TOX/2021/22

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

A summary of data on the bioavailability of nicotine and other ingredients from the use of oral nicotine pouches and assessment of risk to users

Introduction

1. The Committee has been requested by the Department for Health and Social Care (DHSC) and the Public Health England (PHE) Tobacco teams to consider the toxicological risks from tobacco-free oral nicotine pouches.

2. The demand for tobacco or tobacco-related products that are less damaging to health is increasing as users look for substitutes to conventional cigarettes (CC) (Fjellner, 2020). Such products include electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes) and the toxicological risks of E(N)NDS for users and bystanders have been extensively evaluated by the COT (COT, 2020).

3. Smokeless tobacco (ST) products are a further example of CC substitutes. ST products have been available for many years and comprise non-combustible products that may be chewed, inhaled or placed in the mouth (ASH, 2020). One of the better-known ST products is “snus” which is produced and sold in Sweden as loose powder or in pre-portioned pouches. Snus has been prohibited for sale elsewhere in the EU since 1992.

4. Tobacco-free versions of pre-portioned snus are also available on the Swedish market. These reflect the more modern oral tobacco-derived nicotine (OTDN) products which are tobacco-leaf free and contain tobacco-derived nicotine and food grade ingredients (Robichaud et al., 2019). Commercially available OTDN products available in the UK and EU include lozenges, gums, and dissolving tablets (Choi et al., 2003; West and Shiffman, 2001; O’Connor et al., 2011). More recently, nicotine pouches have emerged as a new category of OTDN products, including on the UK market. These products are pre-portioned pouches, similar to snus, in which the tobacco leaf is replaced with a non-tobacco filler and tobacco-derived nicotine (Aldeek 2021). The pouch is placed between the lip and gum allowing for the dissolution of nicotine to occur in the saliva before being absorbed in the oral cavity and entering the bloodstream (Hukkanen et al., 2005).

5. This paper provides the publicly available information for the ingredients present in these products and in particular focusses on the oral bioavailability of nicotine to support assessment of any potential risks associated with their use.

Regulatory framework

6. As oral nicotine pouches are tobacco-free products, they fall outside the Tobacco and Related Products Regulations (TRPR) 2016[1], and as no medicinal claims are made and they are not an obvious alternative to an authorised medicinal product, they are not regulated by the MHRA[2]. The regulatory position on them currently is likely to be under the General Product Safety Regulations (GPSR) (2005)[3].

7. Under GPSR, the general safety requirement states that "products should only be sold if their compliance with product safety regulations has been demonstrated appropriately". The GPSR requires all products to be safe in their normal or reasonably foreseeable use and enforcement authorities have powers to take appropriate action when this obligation is not met.

8. Where producers and distributers know that a product poses a risk to the consumer that is incompatible with the general safety requirement, under the GPSR appropriate actions are required to prevent adverse events by informing consumers of the risk that the product presents. In the case of nicotine pouches, nicotine has known addictive effects, and this has to be stated clearly on packaging and an age limit clearly displayed. In addition, due to the potential for reproductive and developmental toxicity, life-stage warnings must be stated.

9. Nicotine has been registered under the EU REACH regulations. It is classified as acutely toxic (category 2) by oral, dermal, and inhalation exposure and has hazard statements H300: fatal if swallowed, H310: fatal in contact with skin, and H330: fatal if inhaled (discussed fully in TOX/2020/59).

Search strategy

10. A broad-based search of SciFinder and PubMed for publications relating to 'nicotine pouches' was conducted on 10/12/2020 and the search of PubMed was briefly updated on 28/01/2021. Searches of 'grey literature' were also conducted. Due to the low numbers of papers identified it was not considered necessary to

[1] HM Government. The Tobacco and Related Products Regulations 2016. https://www.legislation.gov.uk/uksi/2016/507/contents/made [Accessed February 2021]

[2] MHRA Guidance Note 8 Appendix 4 on Alternatives to tobacco products states: "Products that are sold as alternatives to the use of tobacco products and which do not fall within the definition of a medicinal product will not be regulated by the MHRA. Guidance on the regulation of these products may be obtained from Trading Standards Service. Some products such as electronic cigarettes will now fall within the scope of the Tobacco Products Directive (2014/40/EU). Products may be sold as an alternative to tobacco as a temporary measure such as during periods or in places where smoking is not permitted, or as a longer term regime, perhaps on grounds of comparable costs. Products that do not make any cessation claims but, in the opinion of the MHRA, may be viewed by consumers as an obvious alternative to an authorised medicinal product such as transdermal patches, nicotine gum or mouth sprays, are likely to be regarded as medicinal products.

[3] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/949872/Guide-to-gps-regulations-2005-tp.pdf [Accessed February 2021]

develop more specific search terms. Approximately 70 citations were identified and those of relevance are discussed below.

Contents of nicotine pouches

11. Several large tobacco companies currently market tobacco-free nicotine pouches, and the type of pouch and pouch contents are illustrated in Figure 1.

Figure 1: Product packaging, nicotine pouch, and pouch contents of commercially available nicotine pouches (https://techtravelandlife.com/nicotine-pouches-the-way-ahead/ [accessed April 21]).

12. Commercial nicotine pouches are sold with varying nicotine content, with between 4 and 18 mg of nicotine per pouch being offered across all brands. In addition, each of the nicotine content levels has a choice of strength. For example, a commonly used brand of nicotine pouches used in the UK offers nicotine content of 8, 14 and 16 mg per pouch, with at least two levels of strength (between 1 – 4) available for each of the nicotine contents. The perceived strength of the pouch by the user does not necessarily reflect the nicotine content as it is determined by the amount of nicotine released during use which varies with flavour, the presence of other constituents, moisture content and pH, as well as with nicotine content.

13. As with e-cigarette liquids, nicotine pouches are sold in a variety of flavours such as fruit (e.g., black cherry, citrus) and others (e.g., peppermint, coffee). Some of the contents listed on commercially available nicotine pouch products are indicated below, although contents vary between different brands and individual products:

- Nicotine – can be defined as ‘pharmaceutical grade’, ’synthetic nicotine’ nicotine derived from the tobacco plant’, ’tobacco-derived nicotine salt’ or simply ‘nicotine’
- hydroxypropyl cellulose

- microcrystalline cellulose
- maltitol
- gum, Arabic
- sodium carbonate
- sodium bicarbonate
- acesulfame K
- food-grade flavourings
- water
- salt
- sucralose
- citric acid

14. It is unclear from the literature whether a 'standard' source and purity of nicotine is used in pouches as there appear to be many descriptions used (listed above). It is important for risk assessment purposes to identify the presence of potentially toxic impurities in tobacco-derived nicotine; however, that is not possible at this time. The 'other ingredients' listed above are standard ingredients that are considered safe for use in foods and food products and are not considered further here.

15. No studies were identified relating to the analysis of nicotine pouch contents.

Release of nicotine from oral pouches during use

16. Aldeek et al. (2019) evaluated the release of nicotine from 35 pouches offered by one manufacturer ('on!®' pouches). These pouches are available in seven flavours and five different nicotine levels. Release of nicotine into artificial saliva (pH 6.8 ± 0.1 with buffer capacity of 3.4 mM/LpH unit; Miller et al., 2020), maintained at 37^{o}C, was monitored at time intervals between 0 and 60 minutes.

17. The authors noted that the cumulative release profiles of nicotine showed a dose-dependent response, with equivalent nicotine release (%) for all flavours across all nicotine levels. Dissolution of nicotine was most rapid between 0 and 20 min (around 80% of release), with approximately 95% of release being achieved within 40 min, then reaching a plateau. The dissolution rate of nicotine from on!®nicotine pouches was compared with that from another type of OTDN pouch (ZYN®) and from ST products. The nicotine release rates (%) of the OTDN products were similar, with differences attributed to individual product characteristics, including pouch paper and the presence of other ingredients. The authors also reported that on!® nicotine pouches had similar or faster nicotine release profiles than the traditional pouched ST products tested (Aldeek et al., 2019).

Nicotine toxicokinetics

18. The toxicokinetics of nicotine was summarised in COT discussion paper TOX/2019/38. In brief, nicotine is a weak base with pKa 8.0 and is not well absorbed in the ionised state, in acidic conditions. Absorption of nicotine from saliva across the buccal mucosa increases with the pH of the saliva, which in turn is determined by the

relative acid-base buffering capacities of the saliva and nicotine pouch and the pH of the saliva and nicotine pouch before they come into contact. As nicotine absorption is pH dependent, many nicotine replacement therapy (NRT) products (paragraphs 29 to 35) are buffered to pH 7 to enhance absorption.

19. The bioavailability of nicotine has been estimated as 20%, 44%, 55-78% and 50-79% from an oral solution (approximately 3 mg), oral capsule (3-4 mg), gum (2-4 mg) and lozenge (2-4 mg) respectively (Hukkanen et al., 2005; Benowitz et al., 2009). Gisleskog et al. (2020) reported that swallowed nicotine is absorbed in the small intestine but undergoes extensive first-pass metabolism by the liver and has a relatively low (30-40%) bioavailability.

20. Following absorption, nicotine is distributed extensively within body tissues, with the highest affinity to liver, kidney, spleen, lung, and brain tissue. Nicotine accumulates in gastric juice, saliva and breast milk, crosses the placental barrier and accumulates in fetal serum and amniotic fluid. Approximately 70-80% of nicotine is metabolised to cotinine (mediated extensively (90%) by hepatic cytochrome P450 (CYP) 2A6). Cotinine is subsequently metabolised to 3'-hydroxycotinine (mediated exclusively by CYP2A6). Nicotine and metabolites are excreted in the urine. The flavin-containing monooxygenase (FMO)3, uridine diphosphate glucuronyl-transferase (UGT)2B10 also plays a minor role in nicotine metabolism. Plasma nicotine half-life on i.v. infusion is around 2 h, with terminal half-life of 11 h.

21. Nicotine is excreted by glomerular filtration and tubular secretion, with reabsorption depending on urinary pH (higher reabsorption at higher pH).

22. Nicotine pouches are designed to be placed inside the mouth between the inner cheek or lip, and gum. Absorption of released nicotine occurs across various oral membranes, including the buccal mucosa (cheek lining) (Ciolino et al. 2001). Transfer across the oral mucosa occurs via passive diffusion. Unionised/uncharged forms are transferred more readily due to their higher lipid membrane solubility compared with ionised/charged forms. The proportion of unionised/uncharged nicotine present depends on the pH of the medium in which it is found (Ciolino et al. 2001).

23. It is possible that some nicotine pouch manufacturers include nicotine in the form of salts. The COT has discussed information relating to whether the inclusion of nicotine salts in ENDS products can modify the level of internal exposure to nicotine that is achieved by use of the product, in comparison with use of ENDS products containing nicotine in the freebase form (TOX/2020/59, discussed at the December 2020 meeting). The Committee agreed that the use of ENDS products containing nicotine salts is likely to be associated with increased bioavailability of nicotine to users. However, it is not currently possible to quantify any effects, given the limited availability of pharmacokinetic data and the additional factor of the role of vaping topology in nicotine exposure.

Toxicity of nicotine

24. The toxicity of nicotine has been considered by the COT (TOX/2019/38) for all routes of exposure. The following points are of relevance in relation to oral exposure:

- Nicotine is acutely toxic via all routes of exposure, targeting the central and peripheral nervous systems. In humans, the lethal dose has been estimated as approximately 0.6–1.0 mg/kg bw, although a more recent review has proposed a lethal dose in the range of 6.5–13 mg/kg bw. Poisoning cases mostly relate to accidental or deliberate ingestion or dermal exposure.

- LD50 values for nicotine in animals have been reported for oral, dermal, intraperitoneal (i.p.). and i.v. routes of exposure, ranging from around 3 to 188 mg/kg bw (HCN, 2005).

- Nicotine is reported to cause local irritation at the site of administration (e.g. dermal patch, nasal or oral sprays) in humans. A review of nicotine toxicology by the Health Council of the Netherlands (HCN, 2005) concluded that nicotine is a skin irritant and sensitiser. The REACH dossier classed nicotine as Category 2 (irritant) and noted that nicotine was not sensitising in a well conducted study *in vivo* (local lymph node assay).

- Nicotine is an agonist to nicotinic receptors, which are located in the autonomic and peripheral nervous system, brain and spinal cord. In humans, as in animals, nicotine has been shown to produce both behavioural stimulation and depression. Pharmacodynamic studies indicate a complex dose-response relationship, due to both the complexity of intrinsic pharmacological actions and the rapid development of tolerance. Nicotine-associated effects depend on the dose, route/type of exposure, and time elapsed since the exposure (BfR, 2009).

- Some evaluations have been made based on data from studies of NRT as an aid to quitting CC smoking. The Lung Health Study reported by Murray et al. (2009) found that NRT use was not a significant predictor for lung, gastrointestinal, or all cancers over 7.5 years of follow-up. Studies relating to cardiovascular disease are generally of inadequate quality to draw clear conclusions but have not shown evidence of serious cardiovascular events. The COT discussion paper, TOX/2018/45, noted that a few studies reported potential associations of NRT prescription or use during pregnancy with adverse birth outcomes, but findings were difficult to evaluate due to factors including low levels of NRT use and lack of data on levels of continued CC smoking.

- Recent evaluations in the literature have noted that evidence for a genotoxic effect of nicotine is mixed. Most studies using the Ames test, CA and SCE assays in Chinese hamster ovary cells, and the bacterial genotoxicity luminescence test, were negative. However, some recent *in vitro* genotoxicity studies, including Comet assay, chromosomal aberration (CA) or micronucleus (MN) formation assays, produced some positive findings in the

concentration range of 160–650 mg/mL. A review by the US Surgeon General noted that although this range is above that of systemic levels of nicotine achieved using NRT, higher levels than this may occur at local sites of entry such as respiratory tract or oral epithelia. Genotoxic effects at lower concentrations (16 ng/mL) were noted in a small number of studies, such as the cytokinesis-block micronucleus assay and CA assay (HHS, 2014). The review by the US Surgeon General concluded that, overall, definitive studies to determine the genotoxic potential of nicotine in users of nicotine delivery systems are missing (HHS, 2014). Experimental studies in animals have suggested that nicotine is not carcinogenic *per se*, but adequate studies of long-term exposure to assess carcinogenicity are not available.

25. The specific effects of nicotine on oral tissues have not been well defined. In a systematic review, Holliday et al. (2019) evaluated evidence from *in vitro* studies of the effect of nicotine on human gingival, periodontal ligament, and oral epithelial cells. Measures of cell viability were consistent between cell lines and indicated that nicotine applied at the levels typically found in the saliva of CC, NRT, and E(N)NDS users was unlikely to cause cytotoxicity to human gingival and periodontal cells. However, the authors reported that saliva levels of nicotine in ST users may be high enough to achieve cytotoxicity.

26. Lee (2011) evaluated the risk of oral cancer due to the use of different tobacco products, including snus; they reported no association of oral cancer with snus use, with relative risk (RR) of 0.97 (95% CI 0.68–1.37). The development of non-neoplastic oral disease and oral mucosal lesions (including leukoplakia), periodontal and gingival diseases, tooth loss and dental caries, were also evaluated. Oral mucosal lesions were defined as “any abnormal change or swelling on the epithelial lining of the mouth, lips or gums, which do not contain any malignant or pre-malignant cells”. The authors cited a review of data by Kallischnigg et al. (2008) which concluded that the use of snus markedly increases the risk of developing oral mucosal lesions, which disappear when snus use is stopped. Although it is widely reported in the literature that oral leukoplakia is caused by chronic irritation from tobacco, it is unclear which component of tobacco is linked to this effect.

Reference values for nicotine

27. A toxicological review of nicotine (TOX/2019/38) was discussed by COT at the July meeting in 2019, which included the following reference values:

- The European Food Safety Authority (EFSA) was asked to consider the possible health risks related to the presence of nicotine in wild mushrooms at concentrations up to 0.5 mg/kg. For this purpose, EFSA established an acute reference dose (ARfD) of 0.0008 mg/kg bw, based on a lowest observed adverse effect level (LOAEL) of 0.0035 mg/kg bw for slight, transient increased heart rate in human CC smokers on intravenous (i.v.) infusion of nicotine, and using an overall uncertainty factor (UF) of 10 and a correction factor of 0.44 for oral bioavailability of nicotine. EFSA considered that given the short biological half-life of nicotine, the fact that it does not accumulate in

the body, and that the most sensitive effect was considered to be the pharmacological effect on the cardiovascular system, the value set for the ARfD would be suitable to protect from chronic effects and could also be applied as the acceptable daily intake (ADI). Thus, EFSA established an ADI of 0.0008 mg/kg bw/day but noted some deficiencies in the toxicological database (EFSA, 2009).

- The German Federal Institute for Risk Assessment also established an ARfD for nicotine of 0.0008 mg/kg bw/day, based on the study of Lindgren et al. (1999) (BfR 2009).

- A value of 0.0001 mg/kg bw/day was proposed for the ARfD, ADI, and systemic acceptable operator exposure level (AOEL) for nicotine in pesticides. This was based on an estimated lowest observed effect level (LOEL) of 0.01 mg/kg bw/day identified for clinical signs of toxicity in children exposed dermally (Woolf et al., 1997), with an UF of 100 (UK-DAR, 2007). The French Food Safety Agency (AFSSA) endorsed the proposed ADI and ARfD of 0.0001 mg/kg bw/ (AFSSA, 2009). Plant protection products containing nicotine have now been withdrawn from use in the EU (EC, 2008).

28. In the subsequent statement regarding the potential toxicological risks from use of E(N)NDs, the COT had the following conclusions with regards to nicotine exposure via inhalation, some of which are applicable to nicotine exposure from oral nicotine pouches:

- Experienced users self-titrate nicotine intake from ENDS. Systemic exposure levels of nicotine equivalent to those from CC smoking can be achieved. Factors influencing the level of nicotine exposure and retention include ENDS product type, user profile, usage parameters, e-liquid nicotine concentration, and the overall formulation of the e-liquid.

- For people who switch from CC smoking, the risks associated with nicotine exposure from ENDS would be expected to be similar to those from the same nicotine exposures through use of CC.

- It is thus anticipated that nicotine-related health effects could occur with long-term use of ENDS. Risks include effects on a large range of endpoints in users and their offspring.

- Non-users who have never been exposed to nicotine and who take up vaping would be at risk from effects of nicotine to which they would not otherwise be exposed. This also includes the risk of addiction.

- Use of ENDS while continuing to smoke CC (dual use) could potentially lead to increased nicotine exposure compared with that from CC smoking only and may increase the overall risk.

- Bystanders are likely to be exposed to some nicotine in ambient air where ENDS products are used, which may have some associated effects.

Exposure to nicotine following use of NRT

29. No quantitative data were identified, for humans or animals, describing the uptake of nicotine following the use of nicotine pouches. Although nicotine pouches are not marketed as an NRT product, data to estimate exposure levels following their use can be acquired from NRT studies. Many NRT products substitute the nicotine from inhaled tobacco products with uptake via other routes, with a number of products being designed for oromucosal (i.e. buccal and sublingual) absorption (Gisleskog et al., 2020).

30. Nicotine gum was the first NRT product, registered in Switzerland in 1978. This was followed by a number of alternative oromucosal absorption products including mouth sprays, lozenges, sublingual tablets and inhalers. Hartmann-Boyce et al. (2018), through the evaluation of data identified in a Cochrane Tobacco Addiction Group trials register, estimated that use of NRT was associated with an increased relative smoking abstinence rate of 50–70% compared with placebo or non-NRT control groups. NRT is considered an important tool in reducing tobacco use and lung cancer incidence (Shields et al., 2016).

31. In a retrospective analysis, Gisleskog et al. (2020) developed population pharmacokinetic models for nicotine, using data from 930 healthy smokers (46,016 observations) from 29 single- and repeated-dose studies with multiple formulations across intravenous, oral, transdermal and oromucosal routes of administration. The use of oromucosal-route products results in partial delivery of nicotine to the GI tract due to swallowing, with absorption and metabolism as previously discussed (paragraphs 15 to 18). The authors estimated a relatively low bioavailability from this route (30–40%) (Gisleskog et al. (2020).

32. Considered as a group, absorption of nicotine from the buccal cavity was rapid following use of mouth spray, gum, lozenge and inhaler, with peaks occurring shortly after the end of dosing. Many individual profiles showed a second peak of absorption, which was considered by the authors to be due to intestinal absorption of the fraction of the nicotine dose that was swallowed during use. This fraction of the dose was estimated to be 61%, 67% and 69%, for mouth spray, inhaler and lozenge respectively, and 55% for chewing gum. It was also noted by the authors that increasing doses of nicotine were associated with a higher fraction being swallowed, possibly due to irritant effects of nicotine in the oral cavity resulting in increased saliva production.

33. The acceptability of ST and NRT products to consumers is considered to be influenced by the ability to achieve rapid absorption of a sufficient dose of nicotine to mimic delivery from CC use. Blood plasma nicotine levels in CC smokers generally range from 10 to 50 ng/mL, with typical daily trough concentrations of 10 to 37 ng/mL and peaks of 19 to 50 ng/mL, and a mean nicotine boost per 1 CC smoked of 10.9 ng/mL. *Ad libitum* use of NRT products generally provides a plasma nicotine concentration approximately one-third to two-thirds of that achieved by CC smoking. Steady-state plasma nicotine concentrations from transdermal patches are in the

range of 10–20 ng/mL, with a range of 5–15 ng/mL from gum, inhaler, sublingual tablet, and nasal spray. Systemic doses delivered from different nicotine delivery systems are reported to be as follows: smoking 1 CC, 1–1.5 mg; nicotine gum, 2 mg from one 4-mg gum; transdermal patch, 5–21 mg per day; nasal spray, 0.7 mg per 1-mg dose of 1 spray in each nostril; inhaler, 2 mg for a 4-mg dose released from the 10-mg inhaler; lozenge, 1 mg for a 2 mg lozenge; oral snuff (snus), 3.6 mg for 2.5 g held in the mouth for 30 min; chewing tobacco, 4.5 mg for 7.9 g chewed for 30 min (Hukkanen, et a., 2005; Benowitz et al., 2009).

34. Digard et al. (2013) determined nicotine absorption from snus pouches and loose snus ST products (1 g portions containing 11 mg of nicotine) in comparison with a CC (14.6 mg nicotine) and an over-the-counter nicotine gum (4.2 mg nicotine) used as directed by the manufacturer. The authors reported that snus users held pouches or portions in the mouth for between 60-70 min, which is longer than directed on the product packaging (typically 20-30 min).

35. As previously widely reported in the literature, Digard et al. (2013) determined that nicotine plasma levels rose more rapidly following the use of a CC compared with other oral nicotine-containing products. However, over the total sampling period (120 min) the systemic exposure to nicotine was higher for the snus products than for nicotine gum or CC. The authors reported that the AUC_{0-120} for all six test products were ranked as: loose snus (27.1 mg) > pouched snus (14.7 mg) > loose/pouched snus (10.8 mg and 10.7 mg, respectively) > cigarette (14.6 mg) > 4.2 mg nicotine gum. C_{max} followed a similar ranking: loose snus (27.1 mg) > pouched snus (14.7 mg) > cigarette (14.6 mg) > loose/pouched snus (10.8 mg and 10.7mg, respectively) > 4.2 mg nicotine gum. The authors considered that this was due to a higher nicotine content of the snus products and the longer duration of use. These findings indicated that nicotine absorption kinetics were dependent on the quantity of tobacco by weight and the total nicotine content, rather than the product form.

Questions for the Committee

36. Members are invited to comment on the information provided in this paper and to consider the following questions:

i. From the limited evidence base identified, can any conclusions be drawn regarding the risk of nicotine pouch use.

ii. Is it possible to compare the exposure and/or risk from the use of nicotine pouches with NRT products.

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Abbreviations

ADI	Acceptable daily intake
ARfD	Acute reference dose
CC	Conventional cigarette
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
GPSR	General Product Safety Regulations
i.p.	intraperitoneal
i.v.	intravenous
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
NRT	Nicotine replacement therapy
OTDN	Oral tobacco-derived nicotine
RR	Relative risk
ST	Smokeless tobacco
TRPR	Tobacco and Related Products Regulations

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