TOX/2018/19

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (e-cigarettes). Paper 3: Toxicological review of the main constituents, propylene glycol (PG) and vegetable glycerine (VG, glycerol)

Background

1. After discussing a scoping document (TOX/2016/25) on electronic nicotine delivery systems and electronic non-nicotine delivery systems (E(N)NDS) in July 2016, the COT established the following areas as priorities for more in-depth reviews:

- the composition of particles
- bystander exposure to key analytes
- effects of long term inhalation of the main constituents and emissions
- the situation regarding flavourings (exposure, thermal products, toxicity on inhalation)
- exposure to metals from the device components

2. The Committee agreed that further discussion papers should be prepared to address the above questions. Papers on characterisation of the aerosol particle fraction (TOX/2017/49) and exposure to metals from E(N)NDS use (TOX/2018/15) were discussed at the COT meetings in December 2017 and March 2018, respectively. A summary of publications describing the chemical constituents of E(N)NDS liquids and aerosols (excluding metals and flavourings (TOX/2018/16) was also presented at the COT meeting in March 2018. It was agreed at that meeting that the inhalation toxicity of propylene glycol (PG) and vegetable glycerine (VG, glycerol) would be assessed as would the toxicity of nicotine. This present paper reviews published data on the toxicity of the major constituents of E(N)NDS liquids, namely PG and VG. A paper on the toxicity of nicotine will be produced for a future committee meeting.

Introduction

3. E(N)NDS are battery-powered devices containing a liquid (E(N)NDS liquid or ‘e-liquid’). The E(N)NDS liquid is heated on use to produce an aerosol that is inhaled by the user (‘puffing’, ‘vaping’). E(N)NDS were first introduced commercially in China

in 2004 and subsequently in the EU (2005) and USA (2007) as nicotine-delivery devices (Bansal and Kim 2016). The main constituent parts of an E(N)NDS device are a mouthpiece, cartridge (tank) containing E(N)NDS liquid, a heating element/atomizer, a microprocessor, a battery, and sometimes an LED light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes and thus are termed 'cigalikes'. Initial models comprised three principal parts; a lithium-ion battery, a cartridge and an atomizer. However, more recent models mostly consist of a battery connected to a 'cartomizer' (cartridge/atomizer combined), which may be replaceable, but is not refillable. Second-generation E(N)NDS are larger and have less resemblance to tobacco cigarettes. They often resemble pens or laser pointers (hence the name, 'vape pens'). They have a high-capacity rechargeable lithium-ion battery and a refillable atomizer (sometimes referred to as a 'clearomizer'). Third-generation models ('advanced personal vapers', 'mods') are also refillable, have very-high-capacity lithium-ion batteries and are highly customisable (different coil options, power settings, tank sizes). In addition, highly advanced 'fourth generation' E(N)NDS (innovative regulated mods) are now being described[1].

4. Constituents that have been identified in E(N)NDS liquids and/or aerosols include propylene glycol (PG), vegetable glycerine (VG, glycerol), water, nicotine, carbonyls, volatile organic compound (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), metals, ethanol, ethylene glycol, di-ethylene glycol, flavouring compounds, flavour enhancers, sweeteners, and phenolics. The principal constituents, often in the range of 90-95% of the mass, are usually the solvents, PG and/or VG, which can be present in ratios ranging from 0:100 to 100:0 (see TOX/2018/16). The following sections summarise data relevant to the toxicity of PG and of VG, with a focus on exposure by inhalation. This report is concerned specifically with studies that have evaluated PG or VG individually; evaluations of PG/VG as mixtures will be reviewed in a separate paper on toxicity studies of E(N)NDS aerosols.

Search strategies

5. The toxicity of PG has been reviewed by a number of organisations, including:

- The US Agency for Toxic Substances and Disease Registry (ATSDR) in 1997, followed by a literature update in 2008: all routes of exposure were considered, with the aim to set oral and/or inhalation minimum risk levels (MRLs) (ATSDR 1997).

- The U.S. Department of Health and Human Services National Toxicology Program Center For The Evaluation Of Risks To Human Reproduction (NTP-CERHR) carried out a review and evaluation of the scientific evidence on the potential reproductive and developmental

[1] see, http://ecigclopedia.com/the-4-generations-of-electronic-cigarettes/ (accessed 18/12/17)

toxicities of PG: all routes of exposure were considered, (NTP-CERHR 2004)

- The Health Council for the Netherlands (HCN, Gezondheidsraad) in 2007: all routes of exposure were considered, with a focus to set an occupational exposure limit for concentrations in workplace air (HCN 2007)

- The European Medicines Agency (EMA) in 2014: all routes of exposure were considered, with the aim to review the use of PG as an excipient in medicinal products for human use (EMA 2014).

6. The toxicity of VG/glycerol has been reviewed in detail by a number of organisations, including:

- The Organisation for Economic Co-operation and Development (OECD) in 2002: all routes of exposure were considered in producing a screening information dataset (SIDS) assessment (OECD 2002)

- Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) in 2006: all routes of exposure were considered, with a focus to set a maximum permissible air concentration in the workplace (DFG 2006). An updated evaluation in 2016 noted that no new studies had been published (Hartwig 2017)

- The European Food Safety Authority (EFSA) in 2017: only oral exposure was considered, with a focus of re-evaluating glycerol for use as a food additive (EFSA 2017).

7. Relevant literature was obtained from the reviews described in paragraphs 5 and 6. In addition, searches for further literature relating to the toxicity of PG and VG/glycerol by inhalation exposure were carried out as described in Annex A.

PROPYLENE GLYCOL

Chemical properties and uses

8. Unless otherwise indicated[2], information in the following paragraphs is summarised from reviews published by ATSDR (1997), NTP-CERHR (2004), HCN (2007), and EMA (2014).

9. Propylene glycol (propane-1,2-diol; CAS number 57-55-6) (subsequently referred to as PG) is a synthetic substance that, at room temperature, exists as a clear, colourless liquid, with very little odour or taste. It is soluble in water and

[2] Individual references from within these reviews are only cited where this was considered to be specifically relevant.

miscible with alcohol, acetone, chloroform and other organic solvents. PG lowers the freezing point of water, is hygroscopic, and is a good solvent. PG has a melting point of -60 ˚C, boiling point of 188 °C, and vapour pressure of 0.011 kPa at 20 °C. It can exist in air as a vapour (which, due to the low vapour pressure, must be produced by heating or vigorous shaking) or aerosol. Propylene glycol has several industrial uses, including in the synthesis of polyesters, as a solvent in paints, and as an antifreeze and de-icer. It is a common additive in foods, pharmaceuticals (as a drug solvent and as an emollient in medicinal ointments) and cosmetics, and is generally recognised as safe (GRAS) by the U.S. Food & Drug Administration (FDA) for use in food, where it is used as a solvent for colours and flavours. PG is used as a humectant in tobacco. As a mist, PG is used for the production of fog/smoke, for example special-effect fogs for entertainment venues and to simulate smoke in fire-safety training. Common routes of exposure are via ingestion and dermal contact. Populations with potentially high exposure to PG include workers in PG manufacture or use industries, performers and theatrical workers, and fire-fighters participating in frequent training sessions. Inhalation is usually as the mist, as the presence of the vapour is minimal at room temperature due to the low vapour pressure.

Toxicokinetics

10. PG is rapidly absorbed from the gastrointestinal tract, and is distributed to the aqueous compartment, including the brain and fetus. No data are available on absorption through normal, non-abrased skin in humans, but dermal absorption has been reported to occur in patients with burns treated with topical products containing PG. Approximately 1% of PG permeated through intact rat skin in a diffusion cell during 24 h, with a latency period from skin exposure to systemic delivery of approximately 2 h (Yamada et al. 1987, *cited by* HCN (2007)).

11. Very limited data are available on the absorption of PG in humans or animals by inhalation exposure. Inhalation of PG as a vapour is predicted to be minimal at room temperature due to the low vapour pressure. However, absorption is predicted to occur following exposure to PG aerosol (EMA 2014). This is supported by data from a study in which rats and dogs were exposed to PG aerosol by inhalation for 28 days (Werley et al. 2011) (this study is described in more detail in paragraphs 24-30).

12. In mammals, PG is primarily metabolised to lactate in the liver, while unmetabolised PG is excreted by renal clearance (in humans 20-45% is excreted by this route within 48 hours). In patients with impaired renal dysfunction, PG may accumulate in the serum, leading to hyperosmolarity. Renal elimination of PG is low in neonates, where liver metabolism is the principal route.

Toxicity

Inhalation toxicity

Acute toxicity

Human

13. Short-term inhalation exposure to PG as an aerosol may induce acute ocular and upper airway irritation. Wieslander, Norback and Lindgren (2001) exposed 27 non-asthmatic human volunteers to PG mist from artificial smoke generators (geometric mean concentration 360 mg/m^3; range 176-851 mg/m^3) for 1 min. Exposure was associated with decreased tear film stability and increased ocular and throat irritation. Four subjects had cough, dyspnoea, and slight airway obstruction (5% reduced forced expiratory volume in 1 second (FEV_1)). Clinical trials in which 0.1 mL of an anti-rhinitis (flunisolide) nasal spray formulated with either 5% or 20% PG (in combination with other carriers) was sprayed twice per day per nostril for 4 weeks indicated that the 5% PG formulation was associated with less nasal burning, stinging and throat irritation compared with the 20% PG formulation in patients with allergic rhinitis (Greenbaum et al. 1988, Meltzer et al. 1990, *cited by* HCN (2007)).

Non-human

14. Rabbits exposed to a single PG dose (10% aerosol) at a flow-rate of 10 L air/min for 20 and 120 min showed increased numbers of goblet cells in the tracheal lining and mild changes in the ultrastructure of ciliated cells in the trachea indicative of rapid, massive expulsion of mucous. No changes in the regular ciliary border above the epithelium were found. The number of degenerated goblet cells had increased to 69% at 120 min. Nose bleedings were not recorded (Konradova et al. 1978, *reviewed by* HCN (2007)). ATSDR (1997) and HCN (2007) considered that this study was not adequate for use in establishing guidelines for levels of exposure to PG by inhalation.

15. In an acute inhalation study, Beagle dogs (2 males, 2 females) were exposed via face mask and oropharyngeal tube to 1.5-30 mg/L [1500-30000 mg/m^3] PG aerosol for 8-60 min to determine the maximum tolerated dose (MTD). In preliminary exposures, the dogs showed intolerance to doses of 15 and 30 mg/L, and 5 mg/L was chosen as the maximum concentration for 7-day studies (Werley et al., 2011).

16. In the same set of studies, eight-week old rats (3/sex/group) were nose-only exposed to PG concentrations of 14.4, 30.5, or 44.9 mg/L [14400, 30500, or 44900 mg/m^3] for a single 4 h exposure, following which lungs were either collected immediately or animals underwent a 7-day recovery period. PG aerosol mean mass median aerodynamic diameter (MMAD) values were in the range 1.1-1.4 µm and geometric standard deviations (GSD) were 1.1-1.4. Body weight decreased by 5-10% on days 1-3 after the single 4 h PG exposure, then returned to normal. PG exposure was associated with slight localised bleeding around the eyes and nose at

day 7 of recovery. PG lung concentrations post exposure 'increased approximately dose-proportionately'. There was no mortality, and the authors concluded that the 4-h acute lethal concentration (LC_{50}) for the PG aerosol was > 44.9 mg/L [44900 mg/m^3] (Werley et al., 2011).

Repeat-dose toxicity

Human

17. No data are available on the long-term exposure of humans to PG by inhalation. Chronic respiratory symptoms, including wheezing and chest tightness, have been reported in workers exposed to artificial fogs, of which PG is a component.

18. A NIOSH study in 1990 investigated the use of theatrical fog in Broadway theatres, and carried out air sampling and questionnaires for productions with/without theatrical smoke. PG concentrations were < 2.1 mg/m^3. Increased respiratory irritant symptoms (runny nose, stuffy nose, sneezing) were reported by personnel from productions using theatrical smoke (Driscoll et al. 1992, *reviewed by* NTP-CERHR (2004)).

19. A study sponsored by the Actors' Equity Association and the League of American Theaters and Producers examined irritant effects of theatrical fog to the respiratory tract and eyes of 439 actors from 16 musicals over a 2-year period. Effects were assessed by self-reporting and from medical evaluations. PG exposure was not associated with adverse effects on pulmonary function or rates of asthma, but peak exposures upon release of glycol smoke were associated with increased respiratory, throat, and nasal symptoms, and signs of vocal cord inflammation. Recommendations were made to limit peak PG concentrations to 40 mg/m^3 (Moline et al. 2000, *reviewed by* NTP-CERHR (2004)).

Non-human

20. Montharu et al. (2010) evaluated the respiratory tolerance of 30% PG (150 µL of 30% PG in water; control = deionised water) sprayed through an endotracheal tube (Microsprayer®) in Sprague-Dawley rats once a day for 4 days. Median particle size was reported as 64 µm. Broncho-alveolar lavage fluid (BALF) evaluations and histological analyses performed after euthanasia (24 h after the final PG administration) showed similar responses in control and PG-treated animals.

21. Yoo et al. (2018) reported that exposure of rats to PG enhanced the toxicity of sodium metabisulphite (SM). Groups of 6-week-old Sprague Dawley rats were exposed to air, SM[3], or mixtures of SM+PG, including a PG-only control (5.5 mg/m^3 PG; MMAD 1.70-2.75, GSD 1.69-2.08). Rats were whole-body exposed to the aerosol in an exposure chamber, 6 h/day, 5 days/week, for 2 weeks. Half of the

[3] Target concentrations of SM were 0, 5, 20, 100 mg/m^3 in the SM-only arm. In the SM+PG arm, 1% aqueous solutions of PG were atomised along with 1%, 5%, or 20% aqueous solutions of SM.

animals in each treatment group were euthanised 1 day or 7 days (recovery group) post treatment, respectively. Clinical signs, BAL analysis, and histopathology (gross, liver, lung, nasal cavity) were evaluated.

22. In the experiments including PG, the only significant effect observed from PG-only treatment was significantly (albeit slightly) increased total protein on BALF analysis compared with controls in the PG-recovery group. Histopathology evaluation of rats euthanised 1 day after treatment showed squamous metaplasia of the nasal cavity epithelium associated with SM/PG treatment, but no effects were seen in the PG-only treatment group.

23. Laube et al. (2017) reported that exposure to nicotine-containing, PG-based E(N)NDS aerosol via an E(N)NDS device (Joyetech 510-T) reduced mucociliary clearance (MCC) in mice compared with nicotine-free aerosol. C57BL/6 mice underwent whole-body exposure to aerosols of either PG alone or PG/2.4% nicotine, 20 min/day for 1 or 3 weeks. Exposure was achieved from repeat cycles of a 6-s puff (600 µL E(N)NDS solution per puff) + 15-s puff interval into a 1 L exposure chamber during the 20 min period, although exposure concentrations were not reported. After 1 week, mean MCC (%) was not significantly different between unexposed (8.6%), PG-exposed (7.5%), or PG/nicotine-exposed (11.2%) mice. However, after 3 weeks, MCC was significantly higher in the PG-exposed animals (8.6%, 17.2%, and 8.7% in unexposed, PG-exposed, and PG/nicotine-exposed mice, respectively). No differences were seen in tracheal histology. The authors concluded that chronic exposure to nicotine-containing E(N)NDS aerosol slows MCC.

24. Werley et al. (2011) carried out a series of non-clinical safety evaluation studies of PG aerosol in CD rats (nose-only exposure) and Beagle dogs (exposed via face mask and oropharyngeal tube), with exposures up to 28 days. PG aerosols were produced using a capillary aerosol generator (CAG).

25. In the 7-day toxicity evaluation, rats (5/sex/group) were exposed to 20.8 or 41.0 mg/L [20800 or 41000 mg/m^3] PG aerosol (MMAD, 0.9 µm; GSD, 1.4), 4 h/day, for 7 consecutive days. Clinical observations, body weights, PG concentrations in blood and lungs, histopathological evaluation of lungs, lung weights, and necropsy were performed. No treatment-related effects were observed and there were no macroscopic findings at necropsy and no effects on lung weight or histopathology of the respiratory tract. Lung and plasma peak values increased approximately dose-proportionately (7, 1561, and 3508 µg/mL (plasma) and < limit of quantification (LOQ), 693, and 1482 µg/g (lung) in the 0, 20.8, and 41.0 mg/L PG groups, respectively, at day 7. A no observed effect level (NOEL) > 41.0 mg/L [41000 mg/m^3] was determined.

26. Exposure of dogs to 5 mg/L (mean MMAD, 1.9 µm; GSD, 1.45) for 7 days (60 min/day) was well tolerated and there were no effects on pulmonary function, haematology, clinical chemistry, body weight, food consumption, or macroscopic evaluations of tissues and organs. Therefore, 5 mg/L [5000 mg/m^3] PG was considered by the authors to be the MTD in dogs.

27. Twenty-eight-day studies were carried out in rats and dogs. Rats (31/sex/group) were exposed to room air or 30 mg/L [30000 mg/m^3] PG aerosol (mean MMAD, 2.29; GSD, 1.56) for 4, 12, 40, or 120 min per day for 28-days. Targets for deposited dose in the lung were 0.0, 7.2, 21.6, 72.0, and 216.0 mg/kg bw/day (nominal dose targets[4] of 0, 72, 216, 720, and 2160 mg/kg bw/day, and an assumed pulmonary deposition fraction of 10%). The most prevalent finding was 'minimal' laryngeal squamous metaplasia on the ventral floor of the larynx observed only in the 72.0 mg/kg bw/day (4/10 males, 2/10 females) and 216.0 mg/kg bw/day (8/10 males, 6/10 females) lung-deposited-dose groups. Minimal-to-moderate inflammatory cell infiltration was observed but rates were not significantly different from controls (except for pooled treatment groups vs. controls). Lung 'congestion/haemorrhage' was also reported but the highest incidence was found in the control group males exposed to room air. The authors stated that 'no other biologically significant effects were observed by histopathology on the tissues and organs'. The authors identified a NOEL of approximately 20 mg/kg bw/day deposited dose in the lung, based on minimal laryngeal squamous metaplasia.

28. Dogs (4 /sex/group) were exposed to room air (control) or 5 mg/L [5000 mg/m^3] PG aerosol (mean MMAD, 1.34; GSD, 1.45) for 6, 12, or 36 min once per day, or 60 min twice per day, at target deposited doses in the lung of 0.0, 3.0, 6.0, 18.0, and 60.0 mg/kg bw/day (nominal dose targets of 0, 15, 30, 90, and 300 mg/kg/day, assuming a 20% pulmonary deposition fraction). Endpoints indicative of pulmonary and systemic toxicity were evaluated, including clinical signs, body weights, food consumption, ophthalmoscopic and electrocardiography examinations, pulmonary function, haematology, clinical chemistry, urinalysis, necropsy, histopathology, and toxicokinetics. No significant differences were observed in histological findings between treatment groups and there was no apparent lung, kidney, or liver toxicity. Statistically significant decreases in haemoglobin, red blood cells, and haematocrit were observed in females, but not males, in the 18.0 and 60.0 mg/kg bw/day lung-deposited-dose groups. However, the authors noted that these effects were within the normal historical control range for the age and strain of dog. The authors decided a NOEL of 6.05 mg/kg bw /day deposited dose in the lung for this study.

29. Kinetic studies in the 28-day studies in rats and dogs showed that PG was rapidly absorbed, and lung/plasma concentrations rapidly equilibrated. In rats, high systemic concentrations were achieved that were comparable to those from oral exposure, suggesting very efficient pulmonary absorption. PG did not accumulate in the plasma over the 28-day period, but a 20-30% increase occurred in the lung between day 1 and day 28. PG elimination showed saturation at high dose levels in both the rat and dog.

[4] Nominal daily doses (relecting the dose that the lung was exposed to by inhalation/respiration) were calculated from the aerosol concentration, inhalation exposure duration, and respiratory minute volume.

30. Lechasseur et al. (2017) reported that exposure to PG and/or VG aerosol altered genes involved in control of circadian rhythm in mice. Female BALB/c mice were whole-body exposed to aerosol of either 70% PG/30%VG, 100% PG, or 100% VG, without nicotine or flavour, for 8 weeks (2 h/day, 5 days/week). Exposure was achieved as 3x puffs of 80 µL test liquid per minute, mixed with room air by laminar flow at a rate of 3 L/min into an exposure chamber (no further information available). Exposure concentrations were not reported. Microarray gene-expression analysis of various tissues (lung, brain, liver, kidney, skeletal muscle) appeared to show alterations in genes involved in regulation of circadian rhythm in all 3 test groups compared with the room air-exposed control group.

31. In a study by Suber et al. (1989), groups of 19 male and 19 female Sprague-Dawley rats were exposed by nose-only inhalation to mean aerosol concentrations of 160, 1000, or 2200 mg/m^3 PG for 13 weeks (6 h/day, 5 days/week) or room air (control). Nasal haemorrhaging occurred in all groups (< 1%, 64%, 74%, and 75% (males); < 1%, 14%, 71%, and 71% (females) in control, low, medium, and high exposure groups, respectively), with recovery on non-treatment days. Similar trends were observed for ocular discharge. Body-weight reduction (5-7%) and altered leukocyte profile were observed in female rats in the two highest dose groups. There were no treatment-related changes in gross pathology but light microscopy showed thickening of the respiratory epithelium with increased numbers of goblet cells and mucin content in male and female rats in the medium- and high-dose groups. This study was used to provide points of departure (PoDs) for risk assessments carried out by HCN (2007) (NOAEL of 160 mg/m^3 for increased number of goblet cells) and by ATSDR (1997) and the German Committee on Indoor Guide Values (lowest observed adverse effect level (LOAEL) of 160 mg/m^3 for nasal haemorrhaging) (Umweltbundesamtes 2017) (see paragraphs 49, 51 and 52).

32. Robertson et al. (1947) (*reviewed by* ATSDR (1997), HCN (2007)) exposed monkeys and rats to PG (170-350 mg/m^3) by inhalation continuously for periods of 12-18 months. No adverse effects on the respiratory system were reported. Rats exposed for 12 months showed a 50% increase in body weight. Despite being the only chronic-exposure study available, these data were not considered adequate as a basis for deriving safe exposure levels by ATSDR or HCN.

33. Larcombe et al. (2017) reported that exposure to E(N)NDS aerosols led to impairments in pulmonary function, in the absence of pulmonary inflammation, in a mouse model designed to simulate effects of exposure during adolescence. In this study, groups of 12 female BALB/c mice were whole-body exposed between the ages of 4-12 weeks to control air (AIR), CC smoke (SMOKE), or 1 of 4 E(N)NDS aerosols of 'American Tobacco' flavour, as follows: PG with no nicotine (0-PG), PG + 12 mg/mL nicotine (12-PG), VG with no nicotine (0-VG), or VG + 12 mg/mL nicotine (12-VG). Average measured chamber concentrations were reported as 0.014 g/cm^{-3}

for PG and 0.018 g/cm^{-3} for VG [5]. Mice were exposed for 1 h/day, 5 days/week from weeks 4-10, then twice daily for 1 h, 5 days/week, during weeks 11 and 12. After 8 weeks of exposure, all treatment groups weighed significantly less than AIR controls, with the lowest weight gains in the nicotine-exposed mice. Lung mechanics and function were assessed 24 h after the final exposure. Mice exposed to E(N)NDS aerosols showed various differences in pulmonary function compared with AIR controls, including decreased airway resistance at functional residual capacity (FRC) (0-PG and 0-VG), increased tissue damping at FRC (all 4 E(N)NDS groups), increased tissue elastance at FRC (0-PG and 0-VG), decreased lung volume and changes in volume dependence of tissue damping and elasticity (0-PG, 0-VG, 12-PG). Methacholine challenge tests showed that SMOKE and VG-exposed mice were significantly more responsive than AIR or PG-exposed, whether or not nicotine was present in the aerosol. SMOKE but not E(N)NDS exposure was associated with increased pulmonary inflammation (increased BAL cells). Overall, the authors concluded that 1] VG-based E(N)NDS aerosols induced more severe functional pulmonary impairments than PG-based aerosols, and 2] there was little effect of the presence or absence of nicotine.

Reproductive and developmental toxicity

34. McGrath-Morrow et al. (2015) exposed neonatal C57BL/6J mice to aerosol produced from a cartridge containing 400 µL E(N)NDS liquid (PG or PG/1.8% nicotine; no flavouring) in a chamber (13.5 x 9 x 8.7 cm) from days 1-10 after birth (once per day on days 1 and 2, then twice per day). Exposure was achieved from 6-s puffs every 15 s over approximately 20 min. Exposure concentrations were not reported. As compared with room air–exposed mice, total body weight was decreased in both the PG and PG/nicotine groups (by 11.5% and 13.3%, respectively). Exposure to PG/nicotine was associated with impaired alveolar cell proliferation compared with room air, indicating an effect of nicotine on lung growth, but this effect was not seen with PG-only exposure. There were no differences in markers of apoptosis of oxidative stress between the groups.

35. Pregnant C57BL/6J mice were exposed to PG or PG/2.4% nicotine aerosol during gestation, following which the mothers and offspring were exposed from post-natal days 2-16. Exposures were achieved as 6-s puffs every 15 s into a 13.5 x 9 x 8.7 cm chamber, from a total of 600 µL liquid, over a period of approximately 20 min, once per day. Exposure concentrations were not reported. Behavioural tests at 14 weeks showed behavioural alterations in male offspring from the PG/2.4% nicotine group compared with room air, but these changes were not seen in PG-exposed male offspring. Mean pup weight at birth and throughout post-natal development was significantly lower in the PG group than the control or PG/nicotine groups. Mean pup

[5] The Secretariat assumes that the concentrations should be reported as g/cm^3. Exposure concentrations of 0.014 g/cm^3 and 0.018 g/cm^3 are equivalent to 14,000,000 mg/m^3 and 18,000,000 mg/m^3, respectively, which is far higher than other studies reported in this paper so it is possible that there is a reporting error.

weight in the PG/nicotine group was significantly lower than that of controls from post-natal day 7 onwards (Smith et al. 2015).

36. In the long-term inhalation exposure study of PG in rats conducted by Robertson et al. (1947) (see paragraph 32) the animals reproduced normally after exposure to 170-350 mg/m^3 PG for up to 18 months (this observation was noted in the report of HCN (2007)).

Mutagenicity/genotoxicity

37. No data were identified

Carcinogenicity

38. In the long-term inhalation exposure study of PG conducted by Robertson et al. (1947) (see paragraph 32), there was no indication of carcinogenic action in the kidney, lung, liver, spleen or bladder in rats (noted in the report of HCN (2007)).

39. No other studies regarding the carcinogenicity of inhaled PG were identified.

Overview of non-inhalation toxicity

40. PG is approved for use as a food additive in the EU and US, and oral exposure is not usually associated with adverse effects.

Acute and Repeat-dose toxicity

41. Acute toxicity of PG is very low in humans, generally manifesting as central nervous system (CNS) depression. HCN (2007) reported that the acute lethal oral dose in humans is 'probably above 15 g/kg bw'. Very high repeated exposure to PG causes hyperosmolality and metabolic acidosis, renal dysfunction leading to renal failure, and clinical deterioration (HCN 2007, EMA 2014, Lim, Poole and Pageler 2014). Data mostly relate to exposure to PG as an excipient via intra-venous (i.v.) infusion during medical treatments (usually in critically ill patients), with toxicity seen on administration of doses around 1 g/kg bw/day or higher. EMA (2014) concluded that severe toxicity generally occurs in such patients at PG serum concentrations well above 500 mg/dL.

42. Oral lethal dose (LD_{50}) values for PG in animals were summarised by EMA (2014) as 8-46 g/kg body weight (bw) (rat), 25-32 g/kg bw (mouse), 18-20 g/kg bw (rabbit), 19 g/kg bw (dog), and 18-20 g/kg bw (guinea pig), with i.v. LD_{50} values of 5-8 g/kg bw (mouse) and 4-6 g/kg bw (rabbit). The no observed adverse effect level (NOAEL) for long-term oral exposure is reported to be higher than 1 g/kg bw/day in mice, rats, dogs, and cynomolgus monkeys (EMA 2014). Cats are more sensitive to PG toxicity as they are unable to form the glucuronide metabolite, leading to prolonged presence of PG in the blood, resulting in haemotoxicity (HCN 2007).

Irritation and sensitisation

43. PG is a mild skin irritant in humans and application to the eye causes transient effects including stinging, blepharospasm and lacrimation (HCN 2007). PG also appears to have a low sensitising potential (Lessmann et al. 2005, McGowan, Scheman and Jacob 2018). HCN (2007) concluded that overall, PG has weak irritating properties, can provoke allergic reactions in some patients with allergic skin disease, and that allergic sensitisation after skin application cannot be excluded. Similarly, EMA (2014) concluded that sensitisation by PG, although minimal, has been established in humans.

44. In animal tests, PG produces little or no skin irritation, is slightly irritating to the eye and is non-sensitising (ATSDR 1997, HCN 2007, EMA 2014).

Reproductive and developmental toxicity

45. No adverse effects were reported when mice were exposed up to 10 g/kg bw/day PG in drinking water throughout lactation and to 34 weeks of age in a continuous breeding experiment. No developmental toxicity was observed after oral administration of PG at 10 g/kg bw/day to pregnant mice (day 6-15 of gestation), 6.2 g/kg bw/day to pregnant rats (days 10-12 of gestation), and 1.2 g/kg bw/day to pregnant rabbits (days 6-18 of gestation) (*reviewed by* HCN (2007), EMA (2014)). However, a study in which new-born and juvenile mice were exposed to single intra-peritoneal (i.p.) doses of PG revealed dose-dependent apoptotic neurodegeneration ≥ 2 g/kg, with no apoptotic effects observed at 1 g/kg (Lau et al. 2012, *cited by* EMA (2014)). An expert review of reproductive and developmental toxicity of PG by NTP-CERHR concluded that although no human data on reproductive or developmental toxicity were available, data from animal studies and knowledge of the kinetics of PG in humans allowed confidence that human developmental or reproductive risks are of negligible concern (NTP-CERHR 2004).

Genotoxicity and carcinogenicity

46. Published reviews have concluded that there is no evidence that PG would be carcinogenic to humans (NTP-CERHR 2004, HCN 2007, EMA 2014). In a recent report on the 'public health consequences of e-cigarettes', the US National Academy of Sciences noted that PG is unclassified for carcinogenicity due to a lack of data (NAS 2018).

47. PG is generally considered not to be genotoxic, based on *in vitro* and *in vivo* studies. Some studies indicated that high concentrations of PG cause *in vitro* DNA damage (with or without S9 mix), although the concentrations tested were associated with cytotoxicity (HCN 2007, EMA 2014).

Regulations and guidelines

Inhalation

Occupational

48. In the UK, the workplace exposure limits (8-h time-weighted average (TWA)) for long-term exposure to PG are 150 ppm, or 474 mg/m^3, for total vapour + particulates, and 10 mg/m^3 for particulates alone (HSE 2011). No short terms WELs are available.

49. The Dutch Expert Committee on Occupational Standards recommended a health-based occupational exposure limit for PG of 50 mg/m^3 [16 ppm] (8-h TWA) for summed concentration of vapour and aerosol. The TWA is based on a NOAEL of 160 mg/m^3 for increases in numbers of goblet cells in male and female rats, from the study of Suber et al. (1989) (paragraph 31, supported by the fact that changes in goblet cells were also found in the study of Konradova et al. (1978) (paragraph 14). An uncertainty factor of 3 was applied for inter-individual variation. Adjustment for inter-species variation was not considered necessary for local upper-respiratory effects. Given that exposure to aerosol can also have effects that are comparable to those of inhalable and respirable dust, the Committee also recommended that health-based occupational exposure limits for inhalable and respirable dust should be applied to aerosols of PG (HCN 2007).

50. The American Industrial Hygiene Association has a Workplace Environmental Exposure Limit of 10 mg/m^3 (8-h TWA) for PG as an aerosol (cited in HCN (2007)).

General

51. ATSDR (1997) established an intermediate-duration MRL for PG of 0.009 ppm [0.028 mg/m^3]. This was based on nasal haemorrhaging at the lowest dose tested (51 ppm [160 mg/m^3]) in the study of Suber et al. (1989) (paragraph 32, which ATSDR considered as a LOAEL. Adjustment factors were applied for use of a LOAEL (10), inter-species extrapolation (10), intra-individual variation (10) and continuous exposure (24/6 and 7/5). At the time of the evaluation, ATSDR noted that this was the only suitable intermediate-duration inhalation study available. A literature update did not identify additional data relevant to setting MRLs (ATSDR 2008).

52. The German Committee on Indoor Guide Values recently recommended a health precaution guide value (RW I, guideline value I[6]) of 0.06 mg/m^3 for PG. This

[6] RW I represents the concentration of a substance in indoor air for which, when considered individually, there is no evidence that life-long exposure would have an adverse health impact. RW II represents the concentration of a substance that, if reached or exceeded, requires immediate action as this concentration could pose a health hazard. It may be defined as a short-term value (RW II K) or a long-term value (RW II L). For more information, see: https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/german-committee-on-indoor-guide-values#textpart-3 (accessed 06/04/18).

value was based on a health hazard guide value (RW II, guideline value II) derived using the lowest observed adverse effect concentration (LOAEC) of 160 mg/m^3 for nasal haemorrhage in rats from the study of Suber et al. (1989) (paragraph 32 as the PoD, with adjustment factors as follows: 5.6 for time-scaling to continuous exposure, 2 for sub-chronic study, 2.5 for inter-species extrapolation (dynamic), 10 for intra-species variation (kinetic and dynamic) (Umweltbundesamtes 2017).

Non-inhalation

53. WHO set an acceptable daily intake (ADI) for oral intake of PG in food of 25 mg/kg bw/day, citing a level causing no toxicological effect in the rat and in the dog of 2500 mg/kg bw (FAO/WHO 1974, FAO/WHO 2002).

54. EMA specified the following limits for PG (maximum daily dose) as considered to be safe by any duration and route of administration, with the exception of inhalation: neonates up to 28 days (or 44 weeks post-menstrual age), 1 mg/kg; infants 29 days to 4 years, 50 mg/kg; 5 years and above, 500 mg/kg (EMA 2014).

Risk assessments

55. Kienhuis et al. (2015) published a risk assessment of PG exposure from E(N)NDS. Components of commercial, nicotine-free 'shisha pens' were evaluated and the major constituent was identified as a 54%/46% mixture of PG/glycerol, which the authors calculated to produce 0.7 mg/puff PG and 0.6 mg/puff glycerol. Based on a 50-70 mL puff volume, the maximum alveolar concentration of PG after 1 puff was estimated to be 430-603 mg/m^3. Referring to the study of human volunteers reported by Wieslander et al. (2001), in which concentrations of PG in the range 176-851 mg/m^3 PG caused acute eye and upper airway irritation in a small proportion of individual (i.e. considered as LOAEL), Kienhuis and colleagues determined margins of exposure (MOE) for PG in the range 0.3-2.0 [7]. In a published commentary on this study, Farsalinos and Baeyens (2016) criticised the approach of Kienhuis and colleagues, specifically the use of throat irritation symptoms for the PoD (noting that 'throat hit' is in fact a desired effect for some E(N)NDS users) and the use of a PoD determined from 1-min continuous exposure for calculation of an MOE for a single puff (which would have a duration in the range of 1 s). A response to these criticisms by Kienhuis and colleagues was published by Bos, Kienhuis and Talhout (2016).

56. Chen, Bullen and Dirks (2017) reported a health risk assessment of exposure to several E(N)NDS constituents, including PG, based on E(N)NDS use in New Zealand. Average E(N)NDS use was estimated as 11 'vaping sessions' (15 puffs) per day, and average PG exposure, 12.12 mg per vaping session (based on estimates from a report of Geiss et al. (2015), giving total daily exposure of 133.28 mg/day). Authors reported that this value exceeded the US EPA 'inhalation RfD'[8] for

[7] i.e. using the LOAELs of 176-851 mg/m^3 as the range of PoD values, and alveolar PG concentrations from one 50-70 mL puff to be 430-603 mg/m^3

[8] i.e. the RfC

PG of 116 mg/day (for a 70 kg human). However, this value of 116 mg/day, derived from a risk assessment based on findings in rats from the study of Robertson et al. (1947), is in fact taken from a literature review that was prepared for EPA consideration. The EPA Work Group[9] did not actually set an RfC, considering the available data set to be inadequate.

Summary

57. Acute exposure to PG aerosol caused irritation to the eyes and throat in humans, as well as causing cough, nasal burning and stinging. Evaluations of the possible effects of longer term exposures have generally been limited by concomitant exposure to other substances.

58. Experimental studies of short-term exposure to inhaled PG aerosols in rats have shown local irritant effects.

59. Various longer-term experimental studies have been carried out. In 28-day inhalation studies carried out by Werley et al. (2011), animals (rats and dogs) were exposed to a fixed high concentration of PG aerosol for varying short time-periods each day. A NOEL of approximately 20 mg/kg bw/day deposited dose in the lung (from a nominal target dose of approximately 200 mg/kg bw/day, assuming 10% pulmonary deposition fraction[10]) was determined in rats, based on minimal laryngeal squamous metaplasia at higher doses[11].In dogs, a NOEL of approximately 6 mg/kg bw/day deposited dose in the lung (from a nominal target dose of 30 mg/kg bw/day, assuming 20% pulmonary deposition fraction[12]) was determined in Beagle dogs, based on haematological changes at higher doses[13]. However, such effects were within the historical control range for the age and strain of dog.

60. The 13-week inhalation study in rats carried out by Suber et al. (1989) indicated nasal haemorrhaging and ocular discharge in all treatment groups following exposure to 160, 1000, 2200 mg/m^3 PG aerosol for 6 h/day, 5 days/week. There were no treatment-related gross pathology changes, but thickened respiratory epithelium with increased numbers of goblet cells and mucin content (males and females), and body weight decreases and changes in the leukocyte profile (females only) were observed at the top two doses. A NOAEL of 160 mg/m^3 based on increased number of goblet cells was determined by HCN, and a LOAEL of 160 mg/m^3 based on nasal haemorrhaging was determined by ATSDR and German Committee on Indoor Guide Values. This study was used as a basis to set air-concentration exposure guidelines for PG by The Dutch Expert Committee on Occupational Standards (50 mg/m^3 8-h TWA) (HCN 2007), the US-ATSDR (0.028 mg/m^3 intermediate-duration MRL) (ATSDR 1997), and the German Committee on

[9] US EPA RfD/RfC Work Group reviewed health effects data for PG in 1991, but determined that they were inadequate for the derivation of an inhalation RfC.

[10] Rats exposed to 30 mg/L (30,000 mg/m^3) PG aerosol for 12 min/day during 28 days.

[11] Rats exposed to 30 mg/L (30,000 mg/m^3) PG aerosol for 40 or 120 min/day during 28 days.

[12] Dogs exposed to 5 mg/L (5000 mg/m^3) PG aerosol for 12 min/day during 28 days.

[13] Dogs exposed to 5 mg/L (5000 mg/m^3) PG aerosol for 36 or 2x60 min/day during 28 days.

Indoor Guide Values (RW I of 0.06 mg/m^3) (Umweltbundesamtes 2017). In the UK, the workplace exposure limits (8-h time-weighted average (TWA)) for long-term exposure to PG are 150 ppm, or 474 mg/m^3, for total vapour + particulates, and 10 mg/m^3 for particulates alone (HSE 2011).

61. Some recent studies have reported reduced body weight gain in mice whole-body exposed to PG aerosol during post-natal development, as compared with air-exposed controls. However, direct measurements of PG exposure concentrations were not described in the reports of these studies (McGrath-Morrow et al. 2015, Smith et al. 2015, Larcombe et al. 2017). One study also indicated alterations in respiratory function tests in mice exposed to PG aerosol during weeks 4-12 of post-natal development. However, measured PG exposure concentrations in this study appear to be extremely high, which may have been a reporting error (Larcombe et al. 2017).

62. PG toxicity by oral route is considered to be extremely low, and it is classed as GRAS. Very high doses of PG administered to humans, mostly by the i.v. route, have been reported to produce hyperosmolality, metabolic acidosis and renal dysfunction. LD_{50} values range from 8-46 g/kg bw in the rat to 25-32 g/kg bw in the mouse and the NOAEL for long-term oral exposure is reported to be higher than 1 g/kg bw/day in mice, rats, dogs, and cynomolgus monkeys.

63. Data from exposed humans suggests that PG may be irritating to the skin and eyes. PG can produce skin sensitising effects in a small percentage of people. In animals, PG causes little or no skin irritation, slight eye irritation and no skin sensitisation.

64. PG is not considered to be genotoxic or carcinogenic.

GLYCEROL (GLYCERIN(E), VEGETABLE GLYCERIN(E))

Chemical properties and uses

65. Unless otherwise indicated[14], information in the following paragraphs is summarised from reviews published by OECD (2002), DFG (2006), and EFSA (2017).

66. Glycerol (propane-1,2,3-triol; glycerine; CAS number 56-81-5) exists at room temperature as a colourless, odourless, viscous liquid that is miscible with water and with ethanol. It has a melting point of 18 °C, boiling point of 290 °C, and a vapour pressure of 0.000106 at 25 °C. It is usually produced either from naturally occurring fats and oils, or by chemical synthesis from propene. It is used as a humectant, solvent, sweetener, filler and thickening agent in foods, and is a component of many cosmetic preparations and pharmaceuticals, both as an active ingredient (for osmotic

[14] Individual references from within these reviews are only cited where this was considered to be specifically relevant.

effects, e.g. by oral administration for the reduction of intraocular pressure in glaucoma patients) or as an excipient. Glycerol is considered as GRAS for use in food by the FDA, is an authorised food additive (E 422), and is permitted in cosmetic products in the EU. Glycerol can be used as an anti-freeze and cryoprotectant, and industrially as a vibration dampener, ultrasonic couplant and internal combustion fuel. The glycerol in E(N)NDS liquids is often referred to as vegetable glycerine (VG).

Toxicokinetics

67. Glycerol is rapidly absorbed via the gastrointestinal tract. It is metabolised mainly in the liver (80-90%) and kidneys (10-20%), where it is phosphorylated by glycerol kinase, or it may be combined with free fatty acids in the liver to form triglycerides, which are distributed to adipose tissue. In humans, unmetabolised glycerol (7-14%) is excreted in the urine. Dermal absorption in humans from exposure to a saturated solution for a 1-h period was calculated as 51 or 171 mg, respectively, from two different studies, assuming a skin surface of 2000 cm^2 (*reviewed by* DFG (2006)).

Toxicity

Inhalation toxicity

Acute toxicity

Human

68. No data were identified.

Non-human

69. No data were identified.

Repeat dose toxicity

Human

70. No data were identified.

Non-human

71. Lechasseur et al. (2017) reported that exposure to PG and/or glycerol aerosol altered genes involved in control of circadian rhythm in mice. Female BALB/c mice were whole-body exposed to aerosol of either 70% PG/30% glycerol, 100% PG, or 100% glycerol, without nicotine or flavour, for 8 weeks (2 h/day, 5 days/week). Exposure was achieved as 3x puffs of 80 µL test liquid per minute, mixed with room air by laminar flow at a rate of 3 L/min into an exposure chamber. Exposure concentrations were not reported. Microarray gene-expression analysis of various tissues (lung, brain, liver, kidney, skeletal muscle) appeared to show alterations in

genes involved in regulation of circadian rhythm in all 3 test groups, compared with the room air-exposed control group.

72. Renne (1992) carried out two studies in which Sprague Dawley rats were exposed nose-only to glycerol aerosol.

73. In the first study, groups of 10 male and 10 female rats were nose-only exposed to mean concentrations of 0 (filtered room air), 1000, 1930, or 3910 mg/m^3 glycerol aerosol (with mean MMAD values < 1.5 µm) for 14 days (6 h/day, 5 days/week). Blood glucose levels were significantly reduced in all exposed females compared with controls, but there was no significant difference between exposure groups. Body-weight gains were reduced in all animals. There was no effect on lung, liver, kidney, brain and heart weight, or any macroscopic findings reported. Histopathological examination of the respiratory tract, liver, kidneys, and heart of controls and high-dose-group animals revealed that treatment was associated with local irritant effects: minimal to mild squamous metaplasia of the epiglottis (1/10, 13/18, 16/19, and 13/14 animals in the 0, 1000, 1930, and 3910 mg/m^3 treatment groups, respectively).

74. A second study was carried out in which 15 rats/sex/group were exposed nose-only to mean glycerol aerosol (mean MMAD values < 2 µm) concentrations of 0, 33, 167, or 662 mg/m^3 for 13 weeks (6 h/day, 5 days/week). Treatment was associated with decreased plasma triglyceride in males, but there was no dose relationship. No consistent treatment-related effects on haematology, organ weights, or gross pathology were observed. Minimal squamous metaplasia of the epiglottis was observed in 2/25, 1/19, 4/20, and 10/21 rats in the 0, 33, 165, and 662 mg/m^3 groups, respectively. In addition, one rat in the highest dose group had mild squamous metaplasia of the epiglottis. Ultrastructural examination of Clara cells showed no evidence of proliferation on smooth endoplasmic reticulum. No irritation was observed. A NOEL of 167 mg/m^3 was determined based on adaptive responses to mild local irritant effects.

75. Serra et al. (2017) investigated the effects of exposure to glycerol combustion products on the rat respiratory system. This work was carried out in the context of the developing use of residual glycerol from biodiesel production as a combustion fuel to generate heat for industrial purposes. Male Wistar rats whole-body exposed to glycerol combustion gas 5 h/day, 5 days/week, for 13 weeks, showed changes in respiratory system mechanics and respiratory tract histology in comparison to ambient air-exposed controls. However, as it is unclear what the test exposure was[15], this study is not described in more detail.

76. Exposure to E(N)NDS aerosols led to impairments in pulmonary function, in the absence of pulmonary inflammation, in a mouse model designed to simulate effects of exposure during adolescence (Larcombe et al. 2017). Groups of 12 female

[15] The test exposure is described as 'atomized glycerol in a combustion chamber', and may be combusted petroleum liquid containing a small amount of added glycerol.

BALB/c mice were whole-body exposed between the ages of 4-12 weeks to control air (AIR), CC smoke (SMOKE), or 1 of 4 E(N)NDS aerosols of 'American Tobacco' flavour, as follows: PG with no nicotine (0-PG), PG + 12 mg/mL nicotine (12-PG), glycerol with no nicotine (0-VG), or glycerol + 12 mg/mL nicotine (12-VG). Average measured chamber concentrations were reported as 0.014 g/cm^{-3} for PG and 0.018 g/cm^{-3} for VG [16]. Mice were exposed for 1 h/day, 5 days/week from weeks 4-10, then twice daily for 1 h, 5 days/week, during weeks 11 and 12. After 8 weeks of exposure, all treatment groups weighed significantly less than AIR controls, with the lowest weight gains in nicotine-exposed mice. Lung mechanics and function were assessed 24 h after the final exposure. Mice exposed to E(N)NDS aerosols showed various differences in pulmonary function compared with AIR controls, including decreased airway resistance at functional residual capacity (FRC) (0-PG and 0-VG), increased tissue damping at FRC (all 4 E(N)NDS groups), increased tissue elastance at FRC (0-PG and 0-VG), decreased lung volume and changes in volume dependence of tissue damping and elasticity (0-PG, 0-VG, 12-PG). Methacholine challenge tests showed that SMOKE and VG-exposed mice were significantly more responsive than AIR or PG-exposed, whether or not nicotine was present in the aerosol. SMOKE but not E(N)NDS exposure was associated with increased pulmonary inflammation (increased BAL cells). Overall, the authors concluded that 1] glycerol-based E(N)NDS aerosols induced more severe functional pulmonary impairments than PG-based aerosols, and 2] there was little effect of the presence or absence of nicotine.

Reproductive and developmental toxicity

77. No data were identified.

Mutagenicity/genotoxicity

78. No data were identified.

Carcinogenicity

79. No data were identified.

Overview of non-inhalation toxicity

80. Oral exposure to glycerol is not usually associated with adverse effects (EFSA 2017).

Acute toxicity

81. In humans, therapeutic use by oral bolus of glycerol at 1000–1500 mg/kg bw (for treatment of glaucoma) may trigger an increase in plasma osmolality and

[16] The Secretariat assumes that the concentrations should be reported as g/cm^3 Exposure concentrations of 0.014 g/cm^3 and 0.018 g/cm^3 are equivalent to 14,000,000 mg/m^3 and 18,000,000 mg/m^3, respectively, which is far higher than other studies reported in this paper so itis possible that there is a reporting error.

dehydration, with side effects such as headache, nausea and vomiting. In one study, a minimum dose of 125-333 mg/kg bw/h was considered necessary to achieve a therapeutic reduction in intracranial pressure, which would also be likely to be responsible for the side effects experienced (EFSA 2017).

82. In animals, oral LD_{50} values for glycerol are reported as between 4 and 38 g/kg bw, with the majority in the range 23–38 g/kg bw (OECD 2002). LD_{50} values by i.p. or i.v. injection are > 4 g/kg bw in rats and mice, while sub-cutaneous (s.c.) injection is more toxic, with reported LD_{50} values of 91 and 100 mg/kg bw in mice and rats, respectively (OECD 2002).

Irritation and sensitisation

83. Glycerol is not irritating to rabbit skin but may be slightly irritating to the eyes. No irritation was seen when undiluted glycerol was applied to the lining of the oral cavities of rats, rabbits, and dogs (Informatics Inc 1973, *cited by* EFSA (2017)). There was no evidence of sensitisation in animal tests and no, or very low, sensitising potential in humans (OECD 2002, DFG 2006).

Repeat-dose toxicity

84. Available animal studies have not indicated adverse effects from repeated oral exposure to glycerol (OECD 2002, DFG 2006, EFSA 2017). In a short-term study, administration by gavage of 2800 mg/kg bw/day (rats) or 5600 mg/kg bw/day (dogs) 100% glycerol, 3x per day for 3 days led to dose-dependent irritant effects in the stomach and duodenum but no systemic toxicity (Staples et al. (1967), *cited by* EFSA (2017)). The irritant effects were considered by the EFSA (2017) panel to be likely due to the hygroscopic and osmotic effects of large doses of glycerol administered by gavage. No adverse effects were observed in rats fed doses up to 10 g/kg bw/day for 1 year and there was no increase in tumour incidence in rats fed doses up to 5 g/kg bw/day for 2 years.

Reproductive and developmental toxicity

85. Multi-generation reproductive toxicity studies of glycerol have not shown adverse effects. Developmental toxicity was not observed in rats, mice, and rabbits at the highest doses tested (1600, 1280, and 1180 mg/kg bw, respectively). One study showed suppression of spermatogenesis by injection of glycerol into the testes of rats and monkeys. However, a study of 64 male workers in glycerol manufacture did not observe differences in sperm count or form in comparison with a control group without occupational glycerol exposure (*reviewed by* OECD (2002), DFG (2006), EFSA (2017)).

Genotoxicity and carcinogenicity

86. Overall, glycerol was not considered to be mutagenic, genotoxic, or carcinogenic in the evaluations by OECD (2002), DFG (2006), and EFSA (2017). A

series of studies that indicated a possible weak co-carcinogenic effect on lung tumour (adenoma) formation of oral administration of 5% glycerol in drinking water (around 5000 mg/kg bw/day) for 20 weeks in mice after a single s.c. injection with an initiator was considered by the EFSA Panel to be not relevant for the risk assessment of glycerol as a food additive (EFSA 2017). OECD (2002) noted that in these studies, treatment with glycerol alone did not increase the number of tumour-bearing mice relative to controls, and concluded that overall these did not raise concern for carcinogenic potential.

Regulations and guidelines

Inhalation

Occupational

87. In 2006, DFG in Germany derived a maximum workplace concentration (MAK[17]) value of 50 mg/m^3 for glycerol (glycerin), based on a no observed adverse effect concentration (NOAEC) of 165 mg/m^3 for squamous metaplasia in the epiglottis of rats (corresponding to an uptake of about 25 mg/kg bw at an inhaled volume of 10 m^3 and 100% absorption) from the studies by Renne (1992) (paragraphs 72-74) (DFG 2006). This value was re-evaluated in 2015, based on a re-assessment by of the data by Kaufmann et al. (2009) (although it was noted that no new data had been identified), and the MAK value was raised to 200 mg/m^3 (Hartwig 2017), with the following reasons cited:

- glycerol is not an eye irritant
- the minimal/slight metaplasia of the larynx at 662 mg/m^3 in the study of Renne (1992) is not interpreted as adverse, hence 662 mg/m^3 is the NOAEC
- the response did not increase in severity between the 2-week and 13-week studies.

88. In the US, the U.S. Occupational Safety and Health Administration (OSHA) is establishing an 8-h TWA of 10 mg/m^3 for total particulates and 5 mg/m^3 (respirable fraction) for glycerin mist (https://www.cdc.gov/niosh/pel88/56-81.html, accessed 09/04/18).

89. The long-term workplace exposure limit for glycerol (glycerin) mist in the UK is 10 mg/m^3 TWA (HSE 2011). No short term WELs are available.

[17] The MAK value is the maximum permissible concentration of a substance as a gas, vapour or aerosol in the air at the workplace which, according to current knowledge, does not normally affect worker health or cause unreasonable nuisance even with repeated and long-term exposure, usually 8 hours a day, but assuming an average weekly working time of 40 hours (http://www.dfg.de/en/dfg_profile/statutory_bodies/senate/health_hazards/structure/working_groups/derivation_mak/index.html, accessed 28/03/18)

Non-inhalation

90. In an evaluation of the use of glycerol (E 422) as a food additive, EFSA concluded that toxicological studies in animals did not provide any indication for adverse effects, including at the highest dose tested in a chronic toxicity study (10,000 mg/kg bw/day), and that there is no need for a numerical acceptable daily intake (ADI) for glycerol (E 422). However, it was noted that production methods may lead to the presence or formation of contaminants which are of toxicological concern (EFSA 2017).

Risk assessments

91. Kienhuis et al. (2015) published a preliminary risk assessment of glycerol exposure from E(N)NDS. Components of commercial, nicotine-free 'shisha pens' were evaluated and the major constituent was identified as a 54%/46% mixture of PG/glycerol, calculated to produce 0.7 mg/puff PG and 0.6 mg/puff glycerol. Based on a 50-70 mL puff volume, the maximum alveolar concentrations of glycerol after 1 puff were estimated to be 348-495 mg/m^3. An MOE for glycerol was not calculated due to the lack of study data on the effects of inhalation in humans. However, the authors noted that the estimated alveolar concentrations were in a similar range to the LOAEL of 662 mg/m^3 for local irritant effects in rats exposed continuously to glycerol for 13 weeks in the study of Renne (1992). Farsalinos and Baeyens (2016) criticised this approach, noting that the use of continuous exposure studies for risk assessment of glycerol or PG from E(N)NDS use (where an average puff may have a duration of around 1 s) is inappropriate and would probably overestimate any risk: they commented that this approach would be useful if evaluating total absorption for relation to any systemic effects, but would not be of value for local effects considering the important differences in exposure patterns.

Summary

92. Limited data are available regarding exposure to glycerol by inhalation. One study indicated alterations in respiratory function tests in mice whole-body exposed to glycerol aerosol during weeks 4-12 of postnatal development, with glycerol inducing effects that were more severe than those from PG, as compared with room air, however reported exposure concentrations appear to have been extremely high in this study, which may have been a reporting error (Larcombe et al. 2017). Rats exposed to 662 mg/m^3 glycerol aerosol 6 h/day, 5 days/week, for 13 weeks showed no dose-related systemic effects, but minimal-to-mild squamous metaplasia of the epiglottis, from which a NOEL of 167 mg/m^3 was determined (Renne 1992). This value was initially considered as the NOAEC by DFG in Germany to derive a MAK for glycerol of 50 mg/m^3 (DFG 2006). However, a recent re-evaluation took the higher dose of 662 mg/m^3 as the NOAEC, considering that the effects at this dose were not adverse, and thus the MAK value was raised to 200 mg/m^3 (Hartwig 2017). The long-term workplace exposure limit for glycerol (glycerin) mist in the UK is 10 mg/m^3 TWA (HSE 2011).

93. Glycerol is classed as GRAS for use in foods, and oral exposure to high levels is not usually associated with adverse effects. Glycerol is given therapeutically to humans by oral bolus to reduce intraocular pressure in the treatment of glaucoma, with potential side effects of high doses including headache, nausea and vomiting. Oral LD_{50} values ranged from 4 to 38 g/kg bw.

94. Glycerol may be slightly irritating to the eyes, not irritating to the skin and may have very slight skin sensitising potential in humans.

95. Glycerol is not considered to be genotoxic or carcinogenic.

Questions for the Committee

96. Members are asked to consider this paper and in particular:

i. Is there an appropriate health-based inhalation guidance value or can the Committee identify an air concentration which the Committee can use to assess the risk from PG in E(N)NDS aerosol?

ii. Is there an appropriate health-based inhalation guidance value or can the Committee identify an air concentration which the Committee can use to assess the risk from VG in E(N)NDS aerosol?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
April 2018

Abbreviations

ADI	Acceptable daily intake
ATSDR	US Agency for Toxic Substances and Disease Registry
BAL(F)	Broncho-alveolar lavage fluid
CAG	Capillary aerosol generator
CNS	Central nervous system
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
EFSA	European Food Safety Authority
EMA	European Medicines Agency
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
FDA	U.S. Food & Drug Administration
FEV1	Forced expiratory volume in 1 second
FRC	Functional residual capacity
GRAS	Generally recognised as safe
GSD	Geometric standard deviation
HCN	Health Council of the Netherlands
i.p.	Intra-peritoneal
i.v.	Intra-venous
LC_{50}	Lethal concentration 50
LD_{50}	lethal dose 50
LO(A)EC	Lowest observed (adverse) effect concentration
LO(A)EL	Lowest observed (adverse) effect level
LOQ	Limit of quantitation
MAK	Maximum permissible concentration in workplace air
MCC	Mucociliary clearance
MMAD	Mass median aerodynamic diameter
MOE	Margin of exposure
MRL	Minimum risk level
MTD	Maximum tolerated dose
NO(A)EL	No observed (adverse) effect level
OECD	Organisation for Economic Co-operation and Development
OSHA	U.S. Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbon
PEL	Permissible exposure limit
PG	Propylene glycol
PoD	Point of departure
RfC	Reference concentration
RfD	Reference dose
s.c.	Sub-cutaneous
SM	Sodium metabisulphite
TEEL	Temporary emergency exposure limit
TSNA	Tobacco-specific nitrosamine
TWA	Time-weighted average
VG	Vegetable glycerin(e) (glycerol)
VOC	Volatile organic compound

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 3: Toxicological review of the main constituents, propylene glycol (PG) and vegetable glycerine (VG, glycerol)

Details of Literature search carried out by NCET at WRc/IEH-C

Relevant literature was obtained from reviews published by authoritative bodies, as described in paragraphs 5 and 6 of the main report. In addition, searches for further literature relating to toxicity of PG and VG/glycerol by inhalation exposure for the period 01/01/13 – 26/03/18 were identified as described below.

The following literature searchs were performed by NCET at WRc/IEH-C under contract to PHE on 26/03/18 in Scopus and PubMed:

Propylene glycol

Scopus

(((TITLE-ABS-KEY ("propylene glycol" OR "propane-1,2-diol") OR CASREGNUMBER ("57-55-6")) AND PUBYEAR > 2012) AND (TITLE-ABS-KEY (inhal* OR aerosol* OR vapor* OR lung* OR respirat* OR brocho*) AND PUBYEAR > 2012)) AND (EXCLUDE (LANGUAGE , "French") OR EXCLUDE (LANGUAGE , "Chinese") OR EXCLUDE (LANGUAGE , "Spanish") OR EXCLUDE (LANGUAGE , "German") OR EXCLUDE (LANGUAGE , "Danish") OR EXCLUDE (LANGUAGE , "Polish")): 391 refs.

PubMed

((((((("propylene glycol" [Title/Abstract] OR "propane-1,2-diol"[Title/Abstract])) OR "57-55-6"[EC/RN Number])) AND ((inhal* [Title/Abstract] OR aerosol* [Title/Abstract] OR vapor* [Title/Abstract] OR lung* [Title/Abstract] OR respirat* [Title/Abstract] OR broncho*[Title/Abstract])))) AND english[Language] AND ("2013/01/01"[PDat] : "2018/12/31"[PDat])): 148 refs.

A total of 407 references were identified from these searches, of which 9 were selected as of potential relevance to the toxicity of PG by inhalation exposure. Papers reporting studies of PG in E(N)NDS liquids/aerosol mixtures were excluded as these will be addressed in a separate review.

Glycerol (vegetable glycerine)

Scopus

((TITLE-ABS-KEY ("Glycerol" OR "glycerine" OR "propane-1,2,3-triol") OR CASREGNUMBER ("56-81-5")) AND PUBYEAR > 2012 AND (TITLE-ABS-KEY (inhal* OR aerosol* OR vapor* OR lung* OR respirat* OR broncho*) AND PUBYEAR > 2012)) AND NOT (TITLE-ABS-KEY (film* OR cryopreservation OR metabolism) AND PUBYEAR > 2012) AND (EXCLUDE (LANGUAGE , "Chinese ") OR EXCLUDE (LANGUAGE , "Spanish ") OR EXCLUDE (LANGUAGE , "French ") OR EXCLUDE (LANGUAGE , "Polish ") OR EXCLUDE (LANGUAGE , "Danish ") OR EXCLUDE (LANGUAGE , "German ") OR EXCLUDE (LANGUAGE , "Korean ") OR EXCLUDE (LANGUAGE , "Portuguese ")) AND (EXCLUDE (DOCTYPE , "cr")) : 746 refs.

PubMed

(((((((("Glycerol" [Title/Abstract] OR "glycerine" [Title/Abstract] OR "propane-1,2,3-triol"[Title/Abstract])) OR "56-81-5"[EC/RN Number]))) AND ((inhal* [Title/Abstract] OR aerosol* [Title/Abstract] OR vapor* [Title/Abstract] OR lung* [Title/Abstract] OR respirat* [Title/Abstract] OR broncho*[Title/Abstract])) AND english[Language]) AND ("2013/01/01"[PDat] : "2018/12/31"[PDat]))) NOT ((((film* [Title/Abstract] OR cryopreservation [Title/Abstract] OR metabolism[Title/Abstract])) AND english[Language]) AND ("2013/01/01"[PDat] : "2018/12/31"[PDat])): 328 refs

A total of 872 references were identified from these searches, of which 2 were selected as of potential relevance to the toxicity of VG/glycerol by inhalation exposure. Papers reporting studies of VG/glycerol in E(N)NDS liquids/aerosol mixtures were excluded as these will be addressed in a separate review

For completion, the reference lists of selected papers were examined for further relevant publications, and additional *ad hoc* searches were carried out as considered appropriate.