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Advisory values for maximum emission of **nicotine and 6- methylnicotine** from nicotine products without tobacco for inhalation

RIVM letter report 2025-0067
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Colophon

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Synopsis

Advisory values for nicotine and 6-methylnicotine in nicotine products without tobacco for inhalation

There are products for sale that do not contain tobacco, but do contain nicotine. Some of these nicotine products without tobacco are intended for inhalation. Users take up nicotine from substances released by these products, referred to as emissions. Since these products do not contain tobacco, they do not fall under the EU Tobacco Products Directive. Consequently, there are currently no regulations governing these products.

Nicotine is harmful to health. For this reason, the Dutch government aims to introduce requirements for these products, such as setting a maximum limit on the amount of nicotine they can release. RIVM has derived advisory values for the amount of nicotine in emissions. Below these values, no health effects of nicotine are to be expected if someone uses a product fully. RIVM has also derived advisory values for the substance 6-methylnicotine. This substance resembles nicotine and has previously been used in the Netherlands as a replacement for nicotine. In addition, it has compiled a list of other substances that resemble nicotine.

The advisory values are based on the first health effects people experience from nicotine, namely an increased heart rate and irritation of the airways, indicated by the cough stimulus. To protect users' health, the amount of nicotine and 6-methylnicotine in emissions from nicotine products without tobacco must remain below these advisory values.

Nicotine products are often appealing and addictive, particularly for young people. As such, RIVM recommends adopting the lowest advisory values to ensure protection for younger users as well. This translates to an advisory value of 0.028 milligrams of nicotine per product and 0.0030 milligrams of 6-methylnicotine per product. The maximum concentration in emissions without expected adverse effects is 0.07 milligrams per litre of emissions for nicotine and 0.025 milligrams per litre of emissions for 6-methylnicotine..

Keywords: nicotine; 6-methylnicotine; nicotine analogues; emission; health-based advisory values; nicotine products without tobacco

Publiekssamenvatting

Advieswaarden nicotine en 6-methylnicotine in nicotineproducten zonder tabak voor inhalatie

Er zijn producten te koop waar geen tabak in zit, maar wel nicotine. Sommige van deze nicotineproducten zonder tabak zijn bedoeld voor inhalatie. Mensen krijgen nicotine binnen via de stoffen die uit deze producten vrijkomen, de zogenoemde emissie. De producten vallen niet onder de Europese Tabakswet omdat er geen tabak in zit. Er zijn daarom nu nog geen regels voor deze producten.

Nicotine is schadelijk voor de gezondheid. Daarom wil de Nederlandse overheid dat er eisen aan deze producten worden gesteld, zoals een maximum voor de hoeveelheid nicotine. Het RIVM heeft nu advieswaarden bepaald voor de hoeveelheid nicotine in de emissie. Onder deze waarden zijn geen gezondheidseffecten van nicotine te verwachten als iemand een product volledig gebruikt. Deze advieswaarden zijn ook bepaald voor de stof 6-methylnicotine. Deze stof lijkt op nicotine en is in Nederland al eerder gebruikt als vervanger van nicotine. Verder is op een rij gezet welke andere stoffen op nicotine lijken.

De advieswaarden zijn bepaald voor de eerste gezondheidseffecten die mensen van nicotine merken: een hogere hartslag en irritatie van de luchtwegen, de hoestprikkel. Om de gezondheid van de gebruiker te beschermen moet de hoeveelheid nicotine en 6-methylnicotine die vrijkomt uit nicotineproducten zonder tabak voor inhalatie onder de advieswaarden blijven.

Nicotineproducten zijn vaak aantrekkelijk en verslavend, vooral voor jongeren. Daarom adviseert het RIVM om uit te gaan van de laagste advieswaarden die ook jongeren beschermen. Dit betekent een advieswaarde van 0,028 milligram nicotine uit een product en 0,0030 milligram 6-methylnicotine. De maximale concentratie in de emissie zonder verwacht schadelijk effect is 0,07 milligram per liter emissie voor nicotine. Voor 6-methylnicotine is dat 0,025 milligram per liter emissie.

Kernwoorden: nicotine; 6-methylnicotine; nicotine analogen; emissie; gezondheidskundige advieswaarde; nicotineproducten zonder tabak

Contents

Summary

As of January 1st 2025, the scope of the Tobacco and Smoking Products Act (TRW, in Dutch: '*Tabaks- en Rookwarenwet*') has been amended to broaden it to include nicotine products without tobacco. This expansion introduces stricter regulations for nicotine products without tobacco for inhalation, such as heated nicotine sticks and refillable nicotine devices. These measures include a prohibition on the sale of oral nicotine products, age restrictions, advertising bans, and sales limitations. In light of this regulatory update and at request of the Ministry of Health, Welfare and Sport, the RIVM has undertaken an assessment to derive advisory values for the maximum emissions of nicotine and one of its analogues, 6-methylnicotine (6-MN), from nicotine products without tobacco. This assessment also explores a qualitative grouping of other nicotine analogues.

Methodology

A pragmatic approach was used to establish uniform advisory values for the maximum emission of nicotine and 6-MN from nicotine products without tobacco for inhalation. These values are health-based and represent a total amount and concentration of nicotine and 6-MN in the emission at which no adverse health effects are expected. This value is applicable to the complete use of a product, regardless of the users' behaviour such as puff volume and number of puffs. The assessment considered different age groups, including adolescents, as these products are often marketed in ways that appeal to young people, including children, despite regulatory restrictions.

Critical effects

Advisory values were calculated based on two critical health effects: systemic toxicity (a transient and reversible increase in heart rate and blood pressure) and local toxicity (respiratory tract irritation). Due to differences in the dose metrics and point of departures, the resulting advisory values differ in magnitude and units. For systemic toxicity, the advisory value represents the maximum amount in the emission, while for local toxicity, it corresponds to the maximum concentration in the emission. To ensure adequate protection against both systemic and local toxicity, both type of values should be applied to nicotine products without tobacco for inhalation.

Derived advisory values for nicotine

For systemic toxicity, the calculated safe maximum amounts emitted from a product are: 0.028 mg for children aged 11-16 years, 0.038 mg for children aged 16-18 years, and 0.042 mg for adults. For local toxicity, the calculated safe maximum concentration in the aerosol is 0.07 mg/L.

Due to limited toxicological information on 6-MN, a read-across from nicotine was performed. This approach incorporated correction factors to account for differences in molecular weight, toxicokinetics, and potency. Given that 6-MN is more potent than nicotine (based on *in vivo* studies),

a conservative assessment factor was applied to account for uncertainties.

Derived advisory values for 6-methylnicotine (6-MN)

For systemic toxicity, the derived safe maximum amounts emitted from a product for 6-MN are: 0.0030 mg for children aged 11-16 years, 0.0041 mg for children aged 16-18 years, and 0.0046 mg for adults. For local toxicity, the safe maximum concentration in the emission is 0.025 mg/L.

Nicotine analogues

Although deriving advisory values for other nicotine analogues than 6-MN was beyond the scope of this assessment, a qualitative grouping based on chemical structure was performed. Most analogues exhibit lower binding potency to nAChR compared to nicotine, with 6-MN being a notable exception. However, receptor affinity alone does not reliably predict physiological effects such as increased heart rate or addiction, as these depend on complex interactions involving multiple nAChR subtypes. To ensure safety, a precautionary approach with conservative estimates is recommended for all nicotine analogues.

Cumulative exposure

Cumulative exposure must also be considered as nicotine and its analogues share a common mode of action (binding to nAChR) and may exert additive effects. To ensure safety, the fractions of nicotine and its analogues in the emission must remain below the same fractions of their respective advisory values.

Conclusion

RIVM derived advisory values for both systemic and local toxicity to limit potential health risks associated with the use of nicotine products without tobacco for inhalation. Given that these products are often marketed in ways that appeal to young people, the RIVM recommends using the most protective maximum *amount* to also protect young people, in addition to the maximum *concentration*. These values are summarized as follows:

- **Nicotine**: maximum *amount* of **0.028 mg** per product (for children 11-16 years) and a maximum *concentration* of **0.07 mg/L** in the emission.
- **6-MN**: maximum *amount* of **0.0030 mg** per product (for children 11-16 years) and a maximum *concentration* of **0.025 mg/L** in the emission.

1 Introduction

As of January 1st 2025, the scope of the Tobacco and Smoking Products Act (TRW, in Dutch: '*Tabaks- en Rookwarenwet*') was expanded to include nicotine products without tobacco. As part of this amendment, the sale of oral nicotine products (e.g. nicotine pouches) is prohibited, based on previous risk assessments conducted by the RIVM (RIVM, 2021+2024). Nicotine products without tobacco intended for inhalation - such as steam stones, heated nicotine sticks and other refillable nicotine devices - will be subjected to stricter regulations. These new measures include age restrictions, advertising bans and sales limitations. In addition, a ministerial regulation will be drafted to further regulate these products.

On behalf of the Ministry of Health, Welfare and Sport, the RIVM conducted an assessment of nicotine products without tobacco for inhalation. The primary aim was to determine advisory values for the maximum emission of nicotine from these products. The definition of emission is given in Box 1. A secondary aim was to explore the possibility of including nicotine analogues in the assessment. Given that 6-methylnicotine (6-MN) is a commercially relevant and already marketed analogue found in nicotine products without tobacco, this assessment also aims to derive advisory values for maximum emission of 6-MN. Furthermore, a qualitative grouping of other nicotine analogues was performed as first step in the derivation of advisory values.

Information regarding human behaviour, including puff volume, puff duration and puff interval, is limited for nicotine products without tobacco for inhalation and may even differ between individual type of products. Therefore, a pragmatic approach was taken to establish uniform advisory values for the maximum emissions of nicotine and 6-MN, applicable across all types of nicotine products without tobacco for inhalation, regardless of their (use) characteristics. These values represent health-based levels at which no adverse health effects are expected after complete use of one product.

Box 1: Definition of "Emission"

In the context of nicotine products, "emission" refers to all that is released, such as aerosolized particles, gases, or vapours, generated during the use of these products. These emissions are typically created through processes such as heating, combustion or vaporization, depending on the type of nicotine delivery system being used. The emission is intended to be inhaled by the user for nicotine intake.

Outline

Chapter 2 provides information related to the exposure, pharmacology, toxicokinetics and toxicology of nicotine. This chapter also outlines the process for deriving the advisory values for the maximum emission of nicotine. Chapter 3 shifts focus to the analogue 6-MN, including the derivation of the advisory values for its maximum emission. Building

upon this, Chapter 4 broadens the scope to include a qualitative grouping of other nicotine analogues. Finally, Chapter 5 concludes the report with a discussion and key conclusions.

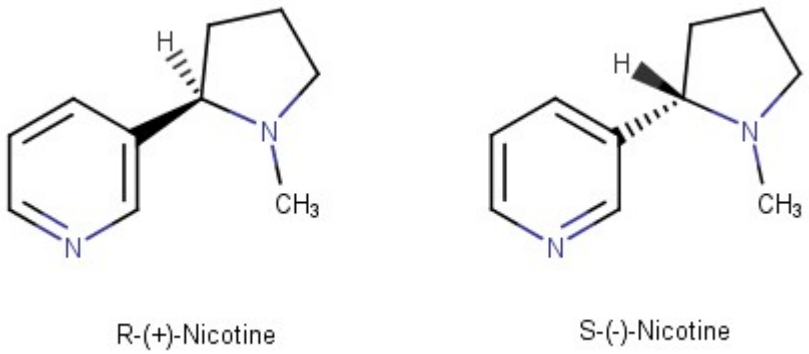
2 Nicotine

It is beyond the scope of the current assessment to perform an extensive literature search. The information presented in this chapter, particularly regarding toxicology, is mainly derived from reviews from well-established international organisations.

2.1 Chemical structure

Nicotine features a bicyclic structure consisting of a pyridine ring connected at the third position to a methyl-substituted pyrrolidine ring (Figure 1). The carbon atom in the pyrrolidine ring that connects to the pyridine ring forms the chiral centre of the molecule (the 2’ position in Figure 1), resulting in the existence of R- and S-enantiomers. S-Nicotine (also denoted as (-)-nicotine, or L-nicotine) is the major enantiomeric form found naturally. Therefore, in section 2, where nicotine is mentioned this is considered to refer to S-nicotine.

Figure 1 Chemical structure of R- and S-nicotine



2.2 Exposure

Exposure to nicotine from inhaling nicotine products without tobacco is a dynamic process, resulting in a highly varying, intermittent exposure profile. This exposure profile is determined by several factors, including the concentration of nicotine in the emission, the duration of exposure, puff topography (i.e., puff volume, puff duration and interval between consecutive puffs which can differ between individuals), as well as the number of nicotine products used per day or the number of inhalation sessions per day. The concentration of nicotine in the respiratory tract fluctuates continuously during respiration. Briefly, when a puff is drawn, the emission is inhaled along with ambient air (i.e., tidal volume) and mixes with the volume of air already present in the lungs (i.e., functional residual capacity). Due to the dead space - referring to the volume in the lungs that does not participate in gas exchange - a maximum of 70% of the inhaled volume reaches the alveoli. It is in the alveoli that gas exchange takes place and where the inhaled chemical can be absorbed (Bos et al., 2021).

Currently, information regarding human behaviour, including puff volume, puff duration and puff interval, is limited for these nicotine products and may even differ between individual products. For the purposes of the current assessment, the default parameter values defined by WHO Intense (WHO, 2012) were used.
Details regarding the pragmatic approach used for the exposure assessment are provided in Appendix I.

2.3 Pharmacology

The pharmacological mechanism of nicotine has been extensively described (Benowitz 2008; Benowitz 2010a). Nicotine is absorbed through the respiratory tract and enters the brain via the bloodstream, where it binds to nicotinic acetylcholine receptors (nAChRs). These receptors exist in various subtypes, each playing a distinct role. Upon activation by nicotine, nAChR triggers the release of different neurotransmitters, depending on the receptor subtype and its location. The effects of nicotine vary depending on the brain region and the types of neurotransmitters released. Nicotine can influence arousal and a range of cognitive functions, such as attention and working memory. It has a particularly high binding affinity for receptors of the α4β2 subtype. Activation of these receptors stimulates the release of dopamine in the frontal cortex and mesolimbic regions of the brain (including the striatum), areas collectively referred to as the brain's reward system. This dopamine release is associated with the reward sensation that contributes to (the development of) (nicotine) addiction.

2.4 Toxicokinetics

The toxicokinetics of nicotine, as described below, are primarily based on the review by Hukkanen et al. (2005):

- *Absorption*: The inhalatory absorption of nicotine across biological membranes is pH-dependent. Nicotine is a weak base with pK_a 8.02 (El Hellani et al., 2015) and is less effectively absorbed in its ionised state, such as under acidic conditions. In the respiratory tract, nicotine is quickly absorbed in the small airways and alveoli, where the pH of lung fluid is approximately 7.4. This results in a rapid rise in blood nicotine concentrations, allowing nicotine to reach the brain within 10–20 seconds of inhalation (Hukkanen et al., 2005). Quantitative data on the extent of nicotine absorption via inhalation are limited but suggest extensive absorption. For example, a study by Armitage et al. (1975) involving four cigarette smokers using ^{14}C-labelled nicotine, reported that approximately 80 to 90% of inhaled nicotine is absorbed during smoking. An absolute bioavailability of 51-56% was found in 14 smokers using a nicotine vapour inhaler (Molander et al., 1996). Absorption of nicotine from environmental smoke has been estimated at 60 to 80%, as observed in 17 nonsmoking women (Iwase et al., 1991).
- *Distribution*: Following absorption, nicotine is widely distributed throughout the body, exhibiting high affinity for liver, kidney, spleen, and lung tissue, with a lower affinity for adipose tissue. Nicotine also has a strong affinity for brain tissue, and crosses the placental barrier easily (Hukkanen et al., 2005).

- *Metabolism*: Nicotine undergoes extensive metabolism in the liver, producing a variety of metabolites, of which cotinine is the primary metabolite. Between 70-80% of nicotine is metabolized into cotinine, with approximately 90% of this conversion mediated by hepatic enzyme cytochrome P450 (CYP) 2A6 (Hukkanen et al., 2005; Yildiz, 2004).
- *Excretion*: Nicotine is excreted primarily via glomerular filtration and tubular secretion. The extent of reabsorption depends on urinary pH. The plasma half-life of nicotine following intravenous infusion or cigarette smoking is approximately 2 hours (Hukkanen et al., 2005).

2.5 Toxicology

The information presented in this section is primarily based on the evaluations of UK's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT, 2020) which offers the most recent review with a specific focus on the inhalation route. This is supplemented with information from the European Food Safety Authority (EFSA, 2009).

2.5.1 Adverse effects

An overview of the adverse effects of nicotine is provided below, with a primary focus on the inhalation route.

2.5.1.1 Acute toxicity

Nicotine is acutely toxic via all routes of exposure. It has a harmonised classification conform the CLP-Regulation (EC. No 1272/2008) as Acute Tox. 2 for the oral, dermal and inhalation route (H300: fatal if swallowed, H310: fatal in contact with skin, H330: fatal if inhaled). However, most human fatality case reports appear to involve oral or dermal exposure. The most significant acute effects of nicotine target the central and peripheral nervous systems, manifesting as symptoms such as dizziness, salivation, and increase in heart rate and blood pressure.

Two human studies investigating acute exposure are considered relevant for the derivation of advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation (section 2.6: Hansson et al. (1994) and Lindgren et al. (1999). Of these, Lindgren et al. (199) was selected by COT (2020) and EFSA (2009) for establishing health-based guidance values (HBGV) for the inhalation and oral routes, respectively (section 2.5.2). These two studies are described in detail below.

Hansson et al. (1994) investigated the acute effects of inhaled nicotine in healthy, nonsmoking human volunteers (14 men, 10 women; mean age 30 y with range 23-45 y). Nicotine was administered as a solution (0-64 mg/mL) via a nebulizer with an output of 0.01 mL/breath. No cardiovascular effects were observed following single-breath inhalation of 64 mg/mL nicotine (i.e. 0.64 mg nicotine total) at time points between 0 and 10 min in five subjects.

Nicotine inhalation induced concentration-dependent coughing in 13 out of 15 subjects after a single-breath inhalation of nicotine (0-64 mg/mL). The mean concentrations of nicotine causing two or more coughs and five or more coughs were determined to be 5.5 mg/mL (95% confidence interval: 3.5-8.7 mg/mL) and 15.8 mg/mL (95% confidence interval: 10.0-25.1 mg/mL), respectively. Increased respiratory resistance was also observed following single-breath inhalation of nicotine in sub-tussive concentrations.

Under a double-blind protocol, eight subjects underwent repeated inhalations of nicotine solutions over a very short time period (21×, every 15 s, up to 5 min) at total doses of 0, 0.42, 0.84, or 1.7 mg nicotine over 5 min. Statistically significant increases in heart rate and systolic blood pressure were observed at all nicotine doses compared to vehicle controls, in a dose-related manner (both $p<0.05$). Additional effects included decreases in skin temperature and headache (Hansson et al., 1994).

In the study conducted by Lindgren et al. (1999) 14 volunteers - all regular smokers who had abstained from nicotine for 12 hours prior to testing – participated. At the start of the study, their plasma nicotine levels were below 4 ng/mL. Under a single-blind protocol (blinded for subjects), the participants received intravenous doses of nicotine at 0, 3.5, 7.0, 14.0, or 28.0 µg/kg body weight (bw) over a 10-minute period. Parallel recordings of spontaneous EEG (electroencephalogram) and heart rate were recorded, along with venous blood sampling, before, during and up to two hours after nicotine administration.

A dose-dependent increase in plasma nicotine levels was observed, with the highest plasma concentration reaching 20 ng/mL following administration of the 28 µg/kg bw dose. Heart rate increased by approximately 10% at the two lowest doses and by about 25% at the highest dose, with levels returning to baseline comparable to the control group after approximately two hours. Nicotine's stimulating effects, as evidenced by changes in the EEG, were most pronounced at the two highest doses (Lindgren et al., 1999).

2.5.1.2 Repeated toxicity

No data have been identified regarding short-term or long-term inhalation exposure to nicotine in humans (COT, 2020). Although tobacco smoking or vaping e-cigarettes results in repeated inhalation exposure to nicotine, this exposure inherently involves a mixture of substances. As a result, studies focussing on smoking or e-cigarette use are not further considered in this assessment.

In a chronic inhalation toxicity study conducted in 1996, female Sprague-Dawley rats were exposed whole body to an analytical verified mean concentration of 0.5 mg/m^3 nicotine (20 hours/day, 5 days/week) for up to 103 weeks. Other than a body weight decrease of approximately 5%, no treatment-related effects were observed. In a 28-day study[1] from 2015, male and female SD rats were exposed nose-only to 50 mg/m^3 nebulised nicotine, either alone or in combination with

[1] according to COT this study is funded by the tobacco industry

pyruvic acid, for 4 weeks (6 hours/day, 5 days/week). Only minor effects were observed. However, it is important to note that both of these repeated inhalation studies included only a single test concentration, limiting the ability to derive a concentration-response relationship. Additionally, relatively low exposure concentrations were used. Further, in a 2018 Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test study (OECD 422), Sprague-Dawley rats were exposed nose-only to 0, 10.87, 14.44, and 20.29 mg/m^3 nicotine for 6 hours/day, 7 days/week, for up to 5 weeks (males) or 10 weeks (females). The lowest test concentration was considered the lowest observed adverse effect concentration (LOAEC) for parental systemic toxicity. This was based on observed clinical signs including mortality and co-occurring liver histopathology, and effects on body weight and feed consumption. For details on the observed effects on reproduction/developmental toxicity in this study, see section 2.5.1.4.

2.5.1.3 Mutagenicity and carcinogenicity

EFSA (2009) concluded that nicotine is not mutagenic. While most studies yielded negative results, a few studies showed positive outcomes. These positive effects are likely linked to oxidative stress, as they were diminishing in the presence of antioxidants (COT, 2020). Nicotine is considered unlikely to be a carcinogen on its own, although the available data are limited (EFSA, 2009; COT, 2020). In animal studies using various exposure routes, including inhalation, no increase in tumour incidence was observed.

2.5.1.4 Reproductive and developmental toxicity

Cigarette smoking during pregnancy has been associated with adverse pregnancy outcomes in humans. However, no human data specifically addressing the adverse effects of nicotine on its own have been identified.

In rodent studies, nicotine has been demonstrated to induce developmental toxicity in several organ systems, particularly the neurological and respiratory systems, following prenatal and/or early postnatal exposure. These studies have used exposure methods such as continuous subcutaneous infusion, administration via drinking water, or bolus injection, rather than inhalation. The observed effects occurred at dose levels in the mg/kg bw range.

For the inhalation route, data are available from an OECD 422 study from 2018. In this study, Sprague-Dawley rats were exposed nose-only to nicotine concentrations of 0, 10.87, 14.44, and 20.29 mg/m^3 nicotine for 6 hours/day, 7 days/week, for up to 5 weeks (males) or 10 weeks (females). Based on the absence of adverse effects on reproduction and development, the no observed adverse effect concentrations (NOAEC) was determined to be 20.29 mg/m^3 for both reproductive and developmental toxicity.

2.5.2 Relevant health-based guidance values (HBGV)

As outlined in section 2.5.1, the available inhalation toxicity data for nicotine are limited, a point also acknowledged by the Health Council of the Netherlands (2004), the Swedish National Institute for Working Life

(NIWL, 2005) and COT (2020). These organisations concluded that a critical effect through inhalation could not be identified for the purpose of deriving HBGVs (Health Council of the Netherlands, 2004; NIWL, 2005). However it is important to note that these organisations primarily focus on occupational inhalation exposure rather than consumer exposure. The Health Council of the Netherlands (2004) identified the NOAEC from the 2-year rat study (being the only concentration tested) as the point of departure (PoD) for deriving its occupational exposure limit (OEL). In contrast NIWL (2005) concluded that no OEL could be established. These assessments specifically addressed long-term exposure and the evaluation of a HBGV for short-term exposure durations was not included.

Subsequent evaluations by EFSA (2009) and COT (2020) provided HBGVs for both short-term and long-term exposure via the oral route. These were based on systemic toxicity (i.e. cardiotoxicity and EEG-changes) which was identified as the relevant PoD in these assessments.

EFSA (2009) established oral HBGVs for nicotine based on the study by Lindgren et al. (1999)(see for details section 2.5.1.1). An oral Acute Reference Dose (ARfD) of 0.8 µg/kg bw was derived, based on a lowest observed adverse effect level (LOAEL) of 3.5 µg/kg bw for a slight increase in heart rate. This value was calculated using an overall uncertainty factor of 10, combined with a correction factor of 0.44 to account for the oral bioavailability of nicotine. Given the mildness of the observed effect, EFSA inferred that the no observed adverse effect level (NOAEL) would be close to the LOAEL. As a result, an overall uncertainty factor of 10 was deemed sufficient to account for both human variability and extrapolation from the LOAEL to NOAEL for the pharmacological effects identified at the LOAEL. Since nicotine does not accumulate in the body and its effects on the cardiovascular system are considered the most sensitive, EFSA equated the Acceptable Daily Intake (ADI) to the ARfD, setting it at 0.8 µg/kg bw per day.

EFSAs ARfD, along with its underlying rationale, has previously served as basis for RIVM's advice regarding safe maximum amounts of nicotine and 6-MN in nicotine pouches (RIVM, 2021; RIVM, 2024).

COT (2020) acknowledged the limited availability of suitable data for deriving an inhalation HBGV for nicotine, particularly in the context of evaluating electronic nicotine delivery systems. Similar to EFSA (2009), they identified the study of Lindgren et al. (1999) to be the most appropriate for this purpose. However, COT (2020) concluded that the observed increase in heart rate did not provide a clear basis for an HBGV. Instead their HBGV was based on changes in the EEG for which they identified a NOAEL of 7 µg/kg bw (albeit with some uncertainty). To account for inhalatory bioavailability, COT (2020) applied a correction factor of 0.55, based on Hukkanen et al. (2005). Additionally, an uncertainty factor of 5 was used to account for human variability, as the effect was considered to be C_{max}-dependent and therefore subject to less toxicokinetic variability than AUC-dependent effects. Using these parameters, COT (2020) derived an HBGV of 2.5 µg/kg bw for acute inhalation exposure to nicotine.

As the endpoint used represents a sensitive pharmacological effect, COT (2020), in line with EFSA (2009), considered this inhalation HBGV adequate to protect against both acute and longer-term effects. However, COT (2020) noted that their HBGV for risk assessment of electronic nicotine delivery systems, based on an intraspecies assessment factor of 5, might not be sufficient to protect nicotine-naïve individuals. For this subpopulation, an additional uncertainty factor of 3 was applied during risk assessment.

2.5.3 Selection of PoD and dose metric

In general, adverse effects from inhalation of toxicants include local toxicity in the respiratory tract, systemic toxicity, or carcinogenic effects, which may manifest as tumours in the respiratory tract or elsewhere in the body. Depending on the type of adverse effect associated with inhalation exposure, an appropriate dose metric should be considered. This could include the inhaled concentration (expressed as mg nicotine/m^3 inhaled air), the absorbed dose (expressed as mg nicotine systemically absorbed/kg bw) or the inhaled dose (expressed as mg nicotine inhaled/kg bw), taking into account the exposure duration (Bos et al., 2021).

Where feasible, the risks of systemic effects are assessed using data from studies involving inhalation exposure. However, when no suitable inhalation data are available, data from studies involving other routes of exposure may be used under certain conditions (i.e. route-to-route extrapolation).

For nicotine, the available evidence suggests that both systemic toxicity (such as cardiovascular effects and adverse effects on development) and local toxicity in the respiratory tract are relevant health effects. This will be further detailed in the following sections.

2.5.3.1 Systemic toxicity

Based on the available data, the LOAEL of 0.42 mg (corresponding to 6.1 µg/kg bw assuming a body weight of 68.8 kg for adults (Te Biesebeek et al., 2014)) from the inhalatory study of Hansson et al. (1994)(see for details section 2.5.1.1) is considered relevant and critical. This LOAEL has been selected as PoD for systemic toxicity. The study applied inhalation exposure, which is the route of interest for the current assessment. This LOAEL is based on a transient and rapidly reversible increase of the heart rate and systolic blood pressure, identifying the critical effect as a systemic effect. For systemic effects, the absorbed dose is generally considered the appropriate dose metric. This will therefore be used for calculating the advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation.

Effects on heart rate were also observed in the study of Lindgren et al. (1999) as described in section 2.5.1.1. This study applied intravenous exposure and a LOAEL of 3.5 µg/kg bw was identified, representing an internal value. Taking into account the fraction of the inhaled dose that reaches the alveoli, that is 0.7 (Snyder et al., 1974), and assuming a worst-case scenario with a fraction absorbed in the alveoli of 1, this LOAEL corresponds to an external value of 5 µg/kg bw (i.e. 3.5 / (1 ×

0.7)). This value is slightly more conservative than the PoD based on the Hansson et al. (1994) study but remains comparable. However, given the uncertainties associated with route-to-route extrapolation and that Hansson et al. (1994) employed a double-blind protocol while Lindgren et al. (1999) used a single-blind protocol in their cardiovascular experiments, the LOAEL identified in the Hansson et al. (1994) study was selected as the PoD for the current evaluation. The findings of Lindgren et al. (1999) are nonetheless considered supportive, as they would result in an advisory value approximately 1.2 times lower than that derived from Hansson et al. (1994).

Regarding the clinical relevance of these cardiovascular effects, Rabenstein et al. (2024) recently emphasized that a persistent elevation in heart rate is associated with increased cardiovascular mortality and morbidity, both in patients with heart disease and in healthy individuals.

It is also noted that results of animal studies indicate adverse effects on development. However, these effects are generally observed at higher dose levels (via oral, dermal, or subcutaneous routes) compared to the selected PoD. Consequently, the PoD chosen for systemic toxicity is also expected to offer protection against adverse developmental effects.

When assessing inhalation exposure, it is important to recognize the importance of dose rate and thus temporal determinants (Bos et al., 2021). The PoD for systemic toxicity is derived from a study in which nicotine exposure occurred over a short time frame, involving 21 repeated inhalations every 15 sec for a duration of up to 5 min. This exposure profile corresponds well with the typical use patterns associated with inhaling nicotine products.

2.5.3.2 Local toxicity

Based on the available data, the effect level of 5.5 mg/mL (95% confidence interval: 3.5-8.7)[2], representing the mean concentration causing two or more coughs from the study of Hansson et al. (1994) is identified as a relevant and critical PoD for local toxicity. To account for uncertainty, the lower limit of the confidence interval (i.e. 3.5 mg/mL or 0.035 mg) has been selected as PoD. This LOAEC is based on respiratory tract irritation. For such effects, the inhaled concentration is generally considered the appropriate dose metric and will therefore be used to calculate the advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation.

Nicotine is likely a direct-acting respiratory tract irritant. Evidence suggests that respiratory tract irritation from nicotine exposure is associated with its binding to nAChR (Lee et al., 2007; Tao et al., 2019).

2.6 Advisory values for maximum emission of nicotine from nicotine products without tobacco for inhalation

As described in previous sections, nicotine induces both systemic toxicity and local toxicity in the respiratory tract upon inhalation. For these effects, PODs are selected: the LOAEL of 6.1 µg/kg bw for effects on

[2] Assuming a single breath of 0.01 mL/breath, this corresponds to 0.055 mg (with 95% confidence interval: 0.035-0.087 mg)

heart rate and blood pressure and the LOAEC of 3.5 mg/mL for the cough response. Both PoDs will be considered in deriving advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation.

For systemic toxicity, the absorbed dose is generally regarded as the relevant dose metric, while for local toxicity, the inhaled concentration (and consequently the local concentration in the lungs) is considered the appropriate dose metric. As a result, two separate calculations will be performed to derive the advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation. For systemic toxicity, the calculation focusses on determining a safe maximum *amount* of nicotine in the emission, whereas for local toxicity the calculation focusses on identifying a safe maximum *concentration* of nicotine in the emission.

Considering the wide variety of possible nicotine delivery systems for inhalation, each with its own usage patterns and characteristics, a pragmatic approach is taken to establish uniform advisory values. These values are applicable to the complete use of a product, regardless of the users' behaviour such as puff volume and number of puffs.

2.6.1 Systemic toxicity

The safe maximum amount of nicotine in the emission of nicotine products without tobacco for inhalation (expressed as mg emitted from the product) is calculated using the following formula:

Safe maximum amount of nicotine in the emission = (PoD × BW) / (AF)

PoD: point of departure [mg/kg bw]
BW: body weight [kg]
AF: assessment factor [-]

For the calculations, the following parameter values are selected:

- *PoD*: The LOAEL of 0.0061 mg/kg bw, representing a slight transient increase in heart rate and systolic blood pressure based on Hansson et al. (1994), is selected as the PoD. Since this is an external value, no correction is needed for inhalation absorption and dead space volume.
- *BW*: Different age groups, including adults and adolescents, are considered in the current assessment. Body weights are taken from the ConsExpo General Fact Sheet (Te Biesebeek et al., 2014), and represent the 25th percentile for each group, as a realistic worst-case assumption. The selected body weights are 45.1 kg for children 11-16 y, 62.2 kg for children 16-18 y, 68.8 kg for adults. It is important to note that body weight is a reverse proportional parameter, meaning that lower body weights results in lower calculated maximum amount (expressed in quantity/product). Therefore, the 25th percentile is used to ensure a conservative approach.
- *AF*: The cardiovascular effects observed are both slight and transient, and the PoD is considered to be very close to the NOAEL. As such, an AF of 10 is deemed sufficient to account for both intraspecies variability and LOAEL-NOAEL extrapolation.

COT (2020) highlighted the potential for nicotine-naïve subjects to exhibit higher sensitivity to the acute effects of nicotine compared to tobacco cigarette smokers. For this subpopulation, COT applied an additional uncertainty factor of 3 during risk assessment. However, the PoD in the current assessment is derived from a study involving nonsmoking subjects. Additionally, findings from Hansson et al. (1994) and Lindgren et al. (1999) - involving nonsmokers and smokers, respectively -, indicate no large differences between these groups concerning the critical effect (i.e. increased heart rate). Therefore, the application of an additional factor for nicotine-naïve sensitivity is not deemed necessary for this assessment.

Table 1 provides an overview of the advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation. These values were calculated using the parameter values and assumptions described above. The critical effect is a transient and rapidly reversible increase in heart rate and systolic blood pressure, which is considered a sensitive pharmacological effect. The advisory value represents the safe maximum *amount* of nicotine in the emission of an inhaled nicotine product without tobacco, regardless of the puff volume or number of puffs taken by the user.

2.6.2 *Local toxicity*

For local toxicity, the inhaled concentration is generally considered the appropriate dose metric. This will be used to calculate the safe maximum concentration of nicotine in the emission of nicotine products without tobacco for inhalation. A pragmatic approach, based on Bos et al. (2021), is applied; further details are provided in appendix I.

The effect level of 3.5 mg/mL (based on a single breath) for the cough response, based on Hansson et al. (1994), is selected as the PoD. One key assumption is that similar concentrations in the respiratory tract are obtained when inhaling nicotine products without tobacco comparable to those obtained under the conditions as described in Hansson et al. (1994). This PoD corresponds to a concentration in the extrathoracic and tracheobronchial airways ($C_{ET,TB}$) of approximately 0.07 mg/L (i.e. 3.5 mg/mL × 0.01 mL / ~500 mL[3]) and an initial alveolar concentration ($C_{alv,initial}$) of 0.023 mg/L (i.e. $C_{ET,TB}$ / 3).

The safe maximum concentration of nicotine in the emission of nicotine products without tobacco for inhalation (expressed as mg/L in the emission) is calculated using the following formula:

Safe maximum concentration in the emission = $C_{ET,BT} \times F_{dilution}$ / AF
$C_{ET,BT}$: concentration in the extrathoracic and tracheobronchial airways [mg/L]
$F_{dilution}$: dilution factor, the ratio between $C_{emission}$ (the concentration in the emission of inhaled nicotine products for inhalation) and $C_{ET,BT}$ [-]
AF: assessment factor [-]

[3] i.e. tidal volume of 500 mL + output of nebulizer of 0.01 mL

For the calculations, the following parameter values are selected:
- $C_{ET,TB}$: 0.07 mg/L (see above)
- $F_{dilution}$: 10 (see appendix I)
- *AF*: An AF of 10 is applied for intraspecies extrapolation. Since the PoD is based on the lower limit of the 95% confidence interval, uncertainties are already included in the PoD, and an additional AF for LOAEC-NOAEC extrapolation is not considered necessary.

Using these parameter values and assumptions, the advisory value for nicotine in the emission of nicotine products without tobacco for inhalation is 0.07 mg/L (i.e. 0.07 x 10 / 10). This value represents a safe maximum *concentration* in the emission of an inhaled nicotine product without tobacco, regardless of the puff volume or the number of puffs taken by a user.

As explained in Appendix I, both the time period between consecutive puffs - during which multiple breathing cycles occur with the inhalation of clean ambient air - and the process of inhalation absorption contributes to a decline in the concentration of nicotine in the respiratory tract. As illustrated in Figure I.1, the extent of absorption also influences the level of the maximum alveolar concentration, with a lower absorption resulting in a higher maximum alveolar concentration.

Quantitative information on the extent of absorption upon inhalation for nicotine appears to be limited but indicates rapid absorption (see section 2.4). For the current assessment of local toxicity, an inhalation absorption rate of 60% is assumed as a realistic worst-case assumption. Under this assumption, the alveolar concentration fluctuates between (practically) zero and the level of the initial alveolar concentration (see Figure I.1). This indicates that drawing multiple puffs will result in a concentration in the respiratory tract similar to that produced by drawing a single puff.

Table 1 Calculated advisory values for maximum emission of nicotine in the emission of nicotine products without tobacco for inhalation

	Advisory values for nicotine in the emission	
Group	**Safe maximum amount (mg emitted from the product)**	**Safe maximum concentration (mg/L in the emission)**
Children, 11-16 year	0.028	0.07
Children, 16-18 year	0.038	
Adults	0.042	

2.6.3 *Considerations on the calculated advisory values for maximum emission of nicotine from nicotine products without tobacco for inhalation*

Advisory values for maximum emission of nicotine from nicotine products without tobacco for inhalation are calculated based on two types of critical effects: systemic toxicity and local toxicity. As different dose metrics and PoDs apply to these effects, the resulting advisory

values differ both in magnitude and unit. Systemic toxicity is determined by the total quantity of nicotine inhaled (and absorbed), whereas local toxicity is determined by the concentration of nicotine in the lungs. These two types of advisory values cannot be directly compared to identify which is most conservative. To ensure adequate protection against both systemic and local toxicity, both type of values should be applied simultaneously to nicotine products without tobacco for inhalation.

3 Nicotine analogue: 6-methylnicotine

New synthetically produced nicotine analogues are being developed and marketed for commercial purposes. One example is 6-methylnicotine (6-MN), which features a methyl group attached to the sixth position of the pyridine ring. This analogue has been marketed in electronic cigarette pod systems and tobacco- and nicotine-free pouches in Europe (Vanhee et al., 2024). As 6-MN is a commercially relevant and already marketed analogue for nicotine products without tobacco, the current assessment also aims to derive advisory values for the maximum emission of 6-MN from nicotine products without tobacco for inhalation, irrespective of enantiomer form.

3.1 Read-across approach

Previously, RIVM determined the nicotine and 6-MN content in nicotine pouches at which no adverse effects occurs (after single product use)(RIVM, 2021; RIVM, 2024). For 6-MN, it was concluded that the available toxicological information on systemic toxicity was too limited to derive a PoD. Therefore a read-across approach using nicotine as the reference compound was applied. This approach involved:

- Assessing the physicochemical properties that determine toxicokinetic behaviour
- Assessing potencies towards the nAChR

For the derivation of advisory values for maximum emission of 6-MN in the emission of nicotine products without tobacco for inhalation, the same read-across approach is followed. For completeness, the underlying justification for this approach is included in this report. In addition to this read-across for the systemic effect, local toxicity is assessed using predictions from Quantitative Structure Activity Relationships (QSARs) for skin irritation.

3.1.1 Toxicokinetics

For 6-MN and other analogues, data on the uptake via inhalation is lacking. To derive a generic advisory value independent of the product or inhalation device, it is sufficient to demonstrate that the physicochemical properties of an analogue are sufficiently similar to those of nicotine to predict similar rapid and complete uptake via inhalation. This uptake is typically in the range of 60% for passive secondary smoke exposure to 90% for nicotine from direct inhalation.

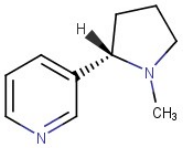
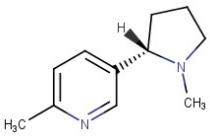
The relevant properties for respiratory uptake are derived from established Physiologically Based Kinetics model approaches for inhalation (Linakis et al., 2020, based on Jongeneelen and Berge, 2011, and Clewell et al., 2001). According to these model equations, the partitioning of a chemical between blood plasma and alveolar air is linearly proportional to the water to air partition coefficient (log $K_{water:air}$), or inversely proportional to Henry's Law constant, see equations 2 and 3 in Linakis et al. (2020). Water solubility and vapour pressure are key properties that determine the $K_{water:air}$.

Additionally, some chemicals may be absorbed into the mucus or otherwise trapped in the upper respiratory tract (Clewell et al., 2001). To account for this uptake component must be included, to compensate for the reduced availability of these substances for respiratory uptake. This compensation is considered linearly dependent on the octanol:water partition coefficient (log $K_{octanol:water}$) of the chemical – see eq.4 in Linakis et al. (2020). The physicochemical properties of nicotine and its analogue, 6-MN, were directly compared to evaluate potential differences or similarities in uptake kinetics.
Since no substance-specific experimental data are available for 6-MN, its relevant physicochemical properties, along with those of nicotine, were estimated using QSAR models available in the US EPA EPISUITE software[4] and ChemAxon[5]. These QSAR analyses were performed using the neutral molecule structures, as the neutral species is considered the form most easily absorbed across the lung epithelial tissue for both nicotine and 6-MN. The findings are presented in Table 2. Experimental data for nicotine were available for log $K_{octanol:water}$, pK_b, vapour pressure, and water solubility, but not for the log $K_{water:air}$. For 6-MN, no experimental data were available for any of these physicochemical properties.

The estimated physicochemical properties of nicotine and 6-MN are highly similar, particularly for log $K_{water:air,}$ which is critical for the uptake of chemicals from alveolar air into the bloodstream. The slightly lower estimated $K_{water:air}$ for 6-MN suggests a marginally lower partitioning of 6-MN into the blood stream compared to nicotine. The log $K_{octanol:water}$ values indicate that partitioning into the mucus of the upper respiratory tract as a competing process to blood uptake will be slightly stronger for 6-MN compared to nicotine. However, because nicotine absorption into the blood is considered rapid and complete (up to 90% of the applied dose), the absorption for 6-MN is also expected to be similarly rapid and complete. This is supported by their almost identical water-air partition coefficients, which are 5 to 6 orders of magnitude larger than the water-octanol partition coefficient driving mucus uptake. As a result, sorption into the mucus is unlikely to significantly influence the rate of blood uptake for either nicotine or 6-MN.

[4] EPISuite https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface
[5] Chemaxon: https://playground.calculators.cxn.io/ Both accessed 24 February 2025.

Table 2 Physicochemical properties relevant for respiratory uptake of S-nicotine and S-6-methylnicotine. These properties are irrespective of the enantiomer form, so they are identical for the S- and R- enantiomer. It is also not known which enantiomer is commercially used in nicotine products without tobacco. As S-nicotine is the major enantiomeric form found naturally, this form is included in this table.

	S-nicotine	**S-6-methylnicotine**
		
CAS number	54-11-5	13270-56-9
Molecular weight (g/mol)	162.24	176.26

	EXP.	**EST.**	**EXP.**	**EST.**	**Model**
log $K_{water:air}$	-	6.91	-	6.87	(I)
log $K_{octanol:water}$	1.17[(a)]	1.00	-	1.55	(II)
pK_b (of the pyrrolidine-N)	8.02[(b)]	8.58	-	8.50	(III)
%neutral (at pH 7.4)	19.3%	6.2%	-	7.3%	(III)
Vapour pressure (Pa, 25°C)	5.07[(c)]	4.26	-	0.715	(IV)
Water solubility (mg/L)	1E6[(c)]	1E6	-	5.6E5	(V)

EXP.: experimental data; EST.: estimated data; (a) Hansch et al. (1995); (b) El Hellani et al. (2015) (c) SRC's PHYSPROP Database as part of US EPA EPISUITE (2024)4; (I) HenryWIN v3.20 as part of US EPA EPISuite (2024); (II) KowWIN v1.68 as part of US EPA EPISuite (2024); (III) ChemAxon pKa calculator5, %neutral at pH7.4 calculated from pH and pKa using the Henderson-Hasselbalch equation; (IV) MPBPVPWin v.1.43 as part of US EPA EPISuite (2024); (V) WSKowWIN v1.42 as part of US EPA EPISuite (2024).

Absorption from the dissolved phase, the chemical passing over the lung epithelial cell membranes, is highly dependent on pH. In their uncharged, 'free' form, the substances are absorbed directly. 6-MN and nicotine have similar estimated pK_b values of 8.50 and 8.58, respectively. As the estimated pK_b of 6-MN is slightly lower than that of nicotine, this would result in slightly better absorption at lung fluid pH. However, the minor increase in the uncharged fraction (7.3% for 6-MN versus 6.2% for nicotine, based on the QSAR estimated pK_b's) is negligible compared to the magnitude of the $K_{water:air}$, which is approximately 10.000.000 to 1 for both nicotine and 6-MN.

The vapour pressure of 6-MN is approximately six times lower than that of nicotine. However, inhalation devices use heat to significantly increase the evaporation rate of nicotine or a potential nicotine analogue, and heating devices can be adjusted to compensate for variations in vapour pressure. Therefore, vapour pressure is considered irrelevant for the advisory value, which is based on the total amount or concentration of the substance in the emission during normal device

use. This advisory value is independent of the evaporation rate at room temperature. Furthermore, for uptake of a substance from the alveolar air into the blood stream, a lower vapour pressure (at body temperature) will actually favour partitioning toward the water (blood serum) phase. While 6-MN has slightly lower water solubility, this results in nearly identical $K_{water:air}$ for nicotine and 6-MN, and thus similar uptake kinetics are expected.

In a review by Yildiz (2004), it is stated that nicotine is metabolized in the liver, forming various metabolites such as nicotine N'-oxide and cotinine N'-oxide, along with other substances through the oxidase system. All metabolic steps discussed occur on the pyridazole ring (5-membered ring), which has the same substitution pattern in both nicotine and 6-MN. No metabolism (oxidation) occurs on the carbon atoms of the aromatic pyridine ring (6-membered ring), meaning substitutions in these positions (such as the methyl group at the 6-position in 6-MN) will likely have minor impact, unless they involve large, bulky substituents. Therefore, 6-MN is highly likely to undergo metabolism similar to that of nicotine, both qualitatively and, to some extent, quantitatively.

Based on these findings, it is anticipated that:

- *Absorption*: respiratory absorption of nicotine and 6-MN will be very similar due to their nearly identical $K_{water:air}$.
- *Distribution*: no differences in distribution are anticipated.
- *Metabolism*: the metabolism of 6-MN is expected to be similar to that of nicotine.
- *Excretion*: no differences in excretion patterns are anticipated.

As a result, no correction factor for differences in toxicokinetic behaviour is considered necessary.

3.1.2 Toxicodynamics: potency towards the nAChR

The relative potency factor of 6-MN toward the nAChR, compared to nicotine, was previously investigated (RIVM, 2024). Results from *in vitro* studies showed varying potency factors, ranging from 0.7 to 3.3, while *in vivo* studies indicated a higher relative potency, varying between a factor of 2 and 5. Based on these findings, it was concluded determining a single definitive value for the potency difference at the nAChR is challenging. Consequently, it was assumed that 6-MN is at least as potent as, if not more potent, than nicotine.

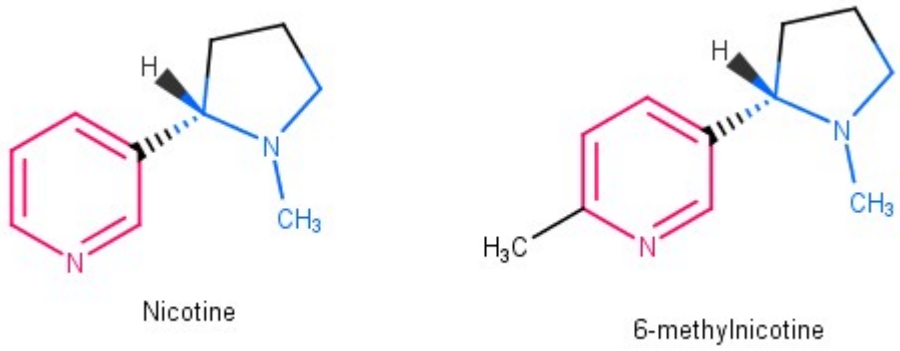
In the current assessment, the highest (worst-case) potency factor of 5 - based on the *in vivo* studies - was applied in deriving the no-adverse-effect levels for 6-MN.This approach deviates from the one previously employed for 6-MN in nicotine pouches (RIVM, 2024). The assessment of other nicotine analogues in Chapter 4 of this report clearly indicates that 6-MN is one of the more potent nicotine analogues. This conclusions was not drawn before. Therefore, the use of a potency factor of 5 appropriately reflects this finding.

3.1.3 *Local toxicity*

There are no data on local irritation effects for 6-MN and other analogues. As described in section 2.5.3.2, local irritation responses might result from direct local irritation of lung epithelium and/or interaction with the nAChR. Qualitative SARs provide some support for the hypothesis that 6-MN will cause similar irritation effects as nicotine. These SAR models are used to predict the potential for skin irritation or corrosion, but they do not able quantitative predictions of irritation potency.

The OECD QSAR Toolbox v2.7 (OECD 2024) profiler "Skin irritation/corrosion Inclusion rules by BfR" (Hulzebos et al., 2005) does not generate skin irritation or corrosion alerts for either nicotine or 6-MN. In contrast, the DEREK expert system (Lhasa ltd, 2024) identifies two structural features present in both nicotine and 6-MN that may contribute to (skin) irritation: the pyridine ring and the alkyl amine embedded within the pyrrolidine ring – see Figure 2.

Figure 2 S-Nicotine and S-6-methylnicotine with substructures indicated that are associated with (skin) irritation and corrosion: pyridine ring (red), and alkylamine (blue)



Several pyridine (red substructures in Figure 2) analogues have demonstrated activity in animal tests for irritation and corrosion, with lower molecular weight analogues bearing small alkyl substituents demonstrating greater potency; examples are 2-methylpyridine, 5-ethyl-2-methylpyridine and 3,5-dimethylpyridine reported to be Category 1 corrosives to rabbit skin under the Globally Harmonised System (GHS). Nicotine and pyridine-4-carbaldehyde (isonicotinaldehyde) are GHS Category 2 irritants to rabbit skin. Additionally, methyl nicotinate is a GHS Category 2 irritant to the guinea pig and human skin, as well as in the EpiDerm(TM) reconstructed human epidermis test.

The mechanism responsible for the skin irritation/corrosion potential of pyridine containing compounds remains unclear; however it is hypothesized to involve the relatively electron-deficient pyridine ring, which is susceptible to nucleophilic attack (Clayden et al., 2001). Skin irritation or corrosion may therefore be initiated by reaction with nucleophilic groups in cellular proteins.

Alkyl amines (blue substructures in Figure 2) have also been shown to induce skin corrosion in rabbits. Examples of corrosive amines include the N-containing ring structures such as 1-methylpyrrolidine (which is the N-containing 5-membered ring in nicotine), piperazine and morpholine, all classified as GHS Category 1A, and piperidine, classified as GHS Category 1B. Additionally, 1-Methylpyrrolidine is corrosive in the Corrositex(R) membrane barrier test method. The mechanism by which alkyl amines induce skin corrosion likely involves the erosion of the stratum corneum (Hulzebos et al., 2005), as these are relatively strong bases. Public data indicate that the vast majority of compounds triggering this alert have a calculated pK_b of 8, or higher for the most basic nitrogen atom in the molecule, which is also the case for nicotine and 6-MN (see Table 2).

All irritation test results for these examples are available in the public ECHA CHEM database of REACH registration dossiers (https://chem.echa.europa.eu/).

3.2 Advisory values for maximum emission of 6-methylnicotine from nicotine products without tobacco for inhalation

3.2.1 Systemic toxicity

The formula used to calculate the maximum amount of nicotine in the emission of nicotine products without tobacco for inhalation (expressed as mg emitted from the product) is also applied to 6-MN, with correction factors for differences in molecular weight, kinetics and potency. Additionally, an assessment factor is included to address remaining uncertainties in the derivation, for example, when correction factor could be established for toxicokinetic behaviour or potency.

Safe maximum amount of 6-MN in the emission =
$(PoD \times BW) / (AF \times CF_{mw} \times CF_{kinetics} \times CF_{potency})$

PoD: point of departure [mg/kg bw]
BW: body weight [kg]
AF: assessment factor [-]
CF_{mw}: Correction factor for difference in molecular weight (MW) ($MW_{nicotine}/MW_{6\text{-}MN}$) [-]
$CF_{kinetics}$: Correction Factor for difference in uptake kinetics [-]
$CF_{potency}$: Correction Factor for difference in potency [-]

Considerations for each parameter are provided below:

- *PoD*: Read-across is performed from nicotine, using the Hansson et al. (1994) study, PoD = 0.061 mg/kg bw.
- *BW*: Same considerations as for nicotine apply.
- AF: An assessment factor of 20 is applied, comprising:
  - A factor of 10 for intraspecies extrapolation and LOAEL-to-NOAEL extrapolation (see considerations for nicotine in section 2.6.1).
  - A factor of 2 for remaining uncertainties related to the read-across approach, which is primarily based on QSAR estimations.
- *CF_{mw}*: the PoD for nicotine (in mg/kg bw) is corrected for molecular weight difference (176.26 g/mol for 6-MN versus

162.24 g/mol for nicotine) to account for the fact that the number of molecules (available for binding to the nAChR) determines the effect, not the weight. CF_{mw}=0.92.
- *$CF_{kinetics}$*: Since the physicochemical properties governing the respiratory uptake are very similar for nicotine and 6-MN, no correction is necessary for differences in kinetics, $CF_{kinetics}$=1.
- *$CF_{potency}$*: Although the available *in vitro* and *in vivo* studies show varying potency factors, this derivation uses the highest potency factor found in the *in vivo* studies. $CF_{potency}$ = 5. See considerations in section 3.1.2.

Table 3 provides an overview of the advisory values for the maximum emission of 6-MN from nicotine products without tobacco for inhalation, as calculated based on the above parameter values and assumptions. These values represent the safe maximum *amount* in the emission of an inhaled nicotine product without tobacco, irrespective of the puff volume or the number of puffs taken by a user.

3.2.2 Local toxicity

The formula used to calculate the safe maximum concentration of nicotine in the emission of nicotine products without tobacco for inhalation (expressed as mg/L in the emission) is also applied to 6-MN. However, it is not possible to derive a correction factor for differences in irritation potency due to the lack of data on irritative concentrations. Nonetheless, QSAR data indicate that 6-MN features structural elements indicative for irritation.

No correction for toxicokinetic behaviour is included in the calculation. Emissions from the nicotine products are directly inhaled and locally deposited at the lung epithelium. Therefore, no correction factor for toxicokinetic behaviour is necessary in the formula below. Additionally, an uncertainty factor is included to account for the lack of quantitative data on irritative potency.

Max. concentration in emission per product =
$C_{ET,BT} \times F_{dilution} / (AF \times CF_{mw})$

$C_{ET,BT}$: concentration in extrathoracic and tracheobronchial airways [mg/L]
$F_{dilution}$: dilution factor, the ratio between $C_{emission}$ (the concentration in the emission of inhaled nicotine products for inhalation) and $C_{ET,BT}$ [-]
AF: assessment factor [-]
CF_{mw}: Correction factor for difference in molecular weight (MW) ($MW_{nicotine}/MW_{6\text{-}MN}$) [-]

For the calculations, the following parameter values are selected:
- *$C_{ET,TB}$*: 0.07 mg/L (see section 2.6.2)
- *$F_{dilution}$*: 10 (see appendix I)
- *AF*: An assessment factor of 30 is applied, comprising:
  - A factor of 10 for intraspecies extrapolation. An additional factor for LOAEC-NOAEC extrapolation is considered unnecessary.
  - A factor of 3 for remaining uncertainties in irritation potential and read-across to the PoD for nicotine.

- CF_{mw}: the PoD for nicotine (in mg/kg bw) is corrected for molecular weight difference (176.26 g/mol for 6-MN versus 162.24 g/mol for nicotine), resulting in CF_{mw}=0.92.

Based on the information for nicotine as a starting point, the derivation of a safe maximum concentration for the emission of 6-MN in the emission of nicotine products without tobacco for inhalation results in an advisory value of 0.025 mg/L. This value represents a safe maximum *concentration* in the emission of an inhaled nicotine product without tobacco, irrespective of the puff volume and the number of puffs taken by a user.

Table 3 Calculated advisory values for maximum emission of 6-MN in the emission of nicotine products without tobacco for inhalation

	Advisory values for 6-MN in the emission	
Group	**Safe maximum amount (mg emitted from the product)**	**Safe maximum concentration (mg/L in the emission)**
Children, 11-16 year	0.0030	0.025
Children, 16-18 year	0.0041	
Adults	0.0046	

3.2.3 *Considerations on the calculated advisory values for maximum emission of 6-MN from nicotine products without tobacco for inhalation*

The current assessment provides a pragmatic approach to deriving advisory values for the emission of 6-MN. It is acknowledged that this approach includes uncertainties and assumptions:

- The acceptability of the read-across approach is primarily based on QSAR estimations.
- Actual physiological effects from using these products cannot be predicted based on binding potency on the nAChR.

Nonetheless, based on the current state of knowledge, the advisory values presented in Table 3 are considered sufficient to protect users from both systemic and local toxicity. Both types of advisory values should apply to nicotine products without tobacco for inhalation.

4 Other nicotine analogues

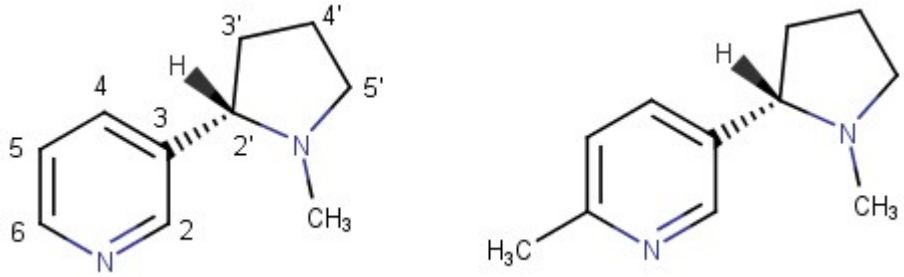
In addition to 6-MN, there are many other nicotine analogues. Several nicotine analogues, such as nornicotine, anatabine, and anabasine, are commonly found alongside nicotine in most tobacco strains (Benowitz et al., 2010b). Numerous other analogues can also be synthetically produced. For the (recreative and/or harmful) effect of a nicotine analogue it is irrelevant whether the analogue (or nicotine) has been synthetically produced or derived from natural resources. A literature search was performed to identify other nicotine analogues. The aim of this assessment was not to provide an exhaustive list but rather to offer a qualitative grouping of other nicotine analogues which can serve as first step in the derivation of advisory values. Additionally, structure-affinity relationships toward the nAChR are qualitatively discussed.

4.1 Grouping of nicotine analogues

4.1.1 Substituted analogues

Substituted analogues feature one or more substitutions of hydrogen atoms on the pyridine and/or pyrrolidine ring with other atoms or groups of atoms. These substitutions can include an alkyl group of various chain lengths, a halogen substituent such as bromo, fluoro or chloro, or another functional group (Figure 3). 6-MN is an example of a pyridine-substituted analogue.

Figure 3 Left; substitution positions of S-nicotine. Right; chemical structure of S-6-methylnicotine



Structure-affinity relationships of nicotine and its substituted analogues with the nAChR have been studied. The position of the substituent appears to be an important factor in receptor affinity, as was indicated in a study by Wang et al. (1998). In this study, the receptor affinity and psychotropic potency of nicotine, determined by the position of an extra methyl group on the ring, is ranked as follows: 6-methyl > 2'-methyl > 5-methyl > 2-methyl > 4-methyl. Additionally, affinity decreased with increasing chain length at the 6th position of the pyridine. This finding was supported by Dukat et al. (2002).

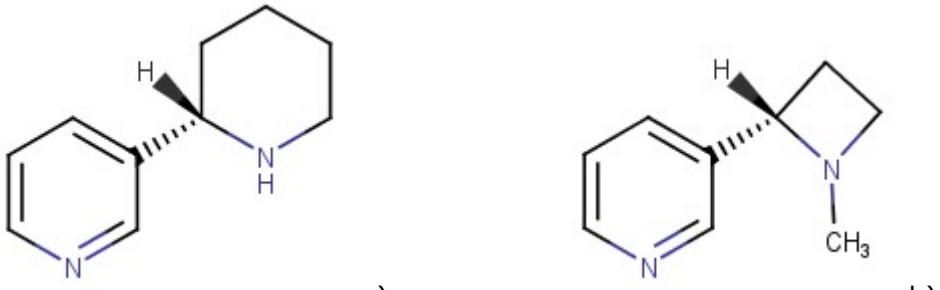
Factors such as the electrostatic and lipophilic nature of substituents might influence the nAChR affinity, but affinity is further modulated by the steric size of the substituent, as noted by Dukat et al. (1999). The electrostatic feature that determines binding potency to the nAChR is the charge on the pyrrolidine-N atom. 6-MN has an almost similar pKb

for this nitrogen atom compared to nicotine (see Table 1), therefore comparable binding potency can be expected for 6-MN.

4.1.2 Azacycloalkyl pyridine analogues

Azacycloalkyl pyridine analogues have a ring size that differs from the 5-membered pyrrolidine ring in nicotine. Examples include the naturally occurring anabasine, which contains a piperidine (6-membered) ring, and 1-methyl-2-(3-pyridyl)azetidine, which has a4-membered azetidine ring (Figure 4). Additionally, azacycloalkyl pyridine analogues may include a variety of substituents on either ring.

Figure 4 Chemical structure of a) anabasine (piperidine ring); and b) azetidine ring analogue to nicotine

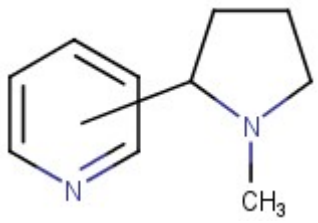


Wang et al. (1998) reported that expanding the 5-membered pyrrolidine ring of nicotine to 6-membered piperidine ring or 7-membered azacycloheptane ring progressively reduced receptor affinity and psychotropic potency, whereas reducing the ring size to the 4-membered azetidine increased both. The addition of an N-substituent to the piperidine group significantly reduces the molecule's affinity, a finding also supported by Zeng et al. (2017).

4.1.3 Ring-shifted nicotine analogues

Ring-shifted nicotine analogues, also called isonicotines, differ in the relative position of the pyridine and pyrrolidine rings within their chemical structure (Figure 5). In nicotine, the third atom (3') of the pyridine ring is connected to the second atom (2) of the pyrrolidine ring. Wang et al. (1998) reported that receptor affinity was ranked as follows: 3,3' > 2-,3' (nicotine) > 4,3. Information on ring-shifted nicotine analogues is limited.

Figure 5 Generic structure of ring-shifted nicotine analogues

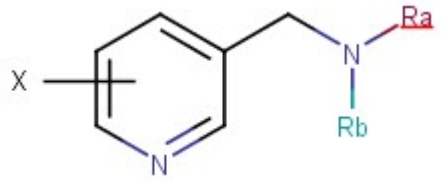


4.1.4 Aminoalkylpyridines

Aminoalkylpyridines are characterized by the substitution of the pyrrolidine ring with an aminoalkyl group (Figure 6). This group may

include various functional groups, such as alkyl groups of various chain lengths, halogen substituents such as bromo, fluoro or chloro, or other functional groups. Generally, this class of substances has been reported to have lower affinities for the nAChR (Wang et al., 1998; Dukat et al., 1999; Dukat et al., 1996).

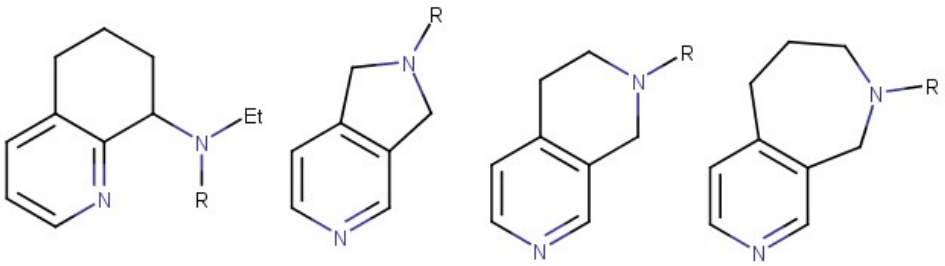
Figure 6 Methylpyridine derivatives (x=nothing) and Aryl-substituted methylpyridine derivatives where X = aromatic ring, Ra and Rb are alkyl substituents



4.1.5 *Conformationally restricted nicotine analogues*

Conformationally restricted analogues include a pyridine ring with an integrated azacycloalkyl or cycloalkyl structure (Figure 7), making them less flexible than nicotine. They may also include various functional groups. Generally, this class of substances has low affinity for the nAChR (Dukat et al., 1996).

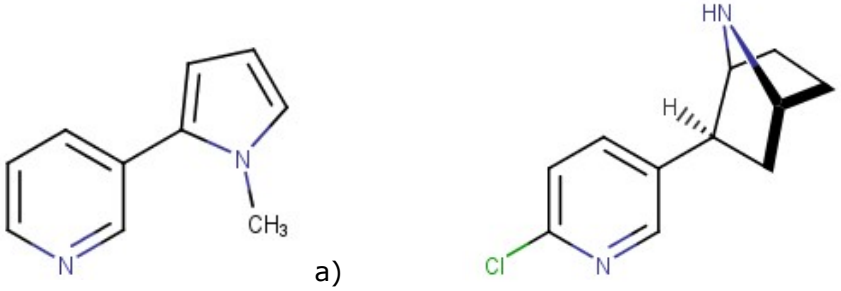
Figure 7 Conformationally restricted nicotine analogues



4.1.6 *Other tobacco alkaloids and related molecules*

There are other nicotine analogues that do not belong to any of the identified analogue groups. For example, nicotyrine (Figure 8a), which features a pyrrole ring instead of a pyrrolidine ring. Another example is epibatidine (Figure 8b), which features an azabicyclo[2.2.1]heptane ring system instead of a pyrrolidine ring and includes a chloro substituent on the pyridine ring. Due to its high affinity for the nAChR, it has gained significant attention in nAChR research (Dukat et al., 2003).

Figure 8 Structure of a) Nicotyrine; and b) Epibatidine



4.2 Considerations on analogues

There are numerous other substances which could be identified as a nicotine analogue. Not all nicotine analogues found in literature are relevant as substitutes for nicotine in commercial products aimed at mimicking the functional (recreational) effects of nicotine. Some analogues, like epibatidine, are so potent that they are considered strong neurotoxins.

Generally, most identified nicotine analogues exhibit lower binding potency for the nAChR compared to nicotine. However, affinity for the nAChR cannot be easily translated into physiological effects, such as the increased heart rate on which the PoD for systemic toxicity of nicotine is based. These effects depend on a complex interplay of activation and deactivation of various nAChR subtypes in different areas of the body and brain. Consequently, the physiological effects, including addictiveness, of nicotine analogues can differ significantly from those of nicotine.

The enantiomeric form could influence potency. For instance, Wang et al. (1998) demonstrated that the S-enantiomer of nicotine exhibits greater potency. However, this effect was less pronounced in the findings of Dukat et al. (2002). For nicotine analogues, data on the potencies of different enantiomeric forms is often unavailable. Moreover, it is still uncertain which enantiomer is used in commercially available nicotine products without tobacco.

The irritation potential of nicotine analogues depends on their chemical structure. Structural alerts, such as a pyridine ring and the alkyl amine group embedded in the pyrrolidine ring, have been identified as indicators of (skin) irritation. Many nicotine analogues contain the pyridine and/or the pyrrolidine ring and could therefore be potential irritants. Additionally, other structural features may affect the irritation potential of these analogues.

As explained in section 2.5.3.2, the exact mechanism of nicotine-induced irritation is not fully understood. There are indications that, in addition to direct effects on the lung epithelium, binding to the nAChR might be involved in coughing responses. This further complicates the assessment of irritation potential of nicotine analogues. With the currently available data, it is therefore not possible to derive a potency factor for the irritation potential of nicotine analogues relative to nicotine.

5 Discussion and conclusion

This report focusses on a pragmatic assessment to derive advisory values for the maximum emission of nicotine, applicable to all kinds of nicotine products without tobacco for inhalation. In addition, the possibility of deriving such advisory values for nicotine analogues is explored, with advisory values for the nicotine analogue 6-MN provided as an example.

The calculated advisory values for the maximum emission of nicotine from nicotine products without tobacco for inhalation are based on adverse health effects (i.e. health-based). Advisory values are calculated for two types of critical effects: systemic toxicity and local toxicity. A transient and rapidly reversible increase in heart rate and systolic blood pressure is identified as the critical effect for systemic toxicity, representing a sensitive pharmacological effect. This critical effect aligns with EFSA's HBGV. For local toxicity, respiratory tract irritation is considered the critical effect. As different dose metrics and PoDs apply to systemic toxicity and local toxicity, the resulting advisory values differ in both magnitude and unit. For systemic toxicity, the calculation of the advisory value focusses on a safe maximum *amount* in the emission, while for local toxicity the calculation focuses on a safe maximum *concentration* in the emission. To protect users from both systemic and local toxicity, both types of values should be applied to nicotine products without tobacco for inhalation. The derived advisory values are applicable to the complete use of a product, regardless of the users' behaviour such as puff volume and number of puffs.

Although both EFSA (2009) and COT (2020) consider their HBGVs protective against longer-term effects, accumulation upon repeated exposure from nicotine products without tobacco for inhalation cannot be excluded. Nicotine is generally eliminated rapidly from the body. However, smoking represents a multiple-dosing scenario resulting in persistent levels in blood serum for 24 hours per day (Hukkanen et al., 2005). This may introduce some uncertainty in the level of protection in case of excessive repetitive use of these nicotine products.

Toxicological information on nicotine analogues is often limited, which complicates the process of setting health-based advisory values. As demonstrated in the current assessment for 6-MN, the available PoDs for nicotine can serve as a starting point for deriving advisory values for other nicotine analogues. This read-across methodology should account for potential differences in toxicokinetic behaviour and potency. Correction factors for these aspects can be included when necessary, and uncertainties can be addressed by applying an additional assessment factor. Deriving advisory values for other nicotine analogues was beyond the scope of this report. However, a qualitative grouping of such analogues was made based on chemical structure.
In general, most identified nicotine analogues exhibit lower binding potency for the nAChR compared to nicotine, with 6-MN being an exception. 6-MN appears to be more potent than nicotine, as demonstrated in *in vivo* studies. However, affinity for the nAChR cannot

be easily translated into physiological effects, such as increased heart rate, which forms the basis for the PoD for systemic toxicity of nicotine. These effects depend on a complex interplay of activation and deactivation of various nAChR subtypes in different areas of the body and brain. Consequently, the physiological effects, including addictiveness, of nicotine analogues can differ significantly from those of nicotine. As a precautionary approach, it is recommended to adopt conservative estimations for nicotine analogues.

When evaluating nicotine and its analogues, cumulative effects should be considered, as nicotine and its analogues have a similar mode of action (i.e. binding to the nAChR). This shared mechanism is expected to result in similar types of effects. Simultaneous presence of nicotine and/or one or more analogues in the emission of nicotine products without tobacco for inhalation will therefore contribute to cumulative exposure through dose- or concentration-addition. To ensure safety, the fractions of nicotine and its analogues in the emission must remain below the same fractions of their respective advisory values. A hypothetical example of a calculation of advisory values for a product containing nicotine and 6-MN is provided in Box 2.

Box 2: Illustrative example of how to account for a mixture of nicotine and/or nicotine analogues in an nicotine product without tobacco for inhalation.

If the emission from a specific product contains both nicotine and 6-MN in a ratio of 70% nicotine and 30% 6-MN, the total amount of nicotine should remain below 70% of the advisory value of emission of nicotine i.e. below 0.7 x 0.028 mg = 0.02 mg emitted nicotine. At the same time, the amount of 6-MN should remain below 30% of the 6-MN advisory value, i.e. 0.3 x 0.0030 mg = 0.0009 mg emitted 6-MN. This approach is also applicable for the maximum concentration in emission

Although the current assessment of nicotine products without tobacco for inhalation focuses solely on nicotine and analogues, potential effects of other components in these products are not taken into account in the current assessment. These products may also contain other intentionally added components that mitigate the irritative effect of nicotine. For example, benzoic acid and propylene glycol are often included in nicotine delivery systems (Leventhal et al., 2021). Furthermore, it is noted that flavourings are regularly added to inhaled nicotine products, such as e-cigarettes and other nicotine products without tobacco for inhalation. Currently only tobacco-flavoured additives are allowed in e-cigarettes in the Netherlands. However, it should be considered that flavourings are typically added to food, and their safety has primarily been evaluated for oral exposure. Their safety for inhalation exposure, in general, has not been extensively assessed.

It is noted that, in the Netherlands, a policy-based age-restriction of 18 years and older applies to the sales of tobacco and related products[6]. However, adolescents are also known to use such products. The use of

[6] https://business.gov.nl/regulation/sales-tobacco/

e-cigarettes (vaping) by adolescents - and even younger children - is a well-known and serious public health concern.[7,8] These products are particularly attractive to younger age groups, which may facilitate increased use among youth. Therefore, the calculations of the maximum emissions are presented for different age groups including adolescents.

In **conclusion**, Table 4 presents the derived advisory values for the maximum emission of nicotine and 6-MN. Given that nicotine products without tobacco are often marketed in ways that appeal to young people, including children. Therefore, the RIVM recommends using the most protective maximum *amount* to also protect young people (i.e. 0.028 mg for nicotine and 0.0030 mg for 6-MN, in addition to the maximum *concentration* of 0.07 mg/L for nicotine and 0.025 mg/L for 6-MN). Hypothetical examples on how to apply the advisory values for the emission are provided in Box 3.

Table 4 Overview of calculated advisory values for the maximum emission of nicotine (A) and 6-MN (B) of nicotine products without tobacco for inhalation.

A: nicotine

	Advisory values for nicotine in the emission	
Group	**Safe maximum amount (mg emitted from the product)**	**Safe maximum concentration (mg/L in the emission)**
Children, 11-16 year	**0.028**	**0.07**
Children, 16-18 year	0.038	
Adults	0.042	

B: 6-MN

	Advisory values for 6-MN in the emission	
Group	**Safe maximum amount (mg emitted from the product)**	**Safe maximum concentration (mg/L in the emission)**
Children, 11-16 year	**0.0030**	**0.025**
Children, 16-18 year	0.0041	
Adults	0.0046	

[7] https://www.cdc.gov/tobacco/e-cigarettes/youth.html
[8] https://www.trimbos.nl/kennis/roken-tabak/e-sigaret-en-shisha-pen/

Box 3: Illustrative examples on how to apply the advisory values for maximum emissions

a) Hypothetical nicotine product without tobacco for single-puff use with a high nicotine dose. Use pattern:
   - 1 puff
   - Puff volume: 55 mL
   - 0.02 mg nicotine emitted from the product

   The amount emitted from the product is 0.02 mg nicotine. This amount is below the lowest advisory value for maximum amount emitted (children, 11-16 year). The concentration of nicotine in the emission is 0.02 mg / (0.055 L x 1 puff) = 0.36 mg/L. This concentration exceeds the safe maximum concentration of nicotine. It can be concluded that this product does not fully comply with the advisory values, as the maximum concentration is exceeded.
b) Hypothetical example of a nicotine product without tobacco with multiple puffs and a low dose per puff. Use pattern:
   - 15 puffs
   - Puff volume: 55 mL
   - 0.04 mg nicotine emitted from the product

   The total amount emitted from the product is 0.04 mg nicotine. This amount exceeds the lowest advisory value for maximum amount emitted (children, 11-16 year). The concentration of nicotine in the emission is calculated as: 0.04 mg / (0.055 L x 15 puffs) = 0.048 mg/L. This concentration is lower than the safe maximum concentration of nicotine. However, it can be concluded that this product does not fully comply with the advisory values, as the maximum amount emitted is exceeded.

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List of acronyms

6-MN	6-methylnicotine
ADI	acceptable daily intake
AF	assessment factor
ARfD	acute reference dose
AUC	area under the curve
BW	body weight
$C_{emission}$	the concentration in the emission of inhaled nicotine products for inhalation
$C_{alv,initial}$	alveolar initial concentration
$C_{ET,TB}$	concentration in the extrathoracic and tracheobronchial airways
C_{max}	maximum concentration
CF	correction factor
COT	UK's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP	cytochrome P450
EEG	electroencephalogram
EFSA	European food safety authority
EST	estimated
EXP	experimental
FRC	Functional Residual Capacity
HBGV	health-based guidance value
LOAEC/L	lowest observed adverse effect concentration/level
6-MN	6-methylnicotine
nAChR	nicotinic acetylcholine receptor
NOAEC/L	no observed adverse effect concentration/level
MW	molecular weight
OEL	occupational exposure limit
pK_b	negative base-10 logarithm of the base dissociation constant Kb; pKb = -log10 Kb
PoD	point of departure
QSAR	quantitative structure–activity relationship
SAR	structure-activity relationship
TRW	Tabaks- en Rookwarenwet (in Dutch)
TV	tidal volume
UF	uncertainty factor

Appendix 1 Exposure assessment upon inhaling nicotine products without tobacco (based on Bos et al., 2021)

The following parameters are used in the approach (see Table I-1): The Tidal Volume (TV) refers to the volume of air inhaled or exhaled during breathing at rest. The volume of air remaining in the lungs at the end of the exhalation phase at rest is called the Functional Residual Capacity (FRC). Default values, considered to reflect average values for an adult human, are 500 mL for TV and 2 L for FRC. Additionally, during a breathing cycle, approximately 30% of the inhaled volume of air does not reach the alveoli, where gas exchange occurs; this is referred to as the dead space volume.

Parameters for human behaviour when inhaling these products are based on WHO Intense-method (WHO, 2012).

Table 1-1. Default parameters for exposure estimation during inhalation of nicotine products without tobacco.

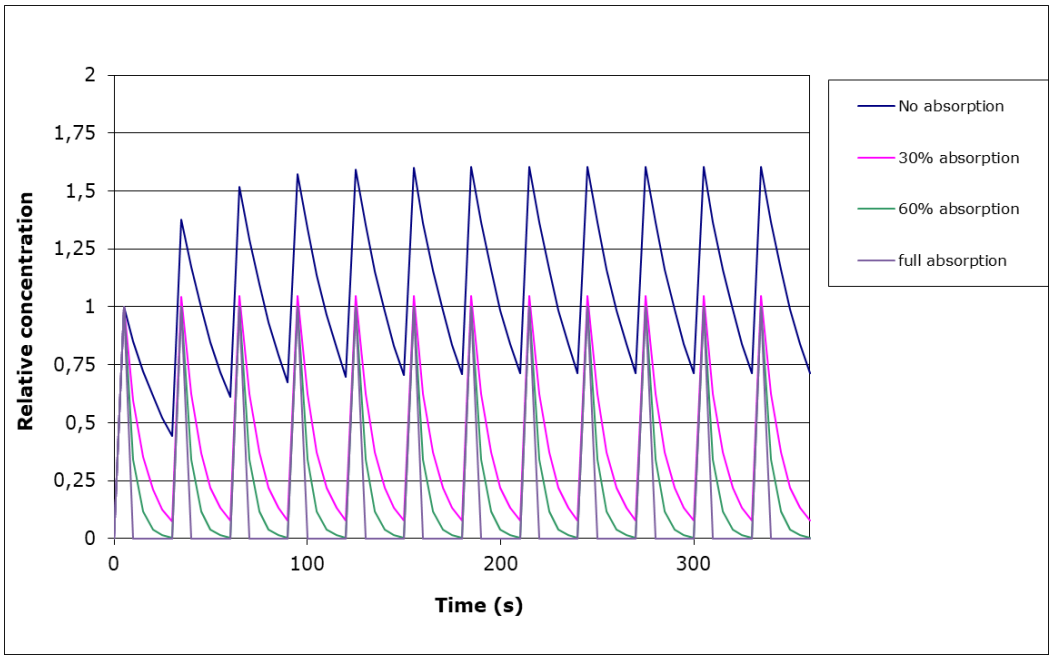
Parameter	**Default value**
Puff volume	55 mL
Puff duration	2 s
Puff interval	30 s
Functional Residual Capacity	2 L
Tidal volume (rest)	500 mL
Breathing rate	12 min^{-1}
Dead space volume (at rest)	30%

Adapted from WHO (2012) and Snyder et al. (1974)

Exposure during inhalation of nicotine products without tobacco is a complex and dynamic process. The concentration of inhaled chemicals in the lungs rapidly increases after taking a puff, followed by several breathing cycles in which clean ambient air is inhaled. The concentration of chemicals in the lungs then decreases until another puff is taken. This decline depends on the extent to which a substance can be absorbed and the time interval between two puffs. At a tidal volume of 500 mL at rest 350 mL (70%) reaches the deeper airways where gas exchange occurs and mixes with 2000 mL of residual air. It is assumed that the alveolar concentration declines with each breath of clean ambient air by approximately 15% (i.e. 100% × 390 mL/2390 mL).

Figure I.1 presents simulations of the relative concentration of an inhaled chemical in the lower respiratory tract during inhalation (topography according to WHO Intense-method) for four scenarios: no absorption, 30% absorption, 60% absorption, and 100% absorption.

Figure 1.1 Estimated relative alveolar concentration (initial alveolar concentration = 1) during inhaling nicotine products without tobacco under different conditions of alveolar absorption (topography following WHO Intense)



If no absorption occurs, a plateau is reached after approximately six puffs with the alveolar concentration varying between 0.71 and 1.6 times the initial alveolar concentration. If some absorption (30%) occurs, the alveolar concentration decreases between two puffs to 0.078 times the initial alveolar concentration and eventually reaches a maximum of approximately 1.05 times the initial concentration. At 60% and 100% absorption, the alveolar concentration varies between (practically) zero and the level of the initial alveolar concentration.

Depending on the type of adverse effect (i.e., local toxicity effects in the respiratory tract, systemic toxicity effects, or carcinogenic effects), an appropriate dose metric should be considered. This could include inhaled concentration (expressed as mg nicotine/m^3), absorbed dose (expressed as mg nicotine systemically absorbed/kg bw), or inhaled dose (expressed as mg nicotine inhaled/kg bw) with consideration of the exposure duration.

Alveolar concentration

When taking a puff, a volume of 55 mL containing a chemical is inhaled. Following the puff, a volume of ambient air equal to the TV of 500 mL is assumed to be inhaled instantaneously, resulting in a total inhaled volume of 555 mL per puff and subsequent inhalation. The concentration of chemicals in the puff ($C_{emission}$) is thus diluted by a factor of 10 (555/55). The exposure concentration in the extrathoracic (ET) and the tracheobronchial (TB) airways $C_{ET,BET}$ can be estimated as $C_{emission}$ divided by 10.

Because of the dead space volume, only 70% of the inhaled volume of 555 mL (i.e. approximately 390 mL) will reach the alveoli. This volume

of 390 mL mixes by diffusion with the air already present in the lungs (i.e., the FRC, approximately 2 L).
Thus, the concentration of chemicals reaching the alveoli is further diluted by a factor of up to six (i.e. 390 mL mixed with 2 L). However, this diffusion process is slow and may not be completed before the next inhalation of ambient air. The chemicals may not be evenly distributed within the lungs, and the concentration will vary. To account for this a more conservative arbitrary factor of three was chosen for estimating a chemical's initial alveolar concentration immediately following the first puff. Using this factor combined with the initial dilution factor of 10, the initial alveolar concentration can be estimated to be a factor of 30 lower than $C_{emission}$.

Between puffs, breathing ambient air leads to mixing of 70% of the TV (i.e. 390 mL) with the air present in the lungs (i.e., FRC of 2000 mL), and a similar volume (390 mL) is subsequently exhaled. Assuming perfect mixing and no absorption, the alveolar concentration of a chemical decreases by approximately 15% (i.e. equal to 100% × 390/(2000 + 390)) with each breathing cycle.

Alveolar absorption will enhance this decline, with greater absorption leading to a more rapid decrease in the alveolar concentration. The lower the alveolar concentration, the lower the risk of potential adverse local effects. An alveolar absorption of 0% is not realistic. As a conservative approach, an alveolar absorption of 30% is assumed when assessing local effects in the respiratory tract. If chemical-specific data on the alveolar absorption are available, these data should be used.

$C_{ET,BET} = C_{emission}/ 10$
$C_{alv,\ initial} = C_{emission} / 30$ or $C_{ET,BET}/3$

Inhaled and absorbed dose

Because of the dead space volume, only 70% of the inhaled volume reaches the alveoli, where gas exchange occurs, and the inhaled chemical may be systematically absorbed. Therefore, a maximum of 70% of an inhaled chemical in the emission can be absorbed systemically. The higher the absorption, the higher the absorbed dose and thus the higher the risk of potential adverse systemic effects.
As a worst-case approach, 100% alveolar absorption is assumed for assessing risks of systemic effects. If chemical-specific data on the alveolar absorption are available, these data should be used.

The total inhaled dose ($D_{inhaled}$ [mg]) can be estimated as per inhaled product: $D_{inhaled}$ = amount per puff × N
N= number of puffs [-]

The total absorbed dose ($D_{absorbed}$ [mg]) can be estimated as per inhaled product: $D_{absorbed} = F_{dead\ space} \times F_{inhalation} \times D_{inhaled}$
$F_{dead\ space}$ = correction for dead space [-]
$F_{inhalation}$ = alveolar absorption [-]

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