German Federal Institute for Risk Assessment

Opinion 013/2026

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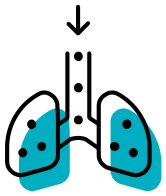
6-methylnicotine in e-cigarettes: Insufficient data available – the BfR participates in investigation of addictive potential

In brief

- Unlike nicotine, which has a high prevalence in tobacco plants, 6-methylnicotine is produced in the laboratory. Only trace amounts are detected in nature.
- E-cigarettes containing 6-methylnicotine are available in Germany, other EU countries, Australia and the USA. These e-cigarettes are advertised as being "non-addictive", "nicotine-free" and safe for “older and younger people”.
- The German Federal Institute for Risk Assessment (BfR) has conducted a comprehensive literature review. The result: the data on 6-methylnicotine is very sparse, and there are hardly any independent studies. The majority of the studies were funded by the tobacco industry. A health risk assessment is therefore not possible at present.
- Some results from the few studies available suggest that 6-methylnicotine could be more addictive than nicotine.
- In order to improve the data available for a health risk assessment, the BfR is participating in the world's first independent clinical study on the addictive potential of 6-methylnicotine. The study is being conducted by Ludwig Maximilian University (LMU) in Munich.

How does 6-methylnicotine from e-cigarettes enter the body?

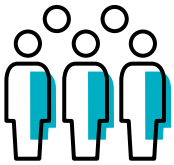


The intake of 6-methylnicotine from e-cigarettes occurs through inhalation via the lungs.

Is there a health-based guidance value for 6-methylnicotine?

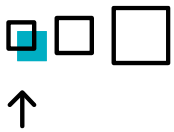
No. There is no health-based **guidance value** that describes a quantity of the substance that poses no health risk during intake. Due to a deficiency in data on pharmacokinetics and inhalative toxicity, there is also no other toxicological value available for assessing health risks.

Is there a health risk associated with 6-methylnicotine in e-cigarettes?



E-cigarettes containing 6-methylnicotine pose a health risk to the general public when vaped. This is particularly true when considering the addictive potential of 6-methylnicotine. This can be a particular risk for adolescents and young adults who consciously avoid nicotine-containing e-cigarettes in order to avoid developing an addiction. Since the physicochemical properties of 6-methylnicotine and nicotine are comparable, it can be assumed that the same amount of 6-methylnicotine is taken up from e-cigarettes as nicotine. Data on receptor binding and functional tests indicate that 6-methylnicotine may be at least as harmful to health as nicotine.

What is the quality of the data on 6-methylnicotine?



The quality of the data is **low**. In particular, there is no relevant, valid data on the pharmacokinetics and inhalative toxicity of 6-methylnicotine. There are only a few studies that were not funded by the industry. The first independent human study, in which the BfR is participating, is currently being conducted.

How can the health risk posed by e-cigarettes containing 6-methylnicotine be reduced?

Consumers can avoid e-cigarettes containing 6-methylnicotine.

1 Subject of the assessment

The German Federal Institute for Risk Assessment (BfR) has conducted an assessment of the health risk associated with 6-methylnicotine in e-cigarettes. In particular, it considers whether 6-methylnicotine poses an increased health risk or has a stronger addictive effect for consumers of e-cigarettes compared to nicotine.

2 Result

For this opinion, a comprehensive bibliographic literature search was conducted on 6-methylnicotine. The search was conducted in various scientific databases and search portals, including PubMed, Scopus, Web of Science, Embase, Science Direct, Wiley, CAS SciFinder, OECD eChemPortal, FDA, WHO IRIS, PubChem, Google Scholar, NTP Technical Report Library, Open Agrar and Chemikalieninfo. The data available on 6-methylnicotine is very limited overall. In particular, there is no relevant, valid data available on the pharmacokinetics and inhalative toxicity of this substance. It is therefore not possible at this time to perform a risk assessment for consumption via e-cigarettes. The comparable physicochemical properties of 6-methylnicotine and nicotine suggest that the transfer rate and inhalative intake of 6-methylnicotine and nicotine from e-cigarettes are comparable. Based on the limited toxicity data and receptor binding data available, as well as functional tests, it is expected that 6-methylnicotine is at least as effective and possibly more effective than nicotine in causing harmful effects. In order to improve the data available and better characterise the addictive potential, a human study is currently being conducted in cooperation with the psychiatric clinic of the Ludwig Maximilian University (LMU) Medical Centre in Munich.

3 Rationale

3.1 Risk assessment

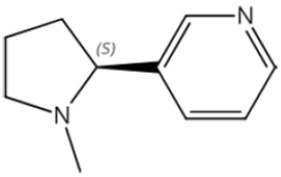
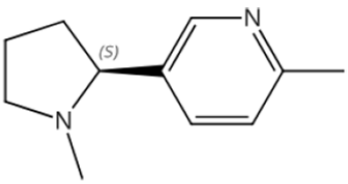
3.1.1 Hazard identification

Nicotine is a natural compound with a high prevalence in tobacco plants (*Nicotiana tabacum*). It consists of two heterocycles, a pyridine ring and a pyrrolidine ring. There are two enantiomers of the nicotine molecule, (S)-nicotine and (R)-nicotine, with (S)-nicotine accounting for approximately 99% to 100% of the total nicotine in tobacco plants (Salam et al. 2023).

Cigarette tobacco contains nicotine in levels of up to 1.5%. Nicotine is used as an ingredient in liquids for e-cigarettes. In the EU, this use is regulated by the Tobacco Products Directive 2014/40/EU, whereby e-cigarettes do not contain tobacco. Nicotine is also used in nicotine pouches, which likewise do not contain tobacco. Furthermore, it is used in medicinal products and medical devices for smoking cessation therapy (BfR, 2022).

6-Methylnicotine is a synthetic nicotine analogue with a methyl group at the 6-position of the pyridine ring. Like nicotine, it has two enantiomers. There are only a few analytical studies on its natural prevalence in tobacco plants. The amount of 6-methylnicotine in tobacco and tobacco leaves has been estimated to be about four orders of magnitude lower than that of nicotine. Since traces form naturally in tobacco plants, 6-methylnicotine has been detected in cigarettes and smokeless tobacco products such as chewing tobacco, snus and smokeless reference products (Pankow et al. 2025).

Table **1** : Chemical structure and physicochemical properties of (S)-nicotine and (S)-6-methylnicotine.

	Nicotine	**6-Methylnicotine**
Chemical structure		
Chemical name (IUPAC)	3-[(2S)-1-methylpyrrolidin-2-yl]pyridine	2-Methyl-5-[(2S)-1-methylpyrrolidin-2-yl]pyridine
Molecular	162.24 g/mol	176.26 g/mol
CAS No.	54-11-5	13270-56-9
pKa	8.58 (calculated)	8.50 (calculated) (RIVM 2024)
Synonyms		Metatine, 6-MN, AltNic, NoNic, "Imotine", "Nixotine", "SFN" (Substitute For Nicotine) (Jordt and Jabba 2024)

Since 2019, there have been reports of various products, such as e-cigarettes and nicotine pouches, containing 6-methylnicotine instead of nicotine.

The occurrence of 6-methylnicotine on the market has been reported in the United States (Erythropel et al. 2024, Jordt and Jabba 2024, Jordt et al. 2025, Sanchez et al. 2025) , Australia (Jenkins et al. 2024) and the European Union (Vanhee et al. 2025) . Various study authors have linked it to attempts to circumvent established nicotine and tobacco regulations.

The physicochemical properties of 6-methylnicotine and nicotine are similar. A poster presented at the CORESTA Smoke Science and Product Technology Conference shows data on the transfer rate of 6-methylnicotine and nicotine from a liquid to the aerosol when using an e-cigarette (Cheetham et al. 2023) . These studies show that the aerosol transfer efficiency of 6-methylnicotine is similar to that of nicotine (82.5 ± 0.6% versus 86 ± 3% for free nicotine). However, these results have not been published in a peer-reviewed journal. Furthermore, the study in question was funded by the tobacco industry.

3.1.2 Hazard characterisation

3.1.3 Mechanism of action and toxicological properties

Nicotine acts as an agonist on nicotinic acetylcholine receptors (nAChR), which are found in the brain, for example. Nicotine affects the nervous system by inducing the release of messenger substances such as dopamine, glutamate and γ-aminobutyric acid. These play a role in the development of dependence (Benowitz 2009) . With regard to the mechanism of action and toxicology of 6-methylnicotine, the available data can be summarized as follows.

In vitro studies

A study conducted by Effah et al. showed that during the thermal decomposition of 6-methylnicotine, a larger amount of reactive oxygen radicals is formed compared to nicotine. This study also compared the cytotoxicity of the substances on human bronchial epithelial cells (Effah et al. 2025) . The study authors concluded that 6-methylnicotine was more cytotoxic than nicotine and that toxicity correlated with the dose administered. In addition, 6-methylnicotine induced the formation of intracellular oxygen radicals to a greater extent than nicotine (Effah et al. 2025, Effah et al. 2025).

In addition, data on the cytotoxicity (neutral red uptake assay), mutagenicity (Ames test) and genotoxicity (micronucleus test) of 6-methylnicotine and nicotine were presented during a poster presentation at the CORESTA Smoke Science and Product Technology Conference (Cheetham et al., 2023). These studies showed that there is no difference between the endpoints examined for 6-methylnicotine and nicotine. However, as mentioned above, these results were funded by the tobacco industry and were not published in a peer-reviewed journal.

There are two conflicting studies reporting on the binding affinity of 6-methylnicotine to nicotinic acetylcholine receptors (nAChRs) in rat brain models. One study concludes that the binding affinity of 6-methylnicotine is slightly lower than that of nicotine, as demonstrated by binding data from radioactively labelled nicotine in rat brain homogenates. The binding affinity (K_i) of 6-methylnicotine to nAChRs was 1.8 nM, while that of nicotine was 1.26 nM (Dukat et al. 1999) . A study by another research team using a rat brain membrane model concluded that the binding affinity (K_i) of 6-methylnicotine is three times higher than that of nicotine, namely 0.3 nM for 6-methylnicotine and 1.0 nM for nicotine (Wang et al. 1998) . The lower the constant K_i, the higher the binding affinity. The studies described above suggest that although binding affinities varied, overall there were no major differences between nicotine and 6-methylnicotine in terms of binding to nAChRs.

A study conducted by an e-cigarette manufacturer investigated and compared the cytotoxicity of nicotine and 6-methylnicotine on human bronchial epithelial cells. The results show that 6-methylnicotine has a higher cytotoxicity than nicotine, with a mean inhibitory concentration (IC_{50}) for nicotine was 12.3 mM and for 6-methylnicotine 5.1 mM. The same study showed that 6-methylnicotine leads to altered expression of tumour-associated proteins (cancer-related proteins) compared to nicotine (Qi et al. 2023).

In vivo studies

One of the first toxicological assessments of 6-methylnicotine was a study conducted by the tobacco industry. The lethal dose (LD_{50}) described in rats was 1.5 to 3 times lower than for nicotine (Rylander 1982, Jordt et al. 2025) , suggesting that 6-methylnicotine may be more toxic than nicotine.

In another study conducted by the tobacco industry, researchers attempted to assess whether laboratory rats would prefer 6-methylnicotine over nicotine in an active substance discrimination test. The authors concluded that rats did not show a preference for either compound up to a certain concentration. However, changes in the behaviour of the laboratory animals were observed as the 6-methylnicotine dose increased, so higher doses could not be systematically investigated. These observations suggest that some properties of 6-methylnicotine and nicotine differ (Philip Morris, Dunn and Levy, 1979) . The results and

observations of the study were described in published industry documents. However, no study protocol with additional information such as the animal model used, a statistical analysis or the dosage of analytes used is available.

In a study with middle-aged female rats that has not yet been peer-reviewed, 6-methylnicotine was administered by inhalation or subcutaneous injection. The experiments with subcutaneous injection showed antinociceptive and thermoregulatory effects as well as reduced spontaneous motor activity (based on the use of a running wheel), comparable to the effects of nicotine. Exposure via inhalation decreased body temperature and increased latency in a tail-withdrawal test, similar to the effects reported for nicotine vapour inhalation. Overall, the authors found that the behavioural effects of 6-methylnicotine were very similar to those of nicotine (Taffe et al. 2025).

Various studies have investigated the effects that occur when nicotine and 6-methylnicotine bind to nAChRs. In a tail flick test to measure analgesic effects in mice, 6-methylnicotine showed up to three times greater potency than nicotine; the ED_{50} for nicotine was 9.9 µmol/kg and for 6-methylnicotine 3.5 µmol/kg (Dukat et al., 2002). In a spontaneous activity assay in mice, it was found that motor activity was more pronounced when 6-methylnicotine was administered compared to nicotine; the ED_{50} for 6-methylnicotine was 1.0 µmol/kg and for nicotine 4.9 µmol/kg (Dukat et al., 2002). In a fatigue test on rats, in which the effects on all four paws were examined, 6-methylnicotine proved to have about five times greater efficacy than nicotine (Wang et al., 1998). It can be concluded that 6-methylnicotine consistently showed a 2- to 5-fold stronger effect than nicotine in functional activity studies in rats and mice (Dukat et al., 1999; Wang et al., 1998).

Overall, the results of *in* vitro and *in* vivo studies suggest that 6-methylnicotine may be more addictive than nicotine. This conclusion has also been reached by international assessment institutes such as the Dutch National Institute for Public Health and the Environment (RIVM) in an assessment of 6-methylnicotine in nicotine pouches (RIVM 2024).

In addition, Xie et al. reported the prevalence of potential biomarkers for exposure to 6-methylnicotine in the urine of mice exposed to the substance intraperitoneally or by inhalation. The study supports the authors' hypothesis that 6-methylnicotine and nicotine form structurally similar metabolites with similar metabolic pathways. They propose 6-methylnicotine-1'N-oxide as a biomarker for exposure to 6-methylnicotine. Furthermore, acute neurotoxic effects of 6-methylnicotine have been reported in mice at doses at which nicotine has no observable effects. Based on these findings, the study authors conclude that 6-methylnicotine poses higher toxicological risks than nicotine (Xie et al. 2025).

Human studies

To date, only two human studies on 6-methylnicotine have been published. In a patent application, ten participants were tested for, among other things, the so-called "throat hit", which refers to the scratchy or irritating sensation in the throat and larynx that occurs during inhalation of nicotine-containing products. According to the patent application, a concentration of 1 mg/ml of 6-methylnicotine produces a comparable level of subjective satisfaction to 3 mg/ml of nicotine (Shanghai Zeno Biotechnology Co Itd, 2020) . It should be noted that this study was conducted by the tobacco industry, the number of subjects was

very small, and no further study data, such as the inhalation protocol or statistical evaluations, were published. Therefore, the significance of these data is severely limited.

Another publication examined urine samples from two participants after consuming an e-cigarette containing 6-methylnicotine. At the same time, the urine of mice exposed to 6-methylnicotine was also examined. A strong cross-species consistency in the metabolism of 6-methylnicotine was demonstrated. In addition, the study pointed to possible human biomarkers for exposure, such as 6-methylcotinine, 6-methyl-3'-hydroxycotinine and 6-methylcotinine N-oxide.

In order to improve the currently poor data on the addictive potential of 6-methylnicotine compared to nicotine when inhaled, an initial human study is currently being conducted by LMU Munich in collaboration with the BfR. This study aims to assess individual smoking cravings and perceived addictive pressure.

3.1.4 Prevalence on the market

Information on the distribution of products containing 6-methylnicotine is very patchy. In 2023, the first e-cigarettes containing 6-methylnicotine came onto the market in the USA and Australia (Jenkins et al. 2024, Jordt et al. 2025) . In 2024, further e-cigarettes and nicotine pouches containing 6-methylnicotine followed on the European and US markets (Jordt and Jabba 2024, Sanchez et al. 2025, Vanhee et al. 2025). These products were advertised as "non-addictive" and "highly safe for older and younger consumers". Between July and October 2024, further new products were reported that, according to advertising, contain 6-methylnicotine, including three brands of disposable e-cigarettes and one brand of refill liquids (Jordt et al. 2025).

A 2024 study from the United States reports that among adolescents and young adults (aged 14-25) who use tobacco products, awareness and usage of 6-methylnicotine products were overrepresented. A significant proportion of adolescents and young adults who had never used tobacco were also aware of nicotine analogues in vaping products (Sanchez et al. 2025).

In Germany, the authorities responsible for tobacco monitoring in the German federal states report on new products on the market, including e-cigarettes containing 6-methylnicotine. This trend is reflected in reports from several Member States of the European Union.

Further information on the BfR website on e-cigarettes

Health risk assessment of e-cigarettes: overview

FAQ: E-cigarettes – anything but harmless

To the BfR podcast (in German)

BfR knowledge comic on e-cigarettes

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About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the German Federal Ministry of Agriculture, Food and Regional Identity (BMLEH). It protects people's health preventively in the fields of public health and veterinary public health. The BfR provides advice to the Federal Government as well as the Federal States ('Laender') on questions related to food, feed, chemical and product safety. The BfR conducts its own research on topics closely related to its assessment tasks.

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Publisher:
German Federal Institute for Risk Assessment
Max-Dohrn-Straße 8-10
10589 Berlin, Germany
T +49 30 18412-0
F
bfr@bfr.bund.de
bfr.bund.de/en

Public institution
Represented by the President Professor Dr. Dr. Dr. h. c. Andreas Hensel
Supervisory authority: Federal Ministry of Food, Agriculture and Rural Affairs
VAT ID number: DE 165 893 448
Responsible for content: Dr Suzan Fiack