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

Scoping paper on the potential risks from electronic nicotine (or non-nicotine) device systems in users and non-users (bystanders): a focused overview

Introduction

1. During a horizon scanning exercise at the February 2016 COT meeting, the Committee considered the subject of the possible human health effects of e-cigarettes or electronic nicotine delivery systems (ENDS) or electronic non-nicotine delivery systems (ENNDS) as a potential item for review (**COT, 2016**). Members considered that the possible human health effects of ENDS/ENNDS was a topic of concern that should be evaluated by the COT. However, it was decided that a full systematic review would not be an efficient way to proceed. The Committee recommended a more focussed review of the following key areas as a way forward:-

i) Additives
ii) Nitrosamines produced by ENDS/ENNDS
iii) Secondary exposure to exhaled products

2. Members should note that this is a scoping document that aims to bring relevant primary studies/key reports on ENDS/ENNDS to the attention of COT in order to set priorities for more in-depth reviews. It briefly considers a selection of original studies chosen on the basis of their clear primary objective to qualitatively and/or quantitatively assess the composition, or toxicity/health impact of exposures to the aforementioned substances/scenarios of interest. Members should note that there are a number of additional studies, not included here, which may also provide relevant data as part of their analysis/sub-analysis and these would be included in any follow-up committee papers as appropriate. Studies were retrieved either from a literature search in Pubmed (using a systematic approach) or via cross-referencing. It is hoped that this background information will provide sufficient detail to help Members establish whether it is possible and/or necessary to examine these and other issues raised in more detail.

Definitions and terminology

3. ENDS or ENNDS are smoking-proxy electronic inhalation devices that use batteries to heat a metal element/coil to evaporate a solution, contained in a reservoir or cartridge, composed of carrier solvents propylene glycol (with or without glycerol), water, flavourings and nicotine (if present). The evaporated solution forms an aerosol (vapour) that is inhaled by the user. Since it does not rely on combustion, no smoke is produced when (non-faulty devices) are used under recommended operating conditions.

4. Substantial heterogeneity exists in the design, operation and composition of these smoking-proxy devices and their liquid formulations. The main types include the cigarette-like 'ciga-like' (first generation), slightly larger and more powerful tank model systems such as 'eGO' (second generation) and the more advanced modified personal vaporisers known as 'MODS' (third generation) that allow customisation of the aerosol quantity, voltage, liquid composition, etc.

5. The terminologies used across studies varied. For the purpose of this paper the following terms are used throughout the review: E(N)NDS denotes ENDS or ENNDS; FHA - Firsthand aerosol; SHA - Secondhand aerosol.

Evidence from published literature

ADDITIVES

6. Additives in E(N)NDS products refer to the raw ingredients that are used to make up the liquid formulations, and are therefore distinct from impurities. These components are presumably added in known quantities, although they are not always listed on product labels. Aside from water and nicotine (which has been extensively studied in relation to its inhalation toxicity and therefore not considered further in this section), the carrier solvents propylene glycol and glycerol, and flavourings represent the main additives that are consistently used.

7. Vaporised propylene glycol (PG) (also known as 1,2-propanediol) and glycerol, collectively known as glycols, create the visible fume/aerosol that resembles cigarette smoke on use of the devices, and is an ideal carrier of a large variety of flavours. PG is a colourless, nearly odourless, clear, viscous liquid and miscible with water, acetone, diethyl ether, and chloroform (Kim & Shim, 2013). It is a solvent used in pharmaceutical products and is generally recognized as safe (GRAS) for use in food. Similarly, glycerol or glycerine (propane-1,2,3-triol) is a colourless, odourless, viscous liquid that is widely used for its sweet-tasting and humectant properties. Glycols are of specific concern as compounds generally not found in conventional cigarettes.

8. Flavour chemicals are present in almost all E(N)NDS liquids currently on the market in the UK and globally. As of early 2014, it was reported that of the 466 E(N)NDS brands available online, they contained 7764 unique flavours with 242 new flavours being added per month (Tierney *et al*., 2015). The vast majority of these flavours are confectionary in nature, for example, chocolate raspberry, cherry cheesecake, cotton candy, vanilla, grape, apple, coffee, bubble gum, as well as beverages, although tobacco and menthol are the most popular.

Analytical methods

9. Studies analysing these additives in E(N)NDS liquids/aerosols have done so with a range of objectives e.g. to

- provide information on their levels (Allen *et al*., 2015; Varlet *et al*., 2015)

- address lack of information provided on labels beyond the level of nicotine, and the inclusion of propylene glycol and/or glycerol (Tierney *et al*., 2015);
- develop fully-validated analytical methods (Kavvalakis *et al*., 2015),
- determine the composition of the liquids/aerosols for quality control/surveillance purposes (Davis *et al*., 2015; Paschke *et al*., 2015)

10. The high solubility of the analytes of interest in PG renders their extraction from replacement liquids of E-cigarettes a challenge. Indeed, the analytical methods used varied across studies and subsequently may contribute to the heterogeneity of results produced. After a relatively simple sample preparation, Kavvalakis *et al*. (2015) used gas chromatography/mass spectrometry (GC/MS) and liquid chromatography (LC/MS) techniques for the simultaneous determination of the components in E(N)NDS liquid. While Farsalinos *et al*. (2015b) modified a previously validated version of a high performance liquid chromatography (HPLC) method used to analyse carbonyl compounds in mainstream cigarette smoke, Paschke *et al*. (2015) developed a headspace solid-phase microextraction (HS-SPME) technique coupled to subsequent GC/MS to characterize different strawberry-flavoured E(N)NDS liquids, and Uryupin *et al*. (2013) measured the homo- and hetero-correlation of chemical shifts of protons and other nuclei to rapidly identify basic components and analyse them quantitatively via nuclear magnetic resonance without prior sample preparation. Hutzler *et al*. (2014) analysed E(N)NDS liquids by GC/MS and used comparisons with known compound-specific MS patterns to tentatively (and qualitatively) identify the presence of 141 flavour chemicals in one or more of the products.

11. Few studies reviewed here assessed the levels of PG or flavours in aerosols. Allen *et al*. (2015) fully discharged the contents of their liquids into a sealed chamber via an automated mass flow controller and the air stream was captured in a glass fibre filter and glass wool plug in front of a dried silica bed. Farsalinos *et al*. (2015b) used a smoking machine to generate aerosols that were collected without the use of a filter pad after being passed through an impinger containing a trapping solution, prior to HPLC analysis.

Levels in E(N)NDS liquids / aerosols

Glycols

12. PG (66%) and glycerol (24%) were the main components in the aromatic E(N)NDS liquid mixture of an Italian brand of e-cigarettes, while flavouring substances comprised less than 0.1% (Pellegrino *et al*., 2012). The same substances were detected in the aerosol in similar proportions. Large amounts of PG and glycerol have been reported in studies testing for these substances. Cheah *et al*. (2014) observed that two of their 20 products contained levels of glycerol more than 3 times those typically found in the other products: 374 mg and 827 mg. These two products contained only relative low amounts of PG. The remaining products contained more than 100 mg of PG per cartridge. The authors commented that the high levels were of particular concern in view of the fact that although these glycols are non-toxic when used as additives in asthma inhalers and nebulisers, the heating of glycols in E(N)NDS is known to generate various potentially toxic carbonyls.

13. The levels of impurities in these glycols have also been assessed. The minimum toxic dose of diethylene glycol is 0.14 mg/kg body weight and the lethal dose is 1 g/kg body weight (Schep *et al*., 2009). Neither ethylene glycol nor diethylene glycol were found in the 20 E(N)NDS liquids of 10 different brands purchased off the internet suggesting that these samples were of satisfactory quality (Etter *et al*., 2013). In contrast, Varlet *et al*. (2015) detected both compounds in a large set of 42 models of E(N)NDS from 14 brands of refill liquids purchased online. The authors noted that levels were within the limits authorised for food and pharmaceutical products. It should be noted that ethylene glycol and diethylene glycol are not authorized as ingredients in food and pharmaceutical products, but maximum residual limits are allowed, as these substances can be found as contaminants in numerous products. None of the liquids showed a concentration of ethylene glycol and diethylene glycol above these limits (1 mg/g according to the US FDA and 620 µg/g according to the US Pharmacopeial Convention in 2007). However, this is based on ingestion rather than inhalation.

Flavourings

14. Very little has been published on the levels of flavour chemicals in E(N)NDS liquids. Flavoured E(N)NDS typically do not list the levels of specific flavour chemicals present, and most do not identify the major flavour chemicals present. Kavvalakis *et al*. (2015) investigated and quantified five flavour ingredients (methyl cyclopentenolone, ethyl maltol, 2,5-dimethylpyrazine, ethyl vanillin, and 3,4-dimethoxybenzaldehyde) in each E(N)NDS liquid sample analysed. The results showed that the detection rates varied between 5.3% for 3,4-dimethoxybenzaldehyde and 30.4% for methyl cyclopentanolone. Measureable levels of eucalyptol (<Limit of detection (LOD)—87 µg/g) and pulegone (<LOD—115 µg/g) were found in the menthol-flavoured varieties for all four manufacturers of 35 E(N)NDS tested for 10 flavour compounds commonly used as additives in tobacco products which included eucalyptol, camphor, menthol, methyl salicylate, pulegone, ethyl salicylate, cinnamaldehyde, eugenol, diphenyl ether, and coumarin (Lisko *et al*., 2015). Menthol concentrations ranged from 3,700 to 12,000 µg/g in menthol-flavoured E(N)NDS liquids, which is similar to levels found in commercial cigarette filler. Menthol was also found at low concentrations in 40% of the tobacco-flavoured non-menthol products tested in this study (6.2–14.7 µg/g).

15. The concentrations of some flavour chemicals in E(N)NDS liquids are sufficiently high for inhalation exposure by E(N)NDS use to be of toxicological concern. Tierney *et al*. (2015) reported that six out of 24 flavour chemicals identified were aldehydes (e.g. benzaldehyde and vanillin) and suggested from calculations using recommended work place exposure limits and self-reported E(N)NDS liquid consumption rates that some were at levels that could cause respiratory irritation (although other studies on vanillin suggest otherwise). Nonetheless, this analysis of 30 products on the US market revealed that 13 liquids were more than 1% by weight flavour chemicals. Most contained the same flavour chemicals: vanillin and/or ethyl vanillin was found in 17 of the liquids as one of the top three flavour chemicals, and/or at ≥ 0.5 mg/mL. Similar findings were obtained in a study that analysed 28 liquids from seven manufacturers (Hutzler *et al*., 2014). Out of 141 flavours identified, vanillin, ethyl maltol, ethyl vanillin and menthol were the four most

frequently found and were present in 79%, 57%, 50% and 43% of the 28 samples, respectively. However, actual concentrations could not be deduced as authentic standards were not used.

16. High ranges of terpenic molecules such as alpha-pinene, beta-pinene, gamma-terpinene and para-cymene were detected in a few flavoured E(N)NDS liquids at levels higher than the oral limits recommended in finished food and drug products (Varlet *et al*., 2015). The implications for inhalation exposure are unclear. Davis *et al*. (2015**)** found that DIY flavouring products, marketed for the sole purpose of flavour enhancement, may contain substantial amounts of nicotine. GC/MS analysis confirmed the presence of nicotine in all four products tested with a 10 µg/mL limit of quantification (LOQ). Nicotine was quantifiable in two bottles, which had concentrations of 14.2 and 95.4 mg/mL. The total nicotine content in these two 5 mL bottles of DIY flavourings was 71 and 477 mg, doses that, if ingested in their entirety, could be fatal to children and, possibly, to adults.

17. Diacetyl (butanedione) is often described as giving a buttery flavour. It is structurally related to acetyl propionyl aka pentane-2,3-dione which is often used along with acetoin as a replacement for diacetyl owing to its caramel or buttery flavour. Diacetyl is of toxicological significance when inhaled owing to occupational evidence of an association with the development of lung damage (see paragraph 31 below). Allen *et al*. (2015) analysed 51 types of flavoured e-cigarettes for their total mass of diacetyl, pentane-2,3-dione and acetoin. Diacetyl was detected above the laboratory LOD in 39 of the 51 flavours tested, ranging from less than the LOQ (0.05 µg/sample) to 239 µg/device. Acetyl propionyl and acetoin were detected in 23 and 46 of the 51 flavours tested at concentrations up to 64 and 529 µg/device, respectively. Diacetyl and acetyl propionyl were also found in 74% of 159 E(N)NDS liquid samples tested from 36 manufacturers in seven countries (Farsalinos *et al*., 2015b). These compounds were detected even in samples that made claims of their absence in the product. It was concluded that 47% of the diacetyl-containing samples and 42% of the acetyl propionyl-containing samples could lead to exposures higher than NIOSH safety limits, although the use of these limits for this purpose needs further consideration. Both the liquid and aerosol samples contained similar ratios of these compounds (1,801 µg/ml and 160 µg/ml for the 5% sample, 3,921 µg/ml and 349 µg/ml for the 10% solution, and 7,546 µg/ml and 606 µg/ml for the 20% propylene glycol:glycerol solution) indicating that both compounds were readily delivered from the liquid to the aerosol.

18. Many of the substances described above were identified at only very low concentrations and obviously any concern will relate to the intensity and duration of these exposures. However, the possibility of additive (or synergistic) effects will also need to be considered.

Toxicity studies

19. There is limited research on the toxicity of glycols and flavouring chemicals used in E(N)NDS. This is largely because manufacturers of E(N)NDS liquids have cited that the ingredients, including the flavour chemicals, are all ‘food grade’, and/or ‘generally recognised as safe’ (GRAS). However, GRAS certification by the Flavour

Extracts Manufacturers Association (FEMA)/FDA pertains to ingestion, not inhalation. Therefore, high doses of some flavour chemicals may be acceptable when ingested, but not safe when inhaled. It is also possible that toxic degradation products can be produced by reaction of the flavour chemicals at the high temperatures present during E(N)NDS use.

In vitro

20. Farsalinos *et al*. (2013) evaluated the cytotoxicity of the aerosol generated from 20 E(N)NDS liquid samples in cultured myocardial cells. The base sample comprised of 50% glycerol and 50% PG, and contained no nicotine or flavourings. The aerosol produced by the "base" liquid was not cytotoxic at any extract concentration used. However, another study reported moderate cytotoxic effects of PG on skin fibroblasts (Ponec *et al*., 1990).

21. Bahl *et al*. (2012) examined the cytotoxic effects of 41 E(N)NDS liquids on human pulmonary fibroblasts, human embryonic stem cells and mouse neural stem cells, and concluded that the stem cells were generally more sensitive to refill fluids than differentiated adult lung cells and that this cytotoxicity correlated with the number and concentration of chemicals used to flavour fluids, especially for cinnamon-flavours. A follow-up study measured levels of cinnamaldehyde, 4-methoxycinnamaldehyde and vanillin in 10 ‘cinnamon’ flavoured liquids (Behar *et al*., 2014). The highest concentrations of the three compounds were ~40, 3 and 8 mg/mL, respectively (~4%, 0.3% and 0.8% by weight or volume). The cinnamon-flavoured refill fluids were cytotoxic with IC50 concentrations below 1% for human pulmonary fibroblasts and human embryonic stem cells. Cinnamon-flavoured refill fluids were also highly volatile, and most produced vapours that were cytotoxic when tested in the MTT assay. The relevance of these *in vitro* findings to users of E(N)NDS and bystanders will need further consideration.

In vivo

22. Studies investigating the effects of E(N)NDS liquids and aerosols on animal cells and tissues *in vivo*, in particular those of the lung are lacking. Consequently, the long-term outcome of chronic E(N)NDS use is difficult to predict

23. A few studies have evaluated the biological effects from inhaled PG (Robertson *et al*., 1947; Suber *et al.,* 1989; Werley *et al.,* 2011) and glycerol (Renne *et al*., 1992) unrelated to E(N)NDS. Robertson *et al*. (1947) found that exposure to significant amounts of PG in air had no adverse effects on the respiratory system in vivo. Indeed, animal studies of PG inhalation for up to several months have revealed little or no toxicity. Renne *et al*., (1992) reported irritation to the upper respiratory tract and squamous metaplasia of the epiglottis following exposure to glycerol at concentrations present in E(N)NDS, although for such comparisons the dose is of more significance given that the rats were exposed continuously for several hours per day, whereas an E(N)NDS user is exposed to only one cartridge at a time.

24. The biological impacts of additives used in E(N)NDS products were evaluated in a 90-day rat study conducted in 5-7 week old rats exposed to smoking-machine

generated aerosols (1 mg/L aerosol) produced from three liquid formulations containing vehicle (23% glycerol and 77% propylene glycol mixture), or vehicle and 2.0% USP grade nicotine; or vehicle, nicotine and 17.6% flavour mixture used in a prototype E(N)NDS device (Werley *et al*., 2016). Animals were exposed individually via a nose-only system to three dose levels (low, medium and high) generated by varying the length of exposure to 16, 48 and 160 min per day to achieve daily targeted aerosol total particulate matter (TPM) doses of 3.2, 9.6 and 32.0 mg/kg/day (equivalent to human exposure doses of approximately 160, 480 and 1600 mg/day of aerosol mass, respectively). This was followed by a 42-day recovery period. Following exposure, treatment-related effects included changes in body weight gain, food consumption and respiratory rate. The greatest attenuation was found for males and females exposed at the high exposure concentration, with body weight differences approaching or greater than 10% necessary to define the maximum tolerable dose. The vehicle exposure groups had the smallest attenuation in body weights. The only dose-related histopathological change observed was an increased incidence of alveolar macrophages in the lungs. The NOAEL, based upon decreased body weights, was the mid-dose level equivalent to a daily TPM exposure dose of approximately 9.6 mg/kg/day for each formulation. Based on various biological measures the authors suggested a possible role for nicotine in which it acted either alone or in combination with the different formulations.

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

25. SCENIHR produced their final opinion on additives used in tobacco products to assist the European Commission in identifying the additives that should be put on the priority list as foreseen by Article 6 of the Tobacco Products Directive 2014/40/EU (SCENIHR, 2016). The priority list was prepared on the basis of unfavourable toxicological characteristics of the compounds in their unburnt form or of pyrolysis/degradation products, and/or based on possible available information about properties resulting in a characterising flavour (one of the factors potentially contributing to attractiveness), facilitating inhalation or increasing nicotine uptake (potentially contributing to addictiveness of the tobacco products).

26. Although this relates to tobacco smoking products, it provides a significant resource for information on toxicity studies (some inhalation) conducted on flavourings that are also used in E(N)NDS products.

Health effects in humans

27. To date, the health effects of E(N)NDS in humans have not been well studied. Relatively little research has been conducted on the human health effects of the additives in E(N)NDS and the effects incurred by long-term use remains unknown. Studies tend to assess the health effects by considering the constituents of the aerosol and their known toxicities and through toxicological evaluation of E(N)NDS liquids and aerosols as described above. To date, research on pulmonary toxicity has focused largely on the nicotine-containing solution (liquid) vaporised by E(N)NDS.

Glycols

28. PG is a constituent of theatre fog and is known to cause eye and respiratory irritation (Wieslander *et al*., 2001). However, in a study by Cohen and Crandall (1964), PG was recommended as a vehicle for routine administration of bronchodilator drugs. No adverse clinical effects were observed after 93 patients with expiratory airflow disorders were exposed for 15 min to an inhalant mist of isoproterenol-HCl containing 40% PG. Despite this, concerns of pulmonary toxicity from inhalation of PG in E(N)NDS abound, particularly for people with asthma or chronic obstructive lung disease, although aside from the Cohen and Crandall study, there is little research on the effects in susceptible populations.

29. An internal technical report commissioned by vapers and vendors of E(N)NDS concluded that estimated levels of exposure to glycols are close enough to threshold-limit values to warrant concern and that the threshold-limit values are based on uncertainty rather than knowledge (Burstyn, 2013). Volunteers exposed to PG mist at a concentration of 0.22 and 0.52 mg/l for 1 min developed a slight airway obstruction and increased self-rated severity of dyspnea (Wieslander *et al*., 2001). Also, the findings of a case control study suggested that long-term exposure to PG may be associated with multiple allergic symptoms in children (Choi *et al*., 2010).

Flavourings

30. No studies were identified that investigated the association between possible adverse health effects and exposure to specific flavouring agents in E(N)NDS.

31. Inhalation exposure to diacetyl, a common flavouring chemical in E(N)NDS, is well known to be associated with a disease that became known as “Popcorn Lung” due to the associations between diacetyl, bronchiolitis obliterans and other severe respiratory diseases observed in workers in a microwave-popcorn processing plant (Allen *et al*., 2015). The disease represents an irreversible loss of pulmonary function that can become so severe that the only treatment option may be a lung transplant. Diacetyl was the most prominent chemical in the butter flavourings. Two other flavouring compounds of interest, acetoin and 2,3-pentanedione, were present in significant amounts (Kreiss, 2002). However, a study that characterised exposures to diacetyl and acetyl propionyl from cigarette smoking via use of a smoking machine found that exposures of both compounds from cigarette smoking were higher than those associated with occupational exposures in settings such as popcorn and flavouring manufacturing (Pierce *et al*., 2014). Mean diacetyl concentrations in mainstream smoke ranged from 250 to 361 ppm for all tobacco products and smoking regimens, and the mean cumulative exposures associated with 1 pack-year ranged from 1.1 to 1.9 ppm-years. It was reported that this exceeded occupational exposures for most food/flavouring workers who smoke. Furthermore, since all of the cohorts evaluated in the Kreiss study had considerable smoking histories, the authors claimed that workplace epidemiology studies involving health effects associated with diacetyl exposure have been significantly confounded due to non-occupational exposure to diacetyl from cigarette smoking. The Pierce study was conducted by authors with links to manufacturers and suppliers of diacetyl and diacetyl-containing flavourings.

32. In summary, the data on health effects to date, studied primarily in healthy people with short-term exposure to E(N)NDS, reveal little or no evidence of severe adverse events. The potential association between respiratory conditions and exposure to either PG aerosols in susceptible individuals and/or diacetyl/ acetyl propionyl inhalation requires further consideration.

TOBACCO SPECIFIC NITROSAMINES

33. Tobacco-specific nitrosamines (TSNAs) are very potent carcinogenic chemicals that occur naturally in tobacco formed during the curing process of green tobacco leaves by the nitrosation of amines. TSNAs are also present in tobacco smoke and it has been suggested that combustion leads to substantial formation of TSNAs although most TSNAs found in mainstream smoke are thought to come from the compounds present in cured tobacco leaves with only a fraction derived from the pyrolytic synthesis.

34. Among the tobacco-specific nitrosamines, nicotine-derived nitrosamine ketone (NNK) and N-nitrosonornicotine (NNN) are the most carcinogenic and have been classified as human carcinogens (Group 1) by the International Agency for Research on Cancer (IARC). Others include nitrosoanabasine (NAB) a weak oesophageal carcinogen in rats and N′-nitrosoanatabine (NAT) which is not carcinogenic and the metabolite of NNK 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) which is a potent systemic lung carcinogen in rats.

35. TSNAs can appear in E(N)NDS as impurities of the nicotine and are reported to be present in minute amounts in EC liquids, at levels comparable to pharmaceutical nicotine products. The levels of TSNAs emitted in tobacco cigarette smoke directly correlate with the levels present in the tobacco leaves. Whether this relationship extends to E(N)NDS, such that levels present in liquid formulations would directly correlate with the levels present in aerosols is unclear. This is partly because it is not known if the heated coil leads to additional formation of TSNAs, although it is argued that such would occur only at unrealistic, very high temperatures that vapers would not use.

36. Six studies were selected for discussion. All sought to quantify the levels of TSNAs in either the liquid (Kim & Shin, 2013**;** Kavvalakis *et al*., 2015) or the aerosol generated from E(N)NDS use (Goniewicz *et al*., 2014) or both (Flora *et al*., 2016; Farsalinos *et al*., 2015a) for various permutations of NNK, NNN, NAT, NAB, and, NNAL. In one study, the type of nitrosamine present in the E(N)NDS liquid was not specified (Varlet *et al*., 2015).

37. Most of the studies listed the model and brand of E(N)NDS device used and also reported the labelled nicotine concentration of the liquids that ranged from 4 to 72 mg/ml. Nicotine levels were additionally measured in a study that reported concentrations of E(N)NDS liquid refill purchased in Greece that were between 5% and 17% lower than the theoretical/labelled levels (Kavvalakis *et al*., 2015).

Analytical methods

38. To date, many of the analytical methods used for the determination of TSNAs have been described in relation to tobacco and mainstream cigarette smoke. Though LC–MS/MS methods have been successful in detecting the TSNAs in cigarette tobacco and mainstream cigarette smoke, it is not fully established whether these could be applied to replacement liquids of E-cigarette with matrices of propylene glycol. This is because the analytes dissolve well in glycols, and therefore more research is needed on their extraction from the matrix and their detection to address the lack of standard validated methods for measuring TSNAs in E(N)NDS. Two studies sought to rectify this by developing complete and fully validated analytical methods. Kim & Shin (2013) used two extraction methods (either solid or liquid-liquid phase) to determine the optimum clean up method with high extraction yield while Kavvalakis *et al*., (2015) optimised several conditions and parameters to develop the most efficient analytical, detection and quantification methods. Given that E(N)NDS liquids are believed to contain only minimal amounts of TSNAs, spiked samples containing known amounts of added TSNAs (e.g. 42.0–53.9 ng/g of each of the TSNAs) have also been used (Farsalinos *et al*., 2015a).

39. Most but not all of the studies reviewed used validated methods to extract and quantify TSNAs either based on the 2005 ICH guideline “Validation of Analytical Procedures: Text and Methodology Q2(R1)” and adapted for e-cigarette liquids according to the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) CRM method 72 or 75 respectively or based on the use of methods with high specificity and sensitivity.

40. Extracted samples from E(N)NDS liquids subsequently undergo chromatographic and spectrometric analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Flora *et al*., 2016; Varlet *et al*., 2015, Kavvalakis *et al*., 2015) and its variants i.e. ultra-performance (UP) LC-MS/MS (Farsalinos *et al*., 2015a) or electro-spray ionisation LC-ESI–MS/MS (Kim & Shin 2013).

41. The general approach used to determine TSNA concentration in E(N)NDS aerosols, involved attaching e-cigarettes to a smoke machine that puffed until battery exhaustion in order to maximise generation and collection of the aerosol from which the particulate matter was extracted and analysed. The puff rates, intervals and duration used varied among studies and ranged from between 1.8-4s, 10-30s and 80-150 puffs respectively. Voltage and temperature settings used were generally not reported. Particulates were collected either via use of filter pads or absorbed in methanol containing gas washing bottles. These were validated although Farsalinos *et al*. (2015a) used a glass fibre filter pad collection method that was validated for tobacco smoke. Samples underwent similar chromatographic and spectrometric analyses as described above for TSNAs in E(N)NDS liquids.

TSNA levels detected

42. LOD and LOQ were often reported to help validate methods and describe the lowest concentration of an analyte that can be reliably measured by an analytical

procedure (of which the latter performance characteristic provides additional assurance of precision and accuracy). A range of LODs/ LOQ values were used in the studies as shown in Table 1.

Table 1. Range of LOD and LOQs used and TSNA levels determined in the studies reviewed

	E(N)NDS liquids (ng/g)*			Aerosols (ng/device)		
TSNA	**LOD**	**LOQ**	**Levels detected (ng/g)**	**LOD**	**LOQ**	**Levels detected (ng/ device)**
NNN	0.02-7.7	0.06-90	0.34–60.08[1,2]	0.046-10	40	0.8-4.3[3]
NNK	0.02-3.7	0.07-90	0.22–9.84[1]	0.134-10	40	1.1-28.3[3]
NAT	0.01-4.6	0.04-9	0.09–62.19[1]	10	-	Nd
NAB	0.02-1.5	0.06-53	0.11–11.11[1,2]	10	-	Nd
NNAL	-	9	nd	-	-	-
TSNAs	1000	-	nd	-	-	-

*For consistency, values expressed as g/l were converted to approximate ng/g (based the density of water).
(-) = Not established; nd= not detected
[1]Kim & Shin (2013); [2]Farsalinos et al (2015a); [3]Goniewicz et al 2014

43. Two studies were affiliated to/ funded by e-cigarette manufacturers and reported that they were unable to detect (or detected only trace amounts) of TSNAs in either the E(N)NDS liquid or aerosol produced (Varlet *et al*., 2015; Farsalinos *et al*., 2015a). In the former study, which was also co-authored by the first author of the latter study, the TSNAs were evaluated as a whole, and the LOD was very high (1 µg/g). In the latter study, Farsalinos and colleagues addressed concerns expressed in the literature about how the heat of evaporation could result in higher levels of TSNAs emitted to the aerosol compared to those present in the liquid. Trace amounts of NNN (7.7 ng/g) and NAB (1.2-2.3 ng/g) were identified in three E(N)NDS liquids obtained from the market, but no nitrosamines were detected in the aerosol. Together with the fact that the expected levels of TSNAs in spiked samples were detected in the aerosol this led the authors to conclude that exposure of E(N)NDS users to TSNAs could be accurately assessed based on the levels present in the liquid, without the need to analyse the aerosol, which contain negligible amounts. However, questions over the validity of the aerosol collection method and the high LOD used (10 ng for aerosol) limit these conclusions. The absence of TSNAs detected in E(N)NDS liquids was supported by two other studies where their declarations of interests were either unknown (Kavvalakis *et al*., 2015) or the investigators had none (Flora *et al*., 2016). Although these studies used relatively high LODs/LOQs in comparison to the two further studies discussed below that detected TSNAs, Flora *et al*. (2016) considered that the LODs were low enough to detect the presence of TSNAs at levels that regulatory bodies consider to be relevant to human exposure. Assuming that 100% of each chemical from the liquid transfers to the aerosol and that the liquid contains the analyte at its LOD/LOQ, the daily exposure to each of these three analytes is well below the regulatory guideline thresholds.

44. After evaluating 12 brands of e-cigarettes obtained from the Polish market a study funded by a pharmaceutical company that produces smoking cessation products (Goniewicz *et al*., 2014) concluded that the aerosols of some e-cigarettes contain traces of the carcinogenic nitrosamines NNN and NNK up to levels of 4.3 and 28.3 ng / device respectively. However, to put things into context, the levels of these TSNAs in cigarette smoke are reported to be 40 to 380-fold higher.

45. By combining high extraction yield with stable and high sensitive ion formation by ESI–MS/MS, Kim & Shin (2013**)** developed an analytical method that permitted sensitive detection of TSNAs. The highest concentrations measured in 105 E(N)NDS liquids imported from 11 companies in China were 60, 62, 11 and 10 ng/g for NNN, NAT, NAB and NNK respectively. The authors noted that the maximum concentrations of total TSNAs in replacement liquids of E-cigarettes were 86.9 ug/L (86 ng/g), which was 10 times more than that published by the Chinese Ruyan E-cigarette Company. With unreported commercial interests the authors concluded that their findings contrasted with claims made by manufacturers that the E-cigarette cartridges contain only trace levels of TSNAs.

BYSTANDER EXPOSURE

46. Second-hand or bystander exposure to E(N)NDS encompasses the following scenarios: (i) direct exposure of non-users to secondhand aerosol (SHA, produced when an E(N)NDS user has exhaled); (ii) prenatal exposure to E(N)NDS; (iii) experimental animal/in vitro models of the above. Exposure from sidestream vapour may also occur from some devices.

47. In the following studies, firsthand aerosol (FHA) is either captured directly from the mouthpiece of the E(N)NDS device or captured after being released into an experimental chamber or air of a room designed to mimic living or public environments in a controlled laboratory setting. It has been suggested that FHA differs from second hand aerosol (SHA) in terms of the concentration of carbonyls (comparable to levels in cigarette smoke) and nicotine (FHA can have up to four times higher levels than SHA (unpublished communication, 2016). Unlike tobacco cigarettes, E(N)NDS do not typically generate sidestream aerosol. For those devices that do not emit sidestream aerosols, the secondhand emissions will therefore consist entirely of what is exhaled after inhalation by the user.

Direct analysis of components present in the exhaled aerosol (SHA) or aerosol emitted through smoking machines (FHA)

48. It should be noted that the conditions used to generate aerosols (e.g. temperature, coil voltage) can have a marked effect on the substances present and a critical evaluation of this will be performed in the full COT paper review of E(N)NDS. Schripp *et al*. (2013) evaluated the composition of a SHA produced by asking a volunteer to use e-cigarettes in a closed chamber. Analysis of the air revealed the presence of formaldehyde, acrolein, isoprene, acetaldehyde, and acetic acid, but at levels 5 to 40 times lower than those generated by a combusted cigarette. Increases after vaping in background levels of fine and ultrafine particles (FP/UFP), VOCs, PG,

glycerol, glyceryl diacetate, flavourings, and traces of nicotine were observed. The authors attributed the rise in FP/UFP to changes in the aerosol size distribution that occurs in the human lung during inhalation of e-cigarette vapour, resulting in an exhalation of smaller particles. The authors noted that this effect was caused by the evaporation of the liquid particles in the lung and also in the environment after exhalation.

49. In contrast, a study funded by the US National Vapers' Club assessed the composition of FHA produced by four high nicotine e-cigarettes and traditional cigarettes for comparison (McAuley *et al*., 2012). The aerosols were collected directly from smoke machines into polyethylene bags and the concentrations of common tobacco smoke by-products i.e. VOCs, carbonyls, PAHs, nicotine, TSNAs, and glycols relative to tobacco cigarettes were found to be either below the limit of detection (most VOCs, PAHs) or lower than levels present in tobacco smoke (ethylbenzene, benzene, toluene, carbonyls, nicotine, TSNAs, particle numbers).

Environmental monitoring of indoor air after vaping

50. The bulk of evidence on the components present in exhaled vapour comes from environmental monitoring of indoor/ambient air quality directly following E(N)NDS use under controlled or natural settings.

Aerosol exhaled from an E(N)NDS user (SHA)

51. Maloney *et al*. (2016) concluded that the majority of chemical constituents sampled were below quantifiable levels despite using standard industrial hygiene collection techniques and analytical methods. Formaldehyde was detected at consistent levels during all sampling periods (i.e. before, during and after vaping). O'Connell (2015) compared measurements of nicotine, VOCs (including low molecular weight carbonyls), PAHs, TSNAs and trace metal levels with human Health Criteria Values to provide a context for potential bystander exposure and concluded that the levels detected were below current regulatory standards that are used for workplaces or general indoor air quality. Similar conclusions were reached by Czogala *et al*., (2014) whose data suggested that use of an e-cigarette in indoor environments did not expose non-users to toxic tobacco-specific combustion products although they may be involuntarily exposed to nicotine. Levels were generally lower than those produced from smoking i.e. with e-cigarette use, the ambient level of nicotine was approximately 10% of that seen with smoking conventional cigarettes (3.3 versus 31.6 $\mu g/m^3$; $p = 0.0081$) and the ambient PM2.5 concentration after e-cigarette use was ≈18% of that seen with cigarette smoking (151.7 ± 86.8 vs. 819.3 ± 228.6 $\mu g/m^3$, respectively; $p = 0.0081$). Use of e-cigarettes did not significantly change toluene levels from background (3.79 ± 2.16 vs. 4.09 ± 2.12 $\mu g/m^3$, respectively; $p = 0.8513$). However, closer inspection of the data revealed that in addition to nicotine, the levels of PM2.5 were significantly higher than background (3.3 and 4.6 times respectively). Interestingly, the levels of PM2.5 generated from a smoking machine (FHA) were substantially less than that generated from the five dual-user subjects (SHA) (44.7 ± 26.4 vs. 151.7 ± 86.8 $\mu g/m^3$).

52. Studies conducted by authors who made declarations of having no links to tobacco or e-cigarette manufacturers generally reported that non-users are exposed to SHA containing pollutants that relative to background levels could be a health concern.

53. Both Ballbe *et al*. (2014) and Soule *et al*. (2016) sought to address the scarcity of evidence about passive exposure to the vapour released or exhaled from e-cigarettes under real conditions. In the former study, airborne nicotine levels in the homes of non-smoker volunteers either living at home with conventional smokers (n=25), living with nicotine e-cigarette users (5), or from control homes (not using conventional cigarettes neither e-cigarettes; n=24) were assessed. Airborne markers were statistically higher in conventional cigarette homes than in e-cigarettes homes (5.7 times higher). The levels of airborne nicotine and cotinine concentrations in the homes with e-cigarette users were higher than control homes (differences statistically significant). Soule *et al*., (2016) measured indoor air quality at a 2-day e-cigarette event held in a large room at a hotel. Measurements of PM2.5 were taken before, during 6 time points when the event was ongoing, where between 59 and 86 active e-cigarette users were present in the event room and the day after. Median PM2.5 concentrations in the event room increased from a baseline of 1.92–3.20 $\mu g/m^3$ to concentrations that ranged from 311.68 $\mu g/m^3$ to 818.88 $\mu g/m^3$ which were higher than concentrations reported previously in hookah cafés and bars that allow cigarette smoking.

54. Schober *et al*. (2014) simulated a real-world scenario (café-like setting) in an environmentally controlled room with predetermined occupancy density and air exchange rate. Concentrations of compounds emitted by e-cigarettes (± nicotine) were measured in nine healthy volunteers who took part in six “vaping” sessions each two hours long in a well ventilated room. The concentration of putative carcinogenic PAHs in indoor air rose by 20% to 147 ng/m^3 during vaping sessions; in addition levels of aluminium more than doubled, rising 2.4-fold. The amount of particulate matter was markedly higher during vaping sessions than at control times. The differences in levels of PAHs (which are mainly products of combustion and are not expected to be emitted from use) may have arisen from changes due to environmental conditions and not due to e-cigarette use (Farsalinos & Polosa 2014). This is because the levels of environmental PAHs show significant diurnal and day-to-day variations and the control environmental measurements were performed on a separate day and not on the same day of e-cigarette use.

55. To summarise, compounds typically reported in SHA include PG and glycerol (comprise bulk volume), metals, VOCs, carbonyls, PMs and nicotine (if the E(N)NDS liquid contains this). Studies upon which the authors either had links to or received funding from the tobacco industry or e-cigarette manufacturers tended to report that indoor levels of toxicants present after vaping (by E(N)NDS users) were not sufficiently higher than background to warrant concern about the apparent risk to bystanders from exhaled e-cigarette aerosols.

Tertiary exposure

56. It has been suggested that nicotine deposited on surfaces can react with airborne chemicals leading to formation of carcinogens (TSNAs) and contribute to thirdhand exposure. This issue was investigated in two studies by the same research team funded by pharmaceutical manufacturers of smoking cessation products. Bush & Goniewicz (2015) measured nicotine on the surfaces of US households of e-cigarette users, cigarette smokers, non-users of nicotine-containing products and reported that although nicotine is a common contaminant found on indoor surfaces there was no significant difference in the amount of nicotine in homes of e-cigarette users and non-users ($p > 0.05$). In a related study, the deposition of nicotine was measured before and after rooms were filled with firsthand aerosols generated from a smoking machine (Goniewicz & Lee 2015). The findings led the authors to conclude that there is a risk of thirdhand exposure to nicotine from e-cigarettes, of which the levels appeared to depend on the surface and the e-cigarette brand. It was suggested that future research should explore the potential risks of thirdhand exposure to carcinogens formed from the nicotine that is released from e-cigarettes.

Environmental hazard (in terms of public health risks from environmental pollution)

57. Concerns over the environmental hazard potential of disposed E(N)NDS products were investigated in two US studies that used quantitative and qualitative methods to detect either heavy metals or oxidant reactivity in leachates of the disposable components of E(N)NDS/e-cigarettes (Krause & Townsend, 2015 and Lerner *et al*., 2015 respectively). High levels of lead and copper were detected in some samples and the oxidant/reactive oxygen species reactivity in e-cigarette aerosols were found to be similar to oxidant reactivity in cigarette smoke.

Toxicological studies

58. No studies were retrieved that used animal or in vitro models to explore the toxicity of SHA. Most of the experimental evidence on the toxic effects of secondary or passive exposure comes from developmental studies examining in-utero exposure to nicotine in e-cigarettes. On the basis of IC50 values, cells harvested from human embryos, and newborn mice were more sensitive to the cytotoxic effects of E(N)NDS liquid refills than adult human pulmonary fibroblasts (Bahl *et al*., 2012). Similarly, adverse effects on cardiac differentiation were apparent in human embryonic stem cells exposed to media containing FHA (Palpant *et al.,* 2015). Both tobacco smoke and FHA exposure led to a decreased expression of cardiac transcription factors in cardiac progenitor cells, which the authors suggested represented a persistent delay in differentiation. As part of the same study, Zebrafish (*Danio rerio*) were used to assess cardiac developmental effects in vivo. Exposure to both types of cigarettes resulted in broad, dose-dependent developmental defects coupled with severe heart malformation, pericardial oedema and reduced heart function. Notably, tobacco cigarettes were more toxic than e-cigarettes at comparable nicotine concentrations. Finally, nicotine exposure from e-cigarettes was considered to be a potential cause of persistent behavioural changes in adult male C57BL/6J mice who received both pre- and postnatal exposure to FHA containing 2.4% nicotine during a period of rapid brain growth (Smith *et al*., 2015). This was based on the potential association between nicotine exposure and the increased likelihood of developing attention deficit hyperactivity disorder in offspring of mothers who smoked during pregnancy.

59. Several studies have investigated the effects of prenatal nicotine on developmental endpoints (irrespective of source). The outcome of these studies, as reviewed by Wong *et al*. *(*2015) and Spindel & McEvoy (2016) suggest that maternal exposure to nicotine during pregnancy through smoking or otherwise may have detrimental effects on reproductive outcomes in pregnancy, and also induce changes in the brain that potentiate addiction in the offspring (Suter *et al*., 2015) as well as impaired respiratory and reproductive health of offspring.

Health effects in humans

60. There are a limited number of studies that explore the health effects of exposure to SHA in bystanders (non-users). Two previously mentioned studies Ballbe *et al*., (2014) and Schober *et al*., (2014) performed comprehensive exposure assessments of e-cigarette emissions by including biological monitoring of exposure and/or effect markers.

61. In addition to measuring airborne levels of nicotine at home, Ballbe *et al*. (2014) also measured biomarker cotinine levels in the saliva and urine of 54 non-smoker volunteers from different homes. Concentrations of both biomarkers among non-smokers exposed to conventional cigarettes and e-cigarettes' aerosols were statistically similar (salivary and urinary cotinine levels in smoking homes was only 2 and 1.4 times higher than vaping homes, respectively). Schober *et al*., (2014) monitored the release of exhaled nitric oxide (an established measure of airway inflammation), and exhaled carbon monoxide in their nine healthy German volunteer vaping participants to reveal acute effects of e-cigarette use on physiological parameters. They also measured the uptake of nicotine and other VOCs via analysis of urinary nicotine metabolites and mercapturic acids. Exhaled nitric oxide increased slightly but significantly (P=0.03) in seven of the nine study participants after using nicotine containing e-cigarettes. Furthermore, the nicotine content of the liquids varied and was 1.2-fold higher than claimed by the manufacturer. The authors proposed that ultrafine particles formed from supersaturated 1,2-propanediol vapour could be deposited in the lung, and aerosolized nicotine appeared capable of increasing the release of the inflammatory signalling molecule NO upon inhalation.

62. However, Flouris *et al*. (2013) was the first to conduct a comprehensive and standardized assessment of the acute impact of active and passive e-cigarette/tobacco smoking on serum cotinine and lung function in a repeated-measures controlled study of Greek smokers and non-smokers. 15 non-smokers were exposed in a ventilated chamber to 1 hour of secondhand cigarette smoke (at a concentration simulating that of a smoky bar) or to e-cigarette aerosol generated by a smoking machine. With regards to passive exposures, e-cigarettes and tobacco cigarettes generated similar serum cotinine levels after passive smoking (2.4 ± 0.9 versus 2.6 ± 0.6 ng/ml) (P>0.001, to allow for multiple comparisons in study). Direct exposure to e-cigarette aerosol had no effect on pulmonary function or white blood cell count. A 1 h passive exposure to e-cigarettes did not significantly affect lung function (p>0.001). In contrast, active (associated with 7.2% reduction in FEV1/FVC; p<0.001) but not passive (indicative: 3.4% reduction in FEV1/FVC; P=0.005) tobacco cigarette smoking undermined lung function. The authors concluded that with regard

to short-term usage, the e-cigarettes studied generate smaller changes in lung function but have a similar nicotinergic impact to tobacco cigarettes.

63. Cooke *et al*. (2015) assessed the physiological effects of nicotine-containing aerosol i.e. seated arterial pressures at rest, and on arterial pressure and functional autonomic control in a controlled randomised study of 20 healthy non-smokers exposed to the aerosol from an e-cigarette fitted with a nicotine-containing cartridge) and placebo (cartridge with no nicotine). After being subjected to hemodynamic challenge associated with orthostatic stress, nicotine inhalation was found to be associated with higher arterial pressures in the seated position, and increased arterial pressures in the head-up positions with no other effects on autonomic control. The authors concluded that vaporized nicotine inhalation was not innocuous.

Risk estimates

64. Few studies have attempted to quantify the risks to health associated with exposure to SHA in bystanders.

65. McAuley *et al*. (2012) used the results from their Vaping Club-funded assessment of the composition of FHA (generated from a smoking machine) to conduct a risk analyses based on dilution into a 40 m^3 room and standard toxicological data. Non-cancer risk analysis revealed “No Significant Risk” of harm to human health for vapour samples from 4 E(N)NDS liquids. In contrast, for tobacco smoke, most findings markedly exceeded risk limits indicating a condition of “Significant Risk” of harm to human health. With regard to cancer risk analysis, no vapour sample from E(N)NDS liquids exceeded the risk limit for either children or adults, while the tobacco smoke sample approached the risk limits for adult exposure. The authors therefore concluded that based on the compounds analysed, their study indicated that there was no apparent risk to human health from e-cigarette emissions.

Future reviews and ongoing studies

Dutch National Institute for Public Health and the Environment (RIVM)

66. A report produced by the Dutch National Institute for Public Health and the Environment (RIVM) assessed the health risks of using e-cigarettes: (Visser *et al*., 2015). It was noted that they will assess the possible health effects resulting from exposure to compounds present in exhaled vapour in a future report.

WHO Study Group on Tobacco Product Regulation (TobReg)

67. The WHO TobReg group carries out research and drafts recommendations for WHO's Member States to establish regulatory frameworks for the design and manufacture of tobacco products. The study group hold meetings on a yearly/ biannual basis. The most recent (8th) meeting took place in December 2015. Issues discussed included a comprehensive systematic review of studies that evaluated

secondhand exposure to E(N)NDS aerosols. This valuable review, which is expected to be published later this year, also discusses the challenges with comparing studies due to the many factors that can influence aerosol production and makes several recommendations on the requirements of future studies to enable comparisons to be made and areas of uncertainty to be addressed.

68. A study presented by Professor Roy Harrison at an e-cigarette summit in London on 13 November 2014 provided evidence for a possible exposure risk to passive smokers in enclosed spaces with limited ventilation if all the emissions from e-cigarettes were exhaled (Torjesen, 2014). Prof Harrison discussed the preliminary findings from chamber experiments that measured the concentration, size, and composition of particles in e-cigarette aerosol, and evaluated how these parameters changed over time, in order to determine their potential effects on non-smokers. The study also calculated the concentration of particulates that a non-smoker would be exposed to in a small room with five e-cigarette users who were inhaling shallowly and producing five puffs of vapour a minute. It was estimated that after 10 minutes the concentration of particulates would be 50 $\mu g/m^3$, which although not considered to be that different to typical background levels in the atmosphere, is associated with human health impact. Nonetheless, these were considered unlikely to be huge in comparison to the background air quality in a large city. It was reported that the particle number concentration was of greater concern given that it was a measure of the nanoparticles.

Significant reports on E(N)NDS by health organisations

Public Health England (PHE)

69. This review by McNeill et al (2015) commissioned by the Health & Wellbeing directorate of PHE, provides a useful starting point /resource for the discussion of E(N)NDS use and public health in Great Britain and the UK (PHE 2015). It updates and expands the evidence considered by two previous reports produced by PHE (PHE 2014ab) and also clarifies the risk of E(N)NDS to health of vapers and bystanders, which it considered was 95% less than from tobacco-based cigarettes. The report was strongly criticised in the scientific literature for basing the risk estimate on ‘perception’ rather than the application of toxicological principles, with many arguing that the report does not represent a comprehensive toxicological assessment of the available evidence.

Royal College of Physicians (RCP)

70. A Report by the Tobacco Advisory Group of the Royal College of Physicians discussed the effects of exposure to nicotine without smoke (RCP, 2016). Topics addressed in this comprehensive report include: nicotine pharmacology and pathophysiology (e.g. toxicity and potential hazards); non-tobacco nicotine products: E-cigarettes (e.g. pharmacokinetics, safety profile, hazards from vapour exposure, generic and chronic effects, ingredients, vaporisation components, passive exposure). The report argues that E(N)NDS are unlikely to be harmless, and that long term use is likely to be associated with long term sequelae, including an

increased risk of chronic obstructive pulmonary disease, lung cancer, possibly cardiovascular disease and some other long term conditions associated with smoking. It suggests that the magnitude of risk however is likely to be very small in relation to that from tobacco smoke. In agreement with the PHE Report 2015, it further maintains that the hazard to health arising from long term vapour inhalation from the E(N)NDS available today is unlikely to exceed 5% of the harm from smoking tobacco.

Dutch National Institute for Public Health and the Environment (RIVM)

71. The Dutch National Institute for Public Health and the Environment (RIVM) produced a report that assessed the health risks of using e-cigarettes (Visser *et al*., 2015). In this study, the RIVM undertook a survey of e-cigarette users (vapers), performed measurements and assessed the possible health risks associated with exposure to substances in e-cigarette vapour. It was reported that considerable differences were observed in the composition of different kinds of E(N)NDS liquid available in the Dutch market and that of the resulting aerosol. Findings from their evaluation of E(N)NDS liquids showed that in some cases the amount of nicotine in the liquid did not match the declared amount on the packaging. Furthermore, the concentration of some compounds was found to be higher in the vapour than in the liquid. The report concluded that aldehydes were formed when the liquids are heated, and metals were released from the atomiser (heating element that produces the aerosol).

European Commission

72. The European Commission (EC) submitted a report to the European Parliament and the Council in May 2016 on the potential risks to public health associated with the use of refillable electronic cigarettes ('e-cigarettes') (EC, 2016). The report was prepared with input from the PRECISE study that analysed the available scientific literature on health risks of refillable e-cigarettes, data from EU poison centres in eight Member States, and performed chemical analysis on e-cigarette samples. It concluded that the use of refillable electronic e-cigarettes, and the potential exposure to E(N)NDS liquids containing nicotine in high concentrations, may pose risks to public health. Recommendations for further research were made particularly on certain aspects of e-cigarettes relevant to refillables, such as emissions testing and the safety of flavours or mixtures of flavours.

Centers for Disease Control and Prevention (CDC)

73. The US Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences are conducting research into E(N)NDS, their liquids and resulting exposures and health effects, and have several manuscripts in the pipeline or under review. CDC have already published papers on the subject of E(N)NDS which include: an analytical study by Lisko *et al*., (2015) that evaluated the chemical composition of 36 E(N)NDS liquids and found that a number of products contained tobacco alkaloids at concentrations that exceed U.S. pharmacopeia limits for impurities in nicotine used in pharmaceutical and food products; and an editorial by England *et al*., (2015) suggesting that poorly characterized, heat-induced partial

decomposition products of a highly complex tobacco extract in propylene glycol and glycerol present potential, but yet unknown, health risks. Further to a visit in April 2016, CDC are seeking collaborative opportunities with PHE and possible joint workshops.

World Health Organisation (WHO)

74. The WHO produced a report in 2014 discussed at the 6th Session of the Conference of the Parties that summarised the public health debate and limited nature of the evidence on the health impacts of E(N)NDS (WHO, 2014). In relation to health risks to users and non-users the report concluded that:

> "*the existing evidence shows that E(N)NDS aerosol is not merely "water vapour" as is often claimed in the marketing for these products. E(N)NDS use poses serious threats to adolescents and fetuses. In addition, it increases exposure of non-smokers and bystanders to nicotine and a number of toxicants. Nevertheless, the reduced exposure to toxicants of well-regulated E(N)NDS used by established adult smokers as a complete substitution for cigarettes is likely to be less toxic for the smoker than conventional cigarettes or other combusted tobacco products. The amount of risk reduction, however, is presently unknown."*

The report assessed the health risk from chronic inhalation of toxicants in aerosol from END users and noted that uncertainty exists over the risk of disease in bystanders exposed to SHA.

Questions for the Committee

75. Members are invited to comment on the available information provided in this paper and to advise on the approach that should be taken in the COT evaluation of E(N)NDS.

a) What would comprise the follow-up? Would this be one or more than one paper? What issues should be covered? Which areas should the review prioritise?

b) Are Members aware of any studies/reports providing data on likely real-world exposures of both users and bystanders, under normal conditions of use?

c) Variation exists in the reported values for a number of analytes identified in secondhand aerosols, which is a likely consequence of methodological differences between studies. Should the full review critically evaluate the design of the studies and the way in which the devices were used (i.e. with respect to temperature, nicotine concentration, etc)?

d) An emerging issue is the nature of particles released from the aerosols produced by E(N)NDS. Discussions with a particle expert, suggest these will be very different from particles found as air pollutants or from normal

cigarettes. Furthermore, the validity of 'read across' from cigarettes and other particle sources is not obvious. Do Members agree that the nature/composition of the fine and ultrafine particles released is a priority that needs addressing?

e) Much of what is presented as health risks in the literature is speculation or extrapolation from non-comparable scenarios. Are Members aware of any studies/reports providing accurate information on health effects (that take account of weight of evidence)? Can Members identify any gaps that need addressing in relation to health effects?

PHE-Supported Toxicology Unit
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Glossary

CDC- US Centres for Disease Control and prevention

CORESTA - Cooperation Centre for Scientific Research Relative to Tobacco

DIY- Do it yourself

EC- European Commission

ENDS - electronic nicotine delivery systems

ENNDS - electronic non-nicotine delivery systems

ESI- electro-spray ionisation

FDA- Food and Drug Administration

FEMA - Flavour Extracts Manufacturers Association

FEV- Forced Expiratory Volume

FHA – Firsthand aerosol

FP - fine particles

FCV- Forced Vital Capacity

GC/MS - gas chromatography/mass spectrometry

GRAS - generally recognized as safe

HCl- Hydrochloric acid

HPLC- High performance liquid chromatography

HS-SPME - headspace solid-phase micro extraction

IARC -International Agency for Research on Cancer

ICH- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

IC - Inhibitory concentration

LC/MS - liquid chromatography

LOD- limit of detection

LOQ- Limit of quantification

MS- mass spectrometry

MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NMR - nuclear magnetic resonance

NAB - nitrosoanabasine

NAT - N′-nitrosoanatabine

NNAL - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol

NNK - nitrosamine ketone

NNN –N-nitrosonornicotine

NOAEL- No Observed Adverse Effect Level

PAHs - Polycyclic Aromatic Hydrocarbons

PG- Propylene glycol

PHE- Public Health England

PM- Particulate matter

ppm- parts per million

RCP- Royal College of Physicians

RIVM -Dutch National Institute for Public Health and the Environment

s- seconds

SCENIHR -Scientific Committee on Emerging and Newly Identified Health Risks

SHA - Secondhand aerosol.

TPM - total particulate matter

TSNAs -Tobacco-specific nitrosamines

UFP –ultra fine particles

UP- ultra-performance

USP- United States Pharmacopeia

VOCs - volatile organic compounds

WHO- World Health Organisation.