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Health assessment of nicotine pouches

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Nicotine pouches are novel, tobacco-free products. They contain a powder consisting of nicotine salts and excipients. The BfR prepared a health assessment of these products based on existing studies and data. In the updated assessment, experimental studies conducted by the BfR (Federal Risk Assessment Institute) were also included. These studies have not been completed, meaning that a comprehensive assessment is not possible as yet. The authorities of the *Länder* (federal states) classify nicotine pouches as novel food.

1 Subject of the assessment

Nicotine pouches are novel products which were described, for example, in the U.S., the UK and Sweden in 2019 [1]. In Germany, they were, for example, the subject of a decision by the German parliament, the *Bundestag* (*Bundestag* document no. 19/20667 dated 1 July 2020).

Nicotine pouches are small pouches which hold a powder containing nicotine. According to the manufacturers' statements, nicotine salts are used which are mixed with microcrystalline cellulose, various salts (e.g. sodium carbonate and sodium hydrogen carbonate), citric acid and flavours, among other ingredients [1]. They do not contain tobacco. The BfR was asked to provide a health assessment of nicotine pouches. Sometimes these products are also called all-white products, and the English term "nicotine pouches" may be used instead of the German "Nikotinbeutel".

In March 2021, the BfR had prepared a preliminary health assessment which was discussed in various meetings. Furthermore, experimental studies using different nicotine pouches were conducted by the BfR, the results of which were included in this assessment.

2 Result

Nicotine pouches are novel, tobacco-free products. The highest amount of nicotine known to the BfR is 47.5 mg of nicotine/pouch. The BfR's studies showed that there were tobacco-specific nitrosamines (TSNA) in some of the nicotine pouches. Pharmacokinetic studies show that at least half of the nicotine in the pouch can be absorbed, whereby these studies were carried out with nicotine dosages of a maximum of 8 mg nicotine/pouch. Relevant blood levels are reached, i.e. nicotine levels are in a range that is also reached after the consumption of traditional cigarettes and of some e-cigarettes.

Some cases of intoxication with nicotine pouches have been observed; however, they did not take a severe course.

Nicotine is a hazardous compound. Is is acutely toxic; for oral exposition, an estimated value of acute toxicity of 5 mg/kg body weight was defined. Nicotine leads to an increase in stillbirths and strongly impacts the cardiovascular system. The long-term effects of the use of nicotine pouches cannot be appraised given the few existing data available.

At present, nicotine pouches are classified as novel food by the authorities of the *Länder* and have been taken off the market as they lacked market approval.

The BfR sees a health risk particularly for the following groups: Children, youth and non-smokers, given that nicotine has an addictive effect. Pregnant and breastfeeding women, due to the effect of nicotine on pregnant women and its passage into breast milk. People with cardiovascular diseases, given that nicotine has strong effects on the heart and on blood circulation.

3 Rationale

Nicotine is a natural component of tobacco leaves and is contained in cigarette tobacco with a proportion of up to 1.5 % [2]. The use of cigarette tobacco, pipe tobacco and chewing tobacco has been known for a long time and is not the subject of this assessment. At the end of this paper, reference will again be made to the effects of cigarette consumption. Nicotine is used as a component of liquids for e-cigarettes. In the EU, this use is governed in Tobacco Products Directive 2014/40/EU, even though e-cigarettes do not contain any tobacco. Nicotine is also used in pharmaceutical/medicinal products for the purpose of replacement therapy for smokers.

In Sweden and some other countries, tobacco is marketed in small pouches which are placed between the upper lip and the gum for a certain time. Usually, these products are also flavoured. In Sweden, this form of tobacco is called snus and has already been used there for many years. In the EU it is prohibited to sell snus outside of Sweden. In the US, there are comparable products usually called "snuff", which is translated as "Schnupftabak" in German but is not identical to "Schnupftabak". There have been novel products for some years which do not contain tobacco in its pouches but rather nicotine salts, excipients as well as flavourings and other additives. The BfR's health assessment is limited to the use of nicotine in such pouches. In this assessment, the authors will also rely on studies and evaluations dealing with oral tobacco products such as Swedish snus, for example. Papers which have evaluated the health risks of smoking tobacco have not been taken into account. This is because tobacco smoke contains not only nicotine but also a multitude of other toxicologically relevant compounds which contribute to the multi-faceted harm of smoking tobacco, as is well known.

3.1 Risk assessment

3.1.1 Identification of hazards

In order to gain first insights into the chemical composition of nicotine pouches, the BfR carried out its own analyses. 44 nicotine pouches were purchased in online trade and were subsequently examined for their weight, nicotine content and pH value. Furthermore, their content of tobacco-specific nitrosamines (TSNA) was analysed. In addition, the labelling on their packages was evaluated. The speed of nicotine release was characterised with the help of in-vitro tests for solubility. A pharmacokinetic study of the nicotine absorption in study subjects using these products is currently being conducted together with a co-operation partner. The BfR is working on the characterisation of the flavourings used. The detailed results of these studies will be published in professional journals.

The median for the weight per pouch was 0.6 g and for the nicotine content per pouch 9.48 mg. The highest nicotine content was 47.5 mg per pouch, the lowest was 1.79 mg per pouch.

In 2020, the Dutch National Institute for Public Health and the Environment (RIVM) published a monograph on nicotine pouches in which it described weights of 0.25 to

0.8 g per pouch. Regarding nicotine content, a range of 1.6 to 32.5 mg per pouch was described [3]. In a study conducted by the US "Center for Disease Control and Prevention″ (CDC), 37 brands of six manufacturers were examined, the highest nicotine content of which was 6.11 mg per pouch [4]. A study carried out by one manufacturer yielded a weight of 0.7 g for four products [5]. The nicotine content of these four products ranged between 4.06 and 11.9 mg per pouch [5]. A study in the US on snus from Northern Europe and from the US showed pouch weights in the range of 0.33 to 1.13 g per pouch, while the nicotine content in the snus samples ranged between 6.81 and 20.6 mg/g [6].

In the pouches examined by the BfR, the median for the pH values of the aqueous extracts was 8.8. It was merely one product where the pH value was in the acidic region. The Henderson-Hasselbalch equation was used to calculate the percentage of uncharged nicotine, also termed free base, on the basis of the pH values. In this state, nicotine may pass through biomembranes such as the oral mucosa more easily, leading to an improved oral nicotine absorption. The median for the percentage of free base was 86 %.

In its monograph on nicotine pouches, the RIVM described pH values ranging from 8.8 to 9.9 [3]. The study by the US CDC using 37 brands yielded a pH range of 6.94 to 10.1. It was converted into percentages of free nicotine base ranging from 7.7 % to 99.2 % [4]. A study conducted by one manufacturer using four products yielded a pH range of 8.5 to 8.7 [5].

Declaration of nicotine strength and labelling on the product packaging:

The nicotine content in mg per pouch or per g was declared clearly on only around one third of the nicotine pouches examined. On the packaging of most products there was a description of the nicotine strength, either on a scale not defined in further detail (for example, strength 3 of 5) or using a notional indication of the nicotine strength (e.g. "easy", "medium", "strong", "extra strong", "ultra", "extreme", "danger strong" or "brutal").

The declared indication of the nicotine strength was compared with the nicotine content per pouch as analysed. Products for which the nicotine strength was indicated as being light had a somewhat lower nicotine content than products for which the nicotine strength was indicated as being medium. In the products where the nicotine strength was indicated to be "medium" all the way to "extra strong", there were many overlaps so that a clear demarcation is difficult. A reason might be that some manufacturers indicate the nicotine content per pouch while others indicate it per gram. However, this is not obvious to the consumer. When changing between products of different manufacturers, it may therefore happen that, while the declared classification of the nicotine strength is the same, the nicotine content per pouch doubles.

In products having descriptors indicative of a nicotine strength higher than "extra strong" (e.g. "ultra", "extreme", "danger strong" and "brutal"), the range of the nicotine content as analysed stretched from 12.1 mg per pouch (product declared as "ultra") to 47.5 mg per pouch (product declared as "brutal").

Almost all products bore a health warning advising minors against consumption. Just about one out of four products bore a health warning regarding its use during pregnancy. Owing to the acute toxicity of nicotine, products having a nicotine content of 2.5 mg/g or more have to be marked with a GHS pictogram 07 (exclamation mark, signal word: warning), and products having a nicotine content of 16.7 mg/g or more have to be marked with a GHS pictogram 06 (scull and crossbones, signal word: danger).

Speed of nicotine release:

For selected nicotine pouches the release kinetics of nicotine was determined. The aim was to examine whether the pouches differ regarding the percentage of released nicotine and the speed of nicotine release.

It was established that there were differences both with regard to the percentage of released nicotine in relation to the total nicotine content in the pouch, and with regard to the release speed. In four samples, more than 70 % of the nicotine contained was released in the first 5 min. In seven further samples, in turn, less than 60 % of the nicotine contained was released within the first 10 min. In summary, the results allow for the conclusion that most nicotine pouches released the majority (> 80 %) of their nicotine content within the test period. Most of the nicotine is released within the first 20 min.

Tobacco-specific nitrosamines in nicotine pouches:

Tobacco-specific nitrosamines (TSNA) include four substances: *N´*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N´*-nitrosoanatabine (NAT) and *N´*-nitrosoanabasine (NAB). These substances are produced by nicotine and the minor tobacco alkaloids nornicotine, anatabine and anabasine during the fermentation of tobacco. Tobacco-based products, particularly conventional cigarettes but also some types of oral tobacco, therefore include considerable amounts of TSNA. For example, approximately 1900 ng NNN and 530 ng NNK per cigarette was found in unburnt cigarette tobacco [7]. A recent comparison of American and Swedish products examining the total of the carcinogens NNN and NNK demonstrated that most products had concentrations of less than 1000 ng/g. In snus, up to 1930 ng NNN and 696 ng NNK were found per g of snus pouch [6]. While nicotine pouches do not contain any tobacco, the added nicotine may have been obtained by extraction from tobacco leaves and may therefore contain traces of TSNA. TSNA were also detected in products that do contain tobacco extracts, for example in e-liquids for e-cigarettes, namely 60 ng NNN and 10 ng NNK per mL e-liquid [8]. It is conceivable that nicotine pouches may also contain TSNA, for example from the tobacco extract possibly added to them or due to the subsequent conversion of the tobacco alkaloids contained in them.

TSNA were detected in more than half of the analysed pouches. The highest contents determined were 13 ng per pouch for NNN, 5.4 ng per pouch for NNK, 2.5 ng per pouch for NAT and 5.6 ng per pouch for NAB. It must further be taken into account that TSNA may also be produced endogenously in the digestive tract, as was described e.g. for NNN in saliva [9].

A study by one manufacturer showed TSNA values of < 10 ng/g for four products. In the same study, three different snus products were analysed for NNN and NNK. For NNN, a range of 560 to 640 ng/g was shown and for NNK a range of 89 to 200 ng/g [5]. The study examined these four products also for further possible ingredients or contaminants: Regarding carbonyle (formaldehyde, acetaldehyde, acrolein and crotonaldehyde), organic compounds (benzo[*a*]pyrene, 1,3-butadiene and benzene), elements (arsenic, lead, cadmium, chrome, nickel and mercury), and aflatoxins (B1, B2, G1 and G2), the contents were below measurable limits [5].

3.1.2 Characterisation of hazards

Nicotine is an alkaloid and a weak base with a pK_a value of 8.0 [2]. It stimulates the nicotinic acetylcholine receptors which exist both in the central nervous system and in the autonomic nervous system. Therefore, exposure to nicotine triggers a number of reactions in the organism (depending on the dose). It leads to an increase in blood pressure and heart rate, among other reactions. Mild symptoms of intoxication include nausea and vomiting. In case of higher exposure, there are additional symptoms such as diarrhoea, increased salivation and a slowing heart rate. Severe intoxication may be characterised by epileptic seizures and respiratory depression [10].

Nitrosamines lead to a base modification of DNA due to their electrophilic properties [11]. They are strongly genotoxic carcinogens with organ-specific effects. So far, a total of seven TSNA have been shown to exist in smokeless tobacco products. Two of the substances, NNN and NNK, were classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogens (causing cancer in humans) [12].

Up until a few years ago, textbooks of pharmacology and toxicology described an oral dose of 60 mg of nicotine per human as a lethal dose. In 2014, this assumption was evaluated by a pharmacologist. Studying the confusingly complex body of sources on the one hand and the accounts of cases of human intoxication on the other hand, he concluded that a lethal oral dose was more than 0.5 g of nicotine per person [13]. In its assessment of nicotine under chemicals legislation in 2015, the Risk Assessment Committee (RAC) of the European Chemicals Agency (ECHA) adopted this view of human toxicity [14]. In ECHA's assessment, the classifications for the acute toxicity of nicotine were re-evaluated. The RAC reached the conclusion that only the studies on the acute oral toxicity in mice and dogs are relevant for the classification as the studies on rats yielded significantly higher LD_{50} values. The LD_{50} values in mice were 3.3 and 24 mg/kg body weight (bw) and in dogs 9.2 mg/kg bw [14], thus in a range that was also calculated in the evaluation of human toxicity with an LD_{50} of 6.5 to 13 mg/kg bw. Consequently, the RAC proposed a classification of nicotine as acute tox. 2 (oral), with the hazard warning "H300: Danger of death after swallowing" and with an estimated value of acute toxicity of 5 mg/kg bw. Meanwhile, this recommendation has been transposed into applicable law in the form of EU regulation 2018/1480.

At the conference of the "Poison Commission" of the BfR in December 2020, representatives of the poison information centres reported some cases of intoxication with nicotine pouches. In one such case, a pouch containing 20 mg of nicotine was swallowed. The person concerned was given activated carbon by the emergency services. Aside from abdominal pain, no further symptoms were developed.

In a study in which a manufacturer took part and which compared the toxicokinetics of nicotine in the use of nicotine pouches versus Swedish snus (see also 3.1.3), further effects on the study participants were also examined [15]. On the one hand, the subjects' heart beat was examined, on the other hand, their perceived feeling of a rush ("head buzz"). For this purpose, nicotine pouches (3 mg and, respectively, 6 mg nicotine per pouch) and snus (8 mg nicotine per pouch) were used for 60 minutes.

Table 1: Effect of nicotine pouches and snus in subjects (from [15])

The heart rate (beats per minute – bpm) and the "head buzz" were studied using a visual analogue scale (VAS) during a 60-minute use of nicotine pouches or Swedish snus with 8 mg nicotine/pouch.

	Max. "head buzz" VAS (mm)		Max. change of heart rate (bpm)	
Product	Median (Q1;Q3)	Range	Median (Q1;Q3)	Range
3 mg nicotine	9 (4;19)	0 - 59	8.5 (5.5; 14.5)	4.0 – 18.0
6 mg nicotine	11 (5; 26)	0 - 63	10.5*(9.5; 16.5)	4.5 – 22.5
Snus (8 mg)	24* (12; 47)	0 - 62	11.0 (4.0; 15.0)	0.0 – 22.0

* A statistically significant difference compared to the group with 3 mg nicotine/pouch ($p < 0.05$), Wilcoxon signed-rank test

The healthy subjects showed a good tolerability of an individual dose of the nicotine-containing pouches. Two cases of mouth dryness were registered as substance-related. The rise in heart rate (see table 1) can be easily explained with the effect of nicotine. Also, the effect was shown to depend on the dose; the changes in the 6-mg-dose group are significantly higher than those in the 3-mg-dose group. As regards the other end point, "head buzz", the Swedish snus product stood out as it yielded clearly higher VAS values than the two nicotine doses of the pouches [15].

In 2009, EFSA derived an acute reference dose (ARfD) for nicotine of 0.0008 mg/kg bw, having used the LOAEL of 0.0035 mg/kg bw. The adverse effect was a rise in heart rate [16]. At this dose, the heart rate rises by approximately 7 beats per minute [17]. The increase of the median by 8.5 beats when using nicotine pouches containing 3 mg nicotine appears to be comparable (see table 1).

In an older study from the U.S., the cardiovascular effects of cigarette consumption were compared with the use of American "snuff" in ten healthy smokers. In one branch of the study, a cigarette was smoked with one puff every 45 seconds for twelve puffs; in another branch of the study, a sachet of American "snuff" weighing 2.5 grams was placed between the upper lip and the gum for 30 minutes. At different points in time, blood samples were taken and the heart rate and blood pressure were measured. The absorbed dose was determined on the basis of the nicotine measurements: it was 1.8 mg nicotine for cigarette consumption and 3.6 mg nicotine for snuff consumption. After cigarette consumption, the heart rate rose by 26 beats per minute, after snuff consumption it rose by 18.2 beats per minute. The increase in blood pressure was 18.6 mm (systolic) and 12.2 mm (diastolic) for cigarette consumption and 15.6 mm (systolic) and 11.4 mm (diastolic) for snuff consumption [18]. It is evident from this study that the consumption of one cigarette and, respectively, one pouch of American "snuff" already leads to a relevant increase in heart rate and blood pressure.

A review paper points out that there is an association between nicotine and type 2 diabetes [19]. Smoking cigarettes is an important risk factor for developing type 2 diabetes. Compared to non-smokers, smokers have an increased insulin resistance; however, there is no indication of any impact on insulin secretion. On the one hand, nicotine may increase insulin resistance by raising the level of insulin antagonists (catecholamines, cortisol and growth hormone). On the other hand, nicotine activates protein kinase which may induce insulin resistance [19].

Reproduction toxicity:

Nicotine passes into the human placenta during the entire pregnancy [20]. In the placenta, the amniotic fluid and the foetal serum, nicotine concentrations are higher than in the maternal serum [20]. After delivery, nicotine concentrations were determined in the maternal serum and in breast milk, the concentration in breast milk being 2.9 times higher than in the serum [21].

In Sweden, a population-based cohort study was carried out which examined the risk of stillbirth. The study included an analysis of the register of births for the years of 1999 to 2006 (n = 610,879). The register of births also includes information on the mother's consumption of tobacco. 7,629 women consumed snus; 41,488 women were described as moderate smokers (1 to 9 cigarettes per day); and 17,014 women were described as heavy smokers (10 cigarettes per day). In the case of 39,734 women, there was no information regarding their tobacco consumption. The risk of stillbirths was determined for these groups in comparison to women who did not consume any tobacco.

Table 2 shows that the consumption of snus leads to a higher risk of stillbirths during pregnancy. Heavy cigarette smokers had an even higher risk of stillbirths [22].

Table 2: Association of tobacco consumption and stillbirths (from [22])

Tobacco consumption is subdivided into cigarette consumption and snus consumption per day. Stillbirths (number of cases), the extrapolation into cases per one thousand pregnant women and the adjusted odds ratio are indicated.

Tob. Consumption	Cases	Ratio (1/1,000)	Adj. OR	(95% CI)
None	1,386	2.7	1.00	
Snus	40	5.2	1.60	1.13 – 2.29
Cigarettes				
1 – 9	172	4.1	1.40	1.17 – 1.67
≥ 10	120	7.1	2.42	1.96 – 2.99

Genotoxicity:

In its monograph on nicotine pouches, the RIVM found no indicators of mutagenic properties of nicotine [3]. Different publications studied the genotoxic properties of nicotine. The analysis in the Ames test yielded negative results [23-25]. A further study yielded negative results in the Ames test and for the sister chromatid exchange (SCE) in cells of Chinese hamster ovaries [26]. In V79 cells, a hypoxanthine-phosphoribosyl transferase (HPRT) mutagenicity test was conducted which yielded a negative result [27]. An in-vitro micronucleus test was carried out in human lymphocytes which also yielded negative results [28].

In a review paper, studies with positive results from SCE tests and chromosome aberration tests in cells of Chinese hamster ovaries were described. The positive results were confirmed *in vitro* both for SCE tests and for chromosome aberration tests in human lymphocytes [29]. In-vitro studies on fibroblasts of human gum showed a significant increase in the micronucleus formation after the cells were treated with nicotine [30].

An in-vivo study of 1 and 2 mg nicotine/kg bw in male mice showed no significant increase of micronucleus formation in bone marrow [31]. In a follow-up study, higher nicotine dosages (up to 16 mg/kg bw) and longer treatment times (up to 36 hours) were used. In the high dosages of 8 and 16 mg/kg bw as well as after 30 and 36 hours, increased micronucleus formation was observed in the bone marrow of female and male mice [32].

In summary, one can observe positive and negative results in the same test system in the in-vitro and in-vivo studies.

Carcinogenicity:

In its monograph on nicotine pouches, the RIVM found no indicators of carcinogenic properties of nicotine [3]. The review paper by Sanner & Grimsrud of 2015 states as a result that no conclusions may be drawn about possible tumor-initiating effects of a long-term treatment with nicotine [29]. A current publication examined the tumor-initiating effect of e-cigarette aerosol on mice [33]. The animals (n = 45) were exposed to the aerosol of e-cigarettes for 54 weeks, 4 hours per day and five days per week. The liquid used had a nicotine concentration of 36 mg per mL. Five animals of the treatment group died or had to be killed. The control groups were exposed either to the vehicle (n = 20) or to filtered air (n = 20). Nine out of 40 treated mice developed lung tumors which were identified as adenocarcinomas. One animal from the control group with filtered air developed an adenocarcinoma. The comparison of the two control groups with the treated group yielded a statistically significant difference [33]. It must be noted critically that only one dose was used in the treated group; that the group size was smaller than specified in the guidelines for long-term studies on carcinogenicity; and that the aerosol of e-cigarettes contains other carcinogenic substances aside from nicotine. A final assessment of the carcinogenic effect of nicotine is therefore not possible.

Addictive effect:

So far, the BfR does not have any specific insights regarding the addictive effect of nicotine pouches. It is assumed, though, that this form of nicotine use causes addiction, too.

3.1.3 Estimate and assessment of exposure

Nicotine may be ingested orally, dermally or by inhalation. In the use of traditional cigarettes or e-cigarettes, absorption by inhalation is decisive. In a review of the literature, the key facts of pharmacokinetics and the metabolism of nicotine were compiled [2]. According to this review, the consumption of a cigarette leads to a systemic absorption of between 1 and 1.5 mg of nicotine. In the EU, the upper limit for the nicotine content in the smoke of one cigarette is one milligram. After inhaling cigarette smoke, nicotine reaches the brain within 10 to 20 seconds [2]. Nicotine is present in the blood stream (with a pH value of 7.4) at a proportion of 69 % (ionic) and 31 % (non-ionic, as free base). Only in a non-ionic state may nicotine pass through cell membranes.

When using nicotine pouches, nicotine is mainly absorbed through the mucosa in the oral cavity. The nicotine pouches are placed between the upper lip and the gum for a period of up to 30 minutes and are then removed. The pouches should not be swallowed.

The pharmacokinetics of nicotine from nicotine pouches was examined in a recent study from Sweden [15]. In the study, financed by a manufacturer, three different nicotine strengths were used in two parts of the study (3 and 6 mg nicotine/pouch in the first part of the study and 8 mg nicotine/pouch in the second part of the study). For the purpose of comparison, snus was studied in the first part of the study (weight: one gram per pouch, 8 mg nicotine/pouch). The subjects (n=17) placed a pouch between the upper lip and the gum and removed it after 60 minutes. Blood samples were taken at the beginning, at different times during the use, and up to five hours after the removal of the pouches, and the nicotine concentration in the blood plasma was analysed. The used pouches were examined to establish their remaining nicotine content. On this basis, the authors calculated the nicotine extraction. While the nicotine extraction for the 3-mg and 6-mg doses was at 56 % and 59 %, the nicotine extracted from snus only came up to 32 % (see table 3).

Table 3: Release of nicotine from pouches and snus (from [15])

The results of the two parts of the study with varying numbers of participants are summarised. As regards Swedish snus, each participant used one pouch containing 8 mg nicotine in the first part and two pouches each containing 8 mg nicotine in the second part.

Product	No. of participants	Nicotine content in mg/pouch	Extracted nicotine in mg/pouch	Extracted nicotine in % of the total
Nicotine pouch	17	3	1.59	55.9
Nicotine pouch	17	6	3.51	59.1
Nicotine pouch	30	8	3.79	50.4
Swedish snus	17	8	2.41	32.0
Swedish snus	30	2 x 8	5.04 (with 2 pouches)	32.6
American snus	30	18	2.99	18.9

The highest concentrations in the blood were at 7.7 ng/mL for the 3-mg dose and at 14.7 ng/mL for the 6-mg dose. For comparison: snus (8 mg nicotine/pouch) yielded 10.6 ng/mL (see table 4). These concentrations were measured 61 min (3-mg dose), 66 min (6-mg dose) and 69 min (snus) after administration. The half-life periods were 152 min (3-mg dose), 140 min (6-mg dose) and 144 min (snus) (see table 4).

In the second part of the Swedish study, the kinetics after the consumption of pouches containing 8 mg nicotine were studied in a larger group of participants (n = 30) and compared with those of Swedish snus and US-American snus. Swedish snus contained 8 mg nicotine per pouch. In the second part of the study, participants used two pouches at the same time so that exposure amounted to 16 mg nicotine. The American product contained 18 mg nicotine per pouch. The nicotine extraction from the 8-mg nicotine pouches was
50 %, from Swedish snus 33 %, and from American snus 19 % (see table 3). As summarised in table 4, the highest concentrations in the blood were 18.5 ng nicotine/mL after 59 min for the 8-mg nicotine pouch, 21.2 ng/mL after 63 min for Swedish snus and 16.9 ng/mL after 65 min for American snus. The half-life period was 109 min (nicotine pouches), 114 min (Swedish snus) and 115 min (American snus) [15].

The paper by Lunell et al. [15] shows that after an administration of 60 minutes, at least half of the nicotine contained in the pouch is absorbed by the body (see table 3). The major portion of the nicotine is absorbed directly through the oral mucosa. A part of

the nicotine might also be dissolved in the saliva and swallowed subsequently. For this fraction, absorption may occur in the gastro-intestinal tract. The two snus products under examination yielded different values for nicotine extraction. Here, the values ranged between 19 % for the American product and 33 % for the Swedish product (see table 3). Some manufacturers of nicotine pouches and snus recommend much shorter periods of use, namely 20 to 30 min. It is safe to assume that this leads to less nicotine being absorbed. However, tobacco-containing pouches (snus) are often used for a period of 60 min [34] and it is possible that this consumption behaviour is also adopted for nicotine pouches.

Table 4: Toxicokinetics of nicotine (from [15])

For snus and nicotine pouches, the results from the two parts of the study with varying numbers of participants are summarised. As regards Swedish snus, each participant used one pouch containing 8 mg nicotine in the first part and two pouches each containing 8 mg nicotine in the second part [15]. For the purpose of comparison, values for traditional cigarettes and for e-cigarettes are indicated.

	Nic. content	C_{max}	T_{max}	$T_{1/2}$
Product	in mg/pouch	in ng/mL	in min	in min
Nicotine pouch	3	7.7	61	152
Nicotine pouch	6	14.7	66	140
Nicotine pouch	8	18.5	59	109
Swedish snus	8	10.6	69	144
Swedish snus	2 x 8	21.2	63	114
American snus	18	16.9	65	115
E-cigarette	n.a.	8.4	5	106
Cigarette	n.a.	15.0	No indication	No indication

The authors compared the values with data from publications on e-cigarettes. There, the highest nicotine concentrations of 8.4 ng/mL were measured after 5 min and the half-life period was determined to be 106 min [15]. In comparison: an earlier study determined top values of 15 ng/mL after the consumption of conventional cigarettes [18]. It can be stated that consumers absorb considerable amounts of nicotine from nicotine pouches. Owing to the higher nicotine extraction compared to snus, the consumer is subject to higher exposition even though the nicotine content in the pouches is the same. However, the study also shows that increasing nicotine doses also lead to increasing nicotine concentrations. The study's range under examination ended at 8 mg nicotine per nicotine pouch. As yet, no statements can be made about the blood concentrations occurring at higher nicotine strengths as there are still no experimental data available.

A further pharmacokinetic study by one manufacturer examined the kinetics of six different products with different flavours, all of which had a nicotine content ranging between 3.30 and 3.82 mg per pouch. All participants used all flavours. In the last part of the study, they were allowed to consume the cigarette brand they typically used. The participants placed the pouches between the upper lip and the gum for 30 minutes. The maximum concentrations (C_{max}) were between 9.0 and 11.5 ng/mL for the nicotine pouches and 16.3 ng/mL for cigarettes. In the use of cigarettes, the maximum concentration was reached after 7.5 min, in the use of nicotine pouches after 30.1 to 34.9 min. The flavouring of the pouches had no influence on pharmacokinetics [35].

Currently, the BfR is studying the pharmacokinetics of nicotine from nicotine pouches in co-operation with a university clinic.

According to the German Association of the Tobacco Industry and New Products (BVTE), its member companies offer products containing up to 20 mg nicotine per pouch in Germany. The analyses of BfR have shown that even products containing up to 47.5 mg nicotine per pouch are available in Germany. It must be expected that the products with higher nicotine doses will also lead to higher nicotine concentrations in the blood. Due to the lack of experimental data for nicotine pouches with a nicotine content of more than 8 mg, there is a great deal of uncertainty in this area. Therefore, the BfR is currently conducting a pharmacokinetic study with a co-operation partner involving higher nicotine concentrations.

There is only very limited data available regarding the use of nicotine pouches in the population. A study by one manufacturer presents the results of consumer surveys in Sweden: Between the first quarter of 2018 and the fourth quarter of 2020, the daily use of nicotine pouches was queried in an interval of three months. In the years of 2018 and 2019, the sample size was still between 20 to 99 persons, while in the year 2020 it ranged between 190 and 238 persons. An average of 8.6 nicotine pouches were consumed per day [5].

Tobacco-free nicotine pouches were launched in the US in 2016; the market share in the segment of smokeless tobacco rose to 4 % by 2019 [36]. Also in the US, there have been analyses of consumer behaviour regarding nicotine pouches. Nicotine pouches were appealing to only a small part of those who had never or formerly consumed tobacco (11-12 %). 36 % of active smokers found the product appealing, compared to 52 % of users of smokeless tobacco. The appeal was strongest (75 %) for people who consumed both cigarettes and smokeless tobacco [37].

A survey conducted in the UK in 2019 among people who consumed cigarettes or e-cigarettes or had consumed them in the past established that a percentage of 4.4 % had ever used nicotine pouches [38]. No comparable scientific studies are available for Germany yet.

3.1.4 Risk characterisation

For nicotine, there is a classification concerning acute toxicity under chemicals legislation, whereby only the acute toxicity after oral administration is of interest in this context. The Risk Assessment Committee of ECHA, relying on different animal studies and taking into account human toxicity, determined an estimated value for acute toxicity of 5 mg nicotine/kg body weight. The CLP regulation provides the following formula for the calculation of the acute toxicity of mixtures in Annex 1, part 3, no. 3.1.3.6.1:

$$\frac{100}{ATE_{mix}} = \sum_{n} \frac{C_i}{ATE_i}$$

The formula is converted into c_i.

$$c_i = (100 \times ATE_i) / ATE_{mix}$$

In this specific case, the value of 5 mg/kg body weight is used for ATE_i, this is the estimate value of acute toxicity of nicotine. For ATE_{mix}, the estimate value of acute toxicity of mixtures, a value of 300 mg/kg bw is used; this is the limit between categories 3 and 4 of the acute oral toxicity (see also table 3.1.1 of the CLP regulation). Then, the formula yields the following value:

$$(100 \times 5)/300 = 1.67\ \%$$

For nicotine pouches, this would be a concentration of 16.7 mg/g per pouch. From a perspective of chemicals legislation, a product with a concentration of 16.6 mg/g pouch would be classified in hazard category 4 and would not have to be marked with skull and crossbones.

From a toxicological perspective, this limit is reasonable. As described under 3.1.2, the use of a nicotine pouch with 6 mg already lead to a significant increase in heart rate by 10 beats per minute. The concentration proposed here is almost three times as much.

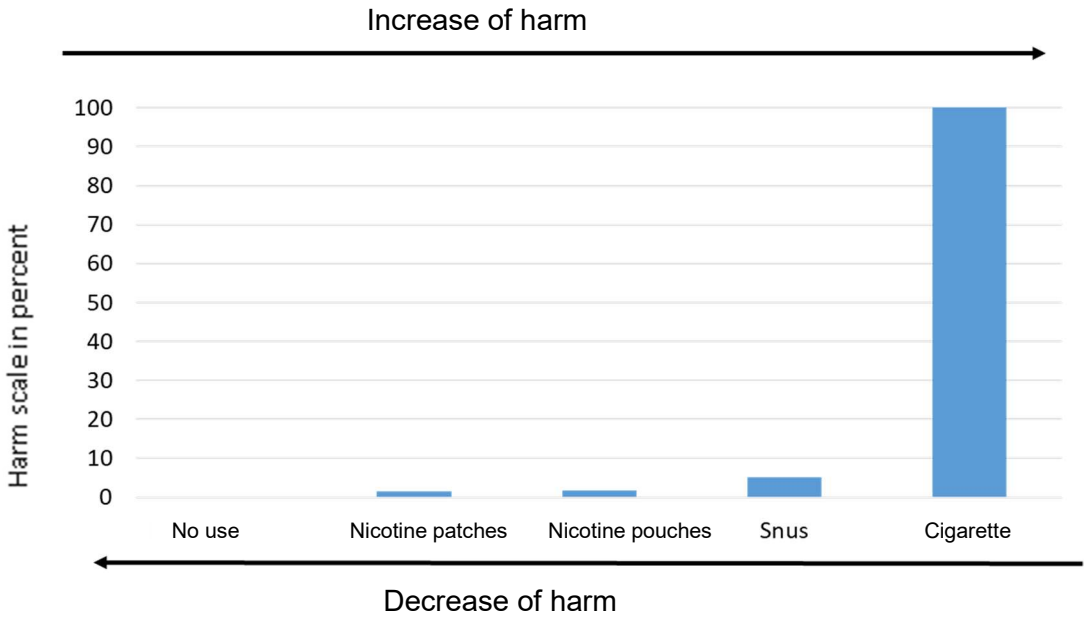
NNN and NNK are genotoxic carcinogens for which no threshold value can be defined. The concentrations of TSNA in nicotine pouches should be below measurable limits.

3.2 Scope of action, recommended measures

At present, nicotine in pouches is classified as novel food by the surveillance authorities. Applying the ARfD value of 0.0008 mg/kg body weight, nicotine pouches containing the amounts of nicotine presented in this paper are taken off the market. The BfR sees a health risk particularly for the following groups: Children, youth and non-smokers, given that nicotine has an addictive effect. Pregnant and breastfeeding women, due to the effect of nicotine on pregnant women and its passage into breast milk. People with cardiovascular diseases, given that nicotine has strong effects on the heart and on blood circulation.
The different intensities of the harmful effects of tobacco and nicotine products have been discussed for some years. There is a focus on creating options which allow a switch to nicotine products that contain or release substances which are harmful to health to a lesser degree [39, 40].

This concept was further developed by Abrams and colleagues, as shown in a modified way in figure 1 [41].

Figure 1: Products along the continuum of harm reduction (modified according to [41]).



The continuum of harm reduction assumes that not all products containing nicotine are equally harmful but instead range from very low harmfulness (e.g. nicotine patches) to very high harmfulness (e.g. cigarettes). Figure 1 shows a selection of products containing nicotine. The most harmful ones are cigarettes, the consumption of which causes the death of approx. 127,000 people every year in Germany [42]. Considerably less harmful is snus of the Swedish type. However, the comparison with non-smokers and non-consumers of nicotine, which are summarised in the figure under "no use", shows that there is an increased risk for users of snus. Evidence for this was recently provided by a pooled analysis of eight cohort studies showing an increased mortality of snus consumers in Sweden [43]. No tobacco is contained in nicotine pouches and therapeutic nicotine replacement products such as nicotine patches. Even nicotine patches may lead to patients being exposed to TSNA [44].

However, the figure should also be viewed from left to right, meaning that any form of nicotine consumption constitutes an aggravation of health risk for people who have previously not smoked or consumed nicotine in any other way.

When taking this concept into account, switching from cigarettes to nicotine pouches could represent a risk reduction for a smoking individual. However, there is still a great deal of uncertainty. There is still no data available about the nicotine level caused in the blood by a nicotine content of 8 mg/pouch or more. It should be avoided that the consumption of nicotine pouches leads to higher nicotine absorption in comparison to other products available on the market. In the BfR's study, TSNA were demonstrated to exist as genotoxic carcinogens in some nicotine pouches. The fact that several products were shown to contain no TSNA illustrates that it is technologically possible to avoid these substances.

From a toxicological perspective, TSNA should not be measurable in nicotine pouches. Moreover, the substances contained in nicotine pouches may sometimes also be swallowed and are subject to the interaction with food particles, saliva as well as gastric and intestinal juices. The formation of nitrosamines occurs under the influence of nitrosating agents such as e.g. nitrite salts, preferably in an acidic environment. This way, an endogenous formation of carcinogenic TSNA in the human intestinal tract, for example, may be conceivable [45]. It is also against this background that the amounts of nicotine measured in the nicotine pouches, some of which were very high, must be viewed critically. In addition to closing the above-mentioned knowledge gaps, it seems to be a reasonable approach to implement quality control via standardisation and regulation measures to achieve harm reduction through nicotine pouches.

3.3 Further aspects

In Sweden, snus has been consumed for many decades, with men using snus clearly more frequently than women. A study from Sweden has shown that snus does not facilitate the entry into cigarette smoking. Rather, Cigarette smokers who start consuming snus tend to stop smoking cigarettes [46]. Whether this is similar with nicotine pouches cannot be appraised yet.

With regard to tobacco-induced diseases, Sweden holds a particular position in Europe. An evaluation of cancer incidences and mortalities in Europe in 2021 showed that Sweden was the only country in Europe where lung cancer did not rank first among cancer mortalities in men [47]. In this study, age-standardised cancer incidences were also calculated. In Sweden, men had the lowest value for lung cancer among 40 countries in Europe, i.e. 28.8 per 100,000, compared to Germany with 57.3 per 100,000 [47]. Consequently, the lung cancer incidence in men is twice as high in Germany as in Sweden.

This data is currently being acknowledged, e.g. also by the treatment network of the Society for Research on Nicotine and Tobacco [48]. However, whether these effects of snus, which has been consumed in Sweden for many years, suggest a potentially achievable smoking cessation by means of nicotine pouches has not been demonstrated so far.

4 References

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The German Federal Risk Assessment Institute (BfR) is an independent scientific body within the portfolio of the German Federal Ministry of Food and Agriculture (BMEL). It advises the Federal Government and the *Länder* on issues of food, chemicals and product safety. The BfR conducts its own research on topics that are closely related to its evaluation tasks.