Committee for Risk Assessment

RAC

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

Adopted

9 December 2016

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9 December 2016

CLH-O-0000001412-86-133/F

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

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

Chemical name: **propane-1,2-diol**

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

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

The proposal was submitted by **Germany** and received by RAC on **23 November 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **7 March 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 April 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 December 2016** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	603-RST-VW-Y	propane-1,2-diol	200-338-0	57-55-6	STOT SE 3	H335	GHS07 Wng	H335	-	-	-
RAC opinion	603-RST-VW-Y	propane-1,2-diol	200-338-0	57-55-6	No classification	-	-	-	-	-	-
Resulting Annex VI entry if agreed by COM	603-RST-VW-Y	propane-1,2-diol	200-338-0	57-55-6	-	-	-	-	-	-	-

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 much lower concentrations 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 a 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 gender differences in the development of ocular symptoms.

All volunteers participated in an acoustic rhinometry and the lung function test. No significant changes in any measurements 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 U.S. 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 another smoke system (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 in this case, it was 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 repeated dose toxicity studies following inhalation 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 a 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 provide a 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 millions 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% upon 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 animal data, RAC is of the opinion that **propane-1,2-diol does not warrant classification as STOT SE 3 (H335, May cause respiratory irritation)**.

Additional references

Dalton P. (2016). Evaluation of respiratory and ocular irritation from propylene glycol in healthy humans. Preliminary results. Submitted as room document at the RAC 39th meeting on 1 December 2016.

Krieg T. (2015). Erfahrungsbericht über die An wendung von Theaternebel bei Notfalltrainings; Cpt. Dipl-Ing. Thomas Krieg, Sea-Med-Care, Basic Safe ty Training Instructor.

ANNEXES:

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

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