Committee for Risk Assessment

RAC

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

propane-1,2-diol

EC Number: 200-338-0
CAS Number: 57-55-6

CLH-O-0000001412-86-133/F

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

Adopted

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Propane-1,2-diol

EC Number: **200-338-0**

CAS Number: **57-55-6**

Index Number:

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Propane-1,2-diol***
EC number:	***200-338-0***
CAS number:	***57-55-6***
Annex VI Index number:	**-**
Degree of purity:	***> 99.9%***
Impurities:	**-**

1.2 Harmonised classification and labelling proposal

Propane-1,2-diol has not been classified and labelled up to now. The proposal is STOT SE 3.

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	none
Current proposal for consideration by RAC	STOT SE 3; H335
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	STOT SE 3; H335

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	none	Not applicable	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	none	Not applicable	Not classified	conclusive but not sufficient for

					classification
2.16.	Substance and mixtures corrosive to metals	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	none	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - dermal	none	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Skin sensitisation	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	None	Not applicable	Not classified	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	**STOT SE 3 H335**			
3.9.	Specific target organ toxicity – repeated exposure	none	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	none	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	none	Not applicable	Not classified	Data lacking
5.1.	Hazardous to the ozone layer	none	Not applicable	Not classified	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram: GHS07

Signal word: Warning
Hazard statements: H335: May cause respiratory irritation

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

No harmonised classification and labelling for Propane-1,2-diol (propylene glycol (PG)) exists in Annex VI of the CLP Regulation.

The majority of notifications proposed no self-classification in the C&L inventory, others classify for Acute Tox. 3, Eye Irrit. 2, Skin Irrit. 2, STOT SE 3, Aquatic Chronic 1 and/or Aquatic Chronic 2.

2.2 Short summary of the scientific justification for the CLH proposal

Among many other uses propane-1,2-diol is commonly used to produce artificial smoke with generators in theatres, discotheques, emergency trainings or is used as a liquid for vaporisation in electronic cigarettes.

A number of internet forums indicate that the inhalation of propane-1,2-diol aerosol/vapour may have adverse effect to the respiratory tract if inhaled, e.g.:

> 'The side effects of prolonged inhalation of propylene glycol found in room deodorizers can cause irritation of the mucous membranes, wheezing, coughing and shortness of breath.'
>
> http://www.livestrong.com/article/234677-propylene-glycol-side-effects/

In other internet chats the potential to harm by its use as theatrical fog is discussed:

> http://www.gutefrage.net/frage/nebel-von-nebelmaschine---gesundsheitsschaedlich

There are numerous reports on irritation and sore throat in internet forums when propane-1,2-diol was used as liquid in electronic cigarette. Health effect related data from its use in electronic cigarette are not considered for this report.

> http://www.bag.admin.ch/themen/drogen/00041/00618/13196/13199/index.html?lang=de&print_style=yes

In general the information from internet communications does not present reliable data for the classification and labelling of a substance. However the dossier submitter has taken them as a reason to assess the science-based evidence on adverse effects on the respiratory tract after short term inhalation of propane-1,2-diol. The need for a harmonised classification is given potentially by the wide exposure of the public and by occupational exposure (actors and other professionals in the entertainment sector).

The presented CLH report is mainly based on published data on human experience. Some limited animal data on acute toxicity are included in the weight of evidence analysis. Data from repeated inhalation studies are also documented and assessed with regards to effects that are indicative for respiratory tract irritation.

In conclusion, a classification as STOT SE Cat 3 due to its properties to cause respiratory tract irritation is proposed.

Although the dossier submitter has noted that there are a number of reports that indicate eye irritation properties, this CLH report is targeted to the respiratory tract irritation: No other route of exposure and no other human health hazard classes than respiratory tract irritation or environmental hazard classes are assessed in this report.

2.3 Current harmonised classification and labelling

No harmonised classification and labelling for propane-1,2-diol exists in Annex VI of the CLP Regulation.

2.4 Current self-classification and labelling

The majority of notifications proposed no self-classification in the C&L inventory, others classify for Acute Tox. 3, Eye Irrit. 2, Skin Irrit. 2, STOT SE 3, Aquatic Chronic 1 and/or Aquatic Chronic 2.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

As stated in the scientific justification already, propane-1,2-diol is commonly used to produce artificial smoke with generators in theatres, discotheques, emergency trainings or is used as a liquid for vaporisation in electronic cigarettes. The need for a harmonised classification is given potentially by the wide exposure of the public and by occupational exposure (actors and other professionals in the entertainment sector). Furthermore, several notifiers used STOT SE 3 in the self-classification, whereas the majority of notifiers proposed no self-classification at all. Therefore the CLH report on propane-1,2-diol was prepared.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

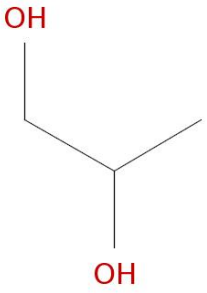
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	200-338-0
EC name:	propane-1,2-diol
CAS number (EC inventory):	57-55-6
CAS number:	57-55-6
CAS name:	1,2-Propanediol
IUPAC name:	1,2-Propanediol
CLP Annex VI Index number:	-
Molecular formula:	$C_3H_8O_2$
Molecular weight range:	76.094 g/mol
Synonym	Propylenglycol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
propane-1,2-diol, EC-Nr.: 200-338-0		> 99.9 %w/w	

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
	Please see confidential annex or technical dossier		

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	strongly hygroscopic colourless liquid	Harlan Laboratories Ltd. 2010a	
Melting/freezing point	< -20°C	Harlan Laboratories Ltd. 2010a	EU Method A.1 (Melting / Freezing Temperature)
Boiling point	184 °C at 100.32 kPa	Harlan Laboratories Ltd. 2010a	EU Method A.2 (differential scanning calorimetry)
Relative density	1.03 at 20 ˚C	Harlan Laboratories Ltd. 2010a	EU Method A.3 (pycnometer method)
Vapour pressure	20 Pa at 25 ˚C	Harlan Laboratories Ltd. 2010b	EU Method A.4 (effusion method: vapour pressure balance)
Surface tension	71.6 mN/m at 21.5 °C Concentration 1.01 g/L	Harlan Laboratories Ltd. 2010a	EU Method A.5 (ring method)
Water solubility	Miscible with water at 20 ˚C and pH = 7.1-7.8	Harlan Laboratories Ltd. 2010a	EU Method A.6 (flask method)
Partition coefficient n-octanol/water	log Pow = -1.07 at 20.5 ˚C and pH = 6.2-6.4	Harlan Laboratories Ltd. 2010a	EU Method A.8 (shake-flask method)
Flash point	104 ˚C at 100.01 kPa.	Harlan Laboratories Ltd. 2010b	Closed cup equilibrium method
Flammability	non flammable	BAM (2013)	Flammability upon ignition (solids, gases): Testing can be waived, substance is a liquid. Flammability in contact with water: The classification procedure needs not to be applied because the substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).
Explosive properties	no explosive properties	BAM (2013)	The classification procedure needs not to be applied because there are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	> 400 °C at 100.01-101.44 kPa	Harlan Laboratories Ltd. 2010b	EU Method A.15 (Auto-Ignition Temperature (Liquids and Gases))
Oxidising properties	no oxidising properties	BAM (2013)	The classification procedure needs not to be applied because the organic substance contains oxygen, which is chemically bonded only to carbon or

			hydrogen.
Granulometry	The study does not need to be conducted, as propane-1,2-diol is a liquid and thus is manufactured and marketed in a non-solid form.		
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	Dissociation constants of alcohol groups present in the molecule are known to be extremely low, alcohols being weaker acids than water. Thus no dissociation of the substance in aqueous solution is expected.		
Viscosity	43.4 mPa*s at 25 ˚C	George J, Sastry NV, 2003	experimental

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Among many other uses propane-1,2-diol is commonly used to produce artificial smoke with generators in theatres, discotheques, emergency trainings or is used as a liquid for vaporisation in electronic cigarettes.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not classified for physico-chemical properties

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not evaluated for this dossier.

4.2 Acute toxicity

The results of the acute toxicity studies will be used as supportive evidence for specific target organ toxicity – single exposure and are not submitted to support classification for acute toxicity. Information given in the registration dossier(s) has been used for preparation of the CLH report.

Table 9: Summary table of relevant acute and short-term toxicity studies in animals

Method	Results	Remarks	Reference
Rabbit (2000 – 3000 g body weight) Inhalation of propane-1,2-diol aerosol Six rabbits were exposed for either 20 or 120 minutes to an aerosol of (10% PG in air – no explanation given if w/v or v/v)). In each experimental group the tracheal epithelium of 3 rabbits was examined.	No mortality was reported. Time-related increase of mucous release and degeneration of goblet cells of the trachea. 20 min exposure induced also minimal ultrastructural alteration (apical small cytoplasmatic blebs) of the ciliated cells. Signs of pathological alterations (cytoplasmic protrusions with destruction of kinocilia) were observed after 120 min. No data are given on mass median aerodynamic diameter.	Supporting study Reliable with restrictions Ultrastructural examinations of the tracheal epithelium. No other tissues examined.	Konrádová et al., 1978
30 Sprague-Dawley rats, (Crl: CD (SD) IGS BR), 8 weeks old, Charles River Laboratories. Males and females were 200-220 g at study initiation. Three groups of three males and three females were exposed nose-only to increasing concentrations of capillary aerosol generator (CAG)-PG aerosol at 14.4, 30.5, and 44.9 mg/L during a 4h inhalation exposure. Immediately following exposures, lungs from exposed animals were collected and frozen in liquid nitrogen for analysis of PG concentration. Recovery animals were maintained for a 7day post-exposure recovery period. Body weights and clinical observations were performed immediately after exposure and just prior to necropsy on post-exposure Day 7	No mortality was observed. On study day 1-3 post-exposure, there were 5-10% decreases in body weight in males and females (no data are given presented to judge concentration-dependency). By study day 7, all animals had returned to normal growth rates and increased body weights. End-of-exposure lung concentration of PG increased approximately dose-proportionately. Clinical signs of ocular and nasal irritation indicated by minor bleeding around the eyes and nose at day 7. PG aerosol showed a mass median aerodynamic diameter (MMAD) of 1.1-1.4 µm .	Supporting study Reliable with restrictions The study did not include histopathological examinations.	Werley et al., 2011
104 Sprague-Dawley rats, (Crl: CD (SD) IGS BR), 8 weeks old, Charles River Laboratories. At study initiation, males weighed 233-260g and females weighed 207-237g. Animals were exposed nose-only for 4h/day for seven consecutive days. The pulmonary and systemic toxicity of PG aerosol was investigated in two groups of five males and five females were exposed to either 20.8 or 41.0 mg/L PG aerosol, respectively. Clinical observations, body	There were no treatment-related clinical observations. Dose-related increase of PG in lung tissue. There were no histopathological findings in the respiratory tract. The NOEL was greater than 41 mg/L under the conditions of this study. PG aerosol showed a mass median aerodynamic diameter (MMAD) of 0.9 µm	Supporting study Reliable with restrictions The absence of findings in the respiratory tract must refer to the lungs only, as no indication was found that the nasal cavity, the nasopharynx and the larynx were included in the microscopical	Werley et al., 2011

weights, PG concentrations in the blood and lungs, histopathological evaluation of the lungs, lung weights, and necropsy were performed during the study.		examinations.	
Beagle dogs (2 m and 2 f) were supplied by Mashall Farms (North Rose, NY, USA). Animals were acclimated for approximately 15 weeks. At study, animals were 8-9 month old, and weighed 9.4-9.6 and 7.4-7.5 kg for males and females, respectively. Dogs were exposed to 1.5-30 mg/l for 8-60 min depending upon toleration of exposure. Clinical signs before, during and after exposure were monitored. Body weights, food consumption, clinical chemistry, hematology, pulmonary function, and necropsy were performed.	Animals were generally intolerant to high exposure concentrations of PG aerosol at 15 and 30 mg/L. Dogs became restless as the exposure concentration to PG aerosol was increased to the nominal concentration. No further reactions and effects were described. maximum tolerated dose (MTD) was determined at 5 mg/L.	Supporting study Reliable with restrictions The study did not mention histopathological examinations.	Werley et al., 2011
Dogs (see above for details) were exposed to 5.0 mg/L PG aerosol for 60 min on seven consecutive days.	Evaluations of pulmonary function, hematology, clinical chemistry, body weight, food consumption, and macroscopic evaluation of tissues and organs at necropsy were all unremarkable. PG aerosol showed a mass median aerodynamic diameter (MMAD) of 1.9 µm	Supporting study Reliable with restrictions The study did not mention histopathological examinations.	Werley et al., 2011

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Not evaluated for this dossier.

4.2.1.2 Acute toxicity: inhalation

The results of acute toxicity inhalation studies will be used as supportive evidence for specific target organ toxicity – single exposure and are not submitted to support classification for acute toxicity.

The effect of propane-1,2-diol on the tracheal epithelium was investigated in rabbits by exposing groups of 3 rabbits for 20 minutes or 2 hours to 10% PG in air. No mortalities were reported. 20 minutes inhalation of 10% PG produced minimal ultrastructural alterations of ciliated cells in the trachea that were reported as apical small cytoplasmatic blebs, sometimes with intact or disintegrated axial complexes of ciliary fibres. Effects more pronounced in tracheal goblet cells where the percentage of unaffected goblet cells were 16.5%, mucous release was seen in 31% of goblet cells and 52% of goblet cells were completely exhausted ones with highly electron dense

degenerated cytoplasm. After 2 hours inhalation of PG signs of ciliated cells' alteration were more severe, cytoplasmic protrusions containing destructed kinocilia were observed. A further drop in the number of intact mucus filled (12%) and mucus discharging goblet cells (19%) was accompanied by an increase in the number of exhausted degenerated ones (69%). The degenerated goblet cells were gradually expelled from the epithelium. After 2 hours inhalation exposure the signs of increased differentiation of new goblet cells were not noticed (Konrádová et al., 1978). It is the dossier submitter's view that the exposed time period is too short to expect increased cell proliferative activity.

In another study on Sprague-Dawley rats, males and females were exposed for 4 hours and single doses of 14.4, 30.5, and 44.9 mg/L PG. Treatment-related clinical signs associated with exposure to capillary aerosol generator (CAG)-PG aerosol included slight localized bleeding around the eyes and nose at day 7. On study day 1–3 post-exposure, there were 5–10% decreases in body weight in males and females. By study day 7, all animals had returned to normal growth rates and had increased body weights. End-of-exposure lung concentrations of PG increased approximately dose-proportionately. There was no mortality; therefore, the acute 4h LC50 for exposure to PG aerosol delivered using the CAG was greater than 44.9 mg/L. PG aerosol particles were considered to be fully respirable in the rat with MMAD values of 1.1–1.4 µm and GSD of 1.1–1.4 (Werley et al., 2011). The study did not include a microscopic examination.

Rats were exposed nose-only for 4h/day for seven consecutive days. Groups of five males and five females were exposed to either 20.8 or 41.0 mg/L PG aerosol, respectively. There were no treatment-related clinical observations. Body weights were unaffected by exposure to PG aerosol. No macroscopic findings were noted at necropsy. There were no effects upon lung weights. No histopathologic findings were observed in the respiratory tract. (As far as the information is given in the publication, only the lungs were examined histopathologically.) Pharmacokinetic analysis on day 7 of treatment indicated that both lung and plasma peak exposures increased approximately dose-proportionately, with a lung-to-plasma ratio close to 0.45. The control group had small but measurable PG concentrations in the lungs, presumably from other environmental sources including the rodent chow diet. PG aerosol particles were considered to be fully respirable in the rat with MMAD values of 0.9 mm and GSD of 1.4. The no-observed-effect level (NOEL) was greater than 41.0 mg/L under the conditions of this study (Werley et al., 2011).

Beagle dogs were exposed via face mask to PG aerosol. Animals were generally intolerant to high exposure concentrations of PG aerosol at 15 and 30 mg/L. There was a clear inverse relationship between the tolerable exposure concentration and the time of exposure. As the exposure concentration to PG aerosol was increased from 1.5 mg /L, dogs became restless and intolerant at concentrations of ≥15 mg/L, and could be exposed only for shorter periods of time. Based upon these observations, it was determined that the highest exposure concentration to PG aerosol should be approximately 5 mg/L to avoid stress in the animals and facilitate exposure (Werley et al., 2011). The study did not include a microscopic examination.

During the 7-day study on dogs, animals were titrated up to the target exposure concentration of 5 mg/L for the first 2 exposures. Aerosol particle size determinations showed that 100% of the PG aerosol particles were less than 4.2 µm and were log-normally distributed with a MMAD of 1.9 µm, indicating that they were fully respirable in the dog. The larger than expected particle size in this study may have been related to the hygroscopic nature of the PG test article. Since the dogs were exposed via flexible tubes connected to face masks approximately 4 feet from the plenum, it was discussed that moisture in the air may have caused particle growth resulting in a somewhat larger particle size delivered to the study animals than normally generated by the CAG using drier air.

Repeated inhalation exposure to PG aerosol at 5 mg/L for up to 60 min duration was well tolerated. Evaluations of pulmonary function, hematology, clinical chemistry, body weight, food consumption, and macroscopic evaluation of tissues and organs at necropsy were all unremarkable at 5 mg /L PG aerosol (data not shown) (Werley et al., 2011). Also in this dog study, no histopathological examination was conducted.

4.2.1.3 Acute toxicity: dermal

Not evaluated for this dossier.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

Human information is presented in 4.3.

4.2.3 Summary and discussion of acute toxicity

No mortality was reported in any investigation of inhalative toxicity.

The study on human volunteers will be summarized and discussed in 4.3.

20 min or 2 hours of inhalation exposure of rabbits to 10% PG in air produced minimal alteration of ciliated cells and an exposure time-related damage of goblet cells that leads to a release of mucous and a degeneration of the goblet cells. An ultrastructural examination of the tracheal epithelium only was conducted (Konrádová et al., 1978).

Several studies in rats and dogs were reported in Werley et al. (2011):

A 4 h inhalation study on rats, using PG concentrations from 14.4 to 44.9 mg/L, observed a 5-10% decrease in body weight during day 1-3 post exposure. At day 7 post exposure all animals had returned to normal growth rates and increased body weight. Slight localized bleeding around the eyes and nose was observed at day 7. It is the dossier submitter's view that the transient decrease in body weight indicates that the single 4 h inhalation must have affected the general health condition of the exposed animals, although no specific clinical observations were reported during and directly after the exposure. The interpretation of the bleeding at day 7 is difficult, if related to the PG exposure, the effects should be expected immediately after the end of inhalation exposure.

A study in dogs demonstrated that animals became restless and were generally intolerant to 8-60 min exposure towards concentrations of PG aerosol at 15 and 30 mg/L. The maximum tolerated dose was determined at 5 mg/L.

The available studies on acute toxicity testing of PG aerosols induced no mortalities and mild effect of general toxicity such as transient body weight reduction. Observed effects in two species provide some evidence for an irritation of the respiratory tract with slight bleeding around the eye and the nose in rats and with an adverse behaviour in dogs exposed towards high concentrations of PG aerosol. Increased mucous release and degeneration of mucous cells are also indicative of the irritative nature of propane-1,2-diol, and altered ciliated cells were observed in rabbits after 20 or 120 min exposure to 10% PG in air.

In none of these studies with acute or short-term inhalation exposure to PG a full microscopic examination of the complete respiratory tract was included.

4.2.4 Comparison with criteria

Not relevant for this dossier.

4.2.5 Conclusions on classification and labelling

The results of the animal studies will be used as supportive evidence for specific target organ toxicity – single exposure.

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

Human data:

Acute exposure (one min) of healthy non-asthmatic volunteers towards PG mist (concentration range 176-851 mg/m^3) at normal aviation emergency training in March 1998 resulted in a sensation of sore and dry eyes, throat dryness and irritative cough. Nine out of 25 volunteers without previous symptoms reported at least one ocular symptom (36%) and 14 out of 23 volunteers (61%) reported throat dryness. Two reported appearance of nasal catharr and one got nasal itching, but none reported sneezing or nasal obstruction after the exposure. None reported appearance of headache, nausea, or breathing difficulties after exposure to PG, and there was no net change in reporting fatigue. There were some indications that women and those with a history of atopy seemed to be more sensitive to exposure to PG, for some types of symptoms, but the number of women (n=5) and subjects with atopy (n=8, 2 women and 6 men) were small. In total, 29% of men and 80% of women reported the development of throat symptoms, but there were no sex difference for development of ocular symptoms. Moreover, 50% of those with atopy, and 11% of those without atopy reported development of least one ocular symptom. Finally, 100% of those with atopy, but only 28% of those without atopy reported development of throat symptoms after exposure to PG.
The authors interpreted this as indicating that women and those with a history of atopy seemed to be more sensitive for some effects by PG inhalation. However, this is a preliminary guess, since the number of women (n=5) and subjects with atopy (n=8) were (too) small.

All volunteers participated in the acoustic rhinometry and the lung function test. One person could not participate in the measurement of tear film stability due to nervous blinking. A significant decrease of tear film stability was found after exposure to PG, with a reduction of mean tear film stability break up time from 38 to 29 seconds (p=0.02), and the decrease of tear film stability was similar in men and women. When comparing tear film stability after the exposure, those who developed ocular symptoms had numerically lower tear film stability (mean 27 s) than those who did not (mean 34 s). This observation may indicate that tear film stability is lowered in subjects reporting ocular symptoms.
The two people with ocular symptoms before the exposure had the greatest decrease of tear film stability (mean decrease 38 s).
No significant changes in any measures of nasal patency (data on nasal dimensions as measures of minimum cross sectional areas and volumes of the nasal cavity measured from 0 and 22 mm and from 23 and 54 mm from the nasal opening) were found after exposure to PG. Most of the lung function values remained unchanged after exposure to PG, but there was a minor numerical decrease of FEV1[1] from 103% to 102% at exposure, and a small but significant decrease of FEV1/FVC (p=0.049). Mean VC[2] was unchanged after the exposure, whereas FVC[3] was slightly

[1] Forced expiratory flow in 1 second

[2] Vital capacity

increased. None of the 27 participants had an initial lung function value (FEV1) below 80% of predicted value, but one got a 77% value for FEV1 after the exposure. The mean decrease of FEV1 and FEV1/FVC was similar in subjects with and without a history of atopy. Moreover, there were no significant association between a decrease in FEV1, and development of mild dyspnoea (measured by the rating scales) in the total material. A few, however, reacted with cough, mild airway obstruction, and mild dyspnoea. There were four subjects (16%) who developed irritative cough after the exposure. All were non-smoking men without any history of allergies. They had an average reduction in FEV1 of 5%, compared with a 0% reduction of FEV1 among those who did not develop a cough. Moreover, those four subjects had an increase in self rated dyspnoea of 13% on the analogue scale, whereas those who did not develop cough only had a 1% increase of dyspnoea, a significant difference between the two groups ($p<0.01$).

The investigation was conducted in an aircraft simulator where PG concentration was repeatedly measured and medical investigations were performed before and after the exposure (within 15 min). The mean exposure measurements showed that there were higher exposures (520 mg/m³) in the afternoon than in the morning, before the lunch break (220 mg/m³). These differences made it possible to evaluate possible dose-effect relations comparing changes from before to after exposure in those nine subjects exposed in the afternoon with those 18 exposed in the morning. A dose-effect relation was found for tear film break up time, with a 6-second average decrease in the low exposure group and a 13-second decrease in the high exposure group. Moreover, 47% in the low exposure group but 100% in the high exposure group reported development of throat dryness, and the self-reported rating of throat symptoms were higher in the highly exposed group. By contrast, no dose-effect difference in the observed symptoms or measurements observed after 220 mg/³ (morning) or 520 mg/m³ (afternoon) were found for other effects such as ocular or nasal symptoms, dyspnoea, nasal patency, or FEV1% (Wieslander et al., 2001). The strengths of these effects were similar at both concentrations.

A study on painters (Wieslander & Norbäck, 2010) using water-based paints yields a supportive argument. PG exposure of 17 painters was in the mean 2.038 mg/m^3 (0,066 to 7.620 mg/m^3). Painters were also exposed to other compounds such as diethylene glycol monoethyl ether (0.458 mg/m^3), diethylene glycol monobutyl ether (0.145 mg/m^3), and 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (0.404 mg/m^3) (all values are arithmetic means). Additionally, painters were also exposed to several microbial volatile organic compounds.

Associations were observed between measured exposure and biomarkers. A significant correlation of 0.37 was found for PG and eosinophilic cationic protein from nasal lavage. The level of eosinophilic cationic protein is a measure of the activity of eosinophilic granulocytes which is cytotoxic and can be destructive to the epithelium of the airways.

However, in contrast to these results a previous paint emission exposure study which included a 4-h exposure to a mixture of volatile organic compounds including 10 mg/m^3 of PG did not find any effect on eosinophilic cationic protein (Ernstgard et al. 2007, cited according to Wieslander & Norbäck, 2010). In summary, the results of this study with a mixed exposure towards several volatile organic compounds support the thesis that PG has an irritation effect on the mucosa of the upper airways. Due to the mixed exposure to different components emitted from the water-based paints the findings cannot be associated with PG as the only origin of irritative effects on the eyes and nasal mucosa.

[3] Forced vital capacity

The NTP report (NTP, 2004) cited an early study of NIOSH that investigated the health effects associated with the use of theatrical smokes:
The National Institute for Occupational Safety and Health (NIOSH, 1992) conducted a study in 1991 on the use of theatrical fog in Broadway theaters. Personal breathing zone and general area air sampling and a questionnaire on irritant effects (130 questionnaires from productions with theatrical smoke, 90 questionnaires from productions without theatrical smoke) were collected from personnel from four productions using theatrical smoke and five productions without theatrical smoke. Air samples collected yielded propane-1,2-diol concentrations < 2.1 mg/m³. However, there was a significant (.05) increase in the reporting of respiratory irritant symptoms such as runny nose, stuffy nose, and sneezing by personnel from productions using theatrical smoke.

In their report NIOSH concluded that

> 'When compared to actors from the non-smoke productions, actors from two or more of the four productions utilizing theatrical smoke reported experiencing significantly greater prevalence of nasal symptoms (sneezing, runny or stuffy nose), respiratory symptoms (cough, wheeze, breathlessness, chest tightness), and mucous membrane symptoms (sore throat, hoarseness, dry throat, itchy, burning eyes, dry eyes) during their performances for the week prior to the survey.'
>
> 'Although some of the constituents of theatrical smoke (primarily the glycols) have irritative properties, the reason for the high symptom prevalence in the productions that use theatrical smoke is not clear since the TWA concentration (*over the whole sampling period, e.g. the 3-h production time*) of the glycols measured during the performances were quite low. It is possible however, that the smoke concentrations could be sufficiently high during the short periods of time that the smoke is generated to contribute to the symptoms reported by the actors.'

Based on this early study on four Broadway productions using smoke compared to five productions without smoke NIOSH concluded that theatrical fogs may contribute to upper respiratory tract problem including sneezing, stuffy noses, coughs, breathlessness, and sore or dry throats. As the TWA of the glycols measured during the performances were quite low and one production used glycol and other smoke systems (mineral oil based mist), NIOSH concluded the etiology remains unclear and decided to continue the investigations.. The glycols detected include ethylene, propylene, 1,3-butylene, diethylene and trietylene glycols. Only the ethylene glycol concentrations were reported to range from non detectable to 21 mg/m³ (TWA).

In conclusion, the increased incidences of respiratory tract irritation in actors of the NIOSH study were associated with the use of theatrical fog. The observed effects are consistent with other studies in their conclusion that glycol may be the cause of irritative effects, but the findings do not allow to identifing propane-1,2-diol as the only source of irritation.

In their report, NTP (NTP, 2004) irritation effects following inhalation exposure to PG were summarized from a subsequent study:

> 'Propylene glycol is a component of theatrical fog and is used for special effects. The Actors' Equity Association and the League of American Theaters and Producers sponsored a study (*conducted in 1997-99*) which included an examination of the health effects of theatrical fog in response to actors' concerns about exposure (Moline et al., 2000). The health endpoints selected for investigation were irritant effects to the respiratory tract and eyes. This study was conducted over 2 years with 439 actors from 16 musicals, and consisted of a baseline questionnaire, daily checklists, and medical evaluation. There was no

> clinically significant adverse impact on pulmonary function or in rates of asthma associated with exposure to propylene glycol. However, "peak exposures to elevated localized air concentrations following release of glycol smoke are associated with increased reporting of respiratory, throat, and nasal symptoms, and findings of vocal cord inflammation." The study authors recommended that exposures to propylene glycol by actors not exceed peak or ceiling concentrations of 40 mg/m³.'

The study also compared the symptom scores with the exposure values for a subset of 218 actors with detailed integrated dose estimation and peak estimation. The study population was characterised (age, attendance time at the ensemble, time as professional actor, time at the production, and possible confounders assessed (medical and smoking history, environmental factors (such as type of home, heating, air conditioning) and performance factors).

As theatrical effects glycol was used in 8 of 16 musicals (actors mean time in current shows were 18.4 months (range 0-186 months)). For specific show effects propane-1,2-diol was used in 7 studies, in 6/7 additional other glycols were used (in 5/7 triethylene glycol, 4/7 butylene glycol and 1/7 diethylene glycol). In one show ('titanic') only propane-1,2-diol was used.
The overall exposure to glycol was low: the average concentrations of total glycols in the preliminary air sampling were in the range of 0.1 to 7.2 mg/m³). Maximum measured short-term exposure concentrations were in the range of 0.37 to 46 mg/m³.

The original report (Moline et al., 2000) summarised the results as following:

> 'There are associations between symptoms reported in the baseline questionnaire and increasing glycol exposure levels, based on the preliminary exposure estimates developed for all 439 study participants. To examine the nature of these associations, symptom reporting was evaluated in the subset of 218 actors for whom detailed integrated dose and peak exposure estimates were measured (using time exposed to two times and five times the Broadway average exposure level as a measure of peak exposure). Based on this analysis, symptom reporting – in particular respiratory, throat, and nasal symptoms – was found to be associated with peak exposures and not with integrated dose. No association was found for pulmonary function tests or voice performance and exposure to glycols.' 'An increase in fiberoptic findings (excess phlegm) was significantly associated with the preliminary and the detailed integrated glycol exposure variable (but details on data were not shown). The pre-show evaluation in those actors with increased glycol exposure (2times or 5times the Broadway average) revealed a significant (peak concentration-dependent) association with signs of inflammation (pharyngitis, laryngitis, tracheitis).

Moline et al. concluded that peak levels of glycol exposure are associated with reported symptoms of mucus membrane irritation. This is consistent with the chemical and physical properties of glycols, since they have irritative and drying properties at high doses. There are consistent, statistically associations between an overall increase in throat symptoms with increasing glycol exposure. Similarly, symptoms such as coated vocal cords, hoarseness, and voice change were associated with increasing glycol exposure, as were symptoms of nasal irritation.'

The dossier submitter noted that the peak concentration associated with irritation of the upper respiratory tract were not attributable to propane-1,2-diol alone as the total concentration of the four glycols were estimated and the majority of show used fogs that contained also other glycols.

NIOSH's conclusion caused the American Chemistry Council's Propylene Oxide/Propylene Glycol Panel to inform about their considerations against use in theatrical fogs (American Chemistry Council, 2001).

Occupational exposure limits were established for propane-1,2-diol by the AIHA WEEL (American Industrial Hygiene Association Workplace Environmental Exposure Level) to be 10 mg/m³ TWA, aerosol (3.2 ppm) and 156 mg/m³ TWA total vapor and aerosol (50 ppm).

Table 10: Summary table of relevant acute toxicity studies in humans

Method	Results	Remarks	Reference
Healthy non-asthmatic volunteers (n=27), 22 men and five women, were examined medically within 15 min before and after exposure to PG mist. The geometric mean concentration of PG in the flight simulator was 309 mg/m^3, arithmetic mean was 360 mg/m^3 (range 176-851 mg/m^3) with an arithmetic mean exposure of 220 mg/m^3 in the morning, and 520 mg/m^3 in the afternoon In total, 30% had a history of atopy, 15% had hay fever, 15% had a history of childhood eczema, but none reported allergy to furry animals. In total, two women and six men had a history of atopy, whereas three women and 16 men did not have atopy, a non-significant but numerically higher occurrence of atopy among the women. None had ever had any respiratory disorders including asthma or chronic bronchitis and none had febrile respiratory infection the week before the investigations. The subjects were naïve, in that none had previous occupational exposure to PG. The investigations were done at normal aviation emergency training, during one week in March 1998, before the pollen season had started in mid-Sweden. The artificial smoke generator was placed in the flight simulator, with a commercial PG solution for smoke generation. The exposure was performed as a part of the regular training schedules for pilots. One general medical questionnaire was used to gather information on personal factors, including medical disorders, medication, occupational data, the home environment, and smoking habits. Information on current symptoms was obtained from a second questionnaire containing 10 rating scales on current ocular, nasal, throat symptoms, dyspnoea, malodour, and systemic symptoms and 23 yes or no questions on	After exposure to PG mist (mean 220 mg/m) for 1 minute tear film stability decreased, ocular and throat symptoms increased, forced expiratory volume in 1 second/forced vital capacity (FEV1/FVC) was slightly reduced, and self rated severity of dyspnoea was slightly increased. No effect was found for nasal patency, vital capacity (VC), FVC, nasal symptoms, dermal symptoms, smell of solvent, or any systemic symptoms. Those exposed to the higher concentrations (mean 520 mg/m) in the afternoon had a more pronounced increase of throat symptoms, and a more pronounced decrease of tear film stability. In four subjects who reported development of irritative cough during exposure to PG, FEV1 was decreased by 5% and also had an increased perception of mild dyspnoea. FEV1 was unchanged among those (n=21) who did not develop a cough.	Key study Reliable with restrictions	Wieslander et al., 2001

different types of symptoms. Both questionnaires were administered before and after the exposure to PG. Tear film stability was estimated by a standardised method. Acoustic rhinometry was applied to measure nasal patency. Respiratory function was studied by dynamic spirometry. History for atopy (defined as having a history of childhood eczema or current history of allergy) and smoking was assessed.			
Active house painters (n=31) were compared to 20 janitors serving as control group. None of the painters had doctor´s diagnosed asthma. None of the janitors had current chemical exposure, none had been working as a painter, and all had predominantly outdoor work. In order to detect effects from the indoor environment, all subjects had worked 1 h up to 6 h prior to clinical examination. Personal 8-h exposure measurements of formaldehyde and (microbial) volatile organic compounds were performed in 17 randomly selected house painters the same day as the medical investigations were performed. The subjects were questioned by a physician about previous diseases, allergy and atopy, asthma, medication, ongoing respiratory infections, earlier occupations, smoking habits, and age. Tear film stability was estimated by a standardised method. Acoustic rhinometry was performed in the working place and prior to lavage. Lavage fluid of the nasal mucosa was quantified for lysozyme, myeloperoxidase and eosinophilic cationic protein. Among painters (n=31), 10% were current smokers, 42% had a history of atopy, 13% hat at least one asthma symptom (wheeze, daytime or night time attacks of breathlessness), and mean age was 32 years. Among jainitors (n=20), 10% were	The clinical study and the exposure measurements in the painters were restricted to days when water-based paints were used. PG exposure of 17 painters was in the mean 2.038 mg/m^3 (0,066 to 7.620 mg/m^3). Painters were also exposed to other compounds such as diethylene glycol monoethyl ether (0.458 mg/m^3), diethylene glycol monobutyl ether (0.145 mg/m^3), and 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (0.404 mg/m^3) (all values are arithmetic means). Additionally, painters were also exposed to several microbial volatile organic compounds. Associations were analysed between measured exposure and biomarkers. A significant correlation of 0.37 was found for PG and eosinophilic cationic protein from nasal lavage.	Supporting study Reliable with restrictions	Wieslander & Norbäck, 2010

current smokers, 30% had a history of atopy, 18% had at least one asthma symptom, and mean age was 38 years.			

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

A reliable study in 27 human volunteers demonstrates ocular and throat symptoms after 1-min exposure toward PG mists in self-ratings 10 min before and after the exposure (Wieslander et al., 2001). Medical investigations performed within 15 min before and after the exposure revealed that the forced expiratory volume in one second (FEV1) was slightly reduced. Four subjects reacted with irritative cough, mild airway obstruction, and mild dyspnoea.

Additionally, some evidence from acute and repeated inhalation studies in animlas is included to support the weight of evidence.

4.3.2 Comparison with criteria

The CLP Regulation defines the following criteria:

(a) *Respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.*

The Wieslander study clearly fulfils these criteria, since throat dryness indicates irritation of the upper respiratory tract and subjects with cough, mild airway obstruction and mild dyspnoea indicate impaired function of the lower respiratory tract. The study reported throat dryness in 61% of volunteers (all without symptoms on the same day shortly before the exposure) and impairment of respiratory function in 16% of volunteers who suffered from irritative cough, mild airway obstruction and mild dyspnoea. Two subjects reported appearance of nasal catharr and one got nasal itching, but none reported sneezing or nasal obstruction after the exposure. The latter is consistent with the absence of measured effects on the cross section and volumes of the nasal cavities.

A cohort study on actors who were exposed to glycol fogs and where peak exposures were associated with respiratory, throat and nasal symptoms, and vocal cord inflammation is considered as giving supportive evidence (Moline et al., 2000). The used fogs contained a mixture of glycols including PG and thus the effects can not solely be attributed to PG.

(b) *Subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids.*

The Wieslander study fulfils these criteria. The slight reduction of forced expiratory volume in one second (FEV1) indicates slightly impaired lung function. These parameters are obtained by objective measurements.

(c) *The symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports*

simply of "irritation" shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation and dryness, which are outside the scope of classification for respiratory irritation.

The Wieslander study carefully asseses the PG-related effects in healthy non-asthmatic volunteers under controlled exposure conditions. Ambiguity could be excluded as the same subjects did the self-reporting immediately before and after the exposure, the same is valid for the medical investigations.

(d) *There are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.*

The acute studies of the Werley paper can be considered in general as supportive for STOT-SE 3 since the rats reacted with slight bleeding around the eyes and nose to the exposure against PG aerosol and the dogs were generally intolerant to high exposure concentrations of PG aerosol at 15 and 30 mg/L. However it remains unclear why bleeding around the eyes and nose were reported on the post-observational day 7 only.

The results of the acute study of Konrádová are supportive for irritation effects and increased mucous production. Electronmicroscopical data of tracheal epithelium from rabbits exposed to 10% PG by inhalation indicated early signs of increased release of mucous and degenerated goblet cells in rabbit tracheal epithelium following 20 or 120 min of PG exposure.

Finally it must be stated that no fully reliable animal study on acute irritation effects on the respiratory tract is available.

In the CLP guidance (3.8.2.5) it is determined that

Category 3 effects should be confined to changes, whether functional or morphological, occurring in the upper respiratory tract (nasal passages, pharynx and larxnx). Localized irritation with associated adaptive responses (e.g. inflammation, epithelial metaplasia, goblet cell hyperplasia, proliferative effects) may occur and are consistent with Category 3 responses.

Squamous metaplasia on the ventral floor of the larynx observed after exposure to 30 mg/l PG aerosol for 40 or 120 min/day for 28 days in rats (Werley et al., 2011) gives supporting evidence that PG has irritation properties[4].

[4] The dossier submitter likes to note that the classification of Category 3 effects are not confined to the upper respiratory tract. The CLP criteria (3.8.2.2.1) defines respiratory tract irritation as 'characterised by localised redness, oedema, pruritis and/or pain) that may impair function with symptoms such as cough, pain, choking, and breathing difficulties'. The clinical symptom 'cough' indicates irritative effects on the lower respiratory tract (trachobroncheal airways).

Increased number/enlarged goblet cells in nasal turbinates primarily in the respiratory epithelium of the nasal turbinates at PG concentrations ≥ 1 mg/L and concentration-related nasal haemorrhages at all doses from 0.16 mg/l during the 90-day exposure period is also supportive for irritation effects (Suber et al., 1989). The observation that the haemorrhage stopped during the week-end non-exposure period indicates an acute, transient effect.

4.3.3 Conclusions on classification and labelling

The criteria for STOT SE 3 (respiratory tract irritant) are fulfilled as shown above. The evidence is mainly based on one human study that demonstrated the respiratory tract irritation in individuals immediate after 1-min inhalation of PG vapour. The study reported throat dryness in 64% of volunteers and impairment of respiratory function, proven by subjects (16% of volunteers) with cough, mild airway obstruction and mild dyspnoea. Furthermore, the Wieslander study presented results obtained by objective measurements such as slight reduction of FEV1. The study included only healthy non-asthmatic volunteers, and those who responded with irritative cough and dyspnoe were non-smokers and had no history of allergies.

Acute inhalation experiments were performed in three species (rat, rabbit and dog) and yielded sparse information on the respiratory tract system. The intolerance and restlessness of dogs at ≥15 mg/L PG that were acutely exposed (for 8 to 60 min) towards increasing PG concentrations supports the classification as respiratory tract irritant. Furthermore, nose bleeding was observed in two rat studies and duration-related increased mucous release and degenerative changes in goblet cells were ultrastructurally observed in the tracheal epithelium of rabbits after 20 or 120 min of exposure to 10% PG in air.

Overall the available animal data from acute inhalation testing can be interpreted as in agreement with the respiratory tract system as target organ due to irritation properties of PG aerosol/vapour at sufficiently high concentrations. The lack of microscopic findings in the respiratory tract of other rat and dog studies are not controversial to the human data: They could be attributed to the lower sensitivity of the test species compared to humans (as indicated by comparison of tested concentrations in animals and those measure/applied in human studies); the fact that a self-reporting of dry throat or other clinical symptoms is limited to humans; the fact that reliable information from comprehensive histopathological examinations on representative tissues of the respiratory tract (comparable to the standards of OECD test guideline 412 or 413) and the application of objective and standardised measurements to substantiate the irritation effects by modern techniques (see Wieslander studies) is lacking in all acute animal studies.

Some supporting evidence on the irritative nature of the effects came also from the repeated dose inhalation studies. Following 28 days of exposure to 30 mg/l for 40 min or 120 min/day induced laryngeal metaplasia in rats (Werley et al. 2011): The only study with microscopic examination on the nasal turbinates revealed increased numbers of goblet cells/increased mucin content at 1 and 2.2 mg/l PG for 6 hours/day, 5 d/week during 90 days (Suber et al., 1989). In this study concentration-related increased incidences of nasal haemorrhages were confined to the treatment days from 0.16 mg/l onwards and stopped during the weekend.

In conclusion, there is sufficient evidence for the classification and labelling of PG as STOT SE 3 (H335, May cause respiratory irritation).

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

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed to classify propane-1,2-diol for specific target organ toxicity (single exposure) Category 3 (STOT SE 3; H335: May cause respiratory irritation) based on transient respiratory tract irritation caused by this substance in several animal and human studies at concentrations much lower than those tested in animals for setting the LC_{50}.

According to the DS, propane-1,2-diol (propylene glycol) does not warrant classification for systemic acute inhalation toxicity. At concentrations much higher (14.4 - 44.9 mg/L) than the LC_{50} values for category 4 for acute inhalation toxicity (LC_{50} ≥1,0 mg/L and ≤ 5,0 mg/L) it does not cause lethal or other severe toxic effects in animals.

To justify the classification as STOT SE 3; H335, the DS provided results from several human and animal studies, which were presented in the CLH report and are summarised in this section.

Human data

1. Acute exposure (one minute) of healthy non-asthmatic volunteers to propane-1,2-diol alone (aerosol [mist] concentration in the range 176 - 851 mg/m^3) at normal aviation emergency training in March 1998 resulted in a sensation of sore and dry eyes, throat dryness and irritative cough (Wieslander *et al*., 2001). Nine out of 25 volunteers (36%) without previous symptoms reported at least one ocular symptom, and 14 out of 23 volunteers (61%) reported throat dryness. Two volunteers reported appearance of nasal catarrh and one had nasal itching, but none reported sneezing or nasal obstruction after the exposure. Further, there were no reports of headache, nausea or breathing difficulties after exposure to propane-1,2-diol, and there was no net change in reporting of fatigue. There were some indications that women and those with a history of atopy seemed to be more sensitive to exposure to propane-1,2-diol for some types of symptoms, but the number of women (n=5) and subjects with atopy (n=8, 2 women and 6 men) were small. In total, 29% of men and 80% of women reported the development of throat symptoms, but there were no sex differences for development of ocular symptoms.

All volunteers participated in the acoustic rhinometry and the lung function test. No significant changes in any measures of nasal patency (data on nasal dimensions as measures of minimum cross-sectional areas and volumes of the nasal cavity measured from 0 and 22 mm and from 23 and 54 mm from the nasal opening) were found after exposure to propane-1,2-diol.

Most of the lung function values remained unchanged after exposure to propane-1,2-diol, but there was a minor numerical decrease of forced expiratory volume in 1 second (FEV1) from 103% to 102% at exposure, and a small but statistically significant decrease of FEV1/FVC (forced vital capacity) (p=0.049).

The mean decrease in FEV1 and FEV1/FVC was similar in subjects with and without a

history of atopy, and there was no significant association between a decrease in FEV1, and development of mild dyspnoea (measured by the subjective rating scale). A few reacted with cough, mild airway obstruction, and mild dyspnea and there were four subjects (16%) developing irritative cough after the exposure (all non-smoking men without any history of allergies). They had an average reduction in FEV1 of 5%, compared with a 0% reduction of FEV1 among those who did not develop a cough. Moreover, those four subjects had an increase in self rated dyspnoea of 13% on the analogue scale, whereas those who did not develop cough only had a 1% increase of dyspnoea, a significant difference between the two groups ($p<0.01$) (Wieslander *et al*., 2001).

The investigation was not a controlled exposure chamber test, but a physiological investigation performed during exposure conditions occurring when propane-1,2-diol mist was used in aviation training. A dose-effect relationship was found for tear break-up time, with a 6-second average decrease in the low exposure group (220 mg/m^3) and a 13-second decrease in the high exposure group (520 mg/m^3). Moreover, 47% out of 18 subjects in the low exposure group, but 100% out of 9 subjects in the high exposure group, reported development of throat dryness, and the intensity of throat symptoms on the subjective rating scale was higher in the highly exposed group. No dose-response relationships were found for ocular and nasal symptoms, dyspnoea, nasal patency or FVV1%. The authors concluded that their observations indicate that short exposure to propane-1,2-diol mist from artificial smoke generators may cause acute ocular and upper respiratory airway irritation in non-astmatic subjects (Wieslander *et al*., 2001).

2. Ocular symptoms, tear film stability, nasal patency, and biomarkers in nasal lavage (NAL) in indoor house painters were studied in relation to use of water-based paints (WBP) and personal exposure to volatile organic compounds (VOC) and volatile organic compounds of possible microbial origin (MVOC) during indoor painting with WBP (Wieslander and Norbäck, 2010). A large proportion of the VOC emissions from WBP consists of propylene glycol, diglycol ethers such as diethylene glycol monoethyl ether, diethylene glycol monobutyl ether, and 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (Texanol).

All house painters from three major companies (n=31) and unexposed controls (janitors from one company; n=20) participated. Tear film break-up time, nasal patency by acoustic rhinometry, and biomarkers in NAL were measured at work, and health status was assessed based on a questionnaire provided by a doctor. Personal sampling (8 h) of formaldehyde, VOC, and MVOC was performed in 17 house painters using WBP (Wieslander and Norbäck, 2010).

The house painters had an increase in ocular symptoms, decreased tear film break-up time, and higher levels of lysozyme in nasal lavage when compared to controls. Painters reporting mucosal irritation from water-based paints had less nasal patency and higher level of myeloperoxidase in nasal lavage.

A large proportion of the VOC measured in the breathing zone of painters consisted of propylene glycol, diglycol ethers, and Texanol. There was an association between 8-h exposure to propylene glycol and level of eosinophilic cationic protein in nasal lavage.

The inhalation exposure of indoor house painters to propylene glycol calculated as the geometric mean amounted to 0.9 mg/m^3 while exposure to other VOC was much lower: diethylene glycol monoethyl ether - 0.05 mg/m^3, diethylene glycol monobutyl ether - 0.04 mg/m^3, and 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (Texanol) - 0.1 mg/m^3. According to the study authors, associations were found in patterns of paint use, and degree of airway irritation with WBP. Associations were also seen between biomarkers and measured exposures to specific compounds, including propylene glycol, 2-phenoxyethanol, sum of aliphatic glycol ethers, and one MVOC (1-octen-3-ol). This suggests that painters using WBP are exposed to compounds that could cause both impaired tear film stability, and eosinophilic and neutrophilic inflammation in the nasal mucosa, and that some painters could have an increased mucosal reaction to paint emissions (Wieslander and Norbäck, 2010). In summary, the results of this study with a mixed exposure toseveral VOCs support the DS hypothesis that propane-1,2-diol may have an irritation effect on the mucosa of the upper airways. However, due to the exposure to various compounds released from the WBPs, the findings cannot be attributed to propane-1,2-diol as the only source of irritative effects on the eyes and nasal mucosa.

3. The National Institute for Occupational Safety and Health (NIOSH) conducted a study in 1991 (Burr *et al.*, 1994) on the use of theatrical fog in Broadway theaters. Personal breathing zone and general area air sampling, and a questionnaire on irritant effects (130 questionnaires from productions with theatrical smoke, 90 questionnaires from productions without theatrical smoke) were collected from personnel from four productions using theatrical smoke and five productions without theatrical smoke. Air samples collected yielded propane-1,2-diol concentrations < 2.1 mg/m³. However, there was a significant increase in the reporting of respiratory irritant symptoms such as runny nose, stuffy nose, and sneezing by personnel from productions using theatrical smoke.

Based on this early study on four Broadway productions using smoke, compared to five productions without smoke, NIOSH concluded that theatrical fogs may contribute to upper respiratory tract problem including sneezing, stuffy noses, coughs, breathlessness, and sore or dry throats. As the Time-Weighted Average (TWA) of the glycols measured during the performances were quite low and one production used glycol and other smoke systems (mineral oil based mist), NIOSH concluded that the aetiology remains unclear and decided to continue the investigations. The glycols detected include ethylene, propylene, 1,3-butylene, diethylene and triethylene glycols. Only the ethylene glycol concentrations were reported to range from undetectable to 21 mg/m³ (TWA). In conclusion, the increased incidences of respiratory tract irritation in actors in the NIOSH study were associated with the use of theatrical fog. The study conclusion is consistent with other studies which have concluded that glycols may be the cause of irritative effects, but it is not possible to identify propane-1,2-diol as the only source of irritation.

4. In a report from the US National Toxicology Programme (NTP, 2004) a study by Moline *et al*. (2000) is summarised as follows:

'Propylene glycol is a component of theatrical fog and is used for special effects. The Actors' Equity Association and the League of American Theaters and Producers sponsored a study (conducted in 1997-99) which included an examination of the health effects of theatrical fog in response to actors' concerns about exposure (Moline et al., 2000). The

health endpoints selected for investigation were irritant effects to the respiratory tract and eyes. This study was conducted over 2 years with 439 actors from 16 musicals, and consisted of a baseline questionnaire, daily checklists, and medical evaluation. There was no clinically significant adverse impact on pulmonary function or in rates of asthma associated with exposure to propylene glycol. However, "peak exposures to elevated localized air concentrations following release of glycol smoke are associated with increased reporting of respiratory, throat, and nasal symptoms, and findings of vocal cord inflammation." The study authors recommended that exposures to propylene glycol by actors not exceed peak or ceiling concentrations of 40 mg/m³.'

For theatrical effects propylene glycol was used in 8 out of 16 musicals. The actors mean time in current shows were 18.4 months (range 0-186 months). For specific show effects propane-1,2-diol was used in 7 studies, while in 6/7 studies other glycols were also used (in 5/7 triethylene glycol, in 4/7 butylene glycol and in 1/7 diethylene glycol). In one show ('Titanic') only propane-1,2-diol was used.

The overall exposure to glycols was low: the average concentrations of total glycols in the preliminary air sampling were in the range of 0.1 to 7.2 mg/m³). Maximum measured short-term exposure concentrations were in the range of 0.37 to 46 mg/m³.

The DS noted that the peak concentrations associated with irritation of the upper respiratory tract were not attributable to propane-1,2-diol alone as the total concentration of the four glycols were estimated and the majority of the shows used fogs that also contained other glycols.

Animal studies with single inhalation exposure

1. The exposure of rabbits by inhalation to aerosol containing 10% propane-1,2-diol in air (with no explanation of whether it was w/v or v/v) for 20 or 120 minutes caused an exposure time-related increase in mucus release and denegeration of globlet cells of the trachea. The 20 min exposure also induced (minimal) ultrastructural alterations (apical small cytoplasmatic blebs) of the ciliated cells. Signs of pathological alterations (cytoplasmic protrusions with destruction of kinocilia) were observed after 120 min. Ultrastructural examinations were only performed on the tracheal epithelium. No other tissues were examined. No data were given on mass median aerodynamic diameter of the particles in the mist (Konrádová *et al*., 1978).

2. No mortality was observed in three groups of rats (three males and three females per group) exposed (nose-only) to capillary aerosol generator (CAG)- propane-1,2-diol (CAG-PG) aerosol at 14.4, 30.5, and 44.9 mg/L for 4h. On study days 1-3 post-exposure, there were 5-10% decreases in body weight in males and females (no data were given to assess whether the findings were concentration-dependent). By study day 7, all rats had returned to normal growth rates and body weights were increased.

No treatment-related clinical signs were observed during or immediately after after inhalation exposure of rats at very high concentrations, but minor bleeding around the eyes and nose was noted at examination of animals performed on day 7 after exposure. However the number of animals affected by the slight localized bleeding around the eyes and nose was not provided (Werley *et al*., 2011).

3. The pulmonary and systemic toxicity of inhaled propane-1,2-diol aerosol was investigated in 2 groups of 5 male/5 female rats exposed for 4 h/day for 7 consecutive days to either 20.8 or 41.0 mg/L propane-1,2-diol aerosol, respectively. Clinical observations, body weights, propane-1,2-diol concentrations in the blood and lungs, histopathological evaluation of the lungs, lung weights, and necropsy were performed during the study. There were no treatment-related clinical observations. Body weights were unaffected by exposure to CAG-PG aerosol. No macroscopic findings were noted at necropsy. There were no effects on lung weights. No histopathological findings were observed in the respiratory tract, however the trachea and nose were apparently not histopathologically examined in this study. Pharmacokinetic analysis indicated that both lung and plasma peak exposures increased approximately dose-proportionately, with a lung-to-plasma ratio close to 0.45. The control group had small but measurable propane-1,2-diol concentrations in the lungs, presumably from other environmental sources, including the rodent chow diet. Propane-1,2-diol aerosol particles were considered to be fully respirable in the rat with mass median aerodynamic diameter (MMAD) values of 0.9 µm and geometric standard deviation of 1.1-1.4. The no-observed-effect level (NOEL) was greater than 41.0 mg/L under the conditions of this study (Werley *et al*., 2011).

4. The pulmonary and systemic toxicity of inhaled propane-1,2-diol aerosol was investigated in 2 male and 2 female Beagle dogs exposed via a face mask to 1.5–30 mg/L either in the ascending phase for 8–60 min depending upon how the exposure was tolerated, or to 5.0 mg/L propane-1,2-diol aerosol for 60 min during the repeated dose phase. Clinical signs before, during and after exposure were monitored; body weights, food consumption, clinical chemistry, haematology, pulmonary function, and necropsy were performed during the dose-ascending and repeated exposure phases. The maximum tolerated dose (MTD) was determined to be 5 mg/L. Animals were generally intolerant to high exposure concentrations of propane-1,2-diol aerosol at 15 and 30 mg/L. Dogs became restless as the exposure concentration to propane-1,2-diol aerosol was increased to the nominal concentration. No further reactions and effects were described. Based on these observations, it was determined that the highest exposure concentration to propane-1,2-diol aerosol should be approximately 5 mg/L to avoid stress in the animals and facilitate exposure (Werley *et al*., 2011). The study did not include a microscopic examination.

Animal studies with repeated inhalation exposure

The DS provided the results of the repeated dose toxicity studies after inhalative exposure as supportive information for STOT SE. They were not submitted to support a classification for specific target organ toxicity – repeated exposure. Information from the registration dossier(s) has been used for preparation of the CLH report. Summaries of the studies which contributed to the proposed classification of propane-1,2-diol as STOT SE 3 are provided below:

1. A 28-d repeated dose toxicity study was performed with Sprague-Dawley rats that were exposed to 30 mg/L propane-1,2-diol aerosol for 4, 12, 40 or 120 min/d. Nominal daily doses were calculated from CAG-generated propane-1,2-diol aerosol concentration, inhalation exposure duration and respiratory minute volume, to reflect the doses that the lung was exposed to by inhalation/respiration. From that nominal dose, the (pulmonary) deposited daily dose was estimated assuming a pulmonary deposition fraction of 10% in

the nose-only exposed rat.

The measured MMAD for propane-1,2-diol aerosol sampled from the plenum and used to expose each treatment group was 2.29 µm with a geometric standard deviation (GSD) of 1.56.

Histopathology investigations revealed the following results:

The most prevalent finding was laryngeal squamous metaplasia, described as "minimal", on the ventral floor of the larynx, in the 40 min/d and high dose (120 min/d) groups. The normally cuboidal cells were flattened, to layers of squamous epithelium. Inflammatory cell infiltration ranging from minimal to moderate was observed in the lungs of both sexes, but this was not statistically significantly higher than the control group, even though the pooled incidence for "minimal", "mild", and "moderate" inflammatory cell infiltrate in treatment groups was greater than observed for the controls. No other biologically significant effects were observed by histopathological investigations conducted on the tissues and organs. The NOEL for the 28-d rat study was determined to be 30 mg/L for 12 min/d (Werley *et al*., 2011).

2. A 28-d repeated dose toxicity study was performed in Beagle dogs that were exposed to 5 mg/L propane-1,2-diol aerosol for 6 min/d, 12 min/d, 36 min/d or 60 min/twice a day (4 animals/sex/group). Target exposure concentrations and durations were selected to attain the following doses deposited in the lung: 3, 6, 18 and 60 mg/kg bw/d. The measured MMAD for the CAG-generated PG aerosol sampled from the plenum and used to expose each treatment group was 1.34 µm with a GSD of 1.45.

Histopathology investigations revealed the following results:

Sporadic findings of squamous hyperplasia of the larynx, inflammatory cell infiltration in the trachea and alveolar lung, alveolar macrophage accumulation, and congestion/haemorrhage in the lung were reported. None of these findings were significantly higher than air-exposed controls, and there appeared to be no clear treatment- or dose-related pattern in the findings. Indeed, the study director indicated that changes reported were "considered to be typical of spontaneously arising background findings, which are common in inhalation exposure studies in dogs at this laboratory". No other biologically significant effects were observed by histopathology on the tissues and organs. In the 28-d study, the NOEL was determined to be 5 mg/L for 12 min in the Beagle dog (Werley *et al*., 2011). However, this is not conclusive regarding that no dose-related effect was observed after 60 min/twice a day exposure duration. The daily exposure time was very short.

3. A subchronic inhalation toxicity study with rats exposed to propane-1,2-diol aerosol at dose levels of 0.0, 0.16, 1.0 and 2.2 mg/L air for 6 hr/day, 5 days/week for 90 days was reported by Suber *et al*. (1989). A treatment-related effect was reported as nasal haemorrhage which began during the second week of exposure and persisted throughout the study; recovery from these clinical signs occurred during the non-exposure weekend periods.

The frequency of this reported nasal haemorrhage remained constant throughout the study (but as stated above, disappeared during the non-exposure weekend periods) and

was highest (65-75%) in the medium-and high-concentration groups.

Similar trends were observed for ocular discharge, with incidences of 16% in low-exposure males, 40% in medium- and high-exposure males and 5% in controls. There was generally less ocular discharge in females, who had incidences of 8% in controls, 14% in the low-exposure group, 28% in the medium-exposure group and 35% in the high-exposure group. Minute volume, tidal volume and respiratory rates were not significantly altered at any dose levels.

No adverse changes in gross pathological and histopathological examinations were noted, except for an increase in the number of goblet cells or an increase in the mucin content of the goblet cells present, observed in the nasal turbinates of both male and female rats at ≥1 mg/L. In addition, white blood cell counts revealed a concentration-related decrease in total white blood cells in mid- and high-concentration females, a decrease in banded neutrophils in mid-concentration females and high-concentration males and females, and a decrease in lymphocytes in mid- and high-concentration females.

Based on the reported nasal hemorrhage and ocular discharge at all dose levels (Suber *et al*., 1989), the lowest dose level of 0.16 mg/L is considered to be a LOAEC for local effects. The reported nasal "haemorrhage" observed during the exposure period was not confirmed by microscopic evidence of tissue damage after 90 days. The increased number of goblet cells and/or increased mucin content in the mid- and high-dose groups were interpreted by the authors to be a result of physical irritation of propane-1,2-diol upon the nasal epithelium in the rat.

Comments received during public consultation

Two MSCAs supported the proposal of the DS to classify propane-1,2-diol as STOT SE 3; H335: May cause respiratory irritation.

Forty-two individuals, 7 industry or trade associations, 5 companies/manufacturers, 5 companies/downstream users and three NGOs disagreed with the proposed classification of propane -1,2-diol. Some of the arguments against respiratory irritant effects of propane-1,2-diol provided during public consultation are summarised below:

- Propane-1,2-diol was used as carrier for the aerosolisation of cyclosporine, which is used as an anti-rejection drug, in two studies with lung transplant patients (Burckart *et al*., 2003; Corcoran *et al*., 2014). The method was assessed as successful in effective delivery of this drug to the lung of transplant patients in the early postoperative period. None of the publications gave any information on the effects of propane-1,2-diol alone.

- Propane-1,2-diol was used as carrier for aerosolisation of cyclosporine in a study in Beagle dogs, aimed at evaluation of safety and toxicology of cyclosporine after 9 month aerosol exposure. This study did not contain a propane-1,2-diol vehicle group. According to the study, the animals received a dose of 90 mg propane-1,2-diol/kg bw/d by inhalation. However, gross pathological investigations and microscopic investigations did not show findings of any type associated with the respiratory tract (Niven *et al*., 2011).

- One individual noted that the classification proposal is entirely based around the specific

use of the substance monopropylene glycol (MPG, propane-1,2-diol) in two very specific and minor applications (in tonnage terms) - as a carrier in e-cigarettes and in generating theatrical fogs. However, no evidence is offered that any adverse effects resulted from vapour exposure. Therefore, it was proposed to submit, instead of a classification proposal, an Annex XV restriction proposal under REACH covering these two identified uses. According to this commenter this would be a far more targeted approach that would allow a proper consideration of the hazard data against the socioeconomic benefits and the hazards of likely alternatives.

- One individual noted that billions of users of electronic cigarettes have been inhaling propane-1,2-diol daily, for many years, without experiencing any adverse effects, and that this experience showing that propane-1,2-diol is not dangerous for the respiratory system should be taken into account.

- One Company/Downstream user noted that there is the evidence that in emergency trainings, carried out since 1985, comparable to those described in the Wieslander (2001) study with a total of about 54,000 people, no irritation/adverse effects have occurred. Occasional adverse effects seen could be attributed to psychosomatic causes rather than to substance-based effects of propane-1,2-diol. (Krieg, 2015)

- One industry or trade association noted that according to the ECHA dissemination webpage only a minor percentage (0.16%) of notifiers have reported STOT SE 3; H335 for propane-1,2-diol (C&L inventory: 4966 notifiers notified no-self classification and only 8 notifiers notified STOT SE 3; H335). In addition, propane-1,2-diol is a registered substance under REACH for a high tonnage band (tonnage band 100 000 – 1 000 000 tonnes per annum). The 68 registrants argued that propane-1,2-diol should not be classified as STOT SE 3; H335, based also on a sub-chronic nose inhalation study in Sprague-Dawley rats (Suber *et al*., 1989). According to this comment, the REACH registration dossier of propane-1,2-diol was not considered in the dossier submitter's proposal.

- One individual noted that propane-1,2-diol has been, and still is, one of the main ingredients of well-known and approved medicinal inhalers. Furthermore, it is also widely used as a suspension agent for water soluble flavorings, an antibacterial agent for beauty products such as soap, shower gels, shampoos, conditioners, moisturising creams, etc.

- One industry or trade association noted that the currently available evidence is not convincing for propane-1,2-diol as a causative for respiratory tract irritation. There are no credible histopathology reports in the animal studies that document propane-1,2-diol-induced cytotoxicity or inflammation in the respiratory tract of inhalation-exposed laboratory animals. The effects reported for propane-1,2-diol in humans and animals do not indicate irritation responses and are more likely indirect effects of the local drying of the airway mucosa due to the hygroscopic nature of this substance. These effects are not harmful or adverse, and are rather adaptive to the minor physiological change. According to this trade association, out of four analyzed studies only the study of Wieslander *et al*. (2001) was able to show some association of any observed respiratory irritant effects with propane-1,2-diol exposure.

The effects in the Wieslander study could fulfil the criteria for respiratory irritant effects

because the study demonstrated that short exposure to propylene glycol leads to a reduction in FEV1 from 103% to 102% at exposure, and to a small but significant decrease of FEV1/FVC (p=0.049). However, the commenting industry/trade association noted that this 1% change in FEV1 post-exposure is neither statistically or clinically significant, especially since post-exposure values were even 102% of predicted for this health cohort. The small, albeit significant, decrease in the FEV1/FVC ratio is also not indicative of impairment of lower airways as the ratio was greater than 80% both pre- and post-exposure, indicating an absence of any obstructive defect (American Thoracic Society, 2005). A 5% decrease in FEV1, shown by only 4 out of 27 volunteers, cannot be considered significant or indicative of lung impairment due to exposure to a respiratory irritant, as this decrease is well within the normal variation expected with repeated spirometric measurements. Such variability is inherent in the spirometry test procedure, which relies completely on the willingness of the subject to expend maximal effort in test trials. The Society guidelines for interpretation are clear that even a 'statistically significant change may be of no clinical relevance' and that the 'largest errors occur when attempting to interpret serial changes in subjects without disease because test variability will usually far exceed any true decline' (American Thoracic Society, 2005).

As to the subjective reports of 'throat and ocular dryness' in the study of Wieslander *et al*. (2001), it should be noted that "the sensation of smell, unpleasant taste, tickling sensation and dryness…." is outside the scope of classification for respiratory irritation. Thus, as concluded in the comment from this industry/trade association, on the basis of the EU criteria reports of 'dryness' cannot be considered as indicative of respiratory irritation. Therefore, the available scientific human data do not support the classification of propane-1,2-diol as a respiratory irritant in humans.

In the comment from this industry/trade association, the available animal data were not considered to support the classification of propane-1,2-diol as a respiratory tract irritant either. The published papers by Robertson *et al*. (1947) and Konrádová *et al*. (1978) were not considered to be of sufficient quality, due to their limited experimental designs and methodologies, the limitations including a small numbers of animals/group, lack of adequate control animals, lack of rigorous statistical analysis, poor or no standardized and unbiased histopatological examination, approaches that are mandated in current animal toxicology and safety assessments. According to this comment there are no microscopic findings in the respiratory target organs of laboratory animals exposed by inhalation to propane-1,2-diol aerosol that could be labeled as a histopatological finding or morphologic adverse outcome in the targeted tissues.

In this comment it was noted that propane-1,2-diol is strongly hygroscopic and miscible with water under normal physiologic conditions (ATSDR, 1997). Many of the propane-1,2-diol uses take advantage of its physico-chemical hydroscopic properties, therefore this property would similarly be anticipated to potentially dehydrate moist mucus membranes that may impart sensory symptoms and tissue adaptation responses. These same symptoms occur in low humidity climates to which adaptation occurs. Thus, the effects are not harmful or adverse and instead adaptive to the minor physiological change. When deposited as a vapor or aerosol on the apical surface of the airway mucosa, propane-1,2-diol will rapidly absorb water from the protective epithelial lining layer. The likely result of this is a rapid local increase in osmolarity. The drying effect of propane-1,2-diol is analogous to breathing dry air, which can result in decreased cell volume (Van Oostdam

et al., 1986) and may result in epithelial changes (Chalon *et al*., 1972; Freed *et al*,. 1994; reviewed by Anderson and Holzer, 2002). Sensory nerve endings lining the conducting airways are sensitive to changes in osmolarity (Pisarri *et al*., 1992) and cell volume as evidenced by the cough that occurs in healthy human subjects inhaling nonisotonic aerosols (Eschenbacher *et al*., 1984; Higenbottam, 1984). The drying effect of inhaled propane-1,2-diol may be the underlying basis for the reported cough and feeling of airway irritation and a feeling of dyspnea reported in volunteers exposed to high concentrations (220 and 520 mg/m^3) of propane-1,2-diol and/or other hydroscopic substance aerosol (Wieslander *et al*., 2001) as well as in stage actors and show personnel exposed to glycols in theatrical fogs (Moline *et al*., 2000; Burr *et al.*, 1994). In the NIOSH study, the fogs were generally composed of a mixture of glycols, with less than 2.1 mg/m^3 of propylene glycol and the reported concentrations were reported as TWA from personal and area monitors. While these exposures were associated with self-reporting of nasal symptoms (sneezing, runny or stuffy nose), respiratory symptoms (cough, wheeze, breathlessness, chest tightness), and mucous membrane symptoms (sore throat, hoarseness, dry throat, itchy, burning eyes) during their performances, no objective analytical measures were linked to these reports and the possibility of transient high exposure concentrations could not be ascertained from the reported TWA values.

An increase in osmolarity can also result in hypersecretion by mucous goblet cells of the surface epithelium and submucosal seromucous glands (Dwyer and Farley, 1997). The physical drying effect of inhaled propane-1,2-diol aerosol is the likely mechanism leading to the observation of rapid hypersecretion of mucins from mucous goblet cells in the trachea of rabbits exposed for 20 or 120 minutes to 10% propylene glycol aerosols (Konradova *et al*., 1978). In this ultrastructural study propane-1,2-diol exposure resulted in an increase in partially or fully discharged goblet cells. No recovery group was included in this study so the persistence of the morphologic alterations cannot be determined. The data from repeat exposure studies, however, suggest that exposure to high aerosol concentrations of propane-1,2-diol do not induce epithelial injury or inflammation. Suber *et al*. (1989) exposed male and female Sprague Dawley rats to 0, 160, 1000, or 2200 mg/m^3 of propane-1,2-diol aerosol 6 h/day, 5 days/week for 90 days. Rats exposed to the two highest concentrations of propane-1,2-diol developed mucous cell hypertrophy/ hyperplasia in the nasal respiratory epithelium as evidenced by an increase in the amount of stored AB/PAS (Alcian Blue / Periodic Acid Schiff) stain sequence positive glycoproteins in mucous goblet cells. This is suggestive of an adaptive response to protect the epithelium from the repeated drying effects of high concentration propylene glycol aerosol exposure. There were reports of nasal haemorrhage and ocular discharge in a high proportion of the animals, however, there was no histopathologic evidence of nasal epithelial injury and there was no evidence of haemorrhage or ocular discharge on weekends when the animals were not exposed. This suggests that the observations, if not just porphyrin staining, were likely due to increased nasolacrimal discharge resulting from the drying effects of the propane-1,2-diol aerosol.

Therefore, the available evidence suggests that the reported findings in human and animal studies associated with exposure to high levels of propane-1,2-diol aerosol are the result of the physico-chemical properties of propane-1,2-diol (e.g. hygroscopic and highly water soluble) and not the result of chemical toxicity. Furthermore, there is no evidence that propane-1,2-diol is a sensory irritant. Suber *et al*. (1989) reported that male and female rats exposed to 160, 1000 or 2200 mg/m^3 of propane-1,2-diol had no change in

breathing frequency, minute volume or tidal volume. A decrease in breathing frequency in rodents is typical of a sensory irritant and serves to limit exposure to noxious xenobiotics by reducing the total inhaled dose.

Overall, according to this commenting party, the data demonstrate a lack of direct epithelial toxicity and rather suggest an adaptive response often associated with nontoxic irritant vapors and aerosols. The lack of reported airway epithelial injury or inflammation suggest that any perceived irritating effects of high concentration propane-1,2-diol aerosols are indirect effects of the local drying of the airway mucosa due to the hygroscopic nature of propane-1,2-diol. The Guidance on the application of the CLP criteria (ECHA, 2015) clearly states that 'the sensation of smell, unpleasant taste, tickling sensation and dryness….' are outside the scope of classification for respiratory irritation'. It was also announced by the commenting party, that a new study is planned that will clarify propane-1,2-diol's effects on the human respiratory tract. The major producers of propane-1,2-diol are sponsoring a new human study to objectively assess the potential for propane-1,2-diol aerosols to cause respiratory tract irritation. The preliminary results of that study titled as "Evaluation of respiratory and ocular irritation from propylene glycol in healthy humans" (Dalton, 2016) were distributed as a room document at the RAC 39th meeting on 1 December 2016. The results suggested that inhalation exposures of healthy persons to propane-1,2-diol at concentrations of 20 mg/m^3 or 100 mg/m^3 for 4 hours or at concentration of 200 mg/m^3 for 30 minutes does not cause changes in FEV1 or the FEV1/FVC ratio or in ocular hyperaemia, although small exposure-related change in subjective symptoms such as dryness of eye, nose and throat was reported. According to the author of the study (Dalton, 2016) the results indicate that, at the concentrations and durations tested, propane-1,2-diol is not a respiratory or ocular irritant.

Assessment and comparison with the classification criteria

According to the CLP Regulation, STOT SE 3 only covers narcotic effects and respiratory tract irritation. The effects warranting classification of the substance in category STOT SE 3 are the effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

No narcotic effects were observed in animal and human studies, therefore only symptoms related to the respiratory tract can be considerd in this evaluation.

The respiratory symptoms observed in animal and human toxicity studies, as summarised above, do not demonstrate that transient effects caused by propane-1,2-diol meet the criteria for classifying substances as Category 3 for respiratory tract irritation as specified in point 3.8.2.2.1 of CLP Regulation:

(a) respiratory irritant effects (characterized by localized redness, oedema, pruritis and/or pain) that impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. This evaluation will be based primarily on human data.

(b) subjective human observations could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers

of inflammation in nasal or bronchoalveolar lavage fluids).

(c) the symptoms observed in humans shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. Ambiguous reports simply of "irritation" shall be excluded as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation.

(d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.

(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed.

Human studies

Out of the human studies reviewed by the DS, only in the study of Wieslander *et al*. (2001) were humans exposed to propane-1,2-diol alone. However, a very low concentration of formaldehyde (29 µg/m^3) was detected in the flight simulator, where exposure to propane-1,2-diol was carried out. In the other human studies, people were exposed to a mixture of propane-1,2-diol and other glycols or other substances. Regarding the purity of the substance used in the study of Wieslander *et al*. (2001), it is mentioned in the study description that propane-1,2-diol used to produce the artificial smoke in this study was a commercial propane-1,2-diol solution used for theatrical fog/smoke generation.

Twenty-two men and five women (n=27) volunteered to participate in the Wieslander *et al*. study (2001). Most of the subjects were pilots working in civil aviation. The exposure to propane-1,2-diol was performed as part of the regular training for pilots aimed to train them for evacuation at fire emergency situations. The exposure lasted only for 1 minute, but the level of exposure varied from 200 mg/m^3 to 300 mg/m^3 during a time period between 10:20 and 12:00, and then from 13:00 until 14:50 the exposure level was approximately 300 mg/m^3 - 850 mg/m^3. Information on current symptoms before and after exposure was obtained using two questionnaires. One questionnaire sought a subjective rating of ocular, nasal and throat symptoms, dyspnoea, malodour and systemic symptoms, with a possibility to grade responses from "not at all" to "almost unbearable" using an adopted visual analogue rating scale from 0 to 100 mm. A second questionnaire sought information on occurrence or non-occurrence of these symptoms.

An average group rating (n=27) of intensity of three symptoms was significantly increased after 1 minute exposure to propylene glycol, although it remained at a relatively low level on the scale of intensity from 1 to 100. The mean score (±SD) for ocular irritation was increased from 5(10) to 14(13), for throat irritation from 7(9) to 20(14), and for difficulty in breathing from 3(4) to 7(10). Average group scaling of

complains such as nasal irritation, solvent smell, headache, fatigue, nausea, dizziness and intoxication was not significantly changed after exposure to propane-1,2-diol.

In a second questionnaire with possible 'Yes' or 'No' answers for a group of eye ailments, the highest proportion of those developing a particular symptom complained about dry eyes (31%) and sore eyes (19%). There were no complains on eye redness or swollen eyelids. In a group of throat ailments the highest proportion complained about throat dryness (61%), with no increase in complains of sore throat. Four out of twenty-five persons (16%) reported irritative cough, but the proportion of those with difficulties in breathing was unchanged. No increase in complains due to nasal or other ailments was noted. In summary, the dominant and only symptoms with increased incidence were related to dryness of eyes and throat. Such symptoms could have been explained by hydroscopic property of propane-1,2-diol leading to dehydration of mucous membrane in more sensitive people. This property was most probably responsible for the decrease of time of tear film stability after 1 minute exposure to propane-1,2-diol (mean decrease 6 seconds). The measurement of tear film stability is a clinical test used to assess ocular surface dryness. No significant changes were found in any measures of nasal patency indicating lack of significant adverse effects on nasal mucous membranes.

Most of the lung function values remained unchanged after exposure to propane-1,2-diol, but there was a minor numerical decrease of FEV1 from 103% to 102% after exposure, and a small but significant decrease of FEV1/FVC (p=0.049). Mean VC was unchanged after the exposure, whereas FVC was slightly increased. None of the 27 participants had an initial FEV below 80% of predicted value, but one got a 77% value for FEV, after the exposure. The mean decrease of FEV1 and FEV1/FVC was similar in subjects with and without a history of atopy. Moreover, there was no evidence of significant associations between a decrease in FEV1, and development of mild dyspnoea (measured by the rating scales) in the data.

Taking into account variability in results of spirometric test even for the same person, it is highly questionable whether a minor decrease of FEV1 from 103% to 102% after exposure is an indicator of respiratory toxicity of propane-1,2-diol. The variability of the results of the spirometric test was reflected in the description of the methodology of this study: "The measurements were performed three times on each subject, and the highest values were noted. A test was considered adequate when the deviation between the two most reliable tests were less than 5%. The results were expressed as a percentage of expected values based on standardisation for age, sex, height, smoking habits, and body mass using reference values from Uppsala" (Wieslander *et al*., 2001). The statistical analyses performed by the authors of the study (Wieslander *et al*., 2001) did not reveal any statistically significant differences between spirometry values obtained 10 minutes before and 10 minutes after exposure to propane-1,2-diol for all measured functional parameters such as VC, FVC, Peak Expiratory Flow, FEV1. Only a mean FEV1/FVC ratio calculated after exposure – 84.8±6.5 approached statistical difference with a mean ratio FEV1/FVC calculated before exposure of – 86.8±7.3 (two tailed p-value=0.049); however, the clinical and biological significance of this difference is rather low. Overall, noting known variability in spirometry measurements, RAC concludes that these results do not provide sufficient evidence that propane-1,2-diol affected pulmonary functions of exposed persons in the study of Wieslander *et al*. (2001).

In the other studies (Wieslander and Norbäck, 2010; Burr *et al.*, 1994, NTP, 2004) humans were exposed to mixtures, containing in some instances propane-1, 2-diol, therefore they cannot be used for assessment of propane-1,2-diol.

In summary, RAC is of the opinion that the evidence from human studies indicate that single exposure to propane-1,2-diol may induce transient irritation of respiratory and ocular mucosa as indicated by the decreased time of tear film stability or increased frequency of complains related to dryness of eyes and throat. However, these effects do not meet the criteria for classifying propane-1,2-diol as STOT SE, as specified in point 3.8.2.2.1 of CLP Regulation.

Animals studies

In the study of Konrádová *et al*. (1978) a time-related increase in mucus release and degeneration of the globlet cells of trachea were observed in 6 rabbits exposed by inhalation for 20 or 120 minutes to an aerosol of 10% propane-1,2-diol in air (with no explanation of whether it was w/v or v/v). 20 min exposure induced also minimal ultrastructural alteration (apical small cytoplasmatic blebs) of the ciliated cells. However, the results were difficult to compare against the classification criteria because only ultrastructural examinations were performed on the tracheal epithelium and no control animals were examined. It is not known whether these exposures were leading to hyperaemia, oedema, minimal inflammation or thickened mucous layer of trachea as required to support classification. The observed alterations could be reactions to dehydration of the tracheal epithelium due to the hygroscopic property of propane-1,2-diol.

In the acute animal toxicity study (Werley *et al*., 2011) clinical observations immediately after exposure did not revealed any signs of toxicity. Slight localised bleeding around the eyes and nose of some rats (number of affected animals was not reported) which were noticed 7 days after exposure, does not correspond to symptoms of transient respiratory tract irritation, and could be accidental, since occurence of such symptoms was not confirmed in other studies on rats and dogs. Overall, the study does not provide evidence of respiratory irritant effects which could meet the classification criteria. No mortality was observed in male or female rats exposed by inhalation for 4 hours to respirable aerosol (mean MMAD 1.1-1.4 µm with a GSD of 1.1-1.4 µm) of propane-1, 2-diol at concentrations of 14.4 mg/L, 30.5 mg/L and 44.9 mg/L (Werley *et al*., 2011).

In the 7-day inhalation toxicity study in rats, two groups of 5 males/5 females were exposed for 4 h/day for 7 consecutive days to either 20.8 or 41.0 mg/L propane-1,2-diol aerosol,respectively. No histopatological findings were observed in the respiratory tract of rats in this study (Werley *et al*., 2011).

In the 28-d inhalation toxicity study of propane-1,2-diol (Werley *et al*., 2011) thirty-one rats/sex/group were assigned to air control, low, mid-1, mid-2 and high exposure groups. Rats were exposed in a flow-past nose-only exposure chamber to 30 mg/L propane-1,2-diol aerosol for up to 120 min/d. Control group animals were exposed to room air only. Target exposure concentrations and durations were selected to attain the following doses deposited in the lung: 7.2, 21.6, 72.0, and 216.0 mg/kg bw/d. In this study the most prevalent finding was laryngeal squamous metaplasia, described as "minimal" on the ventral floor of larynx, in the mid-2- and high-dose inhalation exposure groups

(corresponding to daily deposits in lungs of 72.0, and 216.0 mg/kg bw/d). The normally cuboidal cells were flattened, to layers of squamous epithelium. Inflammatory cell infiltration ranging from minimal to moderate was observed in the lungs of both sexes, but this was not statistically significantly higher than in the control group, even though the pooled incidence for "minimal", "mild", and "moderate" inflammatory cell infiltrate in treatment groups was greater than observed for the controls. Lung "congestion/haemorrhage" was also reported but the highest incidence was found in the control group males exposed to room air. No other biologically significant effects were observed by histopathology on the tissues and organs. The NOEL for the 28-d rat study was determined to be approximately 20 mg/kg bw/d (Werley *et al*., 2011). According to the authors (Werley *et al*., 2011) in the rat studies, there were no histopathological correlates in the rat lung that showed changes to the tissue mucosa or morphological structure indicative of an inflammation response.

In the MTD study with propane-1,2-diol aerosol (Werley *et al*., 2011), 2 male and 2 female Beagle dogs were allocated to an ascending dose phase and a 7-d repeated dose phase. Dogs were exposed to 1.5–30 mg/L in the ascending phase for 8–60 min depending upon toleration of exposure, and 5.0 mg/L propane-1,2-diol aerosol for 60 min during the repeated dose phase. Evaluations of pulmonary function, haematology, clinical chemistry, body weight, food consumption, and macroscopic evaluation of tissues and organs at necropsy were all unremarkable (data not shown). Repeated inhalation exposure to propane-1,2-diol aerosol at 5 mg/L for up to 60 min duration was well-tolerated in the Beagle dogs, and this was considered to be the MTD.

In the 28-d inhalation toxicity study with propane-1,2-diol in Beagle dogs, 4 males and 4 females per group were assigned to air control, low, mid-1, mid-2 and high exposure groups. Dogs were exposed via a closed face mask to 5 mg/L of propane-1,2-diol aerosol for 3–31 min, except for the high exposure group which was dosed twice per day, from 37 to 49 min per treatment session. Air control group animals were exposed to room air using the face mask. Target exposure concentrations and durations were selected to attain the following doses deposited in the lung: 3, 6, 18 and 60 mg/kg bw/d. Sporadic findings of squamous hyperplasia of the larynx, inflammatory cell infiltration in the trachea and alveolar lung, alveolar macrophage accumulation, and congestion/haemorrhage in the lung were reported. None of these findings were significantly higher than air-exposed controls, and there appeared to be no clear treatment or dose-related pattern to the findings. Indeed, the study director indicated that changes reported were "considered to be typical of spontaneously arising background findings, which are common in inhalation exposure studies in dogs at this laboratory". No other biologically significant effects were observed by histopathology on the tissues and organs (Werley *et al*., 2011).Therefore the authors concluded: "In the dog, no histopathological effects on the laryngeal, tracheal and lung tissues were observed that could clearly be related to exposure to PG aerosol." The observed findings were believed to be spontaneously arising and commonly found in Beagle dogs at this laboratory.

A subchronic inhalation toxicity study with rats exposed to propane-1,2-diol aerosol at dose levels of 0.0, 0.16, 1.0 and 2.2 mg/L air for 6 hr/day, 5 days/week for 90 days (Suber *et al*., 1989) lead to nasal haemorrhaging beginning during the second week of exposure and persisted throughout the study, with transient recovery during weekends

without exposures. However, since effects were seen after repeated exposure only they do not conform with the classification criteria for STOT SE 3 for transient respiratory tract irritation.

In summary, in the opinion of RAC, the results in animal studies do not provide sufficient evidence that a single exposure to propane-1,2-diol by inhalation may induce clinical signs of toxicity (dyspnoea, rhinitis, etc.) and/or histopathological changes (e.g. hyperaemia, oedema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above.

Taking into account the available human and animals data **RAC is of the opinion that propane-1,2-diol does not warrant classification as STOT SE 3 (H335, May cause respiratory irritation)**.

4.4 Irritation

Not evaluated for this dossier.

4.5 Corrosivity

Not evaluated for this dossier

4.6 Sensitisation

Not evaluated for this dossier.

4.6.1 Skin sensititsation

Not evaluated for this dossier.

4.6.2 Respiratory sensitisation

Not evaluated for this dossier.

4.7 Repeated dose toxicity

The results of the repeated dose toxicity studies after inhalative exposure will be used as supportive evidence for specific target organ toxicity – single exposure are not submitted to support classification for specific target organ toxicity – repeated exposure. Information of the registration dossier(s) has been used for preparation of the CLH report.

Table 11: Summary table of relevant repeated dose inhalation toxicity studies

Method	Results	Remarks	Reference
Sprague-Dawley rats, 6-8 weeks old, Charles River Laboratories, Kingston, NY, USA. Rats were exposed nose-only. In a 28-day study 15 male and 15 female rats were exposed daily for 180 min (for the first 3 days) and 90 min thereafter towards a dose of 15,3 mg/L weight/day. Only one dose group was investigated since the study was focused on the investigation of cyclosporine and PG was used as vehicle. An additional control group was exposed to air only. No PG concentrations in air are available.	The animals of the low Cyclosporine and the (vehicle) PG group were noted sporadically and less frequently with rough coat and/or a wet urogenital region following exposure. However the observations could neither be attributed to the group nor are the incidences given. The authors concluded when compared to air, no local or systemic effects of PG were observed.	Supporting study Reliable with restrictions Histopathologic examinations were conducted, information on which organs/tissues were assessed is lacking. At least microscopy on the kidney and the trachea was conducted.	Wang et al., 2007
Beagle dogs (10-14 month old, Ridglan Farms, Inc. Mt. Horeb, WI, USA. Dogs were exposed mouth-only. In a 28-day study five male and five female dogs were exposed daily for 60 min towards a dose of 13.45 mg/L/day. Only one dose group was investigated since the study was focused on the investigation of cyclosporine and PG was used as vehicle. An additional control group was exposed to air only.	Excessive salivation was also noted in all groups including the vehicle group. The incidence of salivation was more predominant in the (Cyclosporin) male and female mid-dose group and high dose females. The authors concluded, when compared to air, no local or systemic effects of PG were observed	Supporting study Reliable with restrictions Histopathologic examinations were conducted, information on which organs/tissues were assessed is lacking. At least microscopy on the larynx, trachea, tonsils, lungs, liver kidney and stomach was conducted.	Wang et al., 2007
Sprague-Dawley rats, (HSD), 5-6 weeks old, Harlan UK Limited. Animals were acclimated to the laboratory for two weeks before assignment to study. They were about 7-8 weeks old at study initiation and weighed 182-245 and 144-206 for males and females, respectively. 31 rats/sex/group were assigned to an air control, low, mid-1, mid-2 and high exposure groups. Rats were exposed in a flow-past nose-only exposure chamber to 30 mg/L PG aerosol for 4 min, 12 min, 40 min or 120 min per day. Control group animals were exposed to room air only. Target exposure concentrations and durations were	The only biologically significant effect was a laryngeal squamous metaplasia, described as “minimal” on the ventral floor of larynx, in the mid-2 (30 mg/L for 40 min/day) and high dose exposure groups (30 mg/L for 120 min/day). The NOEC for this 28day rat study was determined to be 30 mg/L for 12 min/day).	Supporting study Reliable with restrictions The larynx, trachea and lung were included in the histopathological examinations. No information is available on the nasal cavity and nasopharynx.	Werley et al., 2011

selected to attain the following doses deposited in the lung: 7.2, 21.6, 72.0, and 216.0 mg/kg/day. Biological endpoints used to assess potential pulmonary and systemic toxicity included daily clinical signs, body weights, food consumption, ophthalmoscopic and electrocardiography examinations, pulmonary function, haematology, clinical chemistry, urinalysis, necropsy, histopathology, and toxicokinetics			
Twenty male and 20 female beagle dogs (Marshall Farms (North Rose, NY, USA)) were assigned to the study. On commencement of dosing the animals were about 6.25 months old and weighed 6.6–8.5 kg and 5.8–7.6 kg for males and females, respectively. Four male and four female Beagle dogs per group were assigned to an air control, low, mid1, mid2 and high exposure groups. Dogs were exposed via a closed face mask to 5 mg/L PG aerosol for 6 min, 12 min or 36 min, except for the high exposure group which was dosed twice per day for 60 min. Air control group animals were exposed to room air using the face mask. Target exposure concentrations and durations were selected to attain the following doses deposited in the lung: 3, 6, 18 and 60 mg/kg/day. Biological endpoints used to assess potential pulmonary and systemic toxicity included daily clinical signs, body weights, food consumption, ophthalmoscopic and electrocardiography examinations, pulmonary function, haematology, clinical chemistry, urinalysis, necropsy, histopathology, and toxicokinetics.	In this 28 day study, the NOEL was determined to be 5 mg/l for 60 min in the Beagle dog.	Supporting study Reliable with restrictions The larynx, trachea and lung were included in the histopathological examinations. No information is available on the nasal cavity and nasopharynx.	Werley et al., 2011
rat (Sprague-Dawley) male/female subchronic (inhalation) (nose only) 0, 160, 1000, 2200 mg/m^3 (nominal conc.) 0, 0.16 ± 0.04, 1.01 ± 0.11 and 2.18 ± 0.31 mg/l (analytical conc.) Vehicle: air Exposure: 90 days (6 hours/day, 5 days/week) Groups of 19 male and female rats were exposed by inhalation to 0.0,	Nasal haemorrhages from week 2-13 (disappeared during the week-end non-exposure period): concentration-related average incidences were <1% in controls, 64%, 74% and 75% in low, mid and high dose groups in males and <1% in controls, 15% (4% after the fourth week), 71%, and 71% in low, mid and high dose groups of females. Increased number of goblet cells	Supporting study Reliable with restrictions The respiratory tract including nasal passages, lungs, trachea and larynx (and 12 other organs) were	Suber et al., 1989

0.16, 1.0 and 2.2 mg/l air PG for 6 hr/day, 5 days/week for 90 days. The clinical signs, body weights, food consumption, haematological parameters, clinical chemistry and gross and histopathological examinations were performed. Respiratory rates and tidal volumes were measured in 4 rats/sex/group on day 7, and repeated in same animals on day 42 and 84.	or enlarged goblet cells due to increased mucin content in nasal turbinates at ≥ 1.0 mg/l, observed in the posterior portion of the nasal cavity, lining of the septum, the lateral walls, the anterior turbinates and in selected cases in ethmoid turbinates. There was no histological change in the trachea, lungs or larynx and no effect on respiratory rates, tidal and minute volumes. 5-7% decreases in body weights, correlated with reduction in feed consumption in the high-dose females. Average incidences of ocular discharge were 5% in controls, 16%, 40% and 40% for males, <1% in controls, 14%, 71% and 71 % in females for low, mid and high dose groups. $NOAEC_{systemic}$: 2.2 mg/l air No adverse systemic effects observed at the highest tested dose. $LOAEC_{resp\ tract}$: 0.16 mg/l air Based on reported concentration-related nasal haemorrhaging in all test groups.	examined by light microscopy.	

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Not evaluated for this dossier.

4.7.1.2 Repeated dose toxicity: inhalation

The results of the repeated dose toxicity studies will be used as supportive evidence for specific target organ toxicity – single exposure and are not submitted to support classification for specific target organ toxicity – repeated exposure.
Two 28 day studies investigated PG as vehicle for cyclosporine in rats and dogs. Since the Wang-study investigated several doses of cyclosporine only one dose PG was used as vehicle control. The comparison was made against an additional control group exposed to air only. When compared to air, no local or systemic effects of PG were observed (Wang et al., 2007).

A 28 day repeated dose toxicity study was performed with Sprague-Dawley rats that were exposed to 30 mg/L PG aerosol for 4, 12, 40 or 120 min/day. Nominal daily doses are calculated from CAG-generated PG aerosol concentration, inhalation exposure duration and respiratory minute volume, to

reflect the doses that the lung was exposed to by inhalation/respiration. From that nominal dose, the (pulmonary) deposited daily dose was estimated assuming a pulmonary deposition fraction of 10% in the nose-only rat.
The measured MMAD for PG aerosol sampled from the plenum and used to expose each treatment group was 2.29 µm and GSD of 1.56. Histopathologic investigations revealed the following results: The most prevalent finding was larnyngeal squamous metaplasia, described as "minimal" on the ventral floor of larynx, in the mid-2 (40 min/day) and high dose (120 min/day) exposure groups. The normally cuboidal cells were flattened, to layers of squamous epithelium. Inflammatory cell infiltration ranging from minimal to moderate was observed in the lungs of both sexes, but is not statistically significantly higher than the control group, even though the pooled incidence for "minimal", "mild", and "moderate" inflammatory cell infiltrate in treatment groups was greater than observed for the controls. No other biologically significant effects were observed by histopathology on the tissues and organs. The NOEL for the 28 day rat study was determined to be 30 mg/L for 12 min/day (Werley et al., 2011).

A 28 day repeated dose toxicity study was performed in Beagle dogs that were exposed to 5 mg/L PG aerosol for 6 min/day, 12 min/day, 36 min/day or 60 min/twice a day (4 animals/sex/group). Target exposure concentrations and durations were selected to attain the following doses deposited in the lung: 3, 6, 18 and 60 mg/kg/day. The measured MMAD for the CAG-generated PG aerosol sampled from the plenum and used to expose each treatment group was 1.34 µm and GSD of 1.45. Histopathologic investigations revealed the following results: Sporadic findings of squamous hyperplasia of the larynx, inflammatory cell infiltration in the trachea and alveolar lung, alveolar macrophage accumulation, and congestion/hemorrhage in the lung were reported. None of these findings were significantly higher than air-exposed controls, and there appeared to be no clear treatment or dose-related pattern to the findings. Indeed, the study director indicated that changes reported were "considered to be typical of spontaneously arising background findings, which are common in inhalation exposure studies in dogs at this laboratory". No other biologically significant effects were observed by histopathology on the tissues and organs.
In the 28 day study, the NOEL was determined to be 5 mg/L for 12 min in the Beagle dog (Werley et al., 2011). However this is not conclusive regarding that no dose-related effect was observed after 60 min/twice a day exposure duration. The daily exposure time was very short.

A subchronic inhalation toxicity study with rats exposed to PG aerosol at dose levels of 0.0, 0.16, 1.0 and 2.2 mg/L air for 6 hr/day, 5 days/week for 90 days was reported by Suber et al. (1989). A treatment-related effect was reported as nasal haemorrhaging which began during the second week of exposure and persisted throughout the study; recovery from these clinical signs occurred during the non-exposure weekend periods. The frequency of this reported nasal haemorrhaging remained constant throughout the study (but disappeared during the week-end non-exposure periods) and was highest (65-75%) in the medium-and high-concentration groups. Similar trends were observed for ocular discharge, with incidences of 16% in low-exposure males, 40% in medium and high exposure males and 5% in controls. There was generally less ocular discharge in females, who had incidences of 8% in controls, 14% in the low-exposure group, 28% in the medium-exposure group and 35% in the high-exposure group. Minute volume, tidal volume and respiratory rates were not significantly altered at any dose levels.

A reduction in mean body weight by 5-7% was observed in the high-exposure female rats. This reduction correlated with the observed reduction in feed consumption. There was no trend towards reduced feed consumption among male rats, but reduced consumption on selected days for the high-exposure male rats was seen. Inconsistent but statistically significant changes were observed with absolute organ weights, but these changes were not considered to be biologically significant by the

authors when the weights for all of the treatment groups were compared and when the gross histological findings were taken into account. No adverse changes in gross pathological and histopathological examinations were noted, except of an increase in the number of goblet cells or an increase in the mucin content of the goblet cells present, observed in the nasal turbinates of both male and female rats at ≥1 mg/L. In addition, white blood cell counts revealed a concentration-related decrease in total white blood cells in mid- and high-concentration females, a decrease in banded neutrophils in mid-concentration females and high-concentration males and females, and finally a decrease in lymphocytes in mid- and high-concentration females.

Based on the reported nasal hemorrhaging and ocular discharge at all dose levels (Suber et al. 1989), accompanied by the lowest dose level of 0.16 mg/L is considered to be a LOAEC for local effects. The reported nasal "hemorrhage" observed during the exposure period was not confirmed by microscopic evidence of tissue damage after 90 days. The increased number of goblet cells and/or increased mucin content in the mid- and high dose groups were interpreted by the authors as a result of physical irritation of propane-1,2-diol upon the nasal epithelium in the rat.
For systemic effects, a NOAEL of 1 mg/L could be considered based on the 5-7% reduction in body weight and decreased food consumption in high-dose females. As the body weight reduction was reported to correlate with reduced food consumption from day 50 onwards in female rats and no effect was seen in male rats, a NOAEL of 2.2 mg/L for systemic effects may be more appropriate.

A 18-month inhalation study in monkeys exposed to PG vapor published in 1947 Robertson et al., 1947) was not considered for this report, mainly due to infections (including lunge mites) in the animals.

4.7.1.3 Repeated dose toxicity: dermal

Not evaluated for this dossier.

4.7.1.4 Repeated dose toxicity: other routes

Not evaluated for this dossier

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

A standard testing design of 5 hours/day, 5 days/week exposure was only applied in the Suber study, the highest tested concentration using the standard daily exposure duration of 5 hours/day in rats was 2.2 mg/L (Suber et al. 1989). All other studies used very short exposure durations (minutes) per day of inhalation exposure, these studies were of limited value to examine subacute/chronic toxicity. All studies (except the Suber study) had major limitations to assess the effects on the respiratory tract tissues; the microscopic examinations of the respiratory tract were lacking or incomplete (in particular for the nose).

Following 28 days of exposure to 30 mg/l PG for 40 min or 120 min/day induced laryngeal metaplasia in rats (Werley et al. 2011): The only study with microscopic examination on the nasal

turbinates revealed increased numbers of goblet cells/increased mucin content at 1 and 2.2 mg/l PG for 6 hours/day, 5 d/week during 90 days (Suber et al., 1989). In this study concentration-related increased incidences of nasal haemorrhages were confined to the treatment days from 0.16 mg/l onwards and stopped during the weekend.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

Not relevant for this dossier.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Not relevant for this dossier.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Not relevant for this dossier.

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

Not relevant for this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated for this dossier.

4.10 Carcinogenicity

Not evaluated for this dossier.

4.11 Toxicity for reproduction

Not evaluated for this dossier.

4.12 Other effects

Not evaluated for this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated for this dossier.

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8 ANNEXES

Confidential Annex