Systematic Review

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Rendering Oral Nicotine Pouches Harmless—A Biological Appraisal

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Since their introduction to the US market in 2016, oral nicotine pouches have grown in popularity. These small fiber pouches of an appealing taste deliver nicotine to the narrow space between the gum and the lip, precisely at the interface of the mucosa of the vestibulum oris and the oral moisture derived from saliva. This delivery mechanism leads to a systemic effect as the substances bypass gastrointestinal enzymatic degradation and the hepatic first-pass effect. While a portion of the nicotine from these products undergoes enzymatic changes in the liver due to the first-pass effect, some nicotine manages to evade this process. The gut environment appears to influence the first-pass effect within the portal circulation system. Despite their ease of use, these products exhibit variability in nicotine levels and concentrations, potentially leading to poisoning and addiction. The increases in use, marketing, and appeal of nicotine pouches have notably elevated their popularity among younger people. However, it is crucial to note that oral nicotine pouches are not risk-free. Given their potential for overdose, a review of medical literature was conducted to explore whether the microbiota could play a role in influencing nicotine overdose among users of oral nicotine pouches.

Keywords: first-pass effect; immunity; microbiota; oral nicotine pouches; PBPK modeling; probiotics; overdose

Introduction

Nicotine (3-[(2S)-1-methylpyrrolidin-2-yl] pyridine) is a naturally produced primary active alkaloid in tobacco leaves. It is still widely recreationally used as a stimulant (sympathomimetic) and anxiolytic [1]. In some countries, nicotine is still used as an insecticide. The European Union (EU) has prohibited the use of nicotine-containing products for plant protection [2]. Nevertheless, there is a rising trend in the popularity of tobacco-free oral nicotine pouches. These pouches consist of nicotine, flavorings, sweeteners, and plant-based fibers, distributed in colorful packaging resembling mint containers. They are placed between the cheek and gum, delivering varying doses of nicotine. Typically, these pouches contain concentrations ranging from 1.3 to 7 mg per pouch (Table 1, Ref. [3,4]) [5].

Some potent varieties are commercially available, containing approximately 11 mg/pouch of nicotine [6]. These varieties, due to their commercial availability, importance in the food industry, or relevance in toxicological assessments, may become subjects of forensic expertise, even if samples are not routinely screened [7–10]. Data from previous studies have been gathered and utilized to determine the absorbed nicotine amount (nicotine dose), which can be quantified by measuring the concentration of nicotine or its metabolites in different parts of the body.

Building on that, this manuscript's objective was to use a literature review to provide an overview of the effects of the immunity, first-pass metabolism, and microbiota in oral nicotine pouches. In the context of the results of that review, the possibility of poisoning (overdose) was attentively considered, along with the possible strategies for its prevention.

Effect of the Immunity

In smokers in, leukocyte count is increased while T helper cells-clusters of differentiation 4 (TCD4$^+$) and natural killers (NKs) are in the sharp decrease. Injection of nicotine in rats reduces the secretion of T-dependent antibodies, the proliferation of T responses and inhibition of T cell receptor (TCR) pathway signaling. Conclusively, concentration and route of administration play a significant role in the extent of changes in the immune function. In various experimental models, nicotine induces angiogenesis and cell proliferation in general; however, it does not appear to be carcinogenic on its own. Interaction of nicotine and the immunity is employed in the therapeutic strategy for ulcerative colitis where nicotine lowers the levels of cytokines, produces carbon monoxide (potentially acting as an anti-inflammatory agent), and causes activated immune cells towards tobacco compounds [11]. Its ability to suppress the production and secretion of antibodies and reduce T cell receptor (TCR) signaling nicotine is involved in suppressing the immune system [12].

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Table 1. Toxicokinetic parameters of various nicotine pouches.

Study (reference)	Declared nicotine content (mg)	Extracted nicotine (mg)	Cmax (ng/mL) mean	Tmax (h) median (min–max)
Lunell *et al*. [3]	3		7.7 (6.3–9.0)	1.02 (0.93–1.1)
Lunell *et al*. [3]	6		14.7 (12.3–17.1)	1.1 (0.98–1.2)
McEwan *et al*. [4]	6		17.5 (43.8)	1.08 (0.75–1.25)
McEwan *et al*. [4]	8	3.38 ± 1.92	13.0 (20.2)	1.0 (0.05–1.25)
McEwan *et al*. [4]	9		18.4 (30.1)	1.03 (0.17–2.0)
McEwan *et al*. [4]	10		17.1 (24.0)	1.0 (0.002–1.3)
McEwan *et al*. [4]	10	4.53 ± 2.09	11.9 (26.8)	1.1 (0.75–1.25)

The proper effects of nicotine on the immune system were not explicitly explained. Although diverse ecosystem of microorganisms termed the microbiota is involved in the immune system functioning. Accordingly, dysfunctions of the microbiota lead to gut dysbiosis, implying the alterations in the immune system [13].

Effect of the First Metabolic Pass

The absorption of drugs from the oral cavity into the mucosal tissues is typically a fast event. Dissolved drugs are partitioned into the mucosal membranes, reaching equilibrium within minutes [14,15]. The mouth consists of two regions: the vestibule and the oral cavity proper. The vestibule is the area between the teeth, lips, and cheeks. While nicotine delivery can occur through various routes, the oral route is often overlooked. The buccal route of administration involves holding or applying drugs in the buccal cavity (between the cheek and gums/gingiva) for distribution through the oral mucosa (Fig. 1, Ref. [16]). Once absorbed, those drugs enter the systemic circulation through the jugular vein without reaching the intestines, completely bypassing them [17,18]. The oral route of administration is not only convenient and easily accessible but also benefits from the moist environment of the mouth, which is lined with a mucous membrane. This moisture is provided by the saliva, a viscous liquid that protects the soft and hard tissues locally [19]. These facts explain why the vestibule of the oral cavity (vestibulum oris) is of particular interest—especially its upper part. It forms a slit-like space lined with membranes, externally delimited by the lips and cheeks and internally by the gums and teeth. These membranes are sufficiently large to accommodate a nicotine pouch in one of the two compartments-on the left or right gingival/buccal membranes.

The arterial supply to the area mainly comes from the superior labial arteries on one side and three sources on the gingival side. The supraperiosteal arterioles supply gingival blood, vessels from the periodontal ligament, and arterioles emerging from the crest of the interdental septa. Additionally, the subseptal and subalar arteries also contribute to the gingival blood supply to a lesser extent [20,21]. This method of nicotine delivery provides rapid access to the systemic circulation, bypassing the first-pass hepatic metabolism, leading to higher bioavailability.

The pharmacokinetics of nicotine and its transfer rate to tissues are crucial factors to consider when evaluating exposure and blood plasma concentrations. In the context of oral nicotine pouches, the main compartment in the physiologically based pharmacokinetic (PBPK) model is the one involving the buccal mucosa [22–24]. On the other hand, high throughput toxicokinetics (HTTKs) [25,26] can predict the nicotine tissue concentrations due to exposure via pouches and calculate the bioactivity/exposure ratio. The synergy of these tools is beneficial in chemical risk assessments, facilitating provisional toxicokinetic modeling for many chemicals with limited chemical-specific data [27]. For future reference, the schematic of the pharmacokinetics of the nicotine from nicotine pouches is represented in Fig. 2 (Ref. [28]).

Oral Compartment of the PBPK Model

Nicotine absorption is pH-dependent. With a pKa of 8.0, nicotine is a weak base, and it is not absorbed well under acidic conditions, as found in the mouth and upper alimentary tract [17,29,30].

Studies indicate that during the use of nicotine pouches, 75% of the saliva-nicotine mixture is spit, while only 25% of the nicotine is absorbed in the buccal cavity; thus, users are not required to spit while using them. In oral nicotine products requiring spitting, the nicotine concentration in saliva increases between executions of spitting as fresh saliva becomes more saturated with nicotine. Salivation and persistent swallowing of saliva can lead to some nicotine being carried with the saliva and transferred to the gastrointestinal tract [31,32]. Saliva typically has a pH range of 6.2–7.6, making it weakly acidic. The pH of the resting mouth remains above 6.3 [33]. Nicotine uptake rate can be estimated by monitoring the increase in blood nicotine levels over time [34]. Oral absorption through the mass-flow path occurs gradually. [5,22,35]. The buccal mucosal route leads to nicotine being partially delivered to the gastrointestinal tract. A dissolution model was developed to estimate the rate of nicotine release from the oral cavity to the blood circulation system, considering factors such as the diffusion coefficient of nicotine in tissue, transfer surface area, and tissue thickness [22]. Stasio *et al*. [36] provided data on the thickness of the oral epithelium (Ta-

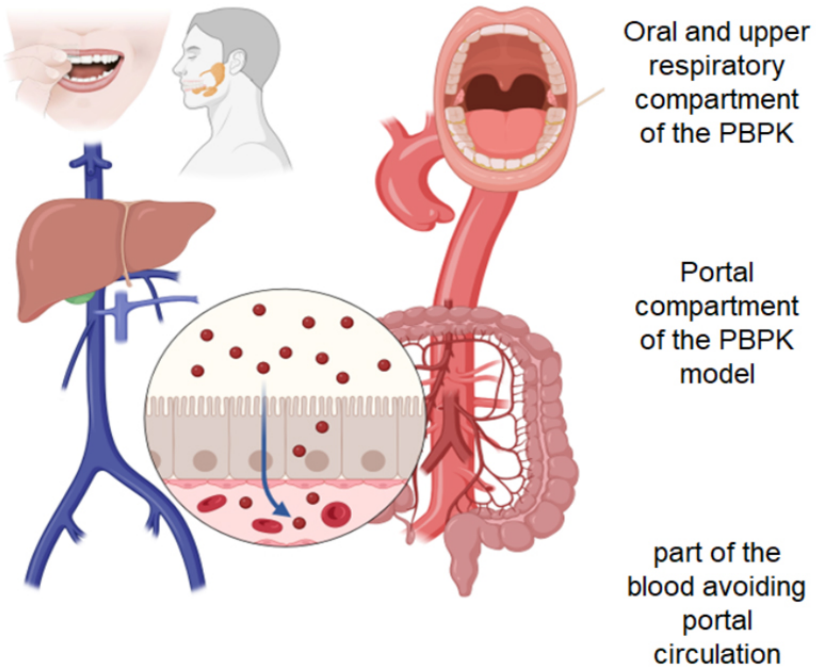

Fig. 1. Illustration of the pharmacokinetics of the nicotine from nicotine pouches. Physiologically based pharmacokinetic (PBPK) model, physiologically based pharmacokinetic model; pharmacokinetic properties of the nicotine pouches vary, descending on multiple factors. Though, toxicokinetic parameters, like Cmax and Tmax, are provided elsewhere in this paper. Nevertheless, the half-life of nicotine is approximately 2–3 hours (based on plasma levels), it and it remains detectable in urine for 11 hours [16]. The picture is made by BioRender (University of Rijeka, Serbia, Croatia, https://www.biorender.com/).

ble 2, Ref. [36]) [15]. This route of nicotine administration, involving interactions between food components, saliva, gastrointestinal juices, and mucosal membranes, shows a relatively low estimated bioavailability of 30–40%.

At the upper end of the stratum granulosum, the epithelium typically has intercellular connections known as "tight junctions", serving as a defense mechanism that prevents the infiltration of harmful components, such as pathogens. However, in the case of buccal epithelium, studies have indicated that this protective barrier does not rely on tight junctions [30,37]. An increase in the presence of 'membrane-coating granules' (MCGs) towards the apical end of the epithelium has been reported to establish a correlation between these granules and absorption across diverse epithelia. These MCGs form neutral lipid (ceramide) sheets within the paracellular region.

The pharmacokinetic model developed by Teeguarden *et al*. [38] simulated kinetic data from various routes of exposure, including oral exposure in rats and humans. However, the model described the intake as a direct infusion into plasma and estimated the absorbed nicotine mass during calibration [22,39]. Nicotine from oral products mixes with saliva, transfers to oral tissue, and induces salivation, suggesting systemic effects even when administered in the form of a pouch [40,41]. In the instance of the ingested saliva-nicotine mixture, mixture undergoes first-pass effect in the liver (due to the enterohepatic circulation).

Portal Compartment of the PBPK Model

PBPK modeling is used to elucidate drug delivery mechanisms without the need for direct computer modeling. The organ compartments in the hepatic portal circulation are summarized as one "portal compartment" to illustrate this process using a simplified model structure (Fig. 1).

When swallowed, nicotine is absorbed in the small intestine but undergoes extensive first-pass metabolism in the liver, resulting in a relatively low (30–40%) bioavailability [34]. In the liver, nicotine is primarily metabolized by the enzymes CYP2A6, UDP-glucuronosyltransferase (UGT), and flavin-containing monooxygenase (FMO). The primary metabolic pathway of nicotine is its conversion by C-oxidation of CYP2A6 into cotinine [29]. Cotinine, with a half-life approximately ten times longer than nicotine (20 h vs. 2–3 h) serves as a more stable marker of exposure over time [42]. Other important metabolites include trans-3′-hydroxycotinine and nornicotine [43,44].

The first-pass effect is a pharmacological phenomenon in which a substance undergoes metabolism at

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Table 2. Values of epithelial thickness within the oral cavity measured by Stasio *et al.* [36] with the aid of optical coherence tomography (OCT).

Anatomic location	Thickness (μm)			
	Mean	Min	Max	SD
Gingiva	28,504	21,830	33,333	±32.98
Labial mucosa	33,983	27,119	40,556	±36.44
Buccal mucosa	54,540	37,375	65,979	±62.45
Ventral surface of the tongue	23,979	15,909	31,800	±37.30
The floor of the mouth	12,409	100.07	14,444	±13.53
Tongue dorsum	47,932	33,333	65,002	±83.56

specific sites in the body before entering systemic circulation. For nicotine, a significant first-pass effect occurs in the liver, lungs, and kidneys [43,45]. The process plays an essential role in the dosage and administration of substances such as nicotine, influencing their peak concentrations. Consequently, drug concentration peaks may occur earlier than with parenteral dosing [29,46]. The importance of the first-pass effect cannot be overstated, prompting understandable concern due to its natural variability among individuals, casting doubt on all pharmacological dosing information.

Regardless of the route of nicotine administration, the liver extensively metabolizes it into at least six primary metabolites [43,44]. Further research is crucial to understand nicotine's impact on the gut microbiota and explore the mechanism of nicotine-induced dysbiosis in the intestinal microbiome. Studies in mice have shown a significant gender-dependent increase in Turicibacteraceae and Peptococcaceae following nicotine treatment by drinking water [47,48]. Nicotine is primarily eliminated through glomerular filtration and tubular secretion, with reabsorption influenced by urinary pH (higher reabsorption at higher pH), ultimately leading to renal excretion [29].

Part of the Gastrointestinal Tract Sidestepping Hepatic First-Pass: Rectal Delivery

The absorption of many drugs, peptides, and low-molecular-weight proteins is increased during rectal administration. This route is used to prevent the acidic and enzymatic degradation of such xenobiotics by avoiding interaction with digestive juices. Additionally, by bypassing the hepatic first-pass effect to some extent through circulation (Fig. 2), this method is advantageous. Nicotine, when ingested, can affect the smooth muscles of the colon, thereby influencing gut motility and altering the rate of movement of digested material through the gastrointestinal system [49]. For years, it has been established that nicotine can influence regular gut-brain communication and alter the activity of the central nervous system (CNS), influencing host behaviors [50]. When administered per rectum, approximately two-thirds of drugs bypass first-pass metabolism due to the systemic venous drainage of the rectum through the middle and inferior rectal veins. At the same time, one-third enters the hepatic portal system via the superior rectal vein (Fig. 2) [51,52]. The physicochemical characteristics of a drug play a crucial role in its absorption through the rectal route. These characteristics include solubility, degree of ionization, partition coefficient, and particle size. Drugs with a low molecular weight (median: 301.34; range: 151.17 to 581.7 g/mol) are typically suitable for rectal administration [53,54]. In this context, nicotine, with a molecular weight as low as 162.2 g/mol, serves as an example [55].

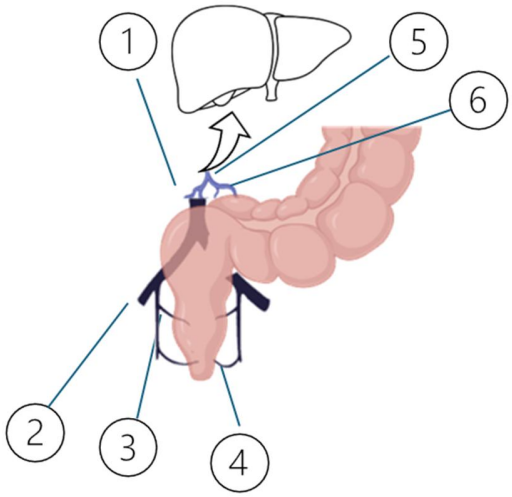

Fig. 2. Venous blood flow in the region involves systemic and portal venous drainage with rectal involvement in both circulations. 1—vena cava inferior (systemic drainage); 2—internal iliac vein; 3—middle rectal vein; 4—inferior rectal vein; 5—inferior mesenteric vein (portal drainage); 6—superior rectal vein [28]. The picture is made by BioRender (University of Rijeka, Serbia, Croatia, https://www.biorender.com/).

Effect of Microbiota

In order to assess the effect of microbiota on the nicotine from oral nicotine pouches, a comprehensive search of existing literature was used. This search aimed to answer a well-defined question-whether microbiota impacts the toxicity of the nicotine released from oral nicotine pouches.

A Systematic Review Strategy and Characteristics of Studies Containing a "Microbiota" AND "Nicotine" in Title/Abstract/Keywords

After registering the study design with the University of York's Centre for Reviews and Dissemination (CRD) and obtaining the registration number 530065, a search strategy was developed. Screening involved deduplicating abstracts and identifying irrelevant studies. In the next stage, full-text retrieval was conducted through online repositories, interlibrary exchanges, automated screening software, and citation management tools. Finally, the relevance of full texts was evaluated concerning the impact of microbiota on nicotine overdose among users of oral nicotine pouches. A Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) protocol, as shown in Figs. 3,4, is used for systematic reviews (**Supplementary Material**). The protocol includes a step-by-step search strategy and outlines what should be included in the search process.

To conduct a systematic literature review following the PRISMA guidelines to explore how microbiota impacts nicotine pouches, we utilized independent academic methods [56]. The identification and evaluation of relevant literature involved searching PubMed, Web of Science—core collection, and the Scopus databases. The search included all studies from database inception to March 30, 2024, including book chapters, research papers, reviews, meta-analyses, case reports, notes, letters, and conference proceedings containing the keywords "microbiota" AND "nicotine".

As shown in Fig. 3, 236 studies were initially identified, included in the review, and assessed for eligibility. Three studies were found ineligible as they were book sections or chapters of an edited book. Subsequently, 78 duplicate studies were identified by automated software. A screening of 155 studies revealed that 23 studies were ineligible due to their exclusive focus on plants, animals, or oral health. Among the remaining 132 studies, full-text was available for 80, with 35 primary publications ultimately included in this paper.

After completing the review from Fig. 3 and excluding the vast majority of articles as being irrelevant, the full text of 35 primary studies and 45 "desk" studies unrecognized earlier were scrutinized for the context of smoking, tobacco, e-cigarettes, and smoking cessation strategies. The results of this analysis were all meticulously screened, and their content was evaluated, as shown in Table 3.

Oral and Upper Respiratory Tract Microbiota

Different terms are used to describe the microorganisms residing in the human oral cavity. Usually, they are called oral microflora, oral microbiota, or the oral microbiome. The mouth, which is another term for the oral cavity, includes several distinct habitats for microbes. These include the teeth along with the gingiva and gingival sulci, tongue, cheek, lip, and palate Fig. 2. Additionally, the oral cavity is contiguous with the tonsils, pharynx, esophagus, Eustachian tube, middle ear, trachea, lungs, nasal passages, and sinuses. Therefore, all microorganisms (comprising 500 to 700 common oral species) present in or on the human oral cavity collectively form the human oral microbiome. Most often, even the contiguous extensions are considered [57–59]. Nutrients, drugs, beverages, saliva, air, and smoke all use the mouth as a gateway to the human body. Microorganisms originating from the oral cavity have been shown to cause several systemic diseases [60–63]. Moreover, contemporary tobacco-free nicotine products have the potential to disrupt the microbial balance in these "perioral" regions. Even smokeless tobacco products carry a variety of bacterial microbiota that differ across products and brands [64].

Dysbiosis can disrupt the local innate immune response, leading to a pathogenetic sequence resulting from interactions among the oral microbiota, respiratory microbiota, and innate immunity [65]. This is similar to the excessive colonization by fermenting microorganisms often seen after disruptions in the intestinal microbiome, which can manifest as symptoms mimicking food allergies or intolerance. Essentially, this presents a clinical scenario of alcohol intoxication, with documented cases in medical literature [66]. When considering nicotine-containing pouches as part of the diverse array of smoking cessation aids, analysis of the salivary microbiota in smokers revealed a notable presence of genera such as Stomatobaculum, Megasphaera, Veillonella, Leptotrichia, Campylobacter, and Treponema compared to former smokers and non-smokers. Neisseria, Lautropia, Haemophilus, and Capnocytophaga were less abundant in the samples [67]. The genera Betaproteobacteria, Gammaproteobacteria, and Flavobacteriia were less abundant in heavy smokers and were directly correlated with the number of years since cessation [68]. It is noted that in the saliva of non-smokers, the nicotine concentrations were significantly higher if they were recently exposed to tobacco smoke [69]. Therefore, a clear distinction between the effects of tobacco and nicotine products is impossible.

Nicotine promotes the formation of S. mutans bacterial biofilm and competes with Streptococcus sanguinis in the oral microbiota [70]. Metagenomic sequencing of the salivary microbiome in non-smokers and smokers revealed differences in the composition of the oral microbiota. A higher abundance of Prevotella and Megasphaera was detected in smokers, whereas the genera Oribacterium, Capnocytophaga, Porphyromonas, and Neisseria were significantly reduced [71]. Normal swallowing does not introduce a theoretical risk of contamination through the carryover of pharyngeal microbiota; thus, standard microbiological culture-based methods indicated a scarce microbiome in healthy respiratory systems [72].

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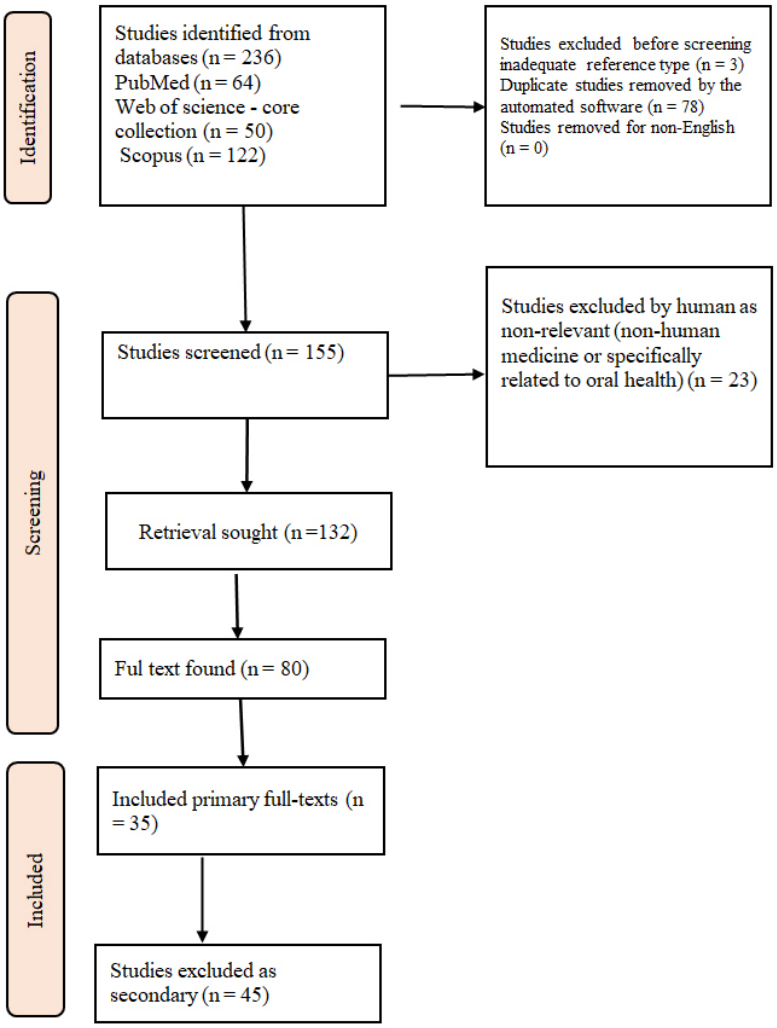

Fig. 3. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol flow diagram for the systematic review of articles containing "microbiota" AND "nicotine" in the title/abstract/keywords. The picture is made by EndNote 20 (Clarivate Analytics, London, England).

Gut Microbiota

Exposure to nicotine can affect the abundance and composition of species comprising the microflora that colonize the oral cavity or the gut. Additionally, it can enhance microbial infiltration through damaged mucosa [47,63].

Oral nicotine pouches deliver varying amounts of nicotine, which is addictive and can negatively impact the commensal gut microbiota [2,5]. Smokers exhibit reduced levels of IgG in serum and saliva, while levels of IgA, which play a role in safeguarding the composition of the gut microbiome, are elevated [73,74]. Within the context of gut-associated lymphoid tissue (GALT), nicotine's potential to disrupt the growth of various microorganisms and potentially transform them into more potent antigenic stimuli raises significant concerns. This mechanism, in combination with the technology of oral pouches, presents a new target for smoking cessation therapy.

Both phyla are rod-shaped bacteria that comprise the most significant part of the gut microbiota and play multiple roles in the human gut [75]. Firmicutes are Gram-positive, while Bacteroidetes are Gram-negative. For instance, Bacteroides xylanisolvens are recognized as effective nicotine degraders [76]. Similarly, nicotine administration leads to changes in gut microbiota reminiscent of a high-fat diet (HFD)-with an increase in the relative abundance of Firmicutes at the expense of typically abundant Deferribacteres, Proteobacteria, and Verrucomicrobia [77,78]. The assessment of normal intestinal homeostasis in humans often involves evaluating the Firmicutes/Bacteroidetes (F/B) ratio [79].

Numerous studies demonstrate the impact of active smoking on the gut microbiota in multiple regions along the digestive tract. From a perspective of nicotine dependence, the presence of Bacteroides xylanisolvens, a bac-

Table 3. Characteristics of 80 studies with available full texts.

Study type			Population		Control	Intervention/outcome	
Primary studies	Original research	12	Human	78	14	Smoking	65
	Case reports	23				Tobacco leaf	10
Secondary studies-not included in the review	Review	36	Non-human	2		E-cigarettes	18
	Other[1]	9				Cessation	37

[1]this includes book chapters, reports, notes, posters, position papers, and patents.

terium found in the human gut, can lead to the efficient degradation of intestinal nicotine. This discovery presents a promising new target for the treatment of patients experiencing nicotine overdoses [80–82]. The accumulation of nicotine in the intestines serves as an indicator of non-alcoholic fatty liver disease (NAFLD) progression and underscores the human intestine's capacity to metabolize nicotine effectively [76,83].

A Systematic Literature Review and Characteristics of Studies Containing "Nicotine" AND "Postmortem" AND "Overdose" AND "Ingestion" in the Title/Abstract/Keywords

At the same time, another systematic review (registered as CRD42024530251 at PROSPERO-International Prospective Register of Systematic Reviews) was carried out. This search followed PRISMA guidelines as well and also included all studies from the database (PubMed, Web of Science, and Scopus) inception to March 30, 2024. Though all book chapters, research papers, reviews, meta-analyses, case reports, notes, letters, and conference proceedings containing the containing "nicotine AND" "postmortem" AND "overdose" AND "ingestion" in the title/abstract/keywords. After initial deduplicating abstracts and identifying irrelevant studies for the exclusion, in the next stage, full-text retrieval was conducted through online repositories, interlibrary exchanges, automated screening software, and citation management tools. Finally, the relevance of full texts was evaluated concerning the impact of microbiota on nicotine overdose among users of oral nicotine pouches.

In this stage, the discrimination of the primary vs. "desk" research was conducted as well, and the whole process, shown as a diagram in Fig. 4.

The search was followed by a critical and meticulous review of the "included" studies, as shown in Table 4. The full text of these 21 studies was searched for contextual relation to the autopsy/postmortem, overdose, lethal outcome, toxicology, ingestion, and oral nicotine pouches. Of these studies, 11 are recognized as secondary studies unrecognized thus far. Eight out of ten primary studies were case reports reporting the fatalities related to nicotine.

Clinical and Postmortem Pathology

Tobacco, a widely used drug, often leads to the misconception that nicotine is merely a harmful chemical associated with chronic diseases rather than a potentially fatal poison causing rapid death. However, nicotine is highly toxic, carrying significant health risks and adverse effects (Fig. 5). Minuscule amounts of nicotine can be fatal to a young child. On the other hand, increased alertness, improved concentration, memory, and reduced anxiety are all the side effects of nicotine with possible value in the treatment of various cognitive problems [84]. Specific health conditions such as Parkinson's disease, Alzheimer's, attention-deficit/hyperactivity disorder (ADHD), and Tourette Syndrome (TS) are treated with nicotine [85,86]. When combined with attractive flavors like fruit or candy in oral pouches, nicotine attracts children and threatens profoundly negative consequences [32,87]. Consequently, toxicologists worldwide are increasingly consulted regarding the toxicity of nicotine-containing products.

Conversely, due to the industry's strategy of replacing tobacco products with smoking cessation aids, nicotine is now delivered at levels similar to those of cigarettes. These alternative products can effectively serve as suitable substitutes for smokers [8,88]. As an illustrative example, a 10 mL bottle of liquid nicotine for e-cigarettes containing 6 mg/mL of nicotine equates to a total of 60 mg of nicotine. This amount is comparable to the nicotine content in two or three packs of cigarettes. Similarly, around 10 "medium-strength" (6 mg) oral nicotine pouches contain an equivalent amount [89–91]. In just the first three-quarters of 2023, the American Association of Poison Control Centers (AAPCC) recorded nearly 6000 cases of nicotine poisoning [92,93]. In addition to this danger, vapes from electronic cigarettes have been found to disrupt lung lipid homeostasis by exerting a detergent effect [94]. Despite these risks, e-cigarettes are currently being promoted as a smoking cessation aid in Europe. However, there is a lack of reliable studies on the safety profile of oral pouches [95]. Consequently, there has been an increase in the number of reports of nicotine intoxication. Acute nicotine intoxication follows a biphasic clinical course. Initially, symptoms stemming from stimulatory effects such as agitation, tachycardia, and vomiting may be observed. These symptoms can then turn into depressive effects, characterized by hy-

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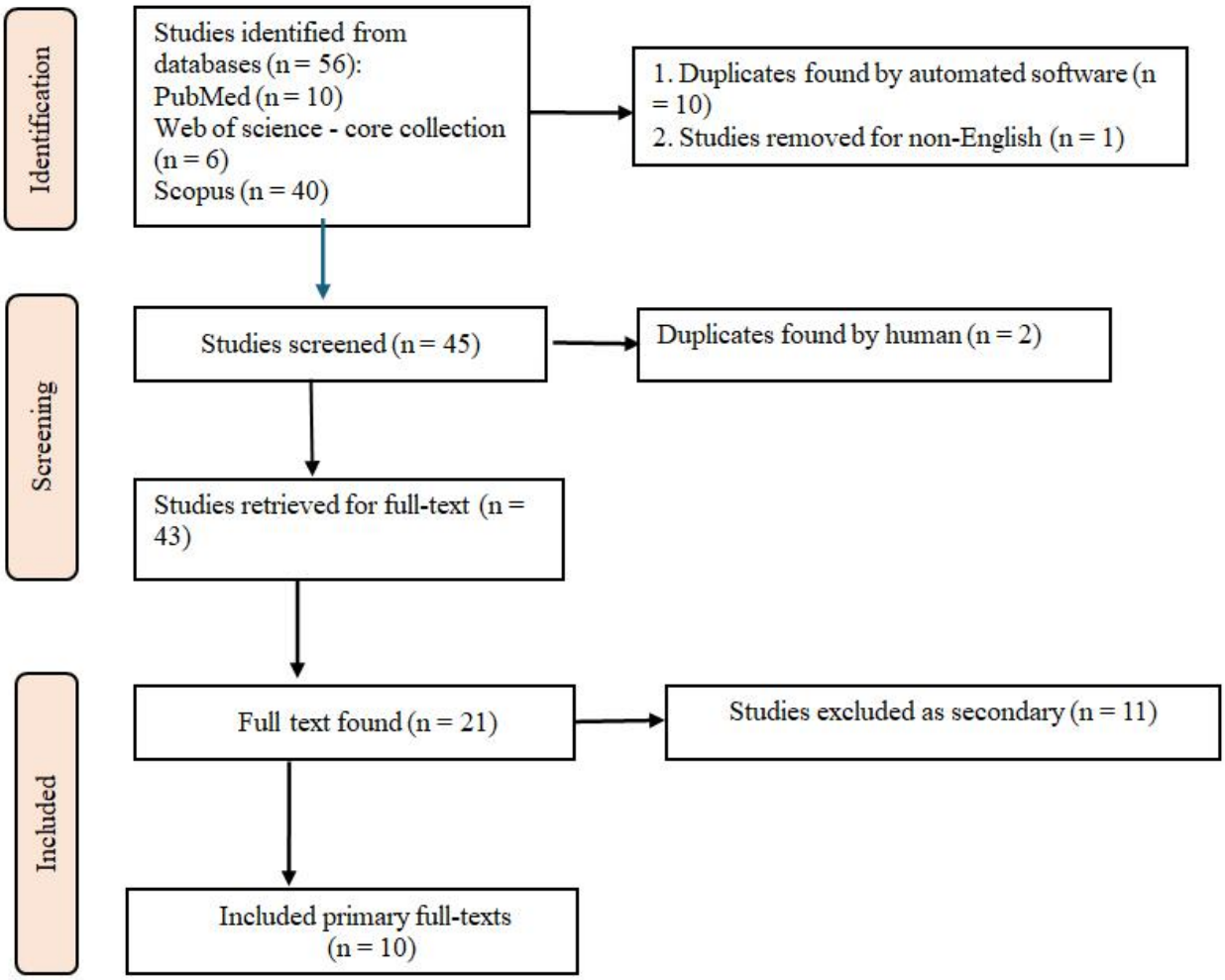

Fig. 4. PRISMA protocol 2020 flow diagram for a systematic review of the literature containing "nicotine" AND "postmortem" AND "overdose" AND "ingestion" in the title/abstract/keywords. The picture is made by EndNote 20 (Clarivate Analytics, London, England).

potension, bradycardia, central nervous system depression, coma, muscular weakness, paralysis, and respiratory difficulties including breathing problems or respiratory failure [96–100] (Fig. 5).

While instances of nicotine poisoning are relatively rare, they are increasing in frequency. There have been only a few reported cases of acute Intoxication involving a known quantity of nicotine, irrespective of its source concentration. Almost three decades ago, Kemp *et al.* [101] first presented data from a case involving attempted suicide with the topical application of multiple transdermal nicotine patches. This incident highlighted the need for further investigation into the pharmacokinetics and potential toxicity associated with the administration through numerous transdermal systems.

In its "pure" pharmaceutical form, nicotine appears as a clear brown liquid with a strong, pungent odor. This liquid is commonly present in the stomach contents observed during autopsies [8]. Additional pathological diagnoses may include: (1) moderate to severe pulmonary and cerebral edema and (2) generalized congestion in the brain, lungs, and abdominal organs [7]. The blood concentrations in those cases were significantly higher than those obtained through other routes, including the proper administration of a single transdermal patch. All 18 patches in Kemp's case [101] were placed over the chest area, leading to the direct absorption of nicotine into the heart. The observed threefold difference between heart and femoral blood levels was potentially attributed to postmortem redistribution. At that time, this particular drug delivery system was highly regarded for its convenience and widespread use for many therapeutic purposes. In relief of pain, the prevention of motion sickness, and, as in this case, assisting in smoking cessation. However, it rarely leads to fatalities. Paralysis of the respiratory muscles and cardiovascular collapse are identified as the mechanisms of death.

In rats and mice, the median lethal dose (LD_{50}) of nicotine is 50 mg/kg and 3 mg/kg, respectively [102]. For

Table 4. Characteristics of 21 studies with full text.

Study type			Population		Control	Intervention/outcome	
Primary studies	Original research	2	Human	21	2	Autopsy/postmortem	4
	Case reports	8				Overdose	8
Secondary studies-not included in the review	Review	10	Non-human	0		Lethal outcome	14
						Toxicology	17
	Other[1]	1				Ingestion	12
						Oral nicotine pouch	0

[1] this includes book chapters, reports, notes, posters, position papers, and patents.

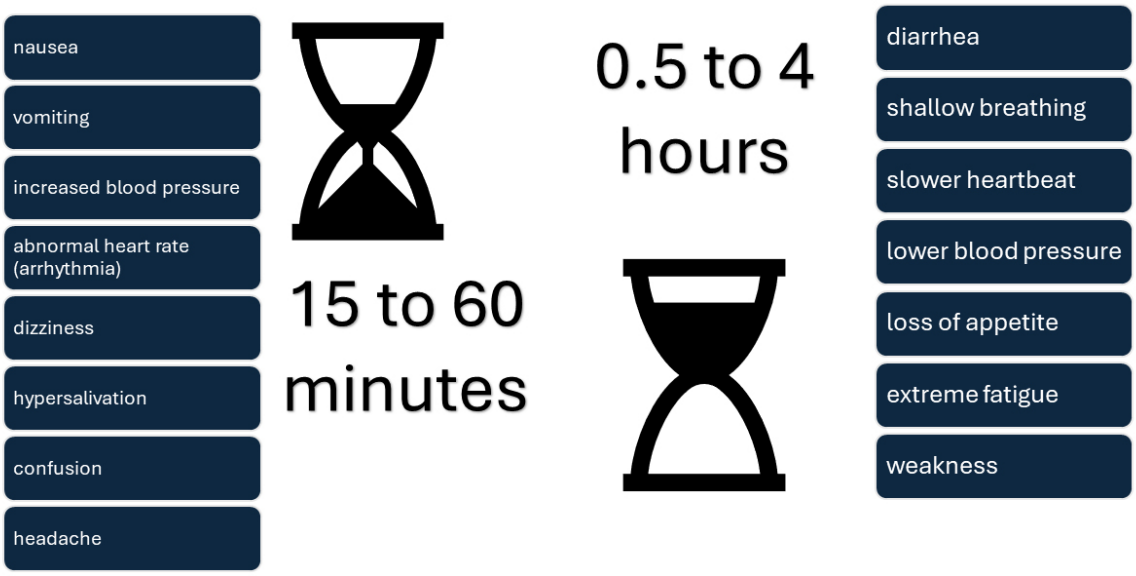

Fig. 5. The symptoms of nicotine toxicity, according to the American Lung Association (ALA). The picture is made by Microsoft®PowerPoint®za Microsoft 365 MSO (64-bit version 16.0.17531.20140, University of Rijeka, Serbia, Croatia).

adult humans, a lethal dosage ranges from 0.5 to 1.0 mg/kg, while for children, it can be as low as 0.1 mg/kg [55]. Notably, ingestion of just 6 mg can be lethal to children [103]. However, the accuracy of this LD_{50} estimate has been questioned due to documented cases of humans surviving much higher doses, leading to a suggested LD_{50} range of 6.5 to 13 mg/kg with oral administration [104]. Conversely, skin contact with a concentrated nicotine solution can result in Intoxication, which can be harmful or fatal [105,106].

It is widely recognized that the oral mucosa can directly absorb xenobiotics, a property utilized in the development of rapid-acting drugs and nicotine pouches [59]. The permeability of the oral mucosa generally decreases from the floor of the mouth to the palate, influenced by variations in thickness and keratinization levels [107]. Non-keratinizing epithelia cover approximately 60% of the oral cavity, while keratinizing epithelia, such as the gingiva, occupy about 25% of its surface. Nicotine's toxicity is characterized by its sympathomimetic nature, stimulating both the autonomic ganglia and central nervous system. In cases of nicotine overdose, a range of symptoms can be observed, including abdominal pain, headache, dizziness, convulsions, coma, and respiratory arrest. Typically, most cases of toxicity result from oral ingestions of nicotine-containing solutions, such as pesticides, or children ingesting used transdermal patches or vaping liquids [8]. Nicotine acts by mimicking acetylcholine's effects through the stimulation of nicotinic cholinergic receptors (nAChRs). Given the abundance of these receptors in the central nervous system (CNS), the diverse effects of nicotine are not surprising [108,109].

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Preventing Overdose

The most effective overall strategy to prevent poisoning (i.e., overdose) is to stop using cigarettes and other nicotine-containing products. Alternatively, less extreme yet highly effective preventive measures include protecting the skin when handling liquids containing nicotine and securely storing nicotine products. It is crucial to always keep these products in their original containers, out of the reach of children or pets [110].

In the case of explicitly modifying the effects of nicotine present in nicotine pouches, the activity of nicotine-degrading enzymes, such as nicotine oxidoreductase (NicA2), is utilized. This enzyme catalyzes the degradation of nicotine into non-psychoactive metabolites. The significance of this flavin-dependent enzyme is increasing, as it is being recognized as a potential injectable therapeutic agent to aid in smoking cessation [111,112].

Discussion

There is substantial evidence indicating that tobacco use can cause alterations in the composition of the gut microbiota. The microbiota-gut-brain axis, lung-gut crosstalk, and skin-gut crosstalk all suggest a potential impact of the microbiota on the health effects of cigarette smoke [113, 114]. Nicotine-degrading microorganisms, primarily bacteria, have been used for biodegradation in tobacco waste [115]. Moreover, bacterial communities in the intestines of sunbirds, specifically Proteobacteria, Firmicutes, and Actinobacteria, are involved in degrading nicotine [115,116]. The cigarette beetle (CB) is another example.

Reducing nicotine levels in the blood and its distribution to the brain might be a key strategy to reduce harm for users of oral nicotine pouches [117]. Incorporating nicotine-degrading bacteria in these pouches offers numerous benefits, especially considering the varying nicotine doses delivered by many oral nicotine products. The potential role of microbiota in nicotine harm reduction has been suggested as a potential tool to address dysbiosis using a range of interventions, from probiotics and prebiotics to more advanced approaches like fecal microbiota transplantation. Notably, while nicotine promotes the proliferation of Firmicutes in the gut microbiota, cigarette smoke has been associated with higher levels of two beneficial probiotics: Bifidobacterium and Lactobacillus [47,82,118,119].

Microbiota significantly impacts the biotransformation of xenobiotics, including nicotine [78,120,121]. Nicotine inhalation can change the gut microbiome by decreasing bacterial diversity and affecting the balance of intestinal microbiota, leading to dysbiosis and systemic disorders [47,50]. Toxicants from cigarette smoke, even when swallowed, can cause dysbiosis through different mechanisms such as antimicrobial activity, impaired mucosal immune responses, and increased permeability of the mucosa [119]. Developing targeted probiotics for smokers may help alleviate the adverse health consequences of nicotine pouches [118].

However, there is a lack of population-level studies providing strong evidence of causality to support the microbiota as a tool to prevent nicotine overdose in users of oral nicotine pouches. Research on bacterial supplementation and microbiota modulation using probiotics remains scarce despite the hope surrounding probiotics explicitly designed for nicotine users [118]. Nicotine has been shown to cause distinct alterations in microbiota composition and can impact chemical signaling in gut-brain interactions, as well as lung-gut/skin-gut crosstalk [50,113,114]. Nevertheless, the exact extent to which the microbiota in the upper airway or gut influences nicotine metabolism remains unclear.

Some of the most apparent limitations of this review pertain to the literature search and study identification protocol. Despite efforts to minimize them, organizational and methodological flaws were not entirely avoided. For instance, this systematic review relied on a limited number of databases (three) to identify potentially eligible studies. The search strategy was detailed; however, an issue known as the risk of selection bias arose due to unclear criteria for selecting studies for inclusion in specific databases.

Data extraction, the possibility of assessing the quality of the study, and qualitative synthesis can present potential limitations. It is hoped that sufficient data from the studies were provided, although a validated checklist was not used. Probably for this reason, operational definitions for specific quality criteria were not explicitly provided, and advanced meta-analysis methods were not applied.

Conclusions

No studies on the safety profile of oral pouches, especially regarding acute Intoxication, were conducted before these products were released. Current knowledge relies on a few case reports and national adverse event reports. This review suggests that probiotics might assist in treating and preventing nicotine overdose. This study also considers nicotine effects in a medicolegal context, potentially neglecting its metabolic functions. Future studies are needed to establish safe pharmacokinetic methods for delivering nicotine.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the author on reasonable request. (ivan.sosa@uniri.hr).

Author Contributions

IŠ made substantial contributions to conception and design of this paper, acquisition of data fot its preparation, and their interpretation. Furthermore, IŠ produced impor-

tant intellectual content, wrote the manuscript; and gave final approval of the version to be published. The author has participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The author declares no conflict of interest. IŠ is a member of the Editorial Board of the Journal of Biological Regulators and Homeostatic Agents. IŠ had no involvement in the peer review of this article and has no access to information regarding its peer review.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243806.368.

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