolute weights (g) (mean value) of B6C3F1 mice after 10 weeks of exposure to ethylene oxide (Snellings, 1984).

Concentration (ppm)	Brain (g)	Testes (g)	Body (g)
250	0.451 (0.020)[#]	0.106* (0.008)	27.5* (2.0)
100	0.457 (0.017)	0.108* (0.009)	29.2 (2.0)
50	0.444 (0.014)	0.108* (0.004)	28.8* (2.8)
10	0.480 (0.063)	0.113 (0.006)	28.8* (2.0)
0	0.454 (0.028)	0.116 (0.006)	31.0 (2.0)

standard deviation
* p<0.05 in comparison to control

Ribeiro (1987) evaluated the effect of inhaling ethylene oxide vapor at 0, 200, or 400 ppm on sperm morphology in mice. Male Swiss Webster mice were exposed 6 h/day for 5 days, and killed 1, 3, and 5 weeks after exposure. Only the head morphology was examined. Ethylene oxide induced concentration-related and statistically significant (p<0.01) increases in the incidences of abnormal spermatozoa, spermatids, and spermatogonial cells in preleptotene compared with the incidences in controls (see Table 59).

Table 59: Frequency of sperm head abnormalities after treatment with ethylene oxide (6h/d) and cyclophosphamide (CPA) at different stages of spermatogenesis (Ribeiro, 1987).

Groups	Treatment		Sacrifice, wk after treatment	Population of treated cells	No of mice	No of cells scored	Sperm abnorm. % (mean+SD)
1	EO	0 ppm	1	Spermatozoa	10	10000	1.76±0.5
	EO	200 ppm			10	10000	3.02±0.5**
	EO	400ppm			10	10000	3.95±0.6**
	CPA	100mg			5	5000	3.12±0.7**
2	EO	0 ppm	2	Spermatid	10	10000	1.62±0.4
	EO	200 ppm			10	10000	3.62±0.6**
	EO	400ppm			10	10000	5.81±1.5**
	CPA	100mg			5	5000	2.60±0.8**
3	EO	0 ppm	3	Spermatogonial cells in preleptotene	10	10000	1.32±0.4
	EO	200 ppm			10	10000	2.32±0.5**
	EO	400ppm			10	10000	5.54±1.4**
	CPA	100mg			4	4000	10.40±1.6**

EO…ethylene oxide
CPA…Cyclophosphamide, 100mg/kg bw i.p., 5 consecutive days
**Significant at 1% level

In an experiment on Cynomolgus monkeys in which 0 ppm, 50 ppm, 100 ppm ethylene oxide were administered by inhalation exposure for 7 hours/day, 5 days/week, for 24 months, a decrease in the number and mobility of spermatozoa was observed. Exposure to 100ppm resulted in significantly decreased body weight (Lynch, 1984b, cited in NEDO, 2004).

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Fischer 344 rats exposed to 0, 10, 33 and 100ppm ethylene oxide vapour (6h/d) on day 6 through 15 of the gestation period showed no treatment related effects for maternal survival, litter size, number of implantation and resorption sited and preimplatation losses. Exposure to 100ppm resulted in a significant depression of body weight in the foetuses and in statistically non-significant variations in ossification of the distal thoracic vertebral centra. No significant difference in crown-rump length. Visceral alteration (renal pelvic dilatation) was observed in 100ppm group and air control group. No treatment related adverse effects were observed for adult females. Maternal body weight gain during gestation was not monitored. An overview on foetal alterations is given in Table 60 (Snellings, 1982b).

Table 60: Summary of observations after exposure to ethylene oxide on GD 6-15 (Snellings, 1982b).

Observations	Exposure group (ppm)				
	100	33	10	Control I (air) 0	Control II [3] (air) 0
Weight male foetuses (g) [Mean of litter means ± SD]	3.1* ± 0.2	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.4	3.3 ± 0.2
Weight female foetuses (g) [Mean of litter means ± SD]	2.9* ± 0.1	3.1 ± 0.3	3.0 ± 0.3	3.1 ± 0.3	3.0 ± 0.2
Crown- rump length (male) (mm)	36 ± 1	36 ± 2	37 ± 1	37 ± 1	36 ± 1
Crown- rump length (female) (mm)	35 ± 1	35 ± 2	36 ± 1	35 ± 2	35 ± 1
Foetuses - one or more gross abnormalities (%)	0	0	0	0	0
Incidence of foetal alterations					
No. of foetuses/No. of litters examined:					
- external examination	154/19	-	-	175/21	149/17
- skeletal examination	75/19	-	-	87/21	74/17
- visceral examination	79/18[1]	-	-	88/21	75/17
% affected, foetuses (litters)					
- external alterations	0 (0)	-	-	0 (0)	0 (0)
- variation ossif. sternebrae	4 (11)	-	-	7 (29)	1 (6)
- variation ossif. vertebrae	11 (42)	-	-	5 (19)	7 (18)
- visceral alterations: renal pelvic dilation	29 (78)	-	-	28 (81)	20 (59)
% foetuses/litter affected, Q_2 (QD)[2]:					
variation ossification sternebrae	0 (0)	-	-	0 (10)	0 (0)
variation ossification vertebrae	0 (12)	-	-	0 (0)	0 (0)
renal pelvic dilation	20 (15)	-	-	33 (13)	17 (20)

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

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

[2] Q_2 50th percentile, QD quartile deviation [=(75th-25th percentile) /2]

[3] two control groups were used so that normal variability between similarly treated concurrent control groups could be evaluated

The study by Snellings, 1982c is reported in detail in Chapter 4.11.1 (effects on fertility) but also some aspects of developmental toxicity have been studied. No statistically significant adverse effects were observed in the 100ppm exposure group when the body weights of the pups per litter were determined at day 4, 14, or 21 post partum. The median number of implantation sites per pregnant female in the 100ppm exposure group was 6, which is significantly lower than the median of 10 or 11 for the air-control groups. The median for the 33- and 10-ppm exposure group was 11. The ratio of the number of fetuses born to the number of implantation sites per female was determined for each litter. The median value of this ratio for the 100ppm exposure group was statistically significantly lower (57) than the value of either air-control group (92 or 100) (see Table 55). There were no statistically significant effects on the survival rate of the F1a generation.

The study report by Hackett (1982) has been published by Hardin (1983). Rats and rabbit have been exposed to 150ppm ethylene oxide, 7h/d for different periods before and during gestation. Results on fertility are presented in the previous chapter. Rat fetal body weight and crown-rump length were reduced in all ethylene oxide exposed groups. External, visceral and skeletal examinations revealed no treatment-related effects other than an increased incidence of reduced skeletal ossification (primary of skull and sternebrae) in all exposure rat groups (without maternal toxicity in group 2 and 3) (see Table 61). No effects were seen in exposed rabbits exposed on GD 1-19 or 7-19. In contrast to rats no pregestation exposure was done in rabbits.

Table 61: Maternal, reproductive and developmental effects in rats after exposure to 150ppm ethylene oxide (Hackett, 1982 cited in US EPA, 2010).

Parameter	Exposure groups			
	Group 1 Unexposed	Group 2 Exposed GD7-16	Group 3 Exposed GD1-16	Group 4 3 weeks premating +GD 1-16
Maternal body weight (g), mean values, (% reduction)				
Pregestation day 21	278	277 (-0.34%)	280 (+0,72%)	267* (-3.96%)
GD 6	298	298 (0%)	293 (-1,68%)	279* (-6.38%)
GD 11	315	314 (-0.32%)	308 (-2.22%)	295* (-6.35%)
GD 16	339	335 (-1.18%)	328 (-3.24%)	317* (-6.49%)
GD 21	382	381 (-0.26%)	378 (-1.05%)	360* (-5.76%)
Reproductive parameters				
No. live litters/no. pregnant	41/41	41/41	41/41	38/39
No. implantation sites/dam	14.7	14.0	14.8	14.3

No. resorptions/litter	0.75	0.71	0.92	1.60*
No. fetuses/litter	13.9	13.5	13.8	12.7
Fetal parameters				
Weight of f (g)	3.56	3.35*	3.23*	3.12*
Weight of m (g)	3.73	3.53*	3.47*	3.34*
Crown-rump length (mm) f	36.1	35.3*	34.7*	34.8*
Crown-rump length (mm) m	36.5	36.1*	35.8*	35.6*
Morphologic alterations (Number of foetuses per number of litters; number in parentheses are percentage of affected litters relative to controls)				
Reduced ossif., skull	3/2 (4.9)	16/9 (22.0)*	10/9 (22.0)*	14/10 (26.3)*
Reduced ossif., sternebrae	69/23 (56.1)	145/36 (87.8)*	159/36 (87.8)*	155/33 (85.8)*

* $p \leq 0.05$, compared with control

Neeper-Bradley (1993, abstract only) exposed groups of 25 pregnant CD rats to 0, 50, 125, 225ppm ethylene oxide for 6h/d on GD 6-15. No maternal mortality was associated with ethylene oxide exposure. There were no exposure-related clinical signs of toxicity. In the 225 ppm group, average gestational body weight was reduced for Days 9, 12, 15, 19, and 21 and were consistent with substantially reduced body weight gains throughout the exposure period. Reductions in food consumption were also observed. In the 125 ppm group, body weight gains were reduced but there were no reductions in food consumption. Relative maternal liver weights were increased in the 225 and 125 ppm groups. There were no effects of exposure at any of the three vapor concentration levels on the number of ovarian corpora lutea and the number, of total, viable, or nonviable (early and late resorption and dead fetuses) implantations/litter, on the percentages of preimplantation loss or live fetuses, or on sex ratio. Fetal body weights were reduced in a concentration-dependent fashion for all ethylene oxide-exposed groups with reductions of approximately 4, 5 and l0% of control values in the 50, 125, and 225 ppm groups, respectively. Increased incidences of 12 skeletal variations (primarily unossified or poorly ossified areas) involving the head region, extremities, and sternebrae were noted in the 225 ppm group. In the 125 ppm group three variations were observed.

The developmental toxicity of short duration exposure to ethylene oxide was examined in Sprague-Dawley rats following inhalation exposure during Days 6 to 15 of gestation (Saillenfait, 1996). Two different exposure regimens were used: (1) exposure for 0.5 hr once a day to 0, 400, 800, or 1200 ppm ethylene oxide; or (2) exposure for 0.5 hr three times a day to 0, 200, or 400 ppm, or 800, or 1200 ppm ethylene oxide. The single short duration exposure showed no effects on maternal weight gain, no adverse effects on resorptions and no external or skeletal malformations. Occurrence of soft tissue malformations (dilated renal pelvis and ureter) at 1x1200ppm was observed but the toxicological significance is doubtful due to the wide variations in the renal development. Repeated short-duration exposure did affect maternal weight gain at 1200ppm and fetal body weights were

significantly reduced ($p<0.01$) at 3x200ppm (not considered toxicological significant due to unusual high weight in the concurrent control group), 3x800ppm and 3x1200ppm. No other signs of fetotoxicity were seen.

Weller (1999) investigated developmental effects of a single exposure to ethylene oxide. Pregnant mice were exposed on GD 7 for 1.5, 3 or 6h via inhalation at 2100 or 2700ppm-h. The study was designed to specifically look at dose-rate (C x t) effects (Haber´s rule). An overview on used concentrations and resulting effects is given in Table 62. Maternal weight loss was observed in all exposed mice as well as maternal death in high exposure groups. Developmental toxicity was exhibited by increased resorptions, significantly decreased fetal body weight, decreased crown-to-rump length, and significantly increased incidences of eye defects (microphthalmia, anophthalmia) after exposure to ethylene oxide. No treatment-related skeletal defects were observed in fetuses from dams exposed to ethylene oxide. This study showed developmental effects at the lowest concentration tested.

The teratogenic potential of intravenously administered ethylene oxide was assessed in the CD-1 mouse at doses of 0, 75, and 150 mg/kg (in sterile 5% dextrose) applied at four periods during gestation: Days 4–6 (Period I), 6–8 (Period II), 8–10 (Period III), and 10–12 (Period IV). Maternal mice were weighted in day 0 of gestation, on each day of treatment and on day17. Maternal animals showed signs of toxicity (weakness, tremors, labored respiration, death) at the 150 mg/kg dose level in Periods I, III, and IV but not in Period II. Significant decrease in mean maternal body weight gain was seen during treatment period I, II and IV. A significant reduction in mean fetal body weight compared to controls occurred in all four treatment periods at the 150 mg/kg level. No changes in the number of implants per litter but a significant reduction in the mean number of live foetuses in III and IV was noted at 150mg/kg bw. A significant increase in the percentage of malformed fetuses/litter was observed in Periods II and IV at this dose level. There was also an increase in group III, but not statistically significant. Approximately 19% of the fetuses in each litter from maternal animals treated with 150 mg/kg ethylene oxide in Period II had some type of malformation (fusion of the cervical and thoracic arches, fusion and branching of ribs). Details on malformations are shown in Table 63 (LaBorde, 1980).

Female mice exposure to 1200ppm ethylene oxide for 1.5h (single exposure) 1, 6, 9, or 25 h after mating also resulted in developmental effects (Rutledge, 1989; abstract only). The exposure times correspond to different developmental stages of the zygote: 1 h, sperm entry; 6 h, early pronuclear stage before DNA synthesis; 9 h, pronuclear DNA synthesis stage; and 25 h, early two-cell stage.
A marked reduction was observed in the number of live fetuses from female mice exposed to ethylene oxide vapor 1 h after mating (6 fetuses per dam versus 9.72 for controls) and 6 h after mating (1.81 fetuses per dam versus 10.11 for controls). In addition, the incidence of abnormal fetuses markedly increased when females were exposed 1 h (14.7% versus 0.2% for controls) and 6 h (39.2% versus 1.7% for controls) after mating. The predominant types of abnormalities were hydrops (different degrees of edema ranging from thick neck to a “balloon-like fetus”) and eye defects. Defects in the limbs and tail occurred in females exposed 6 h after mating. Other abnormalities included abdominal wall defect, cleft palate, exencephaly, and small size.
Two additional groups of female mice were exposed similarly to 0 or 1,800 ppm (3,240 mg/m3) 6 h after mating and were killed serially on GD 11 to 15 to determine the effect on midgestational development. Analysis of the uterine content of females exposed to 1,800 ppm and killed on GD 11 to 15 showed significant increases in fetal deaths, particularly on GD 15 (late deaths). The number of defective living fetuses per dam significantly increased, whereas the number of living fetuses per dam decreased. Most dead fetuses were hydropic (cited in US EPA, 2010).

Table 62: Developmental toxicity in mice after single exposure on GD7 (Weller (1999) cited in US EPA, 2010).

		Maternal effects					Developmental effects						
Conc ppm x h	Exposed (Sperm positive)	Number Deaths (%)	Weigth lost (5)	% with clinical signs 30min	% with clinical signs 24h	No. pregnant (%)	No. implants	No. resorptions (%)	No dead foetuses (%)	Fetal weight (g)	Crown-rump length (mm)	No. offspring (litters)	Eye defects (offspring /litters) [1]
0x1.5	50	0	1.2	2.3	0	28	203	28 (13.8)	0	0.92	19.22	175 (28)	13 (6)
0x1.75	8	0	0.7	12.5	12.5	6	50	3 (6.0)	0	0.97	20.03	47 (6)	5 (3)
0x2	28	1 (3.6)	0.3	0	0	14	95	11 (11.6)	1 (1.1)	0.99	20.70	83 (14)	4 (3)
0x3	38	0	3.4	2.6	0	19	141	15 (10.6)	1 (0.7)	0.93	19.71	125 (19)	5 (4)
0x6	30	1 (3.3)	3.8	6.7	0	19	150	14 (9.3)	0	0.99	19.52	136 (19)	12 (6)
Total	154	2 (1.3)	1.9	4.8	2.5	86(55.8%)	639	71 (11.1)	2 (2.1)	0.96	19.84	566 (86)	39 (22)
C x t = 2100ppm-h													
1400 x 1.5	39	3 (7.7)	7.2	100.0	20.7	8 (22.2)	62	24 (38.7)	17 (27.4)	0.72 (75)	16.89 (85)	21 (8)	7 (3)
700 x 3	41	0	6.6	81.6	5.3	22 (53.7)	168	27 (16.0)	3 (1.8)	0.88 (92)	19.24 (97)	139 (22)	53 (15)
350 x 6	33	0	4.7	53.1	3.1	19 (57.6)	152	13 (8.6)	1 (0.7)	0.97 (101)	19.90 (100)	138 (19)	20 (8)
C x t = 2700ppm-h													
1800 x 1.5	73	41 (56.2)	13.0	100.0	66.2	3 (9.4)	22	14 (63.6)	0	0.70 (73)	16.66 (84)	8 (3)	7 (1)
1543 x 1.75	23	15 (65.2)	13.5	95.7	72.2	1 (12.5)	7	1 (14.3)	0	0.76 (79)	17.83 (90)	6 (1)	6 (1)
1350 x 2	76	27 (35.5)	11.4	100.0	39.7	7 (14.3)	20	9 (45.0)	1 (5.0)	0.86 (90)	18.74 (94)	10 (7)	3 (2)
900 x 3	50	1 (2.0)	8.8	98.0	24.0	11 (22.5)	86	22 (25.6)	5 (5.8)	0.82 (85)	18.42 (93)	59 (11)	34 (9)
450 x 6	41	0 (0)	6.2	95.1	2.4	20 (40.1)	148	28 (18.9)	0	0.97 (101)	19.32 (97)	120 (20)	13 (10)

[1] includes anophthalmia and microthalmia

Table 63: Malformations in CD-1 mice treated with ethylene oxide i.v. (LaBorde, 1980).

Treatment group (days of treatment)	Dose mg/kg bw	No. live fetuses	Total no. foetuses malformed	Observed malformations
I (4-6)	0	246	1	Exencephaly (1)
	75	200	0	-
	150	156	3	Exencephaly (1); thoracic arches, missing (1); thoracic ribs, branched (1); thoracic ribs, misshapen (1), malformed forelimb (1)
II (6-8)	0	148	1	Sternebrae, scrambled and fused (1)
	75	208	0	-
	150	148	60	Exencephaly (2); Cleft palate (3); coloboma, retina (2); Sternebrae, scrambled and fused (4); Cervical arches, fused (17); thoracic arches, missing (2); thoracic arches, fused (7); thoracic ribs, fused (9); thoracic ribs, branched (6); thoracic ribs, decreased number (4), thoracic ribs, misshapen (3); lumbar centra, fused (1);
III (8-10)	0	242	1	Small kidney (1)
	75	178	0	-
	150	74	7	Exencephaly (2), Cleft face (1); thoracic arches, fused (2); thoracic ribs, fused (1); thoracic ribs, branched (1);
IV (10-12)	0	158	0	-
	75	223	4	Cleft palate (1); Small kidney (1); decreased number (1); thoracic ribs, misshapen (1);
	150	22	2	Cleft palate (1); Small kidney (1)

4.11.2.2 Human information

Table 64: Human evidence

Method	Results	Remarks	Reference
Questionnaire/hospital record Female Finnish hospital sterilizing staff	Spontaneous abortion 16,7% versus 5.6% for non-exposed	Typical TWA in a finish sterilization unit ranging from 0.1 – 0.5ppm	Hemminki (1982)
Cross-sectional study Questionnaire Female dental assistants	Age adjusted relative risk: spontaneous abortion: 2.5 (95% CI = 1.0–6.3); pre-term births: 2.7 (95% CI = 0.8–8.8 post-term births: 2.1 (95% CI = 0.7–5.9),	Exposure is based on self-reporting of the used method	Rowland (1996)
Cross-sectional study Questionnaire Hospital sterilizing units	prevalence odds ratio (POR): spontaneous abortion: 20.8 (95% CI = 2.1-199) pregnancy loss: 8.6 (95% CI = 1.8-43.7)	Exposure is based on walk-through surveys, questionnaire collected data and measurements at the time of the study	Gresie-Brusin (2007)
Hospital records/central statistical data Paternal exposure to ethylene oxide (n=10)	increased risk of spontaneous abortion (odds ratio = 4.7; 95% CI = 1.2–18.4)	Exposure status based on occupational titles and industry	Lindbohm (1991)

The findings by Hemminki (1982) indicate that exposure to ethylene oxide in early pregnancy in hospitals correlates with an increased frequency of spontaneous abortions. All sterilizing staff employed in Finnish hospitals in 1980 was included in the analysis (questionnaire, hospital discharge records), with a total of 1443 pregnancies (545 workers exposed during pregnancy). Ethylene oxide concentrations have been measured (but not in the course of this study) in many sterilizing units in Finnish hospitals showing an 8h TWA ranging from 0.1 – 0.5ppm (with peak exposure up to 250ppm). Outcome of the questionnaires showed that exposure to ethylene oxide during early pregnancy was related to an increased frequency of spontaneous abortion (adjusted rate of 16.1% in exposed versus 7.8% in unexposed workers; $p < 0.01$). When data on the pregnancies of the sterilizing staff and the controls obtained from the hospital discharge register were analysed the rate of spontaneous abortions was 22.6% ($p<0.05$) compared to control with 9.2% when exposed to ethylene oxide. These analyses essentially confirm the findings of the questionnaire.

A cross-sectional study by Rowland (1996) on adverse pregnancy outcomes was based on 1320 women whose most recent pregnancy was conceived while working full-time as dental assistant. 32 women reported exposure to ethylene oxide, unexposed comprised the control group. Neither detailed information on timing of exposure during pregnancy nor measurements of exposure are available; no information on the ethylene oxide sterilization system used is available. The occurrence of spontaneous abortion and pre- and post-term delivery is also based on self-reporting. The age-adjusted relative risk of spontaneous abortion among ethylene oxide-exposed women was 2.5 (95% confidence interval [CI] = 1.0–6.3); the relative risks of pre-term 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, ethylene oxide-exposed women 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. Adjustment for unscavenged nitrous oxide exposure and high amalgam use yielded a relative risk of 2.5 (95%CI=1.0-6.1); further adjustment for smoking yielded a relative risk of 2.1 (95% CI = 0.7–5.7).

Gresie-Brusin (2007) investigated the association between ethylene oxide exposure and adverse reproductive outcomes (spontaneous abortions, stillbirth or pregnancy loss) in a province in South Africa. Information on the evolution and outcome of the pregnancy was gathered from the mother using a questionnaire. Information on exposure to ethylene oxide during pregnancy was obtained from three sources, namely walk-through surveys, questionnaire-collected data and measurements of the levels of ethylene oxide in sterilising units at the time of the study (personal and static sampling). The study population consisted of 98 singleton pregnancies. There was a significantly increased risk of spontaneous abortion (prevalence odds ratio POR = 20.8, 95% CI = 2.1-199) and pregnancy loss (POR = 8.6, 95% CI = 1.8-43.7) for pregnancies highly exposed to ethylene oxide compared to low exposed pregnancies. No associations were found between exposure to ethylene oxide and stillbirth.

The effect of paternal exposure to ethylene oxide on spontaneous abortions was assessed by Lindbohm (1991) in the course of a survey on paternal occupational exposure to mutagenic agents. Information was gathered from Hospital Discharge Register, questionnaires and the central statistical office of Finland. Assignment of exposure status was made on the basis of occupational titles and industry. 99 186 pregnancies were included in the analysis but only 10 pregnancies were assigned to paternal ethylene oxide exposure (n=10) resulting in 3 spontaneous abortions (n=3). An increased risk of spontaneous abortion (odds ratio = 4.7; 95% CI = 1.2–18.4 was shown. Other potential confounding factors, such as previous abortions and alcohol and tobacco consumption, were not considered in the analysis.

Due to the size and the solubility of ethylene oxide it can be assumed that it passes the placental barrier. A study by Stedingk (2011) show that the in vivo dose of ethylene oxide in fetal and maternal blood is about the same and that the placenta gives negligible protection of the fetus to exposure.

4.11.3 Other relevant information

No further information available.

4.11.4 Summary and discussion of reproductive toxicity

Effects on sexual function and fertility (see also Table 65):

Fertility of female rats was impaired when exposed to 100ppm ethylene oxide during premating and gestation. A significant reduction of the median number of implantation sited and pubs born was observed (Snellings, 1982c). Exposure only during gestation had no effects on litter size or resorptions (Snellings, 1982b). The same picture is seen by Hardin (1983) were exposure to 150ppm during premating and gestation resulted in significant increase in the incidence of resorptions whereas exposure only during gestation showed no effects. Mice exposed premating for several days to 300 or 1200ppm (Generoso, 1987) showed a significant reduced number of implants at 300ppm and significant elevated percentage of resorptions at 300 and 1200ppm (10.8% and 41.1% respectively). No effects were seen in rabbits at 150ppm exposed during gestation (Hardin, 1983).

Effects on male fertility were seen in rats, mice, guinea pigs and monkeys. Degeneration of tubules of the testes was described in guinea pigs at 357ppm (Hollingsworth, 1956) and rats at 204ppm (Hollingsworth, 1956) or 250ppm (Mori, 1991). Abnormal sperm heads are documented in rats at 250ppm (Mori, 1991) and in mice at 200ppm (Ribeiro, 1987). At 250ppm reduced weights of epididymidis in rats (Mori, 1991 and 1989) and testis in mice (Snellings, 1984) have been observed. Degeneration of sperm cells with germ cell recovery at 13 weeks has been observed by Mori (1989) combined with an increased GST activity in the course of the experiment. In monkeys a decrease in number and mobility of spermatozoa was observed (Lynch, 1984b).

Ethylene oxide as a small molecule is assumed to pass the blood-testis barrier. But Brown (1986) described a reduced concentration of ethylene oxide in the testis (about 50% and 20% of other tissue ethylene oxide concentrations in mouse and rat 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. Consequently it can be assumed that in the above mentioned studies the final concentration of ethylene oxide in the testis was lower than in other tissues.

Elimination of Ethylene oxide in rodents occurs primarily by glutathione conjugation and by hydrolysis to ethylene glycol. In contrast, hydrolysis appears to be the major pathway for metabolism of EO in dogs and rabbits. Humans are known to excrete both ethylene glycol (hydrolysis) and N-acetyl-(2-hydroxyethyl)cysteine (glutathione conj.) (Fennell, 2001). The major amount of ethylene oxide is metabolized in humans 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 (100ppm and above) and non-linearity in metabolic elimination of ethylene oxide (see also Chapter 4.1).

Table 65: Summary of effects on fertility after inhalation of ethylene oxide

Reference	Species	Exposure time	Dose resulting in effects on fertility	Effect seen (significant effects marked with *)	Parental toxicity	NOAEC (fertility)
Female fertility						
Snellings, 1982c	rat	Premating + cohabitation + gestation,	100ppm	Lower fertility index (m, f) Longer gestation	no	33ppm

		6h/d		period * # pubs born↓* # implantation sites ↓ *		
Snellings, 1982b	rat	GD 6-15, 6h/d	-	- (no effects seen)	no (body weight gain was not monitored)	100ppm
Hardin, 1983	rat	Premating + gestation 7h/d _____ GD 1-16 7h/d	150ppm _____	# resorptions ↑ * _____	yes (increased spleen and kidney weights, decreased bw) _____ no	-
Generoso, 1987	mice	Premating, 6h/d	300ppm	# implants ↓ * % resorptions ↑*	- (not reported)	-
Hardin, 1983	rabbit	GD1-19, 7h/d	-	-	no	150ppm
Male fertility						
Hollingsworth, 1956	Guinea pig	~6 months, 7h/d	357ppm	Degeneration of tubules Replacement fibrosis	yes (moderate growth depression)	-
Hollingsworth, 1956	rat	~6 months, 7h/d	204ppm	Small testes, slight degeneration of tubules	Yes (reduced bw)	-
Mori, 1991	rat	13 weeks, 6h/d	250ppm _____ 50ppm	Epididymal weight ↓* Slight degeneration of seminif. tubules Decreased sperm count in tail+body of epididymis * Increased number of abnormal sperm heads * _____ Abnormal sperm	No	-

				heads – teratic type *		
Mori, 1989	rat	up to 13 weeks, 6h/d	500ppm	Testes weight (rel.) ↓* (time dependent) Epididymis weight (rel.) ↓* (time dependent) Degeneration and exfoliation of germ cells GST ↑* Recovery at 13 weeks	No	-
Snellings, 1984	mice	10 weeks, 6h/d	250ppm	Testes weight ↓*	Yes (effects on RBC and HB)	-
Ribeiro, 1987	mice	up to 5 weeks, 6h/d	200ppm	Abnormal sperm heads *	-	-
Lynch, 1984b	monkey	24 months, 7h/d	50ppm	Decreased number and mobility of sperms	No	-

Developmental toxicity in offsprings (Table 66):

Ethylene oxide exposure of rats during GD 6-15 or during premating and gestation resulted in significantly deceased fetal body weights at 100ppm (Snellings, 1982b) and 150ppm (Hardin, 1983) or in a concentration-dependent reduction of fetal weights of 4, 5 and 10% at 50, 125 or 225ppm (Neeper-Bradley, 1993). Non-significant variations in ossification were also seen in these studies.

Short duration exposure of rats during GD 6-15 to high ethylene oxide concentrations revealed reduced fetal body weights at 800 and 1200ppm but not at 400ppm (Saillenfait, 1996). Single exposure on GD7 at 2700ppm-h resulted in decreased fetal body weight, decreased crown-rump length and increased incidence of eye defects (Weller, 1999). Eye defects were also seen in mice exposed once after mating to high concentrations (Rutledge, 1989; Weller, 1999). Intravenous administration in mice at four periods during gestation showed significant reduction in fetal body weight at 150mg/kg bw and significant increase of malformations of the cervical/thoracic skeleton (LaBorde, 1980).

Rabbits showed no adverse effects when exposed to 150ppm (7h/d) during gestation (Hardin, 1983).

Table 66: Summary of studies on developmental toxicity of ethylene oxide

Reference	Species	Exposure time	Dose resulting in effects	Effect seen (significant effects marked with *)	Parental toxicity (at effective	NOAEC (dev.)

					concentration)	
Snellings, 1982b	rat	GD 6-15, 6h/d	100ppm	Fetal bw ↓*, non-signif. variations in ossif.	no (but body weight gain was not monitored)	33ppm
Hardin, 1983	rat	GD7-16 or GD 1-16 or perm+GD 1-16, 7h/d	150ppm	In all exposure groups: Fetal bw ↓*, Crown-rump-length↓* Red. skeletal ossif.	no/yes (Only when exposed prem+GD1-16)	-
Hardin, 1983	rabbit	GD7-19 or GD 1-19 , 7h/d	-	-	no	150ppm
Neeper-Bradley, 1993	rat	Gd 6-15, 6h/d	50ppm 125ppm	Fetal bw ↓ (dose dependent) Skeletal variations at 125 and 250ppm	No (no clinical signs of toxicity) Yes (reduced weight gain, liver weight ↑)	-
Saillenfait, 1996	rat	GD 6-15 (3x0.5h)	800ppm	Fetal bw ↓*	No	400ppm
Saillenfait, 1996	rat	GD 6-15 (1x0.5h)	-	-	No	1200ppm
Weller, 1999	mice	GD7 (single exposure)	2100ppmh or 2700ppmh	All concentrations: resorptions↑, Fetal bw ↓*, crown-rump-length↓*, eye defects↑*	Yes (weight loss, death at high conc.)	-
LaBorde, 1980	mice	i.v. 3 days during GD 6-8	150ppm	Fetal bw ↓, Malformations (cervical/thoracal skeleton) ↑	yes	75ppm
Rutledge, 1989	mice	1h or 6h after mating, (1.5h single exposure on GD 1 or 6)	1200ppm	Number of live foetuses ↓, abnormal foetuses (hydrops, eye defects)↑	-	-

The mechanism by which ethylene oxide induces developmental and fertility toxicity is not known. It is likely that protein and DNA alkylation are involved in inducing developmental toxicity (US EPA, 2010). In testicular toxicity protein alkylation, particular alkylation of enzymes, may be involved (Mori, 1989).

4.11.5 Comparison with criteria

Known or presumed human reproductive toxicant substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Suspected human reproductive toxicant substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. Adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Male reproductive organs were affected in several species after exposure to ethylene oxide for several weeks to months. In male rats exposed to about 200ppm reduced weight of testis and epididymis, small testis, degeneration of seminiferous tubules, abnormal sperm heads were described. At 357 ppm degeneration of seminiferous tubules and replacement fibrosis was seen in guinea pigs, while abnormal sperm heads and reduced testis weight were already seen at 200 ppm and 250 ppm in mice. Slight impairment (reduced number and motility of sperms) in monkeys was noted at 50ppm.

Female rats exposed to 100ppm during premating showed reduction of implantation sites, increased resorptions and number of pubs born. Female mice revealed reduced number of implants and increased number of resorptions at 300 ppm.

After exposure of rats to ethylene oxide reduced fetal body weights at 100ppm, reduced crown-rump length at 150ppm and variations in ossification (at 100ppm) as well as eye defects after single exposure to high concentrations were seen. Short time exposure showed effects at higher concentrations (800ppm). Intravenous exposure also resulted in reduced fetal weight and skeletal malformations (cervical/thoracal).

In rabbits no developmental toxicity or effects on fertility were seen when exposed to 150 ppm during gestation (without premating exposure).

The available studies indicate that rabbits, which metabolise EO mainly via hydrolysis, are less susceptible to EO reproductive toxicity than animals like rat and mouse, which metabolise EO mainly via glutathione conjugation. In humans EO is mainly metabolised via hydrolysis (80%) and to a lesser extent via glutathione conjugation (20%). However, the information on the differences in metabolism and its potential link to reproductive toxicity is insufficient to exclude the relevance of reproductive effects seen in several animal species for humans. Additionally, some reproductive toxicity has also been observed in humans (increased spontaneous abortion in exposed humans), although it has to be noted that the information on EO exposure in the available human studies was not detailed enough to draw a firm conclusion.

4.11.6 Conclusions on classification and labelling

The available data show that ethylene oxide is toxic to male reproductive organs and affects pregnancy outcomes (reduced number of implantations) in female animals at concentrations of 100ppm and above. Developmental Toxicity (increased number of resorptions, reduced number of pubs 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.

Overall it can be concluded that EO has the potential to affect male reproductive organs and female fertility and a potential for developmental toxicity cannot be excluded. These effects are not considered attributable to secondary unspecific toxicity. However, as there are some uncertainties related to the data base, a classification in Category 1B appears not justified, but Category 2 (suspected human reproductive toxicant) is proposed.

Ethylene oxide should be classified as Repr.2, H361fd.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The dossier submitter proposed to classify ethylene oxide as Repr. 2 for fertility effects. This was based on the studies by Snellings *et al.* (1982c) and Hardin *et al*. (1983), where exposure of rats to 100-150 ppm during pre-mating and gestation resulted in the reduction of the median number of implantation sites and pups born (Snellings *et al.*, 1982c) or a significant increase in the incidence of resorptions (Hardin *et al.*, 1983). Also, mice exposed via inhalation to ethylene oxide at pre-mating for several days showed a significantly reduced number of implants at 300 ppm and significantly elevated percentage of resorptions at 300 and 1200 ppm (10.8% and 41.1%, respectively; Generoso *et al.*, 1987). In addition, there are studies showing degeneration of the seminiferous tubules in guinea pigs and rats at the sub-chronic exposures to 200-357 ppm (Hollingsworth *et al.*, 1956, Mori *et al*., 1991). Abnormal sperm heads have been reported in rats at 250 ppm (Mori *et al*., 1991) and in mice at 200 ppm (Ribeiro, 1987). In addition, changes in testis and epididymal weights in rodents have been reported at these dose levels (Mori *et al*., 1991 and 1989; Snellings *et al.*, 1984). On the basis of these data, the DS concluded that ETO has a potential to affect male reproductive organs and female fertility, and that the effects are not attributable to secondary unspecific toxicity. However, since there were some uncertainties related to the studies (e.g. limited data on the parental toxicity), the DS considered a classification as Repr. 2, H361f (suspected human reproductive toxicant) for fertility more appropriate than Repr. 1B.

Developmental effects

The DS proposed to classify ETO as Repr. 2 for developmental effects. This is based mainly

on reductions in foetal body weights seen in three rat studies with exposure to ETO during the gestation period at 100-150 ppm. Also reduced crown-rump length and variations in ossification were described. After single high dose exposure, eye defects were reported in two studies in rodents. One study with intravenous exposure showing reduced foetal weight and skeletal malformations (cervical/thoracic) were used as supportive evidence, as well as data on spontaneous abortions in humans.

Comments received during public consultation

One MSCA supported classification as Repr. 2; H361fd, while one MSCA considered Category 1B more appropriate. One manufacturer did not support the classification proposal for developmental toxicity and considered the fertility effects seen in animal studies to not be conclusive.

Assessment and comparison with the classification criteria

Effects on fertility in animals

Snellings *et al.* (1982c) performed a one-generation study (similar to OECD TG 415) in 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 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 (see table below). 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.

Table: Reproductive effects in rats exposed to ethylene oxide via inhalation from pre-mating to weaning (Snellings *et al.*, 1982c).

Parameter	Exposure (ppm)				
	100	33	10	0 (control 1)[1]	0 (control 2)[1]
Number of females pregnant[a]	17/27 (63%)	25/28 (89%)	25/30 (83%)	24/29 (83%)	19/28 (68%)
Number of males proven fertile[b]	15/22 (68%)	20/23 (87%)	19/23 (83%)	17/21 (81%)	12/20 (60%)
Litters totally resorbed	2	0	0	0	0
Numbers of pups at day 0 postpartum	64	212	237	222	174
Numbers of pups born dead	0	1	3	0	0
Median number of stained implantation sites per pregnant rat	6.0*	11.0	11.0	11.0	10.0

Median number of foetuses born per number of implantation sites (x100)	57*(1)	90	92	92	100

[a] ratio of number of pregnant rats to number mated less number of non-pregnant rats mated for only one mating.
[b] ratio of number of males proven fertile to number mated less number mated for only one of the two matings
* $p < 0.001$ for comparison to control 1 and $0.001 > p > 0.001$[9] for comparison to control 2
*(1) $p < 0.001$ in comparison to either control group
[1] two control groups were used so that normal variability between similarly treated concurrent control groups could be evaluated

Exposure of female rats to 150 ppm of ethylene oxide from three weeks before mating until parturition showed statistically significantly increased incidence of resorptions (Hardin *et al.*, 1983, summarised by Hackett *et al.*, 1982, see table below). This was accompanied by decreases in maternal body weight and increased kidney and spleen weights. When rats were exposed to the same air level of ETO only during gestation (Groups 2 and 3, table 2), no effects were seen. Similarly, in rabbits exposed only during the gestation, no effects were seen.

Table: Maternal and reproductive effects in rats after exposure to 150 ppm ethylene oxide (Hackett *et al.*, 1982).

Parameter	Exposure groups			
	Group 1 Unexposed	Group 2 Exposed GD7-16	Group 3 Exposed GD1-16	Group 4 3 weeks premating + GD 1-16
Maternal body weight (g), mean values, (% reduction)				
Premating day 21	278	277 (-0.34%)	280 (+0.72%)	267* (-3.96%)
GD 6	298	298 (0%)	293 (-1.68%)	279* (-6.38%)
GD 11	315	314 (-0.32%)	308 (-2.22%)	295* (-6.35%)
GD 16	339	335 (-1.18%)	328 (-3.24%)	317* (-6.49%)
GD 21	382	381 (-0.26%)	378 (-1.05%)	360* (-5.76%)
Reproductive parameters				
No. live litters/no. pregnant	41/41	41/41	41/41	38/39
No. implantation sites/dam	14.7	14.0	14.8	14.3
No. resorptions/litter	0.75	0.71	0.92	1.60*
No. foetuses/litter	13.9	13.5	13.8	12.7

*Significantly different from control, $p < 0.01$

Generoso *et al.* (1987) exposed female mice to ethylene oxide at 1200 ppm (2160 mg/m^3)

[9] Reported here exactly as in the CLH report but one of the numbers is presumably 0.0001 or 0.01. ECHA was unable to access the original study report and could not find the info in the CSR in order to confirm.

for 1.5 h/d for 4 consecutive days before mating or at 300 ppm (540 mg/m^3) for 6 h/d for 10 exposures over a 14-d premating period. Exposure to 300 ppm (540 mg/m^3) for 6 h/d for 10 days over mating period resulted in the reductions in the number of implants per female and increased percentage of resorptions (41.1% *vs.* 6.4% in controls). Exposure to 1200 ppm (2160 mg/m^3) for 1.5 h/d for 4 consecutive days before mating resulted in significant, but less pronounced increase in resorptions (10.8% *vs.* 3.0% in controls). Mid-gestational deaths and late foetal deaths were slightly but not statistically significantly elevated; the loss of conceptuses was 15.7% at 1200 ppm and 58.2% at 300 ppm, showing that exposure to the lower concentration for a longer time was more effective than the high concentration for a short time. It should be noted that these dose levels are already rather high and may result in GSH depletion and reduced elimination of ETO.

In addition, there were several studies describing effects of ethylene oxide on the male testis or sperm counts/morphology.

An old study by Hollingsworth *et al.* (1956) described slight degeneration of testes in rats after sub-chronic exposure to 204 ppm of ETO. 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 of ETO for 13 weeks. At 250 ppm, a statistically significant decrease in epididymal weights (1.06 *vs.* 1.32 g), but not 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 ETO. 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 (see table below). 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. In spite of the inhibition of the activity of glutathione reductase at all time points and alterations of glutathione peroxidase activity, GSH concentration in the testes was not affected. Glutathione-S-transferase (GST) activity, the major enzyme detoxifying ethylene oxide in the testis, increased during the course of

exposure.

Table: Effects of ETO on relative testicular eights and epididymal weights (mean ± SD) (Mori, 1989).

Exposure period (week)	Rel. testicular weight (%)		Rel. epididymal weight (%)	
	control	500 ppm ETO	control	500 ppm ETO
2	1.248 ± 0.101 (6)	1.302 ± 0.183 (6)	0.308 ± 0.056 (6)	0.314 ± 0.062 (6)
4	1.129 ± 0.087 (6)	0.924 ± 0.060 (6)*	0.344 ± 0.004 (6)	0.297 ± 0.016 (6)**
6	1.117 ± 0.049 (8)	0.602 ± 0.059 (8)**	0.347 ± 0.018 (8)	0.248 ± 0.035 (8)**
13	1.006 ± 0.066 (8)	0.466 ± 0.113(8)**	0.344 ± 0.042 (8)	0.204 ± 0.029 (8)**

* $p < 0.01$; ** $p < 0.001$

Ribeiro *et al.* (1987), exposed mice for 1, 3 or 5 weeks to 200 and 400 ppm ETO to target the three stages of germ cell development: spermatozoa, spermatid and preleptotene spermatogonial cells and evaluated the frequency of abnormal sperm cells. Statistically significant increases in the number of abnormal sperm were observed at all time points at both doses (see table below). Sperm changes as a result of treatment of spermatogonia in the preleptotene stage may be correlated with the mutagenic potential of ETO. Effects observed as a result of exposure of spermatozoa (1 week before the sacrifice) may be related to the interference of spermatozoa differentiation process.

Table: Frequency of sperm head abnormalities after treatment with ethylene oxide (6 h/d) and cyclophosphamide (CPA, positive control) at different stages of spermatogenesis (Ribeiro *et al.*, 1987).

Group	Treatment		Sacrifice week after treatment	Population of treated cells	No of mice	No of cells scored	Sperm abnorm. % (mean+SD)
1	ETO	0 ppm	1	Spermatozoa	10	10000	1.76 ± 0.5
	ETO	200 ppm			10	10000	3.02 ± 0.5**
	ETO	400 ppm			10	10000	3.95 ± 0.6**
	CPA	100 mg			5	5000	3.12 ± 0.7**
2	ETO	0 ppm	2	Spermatid	10	10000	1.62 ± 0.4
	ETO	200 ppm			10	10000	3.62 ± 0.6**
	ETO	400 ppm			10	10000	5.81 ± 1.5**
	CPA	100 mg			5	5000	2.60 ± 0.8**
3	ETO	0 ppm	3	Spermatogonial cells in preleptotene	10	10000	1.32 ± 0.4
	ETO	200 ppm			10	10000	2.32 ± 0.5**
	ETO	400 ppm			10	10000	5.54 ± 1.4**

	CPA	100 mg			4	4000	10.40 ± 1.6**

**Statistically significant at 0.01 level

There is also one study on *Cynomolgus* monkeys in which 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 (Lynch, 1984, cited in NEDO, 2004, original study report not available).

Developmental effects in animals

Snellings *et al.* (1982b), exposed Fischer 344 rats to 0, 10, 33 and 100 ppm ethylene oxide vapour (6 h/d) on day 6 through 15 of the gestation period. No treatment related effects on maternal survival, litter size, number of implantation and resorption sites and preimplantation losses were seen. Exposure to 100 ppm resulted in a statistically significant depression of body weight (see table below), but no changes in crown-rump length. No statistically significant increases in skeletal or visceral variations were seen; vertebral variations were only slightly (non-significantly) 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. Since no information on maternal weight gain was given, it is unclear if these effects were specific developmental effects or related to maternal toxicity.

Table: Effects of ethylene oxide on foetal body weight after exposure during GD6-15 (Snellings *et al.*, 1982b).

Observations	Exposure group (ppm)				
	100	33	10	Control I (air) 0	Control II [a] (air) 0
Weight male foetuses (g) [Mean of litter means ± SD]	3.1* ± 0.2	3.3 ± 0.3	3.3 ± 0.3	3.4 ± 0.4	3.3 ± 0.2
Weight female foetuses (g) [Mean of litter means ± SD]	2.9* ± 0.1	3.1 ± 0.3	3.0 ± 0.3	3.1 ± 0.3	3.0 ± 0.2
Crown - rump length (male) (mm)	36 ± 1	36 ± 2	37 ± 1	37 ± 1	36 ± 1
Crown - rump length (female) (mm)	35 ± 1	35 ± 2	36 ± 1	35 ± 2	35 ± 1

[a] two control groups were used so that normal variability between similarly treated concurrent control groups could be evaluated

In the study by Hardin *et al.* (1983, reported in Hackett *et al.*, 1982), small reductions in rat foetal body weight and crown-rump length were seen after exposure to 150 ppm ethylene oxide for 7 h/d for different periods of gestation (see table below). Reduced skeletal ossification was also observed. No significant effects on maternal body weight or other signs of maternal toxicity were observed (maternal parameters are summarised in the table in the chapter "Effects on fertility" (above) and in Table 54 of the CLH report).

No effects were seen when rabbits were exposed to the same levels of ETO on GD 1-19 or 7-19 (Hardin *et al.*, 1983, reported in Hackett *et al.*, 1982).

Table: Developmental effects in rats after exposure to 150 ppm ethylene oxide (Hackett *et al.*, 1982). Maternal and fertility effects have been summarised in the table in the chapter "Effects on fertility als" (above).

Parameter	Exposure groups			
	Group 1 Unexposed	Group 2 GD 7-16	Group 3 GD 1-16	Group 4 3 weeks premating + GD 1-16
Foetal parameters				
Weight of female (g)	3.56	3.35*	3.23*	3.12*
Weight of male (g)	3.73	3.53*	3.47*	3.34*
Crown-rump length (mm) female	36.1	35.3*	34.7*	34.8*
Crown-rump length (mm) male	36.5	36.1*	35.8*	35.6*
Morphologic alterations (Number of foetuses per number of litters; number in parentheses are percentage of affected litters relative to controls)				
Reduced ossif., skull	3/2 (4.9)	16/9 (22.0)*	10/9 (22.0)*	14/10 (26.3)*
Reduced ossif., sternebrae	69/23 (56.1)	145/36 (87.8)*	159/36 (87.8)*	155/33 (85.8)*

* $p \leq 0.05$, compared with control

Neeper-Bradley (1993, abstract only) reported concentration-dependent reductions in foetal weight in rats after gestational exposure to 50, 125 and 225 ppm of ethylene oxide. Reductions were approximately 4%, 5% and 10% of control values. Reductions in maternal body weight gain and food consumption were observed at the highest dose. Mid-dose resulted in reductions in maternal body weight gain. 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.

Saillenfait *et al.* (1996) exposed rats either (1) for 0.5 h once a day to 0, 400, 800 or 1200 ppm ethylene oxide; or (2) for 0.5 h three times a day to 0, 200, or 400 ppm, or 800 or 1200 ppm ethylene oxide at GD 6-15. Single daily exposures showed no effects on maternal weight gain, no adverse effects on resorptions and no external or skeletal malformations. Increased incidences of dilated renal pelvis and ureter were observed at 1 × 1200 ppm, but the toxicological significance was doubtful due to the wide variations in renal development. In addition, these findings were not seen in 3 × 400, 3 × 800 or 3 × 1200 ppm groups. Three times per day exposures affected maternal weight gain at 1200 ppm and foetal body weights were significantly reduced ($p < 0.01$) at 3 × 200 ppm (not considered toxicologically significant due to the unusually high weights in the concurrent control group and the fact that at 3 × 400 ppm no effects were seen), 3 × 800 ppm and 3 × 1200 ppm. No other signs of foetotoxicity were seen.

Weller *et al.* (1999) studied the effects of single 1.5, 3, or 6 h exposures to ETO at GD 7 in a study designed specifically to test the applicability of Haber's law in the toxicity of ETO. The test was performed in mice. At dose levels resulting in clinical signs of toxicity in the majority

of maternal animals, increased resorptions, significantly decreased foetal body weight, decreased crown-to-rump length, and significantly increased incidences of eye defects (microphthalmia, anophthalmia) were seen. Doses higher than 1350 ppm × 3 h resulted in significant mortality of the dams. The study shows that the effects observed in foetuses were related to the high acute exposures and did not follow Haber's law. At 350 ppm × 6 h, no significant differences in developmental parameters were identified, whereas e.g. 1400 ppm × 1.5 h resulted in both severe maternal and foetal toxicity. Although foetal toxicity manifested as foetal deaths and decreased body weight gain are likely to be related to maternal toxicity at high short term doses, eye defects were also induced in this study. It is uncertain whether these malformations can be attributed to maternal toxicity. Eye defects observed in this study are summarised in the table below.

Table: Eye malformations caused by single high level exposure on GD7 to ethylene oxide in mice (Weller *et al*., 1999).

Dose ppm × h	Maternal deaths (%)	Maternal weight loss after exposure (g)	% of dams with clinical symptoms at 30 min#	No of Pregnant females n (%)	No of live foetuses n (%)	Mal-formations* n (%)
0	2 (1.3%)	1.9	4.8	86 (56%)	566 (89%)	39 (7%)
1400 × 1.5	3 (7.7%)	7.2	100.0	8 (22%)	21 (34%)	**7 (33%)**
700 × 3	0	6.6	81.6	22 (54%)	139 (82%)	**53 (40%)**
350 × 6	0	4.7	53.1	19 (58%)	138 (91%)	**20 (15%)**
1800 × 1.5	41 (56.2%)	13.0	100.0	3 (9%)	8 (36%)	7 (88%)
1543 × 1.75	15 (65.2%)	13.5	95.7	1 (13%)	6 (86%)	6 (100%)
1350 × 2	27 (35.5%)	11.4	100.0	7 (14%)	10 (50%)	3 (30%)
900 × 3	1 (2.0%)	8.8	98.0	11 (23%)	59 (69%)	**34 (58%)**
450 × 6	0	6.2	95.1	20 (40%)	120 (81%)	13 (11%)

*majority of malformations were eye disorders (anophtalmia, microphtalmia) # depressed movement or arousal, crusty eyes, laboured breathing.

When female mice were exposed to 1200 ppm ethylene oxide for 1.5 h (single exposure) 1, 6, 9, or 25 h after mating a marked reduction in the number of live foetuses was seen after exposure to ETO 1 h after mating (6 foetuses per dam versus 9.72 for controls) and 6 h after mating (1.81 foetuses per dam versus 10.11 for controls) (Rutledge *et al.*, 1989). Also the incidence of abnormal foetuses increased when females were exposed 1 h (14.7% versus 0.2% for controls) and 6 h (39.2% versus 1.7% for controls) after mating. The predominant types of abnormalities were hydrops and eye defects. Defects in the limbs and tail occurred in females exposed 6h after mating. Other abnormalities included abdominal wall defect, cleft palate, exencephaly, and small size. Inhalation exposure to 1800 ppm 6 h after the mating resulted in significant increases in foetal deaths. Also the number of defective living foetuses per dam significantly increased, whereas the number of living foetuses per dam decreased. Most dead foetuses were hydropic.

In the study by LaBorde (1980) in mice, intravenously administered ethylene oxide at days 4–6 (Period I), 6–8 (Period II), 8–10 (Period III), and 10–12 (Period IV) of gestation resulted in signs of toxicity (weakness, tremors, laboured respiration and death) among the maternal animals at the highest dose level of 150 mg/kg bw/d. Also significant decreases in mean maternal body weight gains were seen during treatment periods I, II and IV. Exposure to 150 mg/kg bw/d resulted also in a significant reduction in mean foetal body weight in all four treatment periods and in a significant reduction in the mean number of live foetuses in treatment periods III and IV. A significant increase in the percentage of malformed foetuses/litter was observed in Periods II and IV at this dose level. Approximately 19% of the foetuses in each litter from maternal animals treated with 150 mg/kg bw/d ethylene oxide in Period II had some types of malformations, including fusion of the cervical and thoracic arches, fusion and branching of ribs) (LaBorde, 1980).

Human data

Regardless of wide-spread use of ETO there are only a few studies on the reproductive effects in humans. One early Finnish study (Hemminki *et al.*, 1982) among sterilising staff employed in Finnish hospitals in 1980 showed an increased frequency for spontaneous abortions: 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 to ETO 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.

Rowland *et al.* (1996) performed a questionnaire-based study among 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 pre-term 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, ethylene oxide-exposed women 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.

A third (most recent) study (Gresie-Brusin *et al.*, 2007) was also a questionnaire based study among female workers in sterilising units in South Africa. The study population consisted of 98 singleton pregnancies. Personal and static samplings were performed to assess exposure. 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 highly exposed to ethylene oxide compared to those women with low exposure. No associations were found between exposure to ethylene oxide and stillbirth.

It should be noted 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. Although these studies suggest an association between spontaneous abortions and ETO exposure, the

database is still limited and the role of confounders in different studies cannot be totally ruled out.

The only study evaluating effects of paternal exposure on pregnancy outcome is the study by Lindbohm *et al*. (1991), which evaluated the effects of paternal occupational exposure to different mutagenic agents. An increased risk of spontaneous abortion (odds ratio = 4.7; 95% CI = 1.2–18.4) was shown. 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 analysis.

Comparison with the criteria

In the case of ethylene oxide, the human data does not provide conclusive evidence on the effects of ethylene oxide on fertility. Therefore, the classification criteria for Cat. 1A for fertility effects are not fulfilled. The main evidence on the effects on fertility comes from the one-generation study in rats by Snellings *et al.* (1982c), in which significantly decreased number of implantations and born foetuses per implantation site (indicating post-implantation losses) was observed without any signs of parental toxicity (e.g. decreases in weight gain) at 100 ppm. These findings are supported by the studies by Generoso *et al.* (1987) and Hardin *et al.* (1983) showing increased incidences of resorptions and/or decreased incidences of implantations at 300 and 150 ppm, respectively. Additional support for the fertility effects comes from the studies reporting specific effects on spermatogenesis and sperm morphology. These include the studies by Mori *et al.* (1989, 1991) and Ribeiro *et al.* (1987) and are supported by the monkey study by Lynch *et al.*, 1984 (reported by NEDO, 2004). 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 Cat. 1B for fertility. Although at higher dose levels GSH depletion in rats may have an impact on toxicity, decreases in implantations, increases in post-implantation losses and effects on spermatogenesis and sperm numbers and motility have been seen starting from the dose levels (50-100 ppm), at which no clear GSH depletion has been observed. Ethylene oxide is a well-established mutagen and it is possible that effects observed in one-generation studies are mediated by a genotoxic mechanism. Especially post-implantation losses observed after exposure during the pre-mating period may be due to dominant lethal effect caused by genotoxic insult. Genotoxic insult during the specific stages of spermatogenesis may also affect sperm quality by increasing the number of abnormal sperm as suggested by Ribeiro *et al.*, 1987. However, other mechanisms cannot be excluded. Since there were clear effects on fertility, seen also as a decrease in sperm quality, these are not considered to be covered by a germ cell mutagenicity classification.

Regarding developmental effects small decreases in foetal weights have been seen when pregnant females were exposed to 100-150 ppm. In the case of Snellings *et al.* (1982b), it is uncertain if these were accompanied with decreased maternal body weights. However, in the study by Hackett *et al.* (1982), decreased foetal weights and skeletal variations were seen in the absence of changes in maternal body weights. At higher doses more severe findings were found. Single high dose exposures during the critical periods of organogenesis resulted in foetal deaths and malformations, especially eye disorders (Weller *et al.*, 1999; Rutledge *et al.*, 1989). These were accompanied by slight to severe maternal toxicity. However, 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.

Taking these together and 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 Cat 2. Classification for developmental effects.

RAC concluded that classification of ethylene oxide as **Repr. 1B; H360Fd** is warranted, i.e. 1B for fertility and 2 for development.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated.

6 OTHER INFORMATION

Not relevant.

7 REFERENCES

Alomar A, Camarasa JM, Noguera J, et al. 1981. Ethylene oxide dermatitis. Contact Dermatitis 7:205-207. (as cited in ATSDR, 1990)

Anand VP, Cogdill CP, Klausner KA,, Lister L, Barbolt T, Page BFJ, Urbanski P., Woss (cj, Boyce J (2003). Wiley InterScience (www.interscience.wiley.com) online 7 February 2003.

ATSDR - Agency for Toxic Substances and Disease Registry (1990). Toxicological profile for ethylene oxide. http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=734&tid=133

Aydin G., Ornek D., Kahveci K., Doğer C., Sönmez C., Ozgun G. (2010). Low Platelet Count of a Sterilization Worker: Exposure To Ethylene Oxide; A Case Report. Internet Journal of Occupational Health; Nov2010, Vol. 1 Issue 1 http://ispub.com/IJOH/1/1/5573

Baur X., Bakehe P., Vellguth H. (2012). Bronchial asthma and COPD due to irritants in the workplace – an evidence-based approach. J Occup Med Toxicol. 26;7(1):19.

Biro L, Fisher AA, Price E (1974). Ethylene oxide burns. A hospital outbreak involving 19 women. Archives of dermatology 110:6 Dec pg 924-5.

Blackwood JD Jr, Erskine EB (1938). Carboxide poisoning. U.S. Navy Med Bull 36:44-45.

Bommer J, Barth HP, Wilhelms OH, Schindele H, Ritz E (1985). Anaphylactoid reactions in dialysis patients: role of ethylene-oxide. Lancet 28:1382-1384.

Brashear A, Unverzagt FW, Farber MO, Bonnin JM, Garcia JG, Grober E. (1996). Ethylene oxide neurotoxicity: a cluster of 12 nurses with peripheral and central nervous system toxicity. Neurology Apr;46(4):992-8.

Brown CD, Wong BA, Fennell TR (1996). In vivo and in vitro kinetics of ethylene oxide metabolism in rats and mice. Toxicol Appl Pharmacol. Jan;136(1):8-19.

Bruhin, H., Bühlmann, X., Vischer, W. A. & Lammers, T. (1961). endpoint study record: acute toxicity: oral. Schweiz. med. Wschr., 91, 607.

Bryant H.E., Visser N.D. and Yoshida K. (1989). Ethylene Oxide Sterilizer Use and Short-term Symptoms Amongst Workers. Occup Med (Lond) (1989) 39 (3): 101-106.

Cárdenas-Camarena L. (1998). Ethylene oxide burns from improperly sterilized mammary implants. Ann Plast Surg. Oct;41(4):361-6; discussion 366-9.

Caroli UM, Berner D, Volz T, Röcken M, Biedermann T. Delayed-type hypersensitivity dermatitis to ethylene oxide. (2005). Contact Dermatitis. Nov;53(5):303-4.

Caruana R.J., Hamilton R.W., Pearson F.C. (1985). Dialyzer hypersensitivity syndrome: possible role of allergy to ethylene oxide. Report of 4 cases and review of the literature. Am J Nephrol 5(4): 271-4.

Celanese Chemical Co., Inc. (1972). Primary skin irritation tests with 18 materials in albino rabbits. TSCATS: OTS 84003A, Doc. I. D.: 87-8212151. Owner company: Celanese. Report date: 1972-07-28.

Chapman J, Lee W, Youkilis E, Martis L (1986). Animal model for ethylene oxide (EtO) associated hypersensitivity reactions. Trans Am Soc Artif Intern Organs 32:482-485.

Crystal HA, Schaumburg HH, Grober E, Fuld PA, Lipton RB (1988). Cognitive impairment and sensory loss associated with chronic low level ethylene oxide exposure. Neurology, 38:567–569.

Csanády GA, Denk B, Pütz C, Kreuzer PE, Kessler W, Baur C, Gargas ML, Filser JG (2000). A physiological toxicokinetic model for exogenous and endogenous ethylene and ethylene oxide in rat, mouse, and human: formation of 2-hydroxyethyl adducts with hemoglobin and DNA. Toxicol Appl Pharmacol.; 165(1):1-26.

CSR (chemical safety report) ethylene oxide (2014).

Currier MF, Carlo GL, Poston PL, Weledford WE (1984). A cross sectional study of employees with potential occupational exposure to ethylene oxide. British Journal of Industrial Medicine, 41:492–498.

De Freitas MR, Nascimento OJ, Chimelli L. (1991). [Polyneuropathy caused by ethylene oxide. Report of a case with clinical, electrophysiological and histopathological studies]. Arq Neuropsiquiatr. Dec;49(4):460-4. Portuguese.

Deleixhe P.A., Balsat A., Laurent C. (1986). Acute ethylene oxide intoxication; a report of five cases [French]. Arch. Belg. 44 (11-12): 487-488.

Deschamps D, Rosenberg N, Soler P, Maillard G, Fournier E, Salson D, Gervais P. (1992). Persistent asthma after accidental exposure to ethylene oxide. Br J Ind Med.;49(7):523-5.

DFG – Deutsche Forschungsgemeinschaft (1993). Ethylene oxide. In: Occupational Toxicants, Vol.5, Wiley-VCH, Weinheim.

Dolovich J, Marshall CP, Smith EK, Shimizu A, Pearson FC, Sugona MA, Lee W. (1984). Allergy to ethylene oxide in chronic hemodialysis patients. Artif Organs. Aug;8(3):334-7.

Dolovich J, Bell B (1978). Allergy to a product(s) of ethylene oxide gas: demonstration of IgE and IgG antibodies and hapten specificity. J Allergy Clin Immunol. Jul;62(1):30-2.

Dugue, P., Faraut, C., Figueredo, M., Bettendorf, A. and Salvadori, J.M. (1991) [Occupational asthma provoked by ethylene oxide in a nurse (letter)]. [French]. Presse Medicale 20, 1455

ECHA (2015). Guidance on the Application of the CLP Criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 4.1. ISBN: 978-92-9247-413-3.

ECETOC (1984). Technical report No 11. Ethylene oxide toxicology and its relevance to man. An up-dating of ECETOC technical report No 5. ISSN-0773-8072-11.

Ehrenberg L, Hällström T (1967). Int Atomic Energy Agency Proc Ser, p. 311 [cited by DFG (1993)].

Estrin WJ, Cavalieri SA, Wald P, Becker CE, Jones JR, Cone JE (1987). Evidence of neurologic dysfunction related to long-term ethylene oxide exposure. Arch Neurol. 1987 Dec;44(12):1283-6.

Estrin WJ, Bowler RM, Lash A, Becker CE, (1990). Neurotoxicological evaluation of hospital sterilizer workers exposed to ethylene oxide. J Toxicol Clin Toxicol 28(1):1-20.

FDA (1978). Ethylene oxide, Ethylene Chlorohydrin and Ethylene Glycol. Proposed Maximum Residue Limits and Maximum Levels of Exposure. Docket No. 77N-0424

http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm078413.pdf

Fennell TR, Brown CD (2001). A physiologically based pharmacokinetic model for ethylene oxide in mouse, rat, and human. Toxicol Appl Pharmacol.173(3):161-75.

Finelli PF, Morgan TF, Yaar I, Granger CV (1983) Ethylene-oxide induced polyneuropathy. Archives of Neurology , 40:419–421.

Fujishiro K, Inoue N, Mori K, Ikeda M, Hori H (1989). Excretion of porphyrin and porphyrin precursor during ethylene oxide inhalation. [Article in Japanese] J UOEH. Mar 1;11(1):23-8.

Fujishiro K, Mori K, Inoue N (1990) Chronic inhalation effects of ethylene oxide on porphyrin-heme metabolism. Toxicology , 61:1–11.

Fukushima T, Abe K, Nakagawa A, Osaki Y, Yoshida N, Yamane Y (1986) Chronic ethylene oxide poisoning in a factory manufacturing medical appliances. Journal of the Society of Occupational Medicine, 36:118–123.

Garry VF, Hozier J, Jacobs D, Wade RL, Gray DG (1979). Ethylene oxide: evidence of human chromosomal effects. Environ Mutagen 1:375-382.

Generoso WM, Rutledge JC, Cain KT, Hughes LA, Braden PW (1987). Exposure of female mice to ethylene oxide within hours after mating leads to fetal malformation and death. Mutat Res. Feb;176(2):269-74.

Grammer LC, Roberts M, Nicholls AJ, Platts MM, Patterson R (1984). IgE against ethylene oxide-altered human serum albumin in patients who have had acute dialysis reactions. J Allergy Clin Immunol 74:544-546.

Grammer LC, Paterson BF, Roxe D, Daugirdas JT, Ing TS, Ivanovich PT, Brown CB, Nicholls AJ, Patterson R (1985). IgE against ethylene oxide-altered human serum albumin in patients with anaphylactic reactions to dialysis. J. Allergy Clin Immunol 76(3): 511-4.

Gresie-Brusin DF, Kielkowski D, Baker A, Channa K, Rees D. (2007). Occupational exposure to ethylene oxide during pregnancy and association with adverse reproductive outcomes. Int Arch Occup Environ Health Jul;80(7):559-65. Epub 2006 Dec 13.

Gross JA, Hass ML, Swift TR (1979) Ethylene oxide neurotoxicity: report of four cases and review of the literature. Neurology, 29:978– 983.

Hackett et al (1982). Teratogenic study of ethylene and propylene oxide and n-Butyl acetate. Contract No 210-80-0013.

Hardin, B.D., Niemeier, R.W., Sikov,M.R. and Hackett, P.L. (1983) Reproductive-toxicologic assessment of the epoxides ethylene oxide, propylene oxide, butylenes oxide, and styrene oxide. Scnd. J. Environ. Health 9, 94-102. Publication of study report Hackett et al (1982). Teratogenic study of ethylene and propylene oxide and n-Butyl acetate. Contract No 210-80-0013.

Hanifin JM. 1971. Ethylene oxide dermatitis [Letter]. J Am Med Assoc 217:213. (as cited in ATSDR, 1990)

Hayes J.P. and Fitzgerald M. X. (1994). Occupational asthma among hospital health care personnel: a cause for concern? Thorax 49(3): 198–200.

Hemminki K, Mutanen P, Saloniemi I, Niemi M-L, Vainio H (1982). Spontaneous abortions in hospital staff engaged in sterilising instruments with chemical agents. British Medical Journal, 285:1461–1463.

Hirose T., Goldstein R., Bailey CP (1963). Hemolysis of blood due to exposure to different types of plastic tubing and the influence of ethylene oxide sterilization. J. Thorac Cardiovasc Surg. Feb; 45:245-51.

Hollingsworth, R. L., Rowe, V. K., Oyen, F., McColister, D. D., Spencer, H. C. (1956). Toxicity of Ethylene Oxide Determined on Experimental Animals. A. M. A. Archives of Industrial Health 13, 217-227.

Jacobson, K. H. and Hackley, E. B. (1956). The Toxicity of Inhaled Ethylene Oxide and Propylene Oxide. Archives of Industrial Health 13, 237-244.

Jacson F., Beaudouin E., J., D.A. Moneret-Vautrin D.A. (1991). Allergie au formol, latex et oxyde d'éthylène : triple allergie professionnelle chez une infirmière. Revue Française d'Allergologie et d'Immunologie Clinique. Volume 31, Issue 1, January–March 1991, Pages 41-43.

Joyner, R. E.: Arch. environm. Hlth 8, 700 (1964) [cited by DFG (1993)].

Kerre S, Goossens A. Allergic contact dermatitis to ethylene oxide (2009). Contact Dermatitis. Jul;61(1):47-8.

Klees JE, Lash A, Bowler RM, Shore M, and Becker CE. (1990). Neuropsychological “impairment” in a cohort of hospital workers chronically exposed to ethylene oxide. Clin. Toxicol. 28(1):21-28.

Koelsch F., Lederer (1930), Zbl. Gewerbehyg. 7, 264. (cited in DFG, 1993)

Kuzuhara S., Kanazawa I., Nakanishi T. and Egash T. (1983). Ethylene oxide polyneuropathy. Neurology 33: 377-380.

LaBorde J.B., Kimmel C.A. (1980). The teratogenicity of ethylene oxide administered intravenously to mice. Toxicology and Applied Pharmacology Volume 56, Issue 1, October 1980, Pages 16-22.

Laurent C. (1988). SCE increases after an accidental acute inhalation exposure to EtO and recovery to normal after 2 years. Mutat Res. 204(4): 711-717.

LaMontagne AD, Christiani DC, Kelsey KT. (1993). Utility of the complete blood count in routine medical surveillance for ethylene oxide exposure. Am J Ind Med. 1993 Aug;24(2):191-206.

Leitman SF, Boltansky H, Alter HJ, Pearson FC, Kaliner MA. (1986). Allergic reactions in healthy plateletpheresis donors caused by sensitization to ethylene oxide gas. N Engl J Med. Nov 6;315(19):1192-6.

Lemke H.D. (1987). Mediation of hypersensitivity reactions during hemodialysis by IgE antibodies against ethylene oxide. Artif Organs. Apr; 11(2): 104-10.

Lindbohm, M.-L., K. Hemminki, M.G. Bonhomme, A. Antilla, K. Rantala, P. Heikkilä and M.J. Rosenberg (1991). Effects of paternal occupational exposure on spontaneous abortions. Am. J. Public Health 81: 1029–1033.

Lynch, D. W., Lewis, T. R., Moorman, W. J., Burg, J. R., Groth, D. H., Khan, A., Ackerman, L. J., Cockrell, B. Y. (1984). Carcinogenic and Toxicologic Effects of Inhaled Ethylene Oxide and Propylene Oxide in F344 Rats. Tox. Appl. Pharmacol. 76, 69 - 84.

Lynch D.W., Lewis TR., Moorman WJ., et al. (1984a). Effects on monkeys and rats of long-term inhalation exposure to ethylene oxide: Major findings of the NIOSH study. In: Inhospital ethylene sterilization. Current issues in ET0 toxicity and occupational exposure. AAMI Technology Assessment Report No. 8-84. Arlington VA: Association for the Advancement of Medical Instrumentation, 7-10.

Marshall C, Shimizu A, Smith EK, Dolovich J. (1984). Ethylene oxide allergy in a dialysis center: prevalence in hemodialysis and peritoneal dialysis populations. Clin Nephrol. Jun;21(6):346-9.

Marshall CP, Pearson FC, Sagona MA, Lee W, Wathen RL, Ward RA, Dolovich J. (1985). Reactions during hemodialysis caused by allergy to ethylene oxide gas sterilization. J Allergy Clin Immunol. 1985 May;75(5):563-7.

Matsuoka M.(1988). Effects of chronic exposure of ethylene oxide, especially on heme metabolism. [Article in Japanese] J UOEH Mar 1;10(1):77-88.

McDonald TO, Kasten K, Hervey R, Gregg S, Britton B. (1977). Acute ocular toxicity for normal and irritated rabbit eyes and subacute ocular toxicity for ethylene oxide, ethylene chlorohydrin, and ethylene glycol. Bull Parenter Drug Assoc. 1977 Jan-Feb;31(1):25-32.

Monbaliu D, Van Breussegem A, Onsia A, Vandermeersch E, Segers C, Meert W, Kochuyt AM, Pirenne J, Claes K. (2010). Ethylene oxide allergy in patients on hemodialysis waiting for kidney transplantation: logistical nightmare or challenge? A case report. Transplant Proc. Dec;42(10):4375-7.

Mori K., Kaido M., Fujishiro K., and Inoue N.. (1989). Testicular toxicity and alterations of glutathione metabolism resulting from chronic inhalation of ethylene oxide in rats. Toxicol. Appl. Pharmacol. 101(2):299-309.

Mori K, Inoue N, Fujishiro K, Kikuchi M, Chiba S (1990) Biochemical changes in rat erythrocytes caused by ethylene oxide exposure. Fundamental and Applied Toxicology, 15:441–447.

Mori K, Ohnishi A, Fujishiro K, Inoue N. (1990). [Effects of sexual difference on the toxicity of ethylene oxide. I. Polyneuropathy]. J UOEH. Mar 1;12(1):61-6. Japanese.

Mori, K., Kaido M., Fujishiro K. , Inoue N., Koide O., Hori H, and Tanaka I. (1991). Dose dependent effects of inhaled ethylene oxide on spermatogenesis in rats. Br. J. Ind. Med. 48(4):270-274.

Muller A, Jacobsen H, Healy E, McMickan S, Istace F, Blaude MN, Howden P, Fleig H, Schulte A; EU Working Group on Haemolytic Anaemia (2006). Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regul Toxicol Pharmacol. 2006 Aug;45(3):229-41. Epub 2006 Jun 21. Review.

Nachreiner D.J. (1991). Ethylene Oxide: Acute Vapor Inhalation Toxicity Test in Rats (Four-Hour Test). Project ID54-76. Bushy Run Research Center, Export, PA. (cited in National Research Council, 2010)

Nachreiner D.J. (1992). Ethylene Oxide: Acute Vapor Inhalation Toxicity Testing According to D.O.T. Regulations (One-Hour Test). Project ID54-593. Bushy Run Research Center, Export, PA. (cited in National Research Council, 2010)

National Research Council (2010). Acute Exposure Guideline Levels for Selected Airborne Chemicals, Volume 9. The National Academies Press. www.nap.edu

Neeper-Bradley TL; Kubena MF (1993) Ethylene oxide: developmental toxicity study of maternally inhaled vapor in CD rats. Technical report Bushy run research center.

NEDO (2004). Hazard assessment Report No 36 Ethylene oxide. http://www.pic.int/Portals/5/AIII-Info/Ethylene%20oxide/Japan-212_english_pdf.pdf

NTP (1987): National Toxicology Program, Toxicology and Carcinogenicity Studies of Ethylene Oxide (CAS No. 75-21-8) in B6C3F1 Mice (Inhalation Studies) (NTP Technical Report 326, U.S. Department of Health and Human Services, Public Health Services, National Institute of Health). Research Triangle Park, NC, NTP. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr326.pdf

Ohnishi A, Inoue N, Yamamoto T, Murai Y, Hori H, Koga M, Tanaka I, Akiyama T (1985) Ethylene oxide induces central-peripheral distal axonal degeneration of the lumbar primary neurones in rats. British Journal of Industrial Medicine , 42:373–379.

Ohnishi A, Inoue N, Yamamoto T, Murai Y, Hori H, Tanaka I, Koga M, Akiyama T (1986) Ethylene oxide neuropathy in rats. Exposure to 250 ppm. Journal of the Neurological Sciences , 74:215–221.

Osterman-Golkar S, Ehrenberg L, Segerbäck D, Hällström I. (1976). Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. Mutat Res. Jan;34(1):1-10.

Patch P.C., Hartlage L.C. Neurological and emotional sequelae of exposure to ethylene oxide. Int J Neurosci Jan; 106 (1-2): 101-7.

Pearson F, Bruszer G, Lee W, Sagona M, Sargent H, Woods E, Dolovich J, Caruana R. (1987) Ethylene oxide sensitivity in hemodialysis patients. Artif. Organs 11(2): 100-3.

Popp DM, Popp RA, Lock S, Mann RC, Hand RE Jr. (1986). Use of multiparameter analysis to quantitate hematological damage from exposure to a chemical (ethylene oxide). J Toxicol Environ Health. 18(4):543-65.

PSL (Priority substance list) assessment report – Ethylene oxide (2001). Environment Canada and Health Canada http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/ethylene_oxide/index-eng.php

Ribeiro, L.R., Salvadori D.M., Perera C.A., and Becak W.. (1987). Activity of ethylene oxide in the mouse sperm morphology test. Arch. Toxicol. 60(4):331-333

Röckel A, Klinke B, Hertel J, Baur X, Thiel C, Abdelhamid S, Fiegel P, Walb D (1989). Allergy to dialysis materials. Nephrol Dial Transplant 4:646-652.

Rowland AS, Baird DD, Shore DL, Darden B, Wilcox AJ (1996). Ethylene oxide exposure may increase the risk of spontaneous abortion, preterm birth, and postterm birth. Epidemiology, 7(4):363–368. (as cited in PSL (Priority substance list) assessment report, Environment Canada, 2001. http://publications.gc.ca/collections/Collection/En40-215-51E.pdf)

Rumpf KW, Seubert A, Valentin R, Ippen H, Seubert S, Lowitz HD, Rippe H, Scheler F (1985). Association of ethylene-oxide-induced IgE antibodies with symptoms indialysis patients. Lancet 28:1385-1387.

Rusch, George M. (2009). Establishing a point of departure for risk assessment using acute inhalation toxicology data. Regulatory Toxicology and Pharmacology 54 (247-255).

Rutledge, J.C. and Generoso, W.M. (1989) Fetal pathology produced by ethylene oxide treatment of the murine zygote. Teratology, 39, 563-572.

Saillenfait, A.M., Gallissot F., Bonnet P., and Protois J.C. (1996). Developmental toxicity of inhaled ethylene oxide in rats following short-duration exposure. Fundam. Appl. Toxicol. 34(2):223-227.

Salinas E, Sasich L, Hall DH, Kennedy RM, Morriss H (1981). Acute ethylene oxide intoxication. Drug Intelligence and Clinical Pharmacy, 15:384–386.

Schroeder JM, Hoheneck M, Weis J, Dies H (1985). Ethylene oxide polyneuropathy: clinical follow -up study with morphometric and electron microscopic findings in a sural nerve biopsy. Journal of Neurology, 232:83–90.

Schulte PA, Walker JT, Boeniger MF, Tsuchiya Y, Halperin WE. (1995). Molecular, cytogenetic, and hematologic effects of ethylene oxide on female hospital workers. J Occup Environ Med. Mar;37(3):313-20.

SCOEL (2012) European commission, Employment, Social Affairs & Inclusion. Recommendation from the Scientific Committee on Occupational exposure Limits for ethylene oxide. SCOEL/SUM/160.

Segerbäck D. (1990). Reaction products in hemoglobin and DNA after in vitro treatment with ethylene oxide and N-(2-hydroxyethyl)-N-nitrosourea. Carcinogenesis. 1990 Feb;11(2):307-12.

Setzer JV, Brightwell WS, Russo JM, Johnson BL, Lynch DW, Madden G, Burg JR, Sprinz H. (1996). Neurophysiological and neuropathological evaluation of primates exposed to ethylene oxide and propylene oxide. Toxicol Ind Health. Sep-Oct;12(5):667-82.

Sexton RJ, Henson, E. 1949. Dermatological injuries by ethylene oxide. J Ind Hyg Toxicol 31:297-300 (as cited in ATSDR, 1990).

Sexton RJ, Henson EV. 1950. Experimental ethylene oxide human skin injuries. Ind Hyg Occup Med 32:549-564 (as cited in ATSDR, 1990).

Shaham J, Levi Z, Gurvich R, Shain R, Ribak J. (2000). Hematological changes in hospital workers due to chronic exposure to low levels of ethylene oxide. Occup Environ Med. 2000 Aug; 42(8): 843-50.

Shupack J.L., Andersen S.R., Romano S.J. (1981). Human skin reactions to ethylene oxide. J. Lab. Clin. Med. 98: 723-729.

Smyth H. F. Jr., Seaton J. and Fischer L. (1941). The single dose toxicity of some glycols and derivatives. Journal of Industrial Hygiene and Toxicology 23, 259 - 268.

Snellings, W. M. (1982). Seven- to eight-week vapor inhalation; probe study on rats and mice. Testing laboratory: Bushy Run Research Center. Report no.: 45-139. Owner company: BRRC. Report date: 1982-12-06.

Snellings, W. M., Maronpot, R. R., Zelenak, J. P., Laffoon, C. P. (1982b). Teratology Study in Fischer 344 Rats Exposed to Ethylene Oxide by Inhalation. Tox. Appl. Pharmacol. 64, 476 - 481.

Snellings, W. M., Zelenak, J. P., Weil, C. S. (1982c). Effects on Reproduction in Fischer 344 Rats Exposed to Ethylene Oxide by Inhalation for One Generation. Tox. Appl. Pharmacol. 63, 382 - 388.

Snellings WM, Weil CS, Maronpot RR. (1984). A subchronic inhalation study of the toxicologic potential of ethylene oxide in B6C3Fl mice. Toxicol Appl Pharmacol 76:510-518.

Snellings W.M., Nachreiner D.J., Pottenger L.H (2011). Ethylene Oxide: Acute Four-Hour and One-Hour Inhalation Toxicity Testing in Rats. Journal of Toxicology Volume 2011 (2011), Article ID 910180, Epub 2011 Jul 13. http://dx.doi.org/10.1155/2011/910180

Sprinz, H.; Matzke, H.; Carter, J.(1982) Neuropathological Evaluation of Monkeys Exposed to Ethylene and Propylene Oxide. PB 83-134817. Kansas City, MO: Midwest Research Institute, prepared for NIOSH (cited in Ecetoc, 2010)

Stedingk H, Vikström AC, Rydberg P, Pedersen M, Nielsen JK, Segerbäck D, Knudsen LE, Törnqvist M. (2011). Analysis of hemoglobin adducts from acrylamide, glycidamide, and ethylene oxide in paired mother/cord blood samples from Denmark. Chem Res Toxicol. Nov 21;24(11):1957-65. doi: 10.1021/tx200284u. Epub 2011 Sep 15.

Thiess AM. 1963. [Observations on the health hazards of ethylene oxide.] Archiv Toxiko 20:127-140. (German) (as cited in ATSDR, 1990).

US EPA (2010). Acute Exposure Guideline Levles for selected airborne Chemicals. Volume 9 ISBN: 0-309-15945-8. hhtp://www.nap.edu/catalog/12978.html

Van Sittert NJ, de Jong G, Clare MG, Davies R, Dean BJ, Wren LJ, Wright AS. (1985). Cytogenetic, immunological, and haematological effects in workers in an ethylene oxide manufacturing plant. Br J Ind Med. Jan;42(1):19-26.

Verraes S, Michel O. Occupational asthma induced by ethylene oxide. Lancet. 1995 Nov 25;346(8987):1434-5.

Von Oettingen W. (1939). Ethylene oxide. In: Supplement to occupation and health: Encyclopedia of Hygiene, Pathology, and Social Welfare. Geneva, Switzerland: International Labor Office.

Wass U, Belin L, Delin K. (1988). Longitudinal study of specific IgE and IgG antibodies in a patient sensitized to ethylene oxide through dialysis. J Allergy Clin Immunol. Oct;82(4):679-85.

Weller, E., Long N., Smith A., Williams P., Ravi S., Gill J., Henessey R, Skornik W., Brain J., Kimmel C., Kimmel G., Holmes L., and Ryan L. (1999). Dose-rate effects of ethylene oxide exposure on developmental toxicity. Toxicol. Sci. 50(2):259-270.

WHO (2003). Ethylene oxide, CICAD 54. http://www.who.int/ipcs/publications/cicad/en/cicad54.pdf

WHO (2012). Guidance for immunotoxicity risk assessment. IPCS Harmonization Project Document No. 10 http://www.who.int/ipcs/methods/harmonization/areas/immunotoxicity/en/

Woodard G. and Woodard M. (1971). Toxicity of residuals from ethylene oxide gas sterilization. cited in HIA Technical Symposium. Report date: 1971-10-08.

Yager JW, Benz RD. (1982). Sister chromatid exchanges induced in rabbit lymphocytes by ethylene oxide after inhalation exposure. Environ Mutagen. 4(2):121-34.

Yong LC, Schulte PA, Wiencke JK, Boeniger MF, Connally LB, Walker JT, Whelan EA, Ward EM. (2001). Hemoglobin adducts and sister chromatid exchanges in hospital workers exposed to ethylene oxide: effects of glutathione S-transferase T1 and M1 genotypes. Cancer Epidemiol Biomarkers Prev. May;10(5):539-50.

Zampollo A, Zacchetti O, Pisati G (1984). On ethylene oxide neurotoxicity: report of two cases of peripheral neuropathy. Italian Journal of Neurological Sciences, 5:59–62.

8 ABBREVIATIONS

ALA	δ-Aminolevulinic acid
ATP	Adaptation to Technical Progress
ATSDR	Agency for Toxic Substances and Disease Registry
BM	bone marrow cellularity
CA	Competent Authority
CFU-S/M	Spleen colony-forming units
CSR	Chemical Safety Report
EO	ethylene oxide
GR	glutathione reductase
Hb	haemoglobin
HSA	human serum albumin
Ht	hematocrit
LC	Lethal concentration
LD	Lethal dose
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MNCV	maximum nerve conduction velocity
NTP	National Toxicology Program
PCV	packed cell volume
RADS	reactive airway dysfunction syndrome
RAST	radioallergosorbent test
RBC	red blood cell count
SCOEL	Scientific Committee on Occupational Exposure Limits
WBC	white blood cell count
WHO	World Health Organization