TOX/2018/23

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). Follow up to paper 3: additional information on 13-week inhalation studies in rats of propylene glycol aerosol (Suber et al., 1989) and glycerol aerosol (Renne et al., 1992)

Background

1. As part of a review of the possible human health effects of electronic nicotine delivery systems and electronic non-nicotine delivery systems (E(N)NDS – 'e-cigarettes'), a review of the toxicity of the major constituents of E(N)NDS liquids (e-liquids), propylene glycol (PG) and vegetable glycerine (VG, glycerol), with a focus on inhalation as aerosol, was discussed at the May 2018 COT meeting (TOX/2018/19).

2. This discussion paper identified two key studies, in which the toxicological effects of exposure for 13 weeks to aerosols of PG (Suber et al. 1989) or glycerol (Renne 1992) had been evaluated. It was noted that these studies had been used by other authorities as a basis for setting health-based guidance values (HBGV) for inhalation exposure to aerosols of PG (ATSDR 1997, HCN 2007, Umweltbundesamtes 2017) and glycerol (DFG 2006, Hartwig 2017).

3. Further details of these two studies are provided in the following paragraphs, along with suggestions for possible calculation of HBGVs for use in evaluation of exposures to PG aerosol and glycerol aerosol from E(N)NDS.

Propylene glycol – Suber et al. (1989)

4. Suber et al. (1989) carried out a 90-day study to investigate inhalation exposure to PG aerosol in rats. The full text of this publication is appended at Annex A.

5. Groups of 19 male and 19 female Sprague-Dawley rats were exposed by nose-only inhalation to PG aerosol during 6 hours per day, 5 days per week, for 90 days. Rats in a control group were exposed to humidified, filtered room air[1]. Aerosols were produced using nebulisers and delivered to Battelle nose-only chambers in a manner that the authors described as supplying a uniform individual air supply to

[1] Control exposures were probably given in the same manner as test exposures, but this is not clear from the report of Suber et al. (1989).

each animal at an average flow rate of 1 to 1.5 L/min. One nebuliser provided aerosol to the high-concentration exposure chamber. A different nebuliser supplied the medium-concentration chamber, and then, 'following serial dilution with air', the low-concentration chamber. Variability of aerosol concentration in the chambers was less than 5%. Flow-rate was 35-50 L/min. Target PG aerosol concentrations were 100, 1000, or 2200 mg/m^3 PG, and measured mean concentrations, sampled once per day at 2 individual animal ports per chamber, were 160, 1010, 2180 mg/m^3, respectively). Particle size distributions were measured once per week, indicating mass median aerodynamic diameters (MMAD) of 1.96 µm (geometric standard deviation (GSD), 1.57) for high-concentration aerosol, and 2.22 µm (GSD, 1.44) for medium-concentration aerosol. MMAD values were not obtained for low-concentration aerosol due to the low concentration of particles.

6. Animals were observed daily for evidence of toxicity, and body weights were recorded weekly. Respiratory rates and tidal volumes were measured in 4 rats/sex/group on study days 7, 42, and 84, with no significant differences observed between groups. Clinical chemistry and haematology measurements were carried out on retro-orbital blood obtained before exposures began and before euthanasia.

7. At the end of the 90-day exposure period, rats were anaesthetised with 70% CO_2 in air, exsanguinated by transection of the ventral aorta, and a complete autopsy was performed. Organ weights were recorded for lungs, thymus, spleen, liver, heart, kidneys, adrenals, ovary, urinary bladder, uterus, testes, prostate, and brain. Organs (except lungs) were fixed by immersion in 10% formalin. Lungs were fixed by instillation of 10% buffered formalin through the trachea under pressure, and nasal passages, lungs, trachea and larynx were examined by light microscopy. Respiratory tract tissue slides stained with haematoxylin-phloxine-saffron (HPS) (for polysaccharides and mucus) were examined microscopically.

8. During treatment, nasal haemorrhaging occurred in all groups, with recovery on non-treatment days (< 1%, 64%, 74%, and 75% (males); < 1%, 14%, 71%, and 71% (females) in control, low, medium, and high exposure groups, respectively, between weeks 2-13). Similar trends were observed for ocular discharge (< 5%, 16%, 40%, 40% (males); 8%, 14%, 28%, 35% (females) in control, low, medium, and high exposure groups, respectively). Body-weight reduction was observed in female rats in the two highest dose groups (5-7% body weight reduction in the highest-dose group), associated with reduced feed consumption. A trend towards reduced body weight was seen in male rats but the changes were not statistically significant compared with controls. High-exposure females also showed significant changes compared with controls of increased serum phosphate and decreased mean corpuscular haemoglobin concentration. Medium and high-dose females had decreased leucocyte count, banded neutrophil count and lymphocyte count, and serum protein was increased in medium-dose females only. Serum chloride was elevated in low-dose females. Medium and high-dose male rats had significantly reduced banded neutrophil count (which was considered by the authors not to be

relevant to PG exposure), decreased serum sorbitol dehydrogenase and ɣ–glutamyl transferase. Decreasing trends in serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and ɣ–glutamyl transferase, serum protein, albumin, cholesterol, and inorganic phosphate were reported in males but not females.

9. No treatment-related changes in gross pathology were observed at termination. Absolute organ weights were significantly decreased for lungs (high-exposure females), spleen (low and high-exposure males), liver (medium and high-exposure males), and kidney (medium and high-exposure females and males). Lung weights were significantly increased in low-exposure males. Relative to terminal body weight, changes in organ weights were noted only for spleen (decreased in low-exposure males) and lung (increased in low-exposure males).

10. Microscopic evaluation of the nasal cavity showed thickened respiratory epithelium in the posterior portion of the nasal cavity, with increased numbers of goblet cells and goblet-cell mucin content in male and female rats in the medium and high dose groups. There were no histological changes in the trachea, lungs, or larynx.

Calculation of a health-based guidance value (HBGV)

Previous values calculated by other authorities based on data from Suber et al. (1989)

11. 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 time-weighted average (TWA)) for summed concentration of vapour and aerosol. This was based on a no observed adverse effect level (NOAEL) of 160 mg/m^3 based on increases in numbers of goblet cells in male and female rats in the medium and high dose groups, with supporting evidence that changes in goblet cells were also found in another study in which rabbits were acutely exposed to 10% PG aerosol (Konradova, Vavrova and Janota 1978).

12. An uncertainty factor (UF) of 3 (for inter-individual variation) was applied to the NOAEL to derive the occupational exposure limit. 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[2] should be applied to aerosols of PG (HCN 2007).

[2] A footnote in the HCN (2007) report noted that 'In the Netherlands, MAC values for inhalable and respirable dust existed until January 2007. At the moment, the Dutch Expert Committee on Occupational Standards is re-evaluating the scientific literature in order to recommend health-based occupational exposure limits for inhalable and respirable dust'

13. The German Committee on Indoor Guide Values recommended a health precaution guide value (RW I, guideline value I[3]) of 0.06 mg/m^3 for PG, based on a health hazard guide value (RW II, guideline value II) of 0.6 mg/m^3, derived using a lowest observed adverse effect level (LOAEL) of 160 mg/m^3 based on nasal haemorrhage.

14. The RW II was obtained by dividing the LOAEL by an adjustment factor of 280 (5.6 for time-scaling to continuous exposure, 2 for use of a sub-chronic study, 2.5 for inter-species extrapolation, 10 for intra-species variation (Umweltbundesamtes 2017)).

15. The US Agency for Toxic Substances and Disease Registry, in 1997, established an intermediate-duration minimum risk level (MRL) for PG of 0.009 ppm [0.028 mg/m^3], based on nasal haemorrhaging at the lowest dose tested (51 ppm [160 mg/m^3]) (LOAEL) (ATSDR 1997). The MRL was obtained by dividing the LOAEL by 5600 (10 for use of a LOAEL, 10 for inter-species extrapolation, and 10 for intra-individual variation, and 5.6 to adjust for continuous exposure). 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).

Suggestions for possible calculation of an HBGV for use in evaluation of exposures to PG aerosol from E(N)NDS

16. Three possible options are presented below as options for deriving a specific HBGV for PG, using different points of departure (PoDs) and uncertainty factors (UFs), should the Committee wish to derive one for consideration of E(N)NDS.

17. Option 1: Point of departure (PoD) = NOAEL of 160 mg/m^3 for increased number of goblet cells and goblet-cell mucin content in rats

- Adjustment for continuous exposure (from 6 h/day; 5 days/week): x5.6
- UFs:
  - for inter-species extrapolation: x10
  - for inter-individual extrapolation: x10

$HBGV_{(continuous\ exposure)}$ = 160 / (5.6 x 10 x 10) = 0.286 mg/m^3

[3] 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).

18. Option 2: PoD = LOAEL of 160 mg/m^3 for nasal haemorrhaging in rats

- Adjustment for continuous exposure (from 6 h/day; 5 days/week): x5.6
- UFs:
  - for use of a LOAEL x10
  - for inter-species extrapolation: x10
  - for inter-individual extrapolation: x10

$HBGV_{(continuous\ exposure)}$ = 160 / (5.6 x 10 x 10 x 10) = 0.029 mg/m^3

19. Option 3: PoD = NOAEL of 2180 mg/m^3. Based on discussion at the COT meeting in May 2018, it was not clear that the Committee considered increased number of goblet cells and goblet-cell mucin content at medium and high doses nor nasal haemorrhaging at all doses in rats to be adverse effects of toxicological concern. This option therefore uses the highest dose in the Suber et al. study as the PoD.

- Adjustment for continuous exposure (from 6 h/day; 5 days/week): x5.6
- UFs:
  - for inter-species extrapolation: x10
  - for inter-individual extrapolation: x10

$HBGV_{(continuous\ exposure)}$ = 2180 / (5.6 x 10 x 10) = 3.89 mg/m^3

Glycerol – Renne et al. (1992)

20. Renne (1992) carried out two studies in which Sprague Dawley rats were exposed nose-only to glycerol aerosol. The full text of this publication is appended at Annex B.

21. In the first study, groups of 10 male and 10 female Sprague-Dawley rats were nose-only exposed to target concentrations of glycerol of 0 (filtered room air), 1000, 2000, or 4000 mg/m^3 aerosol for 6 h/day, 5 days/week, during 14 days. Mean measured concentrations were 0 (filtered room air), 1000, 1930, or 3910 mg/m^3, and mean MMAD values were 1.46 µm (GSD, 1.75), 1.45 µm (GSD, 2.11), and 1.36 µm (GSD, 1.91), respectively. Exposures were given in Battelle exposure chambers,

with test and control groups undergoing the same exposure conditions. Aerosol exposure concentrations were measured hourly, and MMAD/GSD measurements were made weekly.

22. Rats were observed twice daily for signs of toxicity, weighed every two to three days, and diet consumption was recorded once per week. At the end of the study period, rats were anaesthetised with 70% CO_2 and euthanised by exsanguination via the brachial artery. Terminal necropsy was performed, with examination of the entire respiratory tract and associated lymph nodes, the liver, kidneys, and heart. Lungs, liver, kidneys, brain, and heart were weighed and then fixed by perfusion with neutral buffered formalin. Sections of nasal cavity, larynx, trachea, lungs, mainstem bronchi and associated lymph nodes, and thymic lymph nodes were examined microscopically after staining with haematoxylin and eosin (H&E), and duplicate slides were stained with Alcian blue/periodic acid Schiff (PAS) to evaluate goblet cell changes. H&E sections of liver, kidneys, and heart were also examined from the high-dose glycerol exposure and control groups.

23. No clinical signs attributable to glycerol exposure were observed. Body-weight gains were reduced in all groups compared with controls, with statistically significant decreases in females. Blood glucose levels were significantly reduced in all exposed females compared with controls, but there were no significant differences between exposure groups.

24. There were no effects of treatment on lung, liver, kidney, brain and heart weight, nor any macroscopic findings reported. Histopathological examination showed a statistically significant increase in minimal-to-mild squamous metaplasia of the epithelium lining the base of the epiglottis in all treatment groups compared with controls (1/10, 13/18, 16/19, and 13/14 rats in the control, low, medium, and high dose treatment groups, respectively). The incidence of microscopic lesions in other organs was low, with no evidence of them being related to glycerol exposure.

25. A follow-up study was carried out for 13 weeks. In this study, 15 rats/sex/group were exposed, in a similar manner as in the 2-week study, to target glycerol aerosol concentrations of 0, 33, 165, or 660 mg/m^3. Study details were similar to the 2-week study, except that body weights were measured weekly, and necropsy included a more extensive collection of tissues.

26. Mean measured exposure concentrations were 0, 33, 167, and 662 mg/m^3, with MMAD (GSD) values of 1.09 µm (1.90), 1.49 µm (1.69), and 1.61 µm (1.75) in the low, medium, and high concentration exposure groups, respectively.

27. No clinical signs attributable to glycerol exposure were observed. No consistent treatment-related effects on haematology, organ weights, or gross pathology were observed. In clinical chemistry analyses, the only difference seen was increased plasma triglyceride in low and medium exposure group males, but there was no dose relationship.

28. Minimal squamous metaplasia of the epithelium lining the base of the epiglottis was observed in 2/25, 1/19, 4/20, and 10/21 rats in the control, low, medium, and high concentration exposure groups, respectively. The increased incidence of squamous metaplasia was statistically significant compared with controls in the high concentration group and was considered to be related to the exposure to 662 mg/m^3 glycerol aerosol. In addition, one rat in this group had mild squamous metaplasia. The incidence of microscopic lesions in other organs was low, with no evidence of it being related to glycerol exposure.

29. Ultrastructural examination of club cells showed no evidence of effects of glycerol exposure, with no proliferation of smooth endoplasmic reticulum or abnormalities in shape of mitochondria. A no observed effect level (NOEL) of 167 mg/m^3 was reported by the authors, based on squamous metaplasia of the epithelium lining the epiglottis, which was considered to be an adaptive response to mild local irritant effects.

Calculation of a health-based guidance value (HBGV)

Previous values calculated by other authorities based on data from Renne et al. (1992)

30. In 2006, DFG in Germany derived a maximum workplace concentration (MAK[4]) value of 50 mg/m^3 for glycerol (glycerin), derived from a NOAEL of 165 mg/m^3 based on 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) (DFG 2006). This value was re-evaluated in 2015, based on a re-assessment 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 NOAEL
- the response did not increase in severity between the 2-week and 13-week studies

[4] The MAK value, set by the German Committee for the determination of occupational exposure limits ('MAK-Commission') 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)

Suggestions for possible calculation of an HBGV for use in evaluation of exposures to glycerol aerosol from E(N)NDS

31. Two possible options are presented below as options for deriving a specific HBGV for glycerol, using different points of departure (PoDs) and uncertainty factors (UFs), should the Committee wish to derive one for consideration of E(N)NDS.

32. Option 1: PoD = NOAEL of 662 mg/m^3 (considering that effect of minimal-to-mild squamous metaplasia of the epiglottis in rats is not adverse)

- Adjustment for continuous exposure (from 6 h/day, 5 days/week): x5.6
- UFs:
  - for inter-species extrapolation: x10
  - for inter-individual extrapolation: x10

$HBGV_{(continuous\ exposure)}$ = 662 / (5.6 x 10 x 10) = 1.18 mg/m^3

33. Option 2: PoD = NOAEL of 167 mg/m^3 for squamous metaplasia of the epiglottis in rats

- Adjustment for continuous exposure (from 6 h/day, 5 days/week): x5.6
- UFs:
  - for inter-species extrapolation: x10
  - for inter-individual extrapolation: x10

$HBGV_{(continuous\ exposure)}$ = 167 / (5.6 x 10 x 10) = 0.298 mg/m^3

Questions for the Committee

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

i. Do Members wish to use an existing HBGV or amend one of the proposed calculations for an HGBV for inhalation exposure to propylene glycol aerosol, in relation to use of this value for future application to the assessment of safety levels associated with exposure to propylene glycol aerosol via E(N)NDS use?

ii. Do Members wish to use an existing HBGV or amend one of the proposed calculations for an HGBV for inhalation exposure to glycerol aerosol, in relation to use of this value for future application to the

assessment of safety levels associated with exposure to glycerol aerosol via E(N)NDS use?

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

Abbreviations/Glossary

ATSDR	US Agency for Toxic Substances and Disease Registry
E(N)NDS	Electronic nicotine or electronic non-nicotine delivery system
GSD	Geometric standard deviation
HBGV	Health-based guidance value
HPS	Haematoxylin-phloxine-saffron
LO(A)EL	Lowest observed (adverse) effect level
MMAD	Mass median aerodynamic diameter
MRL	Minimum risk level
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PAS	Periodic acid Schiff
PG	Propylene glycol
PoD	Point of departure
RWI	Health precaution guide value
TWA	Time-weighted average
UF	Uncertainty factor
VG	Vegetable glycerin(e)

References

ATSDR. 1997. Toxicological Profile for Propylene Glycol. ed. U. S. D. o. H. a. H. S. Agency for Toxic Substances and Disease Registry, Public Health Service. Atlanta, GA.

ATSDR. 2008. Addendum to the toxicological profile for propylene glycol. 15. Atlanta, GA.

DFG. 2006. Glycerin. In *The MAK Collection Part I, MAK Value Documentations 2015,* ed. D. Forschungsgemeinschaft.

Hartwig, A., MAK Commission. 2017. Glycerin / Propane-1,2,3-triol MAK Value Documentation. In *The MAK Collection for Occupational Health and Safety 2017, Vol 2, No 2*.

HCN. 2007. Propylene glycol (1,2-Propanediol); Health-based recommended occupational exposure limit. The Hague:: Health Council of the Netherlands.

Kaufmann, W., R. Bader, H. Ernst, T. Harada, J. Hardisty, B. Kittel, A. Kolling, M. Pino, R. Renne, S. Rittinghausen, A. Schulte, T. Wohrmann & M. Rosenbruch (2009) 1st International ESTP Expert Workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. *Experimental and Toxicologic Pathology,* 61**,** 591-603.

Konradova, V., V. Vavrova & J. Janota (1978) Effect of the inhalation of a surface tension-reducing substance (propylene glycol) on the ultrastructure of epithelium of the respiratory passages in rabbits. *Folia Morphol (Praha),* 26**,** 28-34.

Renne, R. A., Wehner, A.P., Greenspan, B.J., Deford, H.S., Ragan, H.A., Westerberg, R.B., Buschbom, R.L., Burger, G.T., Hayes, A.W., Suber, R.L., Mosberg, A.T. (1992) 2-week and 13-week inhalation studies of aerosolized glycerol in rats. *Inhalation Toxicology,* 4**,** 95-111.

Suber, R. L., R. Deskin, I. Nikiforov, X. Fouillet & C. R. Coggins (1989) Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. *Food Chem Toxicol,* 27**,** 573-83.

Umweltbundesamtes, B. d. (2017) Indoor air guide value for propan-1,2-diol (propylene glycol) : Communication from the Committee on Indoor Guide Values. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz,* 60**,** 1298-1304.

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). Follow up to paper 3: additional information on 13-week inhalation studies in rats of propylene glycol aerosol (Suber et al., 1989) and glycerol aerosol (Renne et al., 1992)

Full text reference of Suber et al. (1989)

Suber, R. L., R. Deskin, I. Nikiforov, X. Fouillet & C. R. Coggins (1989) Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food Chem Toxicol, 27, 573-83.

This paper is attached. It is not being made publicly available for copyright reasons.

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

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). Follow up to paper 3: additional information on 13-week inhalation studies in rats of propylene glycol aerosol (Suber et al., 1989) and glycerol aerosol (Renne et al., 1992)

Full text reference of Renne et al. (1992)

Renne, R. A., Wehner, A.P., Greenspan, B.J., Deford, H.S., Ragan, H.A., Westerberg, R.B., Buschbom, R.L., Burger, G.T., Hayes, A.W., Suber, R.L., Mosberg, A.T. (1992) 2-week and 13-week inhalation studies of aerosolized glycerol in rats. Inhalation Toxicology, 4, 95-111.

This paper is attached. It is not being made publicly available for copyright reasons.

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