Committee for Risk Assessment

RAC

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

Adopted

22 September 2017

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22 September 2017

CLH-O-0000001412-86-164/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ethylene oxide; oxirane

EC Number: 200-849-9

CAS Number: 75-21-8

The proposal was submitted by Austria and received by RAC on 22 August 2016.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at *http://echa.europa.eu/harmonised-classification-and-labelling-consultation/* on 4 October 2016. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 18 November 2016.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: Tiina Santonen

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on 22 September 2017 by consensus.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-023-00-X	ethylene oxide; oxirane	200-849-9	75-21-8	Press. Gas Flam. Gas 1 Carc. 1B Muta. 1B Acute Tox. 3* STOT SE 3 Skin Irrit. 2 Eye Irrit. 2	H280 H220 H350 H340 H331 H335 H315 H319	GHS02 GHS08 GHS06 GHS04 Dgr	H280 H220 H350 H340 H331 H335 H315 H319			U
Dossier submitters proposal	603-023-00-X	ethylene oxide; oxirane	200-849-9	75-21-8	Retain Flam. Gas 1 Press. Gas Carc. 1B Muta. 1B STOT SE 3 Add Repr. 2 Acute Tox. 3 STOT RE 1 Skin Sens. 1 Modify Acute Tox. 3 Skin Corr. 1B Eye Dam. 1	Retain H220 H280 H350 H340 H335 Add H361fd H301 H372 (nervous system) H317 Modify H331 H314 H318	Retain GHS02 GHS08 GHS06 GHS04 Dgr Add GHS05	Retain H220 H280 H350 H340 H335 Add H301 H317 H372 (nervous system) H361fd Modify H331 H314 H318			Retain U
RAC opinion	603-023-00-X	ethylene oxide; oxirane	200-849-9	75-21-8	Retain Flam. Gas 1 Press. Gas Carc. 1B Muta. 1B STOT SE 3 Add Repr. 1B Acute Tox. 3 STOT SE 3 STOT RE 1 Modify Acute Tox. 3	Retain H220 H280 H350 H340 H335 Add H360Fd H301 H336 H372 (nervous system) Modify H331	Retain GHS02 GHS08 GHS06 GHS04 Dgr Add GHS05	Retain H220 H280 H350 H340 H335 Add H360Fd H301 H336 H372 (nervous system) Modify H331			Retain U

					Skin Corr. 1 Eye Dam. 1	H314 H318		H314			
Resulting Annex VI entry if agreed by COM	603-023-00-X	ethylene oxide; oxirane	200-849-9	75-21-8	Flam. Gas 1 Press. Gas Carc. 1B Muta. 1B Repr. 1B Acute Tox. 3 Acute Tox. 3 STOT SE 3 STOT SE 3 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H220 H280 H350 H340 H360Fd H331 H301 H335 H336 H372 (nervous system) H314 H318	GHS02 GHS04 GHS05 GHS06 GHS08 Dgr	H220 H280 H350 H340 H360Fd H331 H301 H335 H336 H372 (nervous system) H314			U

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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).

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, neurobehavioural 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.

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. 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 subcategorisation. 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 subcategorisation 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.

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.

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 sensitisers 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.

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 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 (1) Skin prick test with ethylene oxide-human serum albumin conjugate (2) 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:	Marshall *et al.*,

	22/25 patients with acute allergic reactions (88%) 5/37 patients without allergic reaction (13%) Normal control were negative	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
(1) Clinical surveillance in patients with allergic reactions (2) 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 subcategorisation 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.

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 Case 4: 2 months	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, 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	Supporting study No information on exposure concentrations available. 700 ppm, as estimated by the authors as the workers could smell the chemical Possibility of short time exposure to high levels of ethylene oxide no assessed	Gross, 1979
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

Subjects and exposure	Results	Remarks	Reference
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 wlimited 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 heme 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.

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 (x 100)	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 $0.001 > p > 0.001$[1] 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%)

[1] 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.

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) 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 womenwith 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 Category 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 Category 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 Category 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).