Fontem Ventures

Response to CLP Report
Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Introduction

Fontem Ventures is dedicated to developing and growing a portfolio of innovative non-tobacco products including electronic cigarettes (e-cigarettes). A 100% subsidiary of Imperial Brands plc, we nevertheless operate at arm's length from our parent company. Currently Fontem Ventures manufactures the e-cigarette brand blu™ (France, Italy, UK and US).

Fontem Ventures supports sound, evidence-based and proportionate regulation of vaping products.

Should you have any questions on Fontem Ventures' comments, please contact:

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Background

Prior to placing chemicals (single substances or mixtures) on the EU market, industry must comply with EU regulation 1907/2006 (Registration, Evaluation, Authorization and Restriction of Chemicals "REACH") and EU regulation 1272/2008 (Classification, Labelling, Packaging "CLP"). The aim of these two regulations is to protect human health and environment from hazards posed by chemicals and ensure that they are clearly communicated to workers and consumers in the European Union through classification and labelling based on the United Nations' Globally Harmonised System ("GHS").

Industry has to classify each substance and mixture based on their route of exposure. The CLP regulation defined two hazard classes for the single exposure (inhalation route): "Acute toxicity" and "STOT-SE (Specific Target Organ Toxicity – Single Exposure)". Acute toxicity generally occurs where lethality is evidenced (e.g. with an LD50/LC50 value), or, where the potential to cause lethality can be concluded from evidenced toxicity (e.g. from the fixed dose procedure). STOT-SE generally occurs where there is clear evidence of toxicity to a specific organ, even absent of lethality.

For some substances, a "harmonized" classification exists which is mandatory for industry and authorities (and then contained in Annex VI of CLP). Propane-1,2-diol (Propylene Glycol) does currently not have a harmonised classification, and is not part of Annex VI of CLP regulation. Currently Propylene Glycol is not classified in the REACH dossier.

Harmonized classification Request submitted to the ECHA

The German Federal Institute for Occupational Safety and Health (BAuA) submitted to the European Chemical Agency ("ECHA") a proposal for Harmonized Classification and Labelling (CLH dossier dated October 2015) of Propylene Glycol as STOT SE 3, with the hazard phrase H335: May cause respiratory irritation. The author of the CLH dossier justifies this classification proposal based on the following 3 key points:

1- **The human study data published by Wieslander *et al.* (2001) which do, in the view of the author of the CLH dossier, provide evidence supporting the classification as required by the CLH dossier submitter;**

2- **The comparison of the findings of the Wieslander *et al. (2001)* publication with the STOT SE3 criteria as defined by the Guidance on CLP Application and for support, the evaluation of animal data;**

3- **An evaluation of ECHA CLP inventory data available for the substance.**

Review on the CLH dossier as submitted to ECHA by the BAuA

According to chapter 2.2., the CLH dossier is mainly based on published data on human experience with some limited animal data on acute toxicity 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.The data on human experience with respect to Propylene Glycol as such mainly refer to a publication by Wieslander *et al.* (2001).

Evaluation of the data published by Wieslander *et al.* (2001):

The aim of the investigation by Wieslander *et al.* (2001) was to study effects of an experimental exposure to Propylene Glycol mist, at exposure levels occurring during normal aviation emergency training.

In our view, the aviation emergency training does not necessary reflect usual exposure conditions to Propylene Glycol in the sense of the Guidance on CLP Application.

Regarding the test material used for the experiment, information is missing, especially in terms of analytical purity, since this was not provided in the publication.

As noticed by the authors of the publication, the investigation was not a controlled exposure chamber test and the parameters considered were described as physiological effects including tear film stability, nasal patency, and lung function, as well as subjective symptoms.

Referring to the study design, the experiment in our opinion is to be seen rather as a pilot than as a main study.

The study population was described as consisting of 27 healthy non-asthmatic volunteers (22 men and 5 women), most of whom were pilots working in civil aviation.

Regarding the findings published by Wieslander *et al.* (2001), these were summarized in the CLH dossier as follows:

"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 catarrh 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."

With respect to the percentages reported above, these need to be considered with caution, keeping in mind that 100% refers to only 27 subjects. We are wondering whether the number of volunteers and the fact that nearly all volunteers were working as pilots can be seen as representative of a normal population, in terms of workers (e.g., manufacturing) as well as consumers (e.g. patients using pharmaceutical preparations containing Propylene Glycol, e-cigarette consumers). In fact, we rather share the opinion of Farsalinos and Polosa (2014) that clinical trials can be very informative, but they require monitoring of hundreds of users for many years to adequately explore the safety and/or risk profile of the products under investigation.

In our opinion, the population as described in the publication cannot be considered to be a representative one and is not suitable for extrapolation to the general population.

A further relevant aspect to be considered is that mean exposure measurements showed that there were much higher exposures in the afternoon than in the morning (520 versus 220 mg/m^3 as reported in the publication); nine of the volunteers being exposed in the afternoon versus 18 exposed during morning. Also the author noticed that the mean exposure concentration of Propylne Glycol of 309 mg/m3 is quite high compared to measurements data within a work environment; as mentioned in the CLH dossier, ooccupational exposure limits were established for Propylene Glycol 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).

Hereby, no attempt was undertaken by the author to explain the conspicuous variability of the measured Propylene Glycol concentrations as illustrated graphically in the publication, with values ranging from 171 mg/m³ up to 851 mg/m³.

Moreover, within the concluding part of the publication, the authors referred to the limitations of the study with respect to the informational value due to short exposure and very specific, non-usual, exposure conditions.

Thus, the data do not provide sufficient evidence to warrant CLP classification with respect to respiratory irritation.

A further approach by the author of the CLH dossier, was to review in a comparative manner the findings of the publication in view of the criteria for respiratory tract irritation as reported in chapter 3.8.2.3 of the Guidance on the Application of the CLP criteria, (Annex 1: 3.8.2.2.1, page 433, criteria (a, b, c, d, e).

With respect to point (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.*

According to the author of the CLH dossier, "*the criteria for STOT SE 3 (respiratory tract irritant) are fulfilled*". The author justifies this interpretation with the fact that throat dryness reported by Wieslander *et al*., (2001) "*indicates irritation of the upper respiratory tract and cough, mild airway obstruction and mild dyspnoea indicate impaired function of the lower respiratory tract*" and that "*the slight reduction of forced expiratory volume in one second (FEV1) indicates slightly impaired lung function*". However, the Guidance on the Application of the CLP criteria also clarifies that for STOT SE 3 (respiratory tract irritation) "*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*". In fact, the slight throat dryness reported by Wieslander *et al*., (2001) as well as the coughing in a few individuals are rather to be seen as a reversible short-term unspecified effect/reaction to the exposure to excessive concentrations of aerosols of any hygroscopic substance, such as e.g. Propylene Glycol. In the present case, Propylene Glycol is known to be hygroscopic, and this function contributes to the absorption of moisture from its surroundings (Werley *et al*, 2011). Slight airway obstruction was reported with no significant association with decreased lung function (forced expiratory volume in 1 s, FEV1) (Wieslander *et al*., 2001), thus the findings as reported in the publication cannot be seen as indicative of any functional impairment specifically related to the compound.

The Guidance further clarifies that "*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*".

Wieslander's study was conducted with only 27 volunteers. Of these, 6 were smokers and 12 were ex-smokers. In addition, 30% of the subjects exhibited a history of atopy. Thus, the majority of the study subjects were individuals potentially more sensitive to respiratory tract irritation due to their medical "history".

The author of the CLH dossier also refers to a publication by Moline *et al.* (2000); according to the CLH dossier, *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 [Propylene Glycol] and thus the effects can not solely be attributed to PG*. From our point of view, there is no supportive evidence to be gained from this publication, since, as correctly noticed in the CLH

dossier, the actors were exposed to various theatre fogs generated from mixtures of glycols and no effect could be solely attributed to Propylene Glycol.

With respect to point (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.*

According to the author of the CLH dossier, the publication of Wieslander *et al.* (2001) *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.*

We agree that the dynamic spirometry as applied in the publication falls under the scope of this point; however, the following results were reported in the publication: "Most of the lung function values remained unchanged after exposure to PG [Propylene Glycol], but there was a minor numerical decrease of FEV1 from 103% to 102% at 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 (table 4). None of the 27 participants had an initial lung function value (FEV1) below 80% of predicted value, but one had 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."

In our view, the findings as reported above do not provide any strong evidence that could trigger a STOT SE 3 classification as required by the author of the CLH dossier.

With respect to point (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.*

According to the author of the CLH dossier, the *Wieslander study carefully assess 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.*

In fact, here again it is necessary to remember that the study by Wieslander *et al.* (2001) was conducted with 27 volunteers, with following particularities: 6 were smokers and 12 were ex-smokers, and 30% of the subjects exhibited a history of atopy. Furthermore, the exposure conditions cannot be defined as controlled, as even noticed by the authors of the publication themselves in the discussion part of the publication, and also in view of the high variability reported with respect to the measured exposure concentrations ranging between 170 up to more than 800 mg/m³. Furthermore, it needs to be reiterated that nine of the volunteers were exposed in the afternoon versus 18 during morning, with the exposure concentrations varying between morning and afternoon.

With respect to point (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, and 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.*

In this context, the author of the CLH dossier refers to animal data: *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. Electron microscopical 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.

The publication by Konradova *et al.* (1978) was mentioned in the REACH dossier last modified 18 March 2016 with respect to acute inhalation toxicity. According to the ECHA entry, six rabbits were exposed for either 20 or 120 minutes to an aerosol of monoPropylene Glycol (10% in air). In each experimental group the tracheal epithelium of 3 rabbits was examined. According to the ECHA entry, a LC50 of 317 mg/L after 2 hours of exposure was reported; converting this value according to Haber's Law, the LC50 for a 4 h becomes 159 mg/L, corresponding again to 51091 ppm.

Furthermore, it was reported that following 20 minutes' exposure, 10% Propylene Glycol produced minimal alteration of ciliated cells whereas referring to the goblet cells, it was noticed that 16.5% of goblet cells were filled with large pale mucous granules, 31% were discharging mucus and 52% were completely exhausted with highly electron dense degenerated cytoplasm. After 2 hours inhalation signs of ciliated cells' alteration were more pronounced and a slight 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. **Thus, from an ultrastructural point of view, clear cellular alterations were noticed which however occurred at a concentration level which was described as inducing mortality (LC50 value).**

The CLH dossier also mentions long-term inhalation studies with Propylene Glycol, referring to Suber *et al.* (1989) and Werley *et al* (2011).

Regarding long-term exposure, the REACH dossier refers to a valid publication by Suber *et al.* (1989). Groups of 19 male and female rats were exposed by inhalation to 0.0, 0.16, 1.0 and 2.2 mg/l air Propylene Glycol for 6 hr/day, 5 days/week for 90 days. The test compound was described as Propylene Glycol, USP, with an analytical purity > 99%. The test conduct was in principle similar to the OECD TG 408 and the animals were nose-only exposed 6 hours/day, 5 days/week. The test concentrations were 0, 160, 1000 and 2200 ppm (nominal values). No significant differences in respiratory rates, tidal volumes or minute volumes between the control group and any of the treatment groups were noticed, nor did respiratory rates within groups decrease as the animals became acclimatized to the nose-only exposure conditions. Treatment-related effects were reported to be nasal haemorrhaging, which began during the second week of exposure and persisted throughout the study; recovery from nasal haemorrhaging occurred during the non-exposure weekend periods. Ocular discharge was also mentioned, particularly affecting the males.

We would draw attention to the fact that the Suber et al study focused on rats only, and that the reported nasal haemorrhaging is therefore considered a species-specific response to continuous exposure.

Microscopic examination of the nasal cavity revealed a thickening of the respiratory epithelium, noted as an increase in the numbers of goblet cells or as an increase in the mucin content of the goblet cells in the medium and high-exposure male and female rats. The increased number of goblet cells was observed in the posterior portion of the nasal cavity, lining of the septum, the lateral walls, the anterior turbinates and in selected cases the ethmoid turbinates. The subjective judgement of the proliferation of goblet cells in the posterior nasal cavity was accompanied by an increased volume of mucus in the individual goblet cells. There were no histological changes in the trachea, lungs or larynx. Based on the findings, the LOEC was set at 160 ppm based on nasal haemorrhaging; with respect to systemic toxicity, the NOAEC was set at 1 000 ppm (body weight changes in females).

As noticed by in the CLH dossier, (page 27), according to the Guidance on CLP Application (chapter 3.8.2.2.1), with respect to the classification as STOT SE Cat3 H335, the CLP criteria 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*)". With respect to the present study (Suber *et al.*, 1989), effects were rather confined to the nose, i.e., the upper part of the respiratory tract, without any signs of irritation and/or respiratory alteration evidenced in the low respiratory tract; no respiratory impairment was reported; regarding the effects seen in the nose, these were defined as portal of entry effects by the U.S. EPA within a Hazard Characterization Document dated 2009; thickening of the respiratory epithelium, with increased goblet cell counts or increased mucin contents of goblet cells were reported for the higher dosed groups of rats (1000 and 2200 ppm) whereas no changes were seen in the trachea, lungs or larynx; moreover, the nasal haemorrhages represented a reversible finding since recovery was noticed during the non-exposure weekend periods.

The CLH dossier mentions a publication by Werley *et al.* (2011), with emphasis set on the squamous metaplasia seen on the ventral floor of the larynx of Propylene-Glycol-treated rats (30 mg/L Propylene Glycol aerosol exposure for 40 or 120 min/day during 28 days).

The author of the CLH dossier refers to the Guidance on the Application of the CLP criteria, chapter 3.8.2.5: *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;*

In fact, the systemic toxicity of repeated exposure to Propylene Glycol aerosol over a period of 28 days was tested in rats and Beagle dogs within a a battery of non-clinical studies preceding ICH-compliant "first-time-in-man" studies (Werley *et al.*, 2011).

With respect to the rats, the mass median aerodynamic diameter (MMAD) was reported to be 2.29 µm (1.56 geometric standard deviation GSD). The only biologically relevant findings included clinical signs of ocular and nasal irritation indicated by minor bleeding around the eyes and nose, and minimal laryngeal squamous metaplasia; no alteration in lung tissue was noticed. Referring to the minimal laryngeal squamous metaplasia, the authors noticed that this is an effect commonly observed in inhalation studies in the rat, and likely related to the unique sensitivity of the tissue, as well as the circuitous airflow pathway through the larynx which increases particle deposition (Werley *et al.* 2011).

With respect to the dog study, MMAD was 1.34 µm (1.45 GSD); the MMAD values were consistent with expected particle size exposures in man according to the authors. No clinical findings such as described above for the rat were observed in dogs, thus supporting the assumption of a species (rat, or rodent)-specific finding. In fact for dogs, systemic toxicity was noticed as indicated by decreases in hemoglobin, red blood cells and hematocrit observed in females in the two highest exposure levels and in male dogs at the highest exposure level; no effects in lung were noticed.

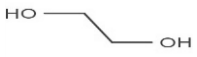
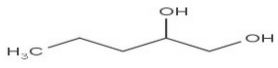
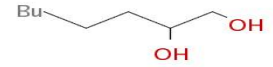
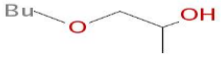
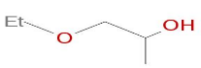
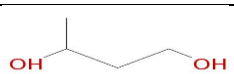
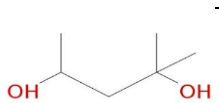
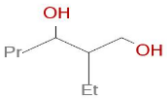
Nasal haemorrhaging and minimal laryngeal squamous metaplasia reported in rats' inhalation studies are common local effects observed and are mostly species-related (Renne *et al.*, 2007). Furthermore, they were not observed in dogs (Werley *et al.*, 2011).

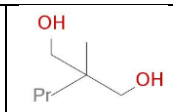
With regard to squamous metaplasia, we agree with the CLH dossier's author that squamous metaplasia gives supporting evidence that Propylene Glycol has irritation properties. However, in the view of the Werley *et al.* (2011), this effect is a typical one observed in rats subjected to repeated inhalation exposure to chemicals, and according to Sahota PS, Popp JA, Hardisty JF and Gopinah C. (2013), this change is most commonly seen in the rodent larynx and is described as a non-adverse, adaptative response to (repeated) irritation that typically does not progress and will recover given a suitable off-dose period (Renne and Gideon, 2006). Furthermore, the incidence of this change in rats is higher than in humans, primates, or dogs, which, according to Lewis (1981) and Renne and Gideon (2006) as cited in Sahota PS, Popp JA, Hardisty JF and Gopinah C. (2013), is likely related to the presence of sensitive epithelia in regions of high particle deposition in the rat larynx.

This kind of findings is further also documented in a publication by Gamer *et al.* (2008) dealing with the systemic and respiratory tract toxicity of di- and triethanolamine in rat following repeated dose exposure under testing conditions in accordance to OECD guidelines. Hereby it has to be noticed, that neither di- nor triethanolamine is classified STOT SE3 H335 with respect to respiratory irritation, despite of findings which have been described as "*laryngeal epithelial changes for DEA after 90 days of exposure (reversible metaplasia at the base of the epiglottis, inflammation at higher concentrations extending into the trachea).*"

Thus, nasal haemorrhaging and squamous metaplasia can be seen as indices for irritation, but based on the facts as reported above, these findings do not provide sufficient evidence that would support a classification as STOT SE 3H 335.

As the third key point considered by the author of the CLH dossier as justification with regard to the STOT SE 3 H335 classification of Propylene Glycol, the author claims that "*several*" notifiers used STOT SE 3 in the self-classification, whereas "*the majority of notifiers proposed no self-classification at all.*" (see CLH dossier, chapter 3, page 9/38). In fact, 8 of 4966 notifiers do support the classification proposal as STOT SE 3 H335, and of the 4966 notifiers, 4651 consider that there is no need for classification; in terms of percentage, this means that only 0.16% of the notifiers support the STOT SE3 H335 classification. This percentage is in our opinion far from being significant to justify the CLH proposal submitted to the ECHA. In this context we moreover have investigated several ECHA registered substances which we consider close enough to Propylene Glycol, and thus suitable for grouping strategy approach. The substances are listed in the following table; it should be mentioned that the substances were evaluated with respect to their classification as STOT SE3 H335 solely.

Substance	**CAS**	**Molecular formula**		**STOT SE3 H335**	**STOT SE3 H335 proposals (%)***
Propylene Glycol	***57-55-6***	C3H8O2		STOT SE 3 H335	8/4966 (0.16%)
Ethane-1,2-diol	107-21-1	C2H6O2		no	0/ 5098
Pentane-1,2-diol	5343-92-0	C5H12O2		no	0/638
Octane-1,2-diol	1117-86-8	C8H18O2		no	0/ 359
1-butoxypropan-2-ol	5131-66-8	C7H16O2		no	
Propylene Glycol monoethyl ether	1569-02-4	C4H10O2		STOT SE 3 H335	3/475 (0.6%)
1-methoxypropan-2-ol	107-98-2	C4H10O2		STOT SE 3 H335	3/ 1681 (0.17%)
Butane-1,3-diol	107-88-0	C4H10O2		STOT SE 3 H335	48/1522 (3%)
2-methylpentane-2,4-diol (hexylene glycol)	107-41-5	C6H14O2		no	0/3735
2-ethylhexane-1,3-diol	94-96-2	C8H18O2		no	0/626

2-methyl-2-propylpropane-1,3-diol	78-26-2	C7H16O2		no	0/12
propane-1,3-diol	504-63-2	C3H8O2		no	0/341
butane-1,2-diol	584-03-2	C4H10O2		no	0/95
** number of notifiers supporting STOT SE3 classification/total number of notifiers*					

The evaluation of the classification data available from the ECHA CLP inventory for Propylene Glycol (0.16% notifiers supporting STOT SE3 H335) in addition to the evaluation of STOT SE 3 H335 classification data from a series of similar substances, does not provide any supportive evidence that would justify the classification as proposed in the CLH dossier on Propylene Glycol submitted to the ECHA.

Propylene Glycol (1-2 propanediol; CAS No 57-55-6) is a low toxicity compound widely used as a food additive, in pharmaceutical preparations, in cosmetics, and in the workplace—for example, water based paints, de-icing fluids, and cooling liquids. Exposure to Propylene Glycol mist may occur from smoke generators in discotheques, theatres, and aviation emergency training (Wieslander *et al.* 2001).

Exposure to Propylene Glycol can occur in a Propylene Glycol manufacturing facility, during transport or in the various industrial or manufacturing facilities that use Propylene Glycol. It is produced, distributed, and stored in closed systems. Those working with Propylene Glycol in manufacturing operations could be exposed during maintenance, sampling, testing or other procedures. Propylene Glycol is it is widely used in consumer product formulations such as liquid laundry detergents, pharmaceuticals, shampoos and shaving products, toothpaste, bath and shower products, baby wipes, cosmetics, deodorants, and many other personal care products. As stated in the CLH dossier, Propylene Glycol is also commonly used to produce artificial smoke with generators in theatres, discotheques, emergency trainings or is used as a liquid for vaporisation in electronic cigarettes. Propylene Glycol is listed a direct additive for specified foods and is classified as generally recognized as safe (GRAS). In addition, it meets the requirements of the Food Chemicals Codex and the specifications of the U.S. Pharmacopeia XXII. Propylene Glycol has been reviewed in several robust assessment documents within acknowledged evaluation programs (OECD SIDS, U.S. EPA Evaluation, EFSA Scientific Opinion). All are in agreement with respect to the low toxic level of this substance. All agree that due to low volatility, at room temperature, exposure to vapours is minimal and due to low systemic toxicity, it is unlikely that injury would occur as a result of limited vapor inhalation. With respect to prolonged exposures to saturated vapors of Propylene Glycol, as shown in the ECHA registration dossier, systemic toxicity remains low but such concentrations would likely be irritating to the upper respiratory tract.

With respect to e-cigarettes, according to a recent publication by Farsalinos and Polosa (2014), the liquid used mainly consists of Propylene Glycol, glycerol, distilled water, flavourings (that may or may not be approved for food use) and nicotine. Only few animal studies are available which evaluated the potential harm of humectants in e-cigarette liquids (i.e. Propylene Glycol and glycerol) when given by inhalation. According to Farsalinos and Polosa (2014), concerns have been raised in human use, based on studies of people exposed to theatrical fog (Varughese *et al.* 2005; American Chemistry Council, 2003) or Propylene Glycol used in the aviation industry (Wieslander *et al.* 2001). Irritation of the respiratory tract was found, but no permanent lung injury or other long-term health implications were detected. The authors noticed that it should be recalled that, in these circumstances, non-pharmaceutical purity Propylene Glycol is used and in some cases oils are added, making it difficult to interpret the results. Further, according to Farsalinos and Polosa (2014), it is known that the thermal degradation of Propylene Glycol and glycerol can lead to the emission of toxic compounds such as aldehydes (Antal *et al.* 1985; Stein *et al.* 1983).

Very recently, Werley *et al* (2016) published a toxicological assessment on E-cigarette based on a 90-day inhalation study with rat; the 90-day inhalation study with a 42-day post-exposure recovery period was compliant with good laboratory practice, and the evaluations were conducted according to guidelines established by FDA and the Organization for Economic Co-operation and Development (OECD, 2009). According to the authors, inhalation testing is particularly useful to assess pulmonary function and pathological and/or pathophysiological effects in the respiratory tract, and would also be necessary to assess potential vehicle and flavour toxicity. In this study, two test aerosol formulations were evaluated; both formulations were compared with a vehicle control, which contained 23% glycerol and 77% Propylene Glycol. The MMAD ranged from 1.1 to 1.3 mm in each exposure group for the duration of the study, indicating that the aerosol particles were fully respirable in the rat. Body weights, clinical observations and food consumption were monitored weekly. Plasma nicotine, cotinine and carboxyhaemoglobin levels were measured at days 28 and 90. After days 28, 56 and 90, lung function measurements were obtained. Biological endpoints after 90-day exposure and 42-day recovery period included clinical pathology, urinalysis, bronchoalveolar fluid (BALF) analysis, necropsy and histopathology. At the 90-day necropsy, higher lung weights were noted in high-dose exposed animals from all treatment groups compared with low- and mid-dose levels of the respective groups. Higher lung weights were associated with vehicle-related microscopic findings and the changes resolved during recovery. With respect to systemic toxicity, the NOEL was derived based on effects on body weight, related to food-consumption; it was noticed that the vehicle control groups had the smallest attenuation in body weights. Further, lung weight increases were seen in all treatment groups after 90 days of exposure to any aerosol formulation. These lung weight differences were associated with histopathology changes that were nearly resolved after the recovery period. BALF chemistry and cytology findings were considered to be vehicle related. BALF analysis of biochemical markers and cytology showed lung inflammatory responses in males and females at day 28 in the high-dose vehicle group and by day 90, the lung inflammatory response was noted in all treatment groups at the high dose. The inflammatory responses were generally higher for vehicle control groups, compared with Formulation 1 and Formulation 2 groups at day 90, and resolved at day 132. These data suggest that exposure to any of the formulations caused a lung inflammatory response. However, in spite of these changes described above, there were no correlated histopathological changes indicating morphological tissue changes or damage associated with exposure to any of the test materials (vehicle control, Formulation 1 and Formulation 2). At day 132, all the biochemical differences had resolved, and persistent populations of alveolar macrophages continued to clear the tissue of inhaled test material. The authors noticed that according to the criteria established by the Society of Toxicologic Pathology Working Group and published by Nikula *et al.* (2014), such a response should be considered a normal adaptive response of the lung. With respect to the upper respiratory tract, the authors reported mucous cell hyperplasia in the nasal cavity and laryngeal exudates; no dose-response relationship was evident and the findings were considered to be of unlikely or uncertain toxicological significance.

Thus, whereas the data of the human study by Wieslander *et al.* (2001) do not indicate a basis for concern because of the very specific exposure conditions which do not occur for the general population, studies of laboratory animals remains inconclusive and insufficient with respect to a respiratory tract irritant potential to be expected from Propylene Glycol, and thus the criteria for classification as STOT SE3 H335 of the Guidance on CLP Application are not clearly fulfilled.

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