

7.5.5-1: INITIAL – EXPOSURE - LITERATURE SUMMARY

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List of Abbreviations

3-OHBaP	3-Hydroxybenzo[a]pyrene
AUC	area under the concentration-time curve
B[a]P	benzo[a]pyrene
BOE	biomarkers of exposure
CI	confidence interval
C _{max}	maximum measured concentration
CRE	creatinine
HPHC	harmful or potentially harmful constituent
LS	least squares
MRTPA	Modified Risk Tobacco Product Application
NAT	N'-nitrosoanatabine
NHANES	National Health and Nutrition Examination Survey
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNAL-gluc	NNAL-glucuronide
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N'-nitrosonornicotine
NRT	nicotine replacement therapy
ST	smokeless tobacco
t _{1/2α}	distribution half-life
t _{1/2β}	terminal half-life
TNE	total nicotine equivalents
TSNA	tobacco specific nitrosamine
U.S	United States

7.5.5-1. EXPOSURE LITERATURE SUMMARY

7.5.5-1.1.Introduction and Literature Review Methods

This section of the Modified Risk Tobacco Product Application (MRTPA) addresses exposure to harmful or potentially harmful constituents resulting from smokeless tobacco (ST) use. This section is a review of published literature related to biomarkers of exposure associated with products representative of the general ST category in the United States.

The use of ST products is associated with increased risks for several adverse health outcomes (Zeller, 2015). The assessment of exposure to ST constituents that may play a role in health risks employs three main types of data:

1. chemical characterization of ST products;
2. characterization of how, and how much, ST products are used by consumers;
3. measurements of biomarkers of exposure to ST constituents.

Characterization of the chemical composition of ST products is fundamental for evaluating potential exposure to harmful or potentially harmful constituents from the use of ST products. Researchers have identified many of the chemical constituents in ST products and, based on their toxicological profiles, identified specific constituents that are suspected of playing a role in one or more adverse health effects associated with ST use.

The United States Food and Drug Administration (FDA) established a list of harmful or potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke (Zeller, 2015) including nine constituents of ST products. Table 7.5.5-1-1 presents an abbreviated list of HPHCs in ST.

Table 7.5.5-1-1: Abbreviated List of Harmful and Potentially Harmful Constituents in Smokeless Tobacco

HPHCs in Smokeless Tobacco
Acetaldehyde
Arsenic
Benzo[a]pyrene
Cadmium
Crotonaldehyde
Formaldehyde
Nicotine (total and free)
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
N-nitrosornicotine

A summary of the scientific literature related to the chemistry of ST products is presented in Section 7.5.4-1 and Section 7.5.4-2 of this MRTPA.

Since both the amount of ST product consumers use and how they use the product can influence exposure to harmful or potentially harmful constituents, investigators have researched ST use topography. This information, combined with chemical composition, can be used to estimate constituent intake resulting from ST use. A summary of the scientific literature related to ST use topography is presented in [Section 7.5.8-1](#) and [Section 7.5.8-2](#) of this MRTPA.

Another approach for evaluating exposure to harmful or potentially harmful constituents from the use of ST is to measure biomarkers of exposure. The Institute of Medicine defines a biomarker of exposure as: “a tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose” ([Institute of Medicine, 2001](#)). Studies using biomarkers of exposure associated with ST use have primarily measured nicotine and its metabolites in blood and urine; cotinine in blood, urine, and saliva; and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine. In addition to these commonly used biomarkers of exposure, biomarker studies of other ST chemical constituents have been published. These include biomarkers of polycyclic aromatic hydrocarbons, other tobacco specific nitrosamines (TSNAs), heavy metals, and biomarkers of tobacco combustion compounds, including benzene, acrolein, pyrene, 1,3-butadiene, crotonaldehyde, aromatic amines, acrylamide, and carbon monoxide.

Altria Client Services LLC conducted a comprehensive literature search to identify published information relevant to exposure to harmful or potentially harmful constituents related to the use of ST products. A description of our literature search and review process is presented in [Section 7.5.1](#) of this MRTPA. This review is limited to studies of ST products used in the United States that were published between through December 2014. From this search, a total of 6,742 publications were identified, and, after a comprehensive and in depth critical review, 537 were determined to be in scope. These publications were further reviewed to assess which specific category(ies) in the MRTPA Draft Guidance each article addressed. Reports published shortly after the date of our last search were included in this review when deemed to be significant contributions to this body of research. Sixty-two publications presenting measurements of biomarkers of exposure associated with use of ST products are summarized. An updated literature review was conducted to bridge the original review to 06 February 2017, and the results informing exposure to constituents of ST products are presented in [Section 7.5.5-2](#).

The comparison of biomarker measurements from different studies is limited by the variations in methodology, products, participant characteristics, product use conditions, and the media in which the biomarker is measured. In addition, the units for expressing biomarker data are not consistent across studies. Consequently, for any given biomarker of exposure, data may be reported using several different units of expression, thus complicating comparisons across studies. Finally, as Berman et al. ([2015](#)) have recently noted: “Although methods now exist to assess exposure reduction through biomarkers, there are no valid biomarkers that serve as proxies for tobacco-related disease.” A summary of the methods, participant characteristics, and findings from the studies included in this summary is

presented in [Table 7.5.5-1-7](#). Where possible, results specific to Copenhagen® Snuff Fine Cut are included in [Table 7.5.5-1-7](#).

7.5.5-1.2. Studies on Specific ST Constituents

7.5.5-1.2.1. Nicotine in Blood

Benowitz et al. (1988) evaluated exposures in 10 men after a single use of cigarettes, oral snuff, chewing tobacco, or nicotine gum. Subjects exposed to oral snuff held a 2.5-g dip in their mouth for 30 minutes, at which point it was removed. Blood samples were taken for nicotine analysis. The maximum increase in blood nicotine concentration was similar in smokers and in subjects using ST, averaging 14.3 ng/mL. The investigators noted that the use of ST resulted in a prolonged plasma nicotine concentration elimination curve as compared with that for the use of cigarettes. In this study, the investigators estimated absorbed doses of nicotine from the pharmacokinetic data to be 1.8 mg from cigarettes, 3.6 mg from snuff, 4.5 mg from chewing tobacco, and 1.9 mg from nicotine gum.

In a later study, Benowitz et al. (1989) employed a crossover sequential study design in four 3- or 4-day blocks in a clinical setting. Eight male subjects used their usual brand of cigarettes, snuff, or chewing tobacco as desired, or they abstained from tobacco use. All subjects were smokers who had also used ST. On the last day of the study block, blood samples were taken for nicotine and cotinine analysis. After use of snuff, maximum blood nicotine levels were 32.4 ng/mL, with a range of 11.4 to 61.6 ng/mL. The area under the blood nicotine concentration-time curve was 402.3 ng/mL/h, with a range of 157.3 to 757.5 ng/mL/h (Benowitz et al., 1989).¹

Findings from these studies are consistent with several other studies that report average blood nicotine levels associated with ST use to be approximately 25 ng/mL, ranging from approximately 11 to 60 ng/mL (Campbell, Brown, Jones, Marano, & Borgerding, 2015; J. O. Ebbert, Severson, Danaher, Schroeder, & Glover, 2012; Escher, Tucker, Lundin, & Grabiner, 1998; Fant, Henningfield, Nelson, & Pickworth, 1999; Gray, Breland, Weaver, & Eissenberg, 2008; Gritz, Baer-Weiss, Benowitz, Van, & Jarvik, 1981; Thomas et al., 2006).

7.5.5-1.2.2. Nicotine in Urine

A significant challenge of making comparisons of published values of nicotine in urine is that investigators use different methods of expressing data for this biomarker. Of the five study populations identified in our search in which nicotine in urine was reported in ST consumers, investigators presented values using four different units of measurement: ng/mL, ng/mg creatinine, µmol/24 h, and µg/24 h. Two reports presented nicotine in urine as µg/24 h. The values presented are 3,219.6 µg/24 h (Prasad, Jones, Chen, & Gregg, 2016) and 1,562 µg/24 h (Jacob, Yu, Liang, Shulgin, & Benowitz, 1993).

¹ In the original manuscript by Benowitz, blood nicotine levels were reported as µmol/L. For the sake of presentation in more commonly used units of expression, we converted the original units of µmol/L to ng/mL.

7.5.5-1.2.3. Total Nicotine Equivalents in Urine

Total nicotine equivalents (TNE) is a measure of nicotine exposure that includes measurement of nicotine in urine plus five of its major metabolites: nicotine N-glucuronide, cotinine, cotinine-N-glucuronide, trans-3'-hydroxycotinine, and trans-3'-hydroxycotinine glucuronide (Sarkar et al., 2010). Of the five study populations identified in our search in which TNE in urine were reported in ST consumers, three presented TNE as pmol/mg creatinine (Benowitz et al., 2012; Borgida et al., 2015; Lemmonds et al., 2005). The respective TNE values reported by these investigators are 72.8, 72.8, and 10.5 pmol/mg creatinine. Two of the studies presented TNE as mg/24 h (Prasad et al., 2016; Sarkar et al., 2010). The respective TNE values reported by these investigators are 29.4 mg/24 h and 5.53 mg/24 h.

7.5.5-1.2.4. Cotinine in Blood

Biomarker data from 23,684 adult participants in the 1999 to 2012 National Health and Nutrition Examination Survey (NHANES) were used to assess exposures associated with smoking cigarettes as compared with the use of ST (Rostron, Chang, van Bommel, Xia, & Blount, 2015). Exclusive ST users in the study population were 94.7 percent male, with an average age of 44.2 years. For exclusive ST users (n=476), the geometric mean for blood cotinine was 179.6 ng/mL, with a 95 percent confidence interval of 145.8 ng/mL to 221.1 ng/mL. Given the large sample size, it is reasonable to assume these values are representative of the general population of ST consumers. However, reported levels of cotinine in blood of ST consumers range from 59.2 (Carmella et al., 1990) to 517 ng/mL (J. O. Ebbert, Patten, & Schroeder, 2006), values well outside the 95 percent confidence intervals reported by Rostron.

7.5.5-1.2.5. Cotinine in Urine

Of the 21 study populations of ST consumers identified in our search, 9 reported cotinine in urine as ng/mL, 8 reported cotinine in urine as nmol/mg creatinine, 2 reported cotinine in urine as µg/24 h, 1 reported cotinine in urine as nmol/mL, and 1 reported cotinine in urine as ng/mg creatinine. Average urinary cotinine levels from the 9 study populations reporting levels as ng/mL urine ranged from 1,269 ng/mL (Jacob et al., 1993) to 10,602 ng/mL (Hatsukami et al., 2008), with an overall average of 5,139 ng/mL.

7.5.5-1.2.6. Cotinine in Saliva

Cotinine in saliva is a commonly reported biomarker of ST exposure. Ferketich et al. (Ferketich, Wee, Shultz, & Wewers, 2007b) conducted a study with the objective of developing baseline salivary cotinine concentrations in a group of 199 snuff only consumers enrolled in a ST-cessation study. The study participants were all men with an average age of 34 years. Baseline saliva samples were collected after an initial enrollment questionnaire. Table 7.5.5-1-2 presents salivary cotinine levels.

Table 7.5.5-1-2: Salivary Cotinine Levels in ST Consumers^a

Product	Salivary Cotinine (ng/mL) Mean (SD)	Range
Copenhagen®	644 (381)	78–2469
Skoal	464 (339)	21–1205
Timberwolf	435 (241)	76–937
Red Seal	675 (273)	262–1,082
Other	570 (377)	154–1,484

^a Adapted from Ferketich et al. (2007b).

The salivary cotinine levels reported by Ferketich et al. (Ferketich et al., 2007b) appear to be representative of ST consumers in general because they are comparable to levels reported to be associated with the use of ST by several other investigators (Bauman et al., 1989; Hatsukami, Anton, Keenan, & Callies, 1992; Hatsukami, Jensen, Allen, Grillo, & Bliss, 1996; Mushtaq, Beebe, Vesely, & Neas, 2014).

7.5.5-1.2.7. Tobacco Specific Nitrosamines in Urine

The most commonly evaluated biomarker of TSNA exposure associated with ST use is urinary excretion of NNAL, a metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Of the 26 study populations identified in our search, the following results were noted:

- 13 reported NNAL in urine as pmol/mg creatinine.
- 6 reported NNAL in urine as pmol/mL.
- 2 reported NNAL in urine as pg/mL.
- 2 reported NNAL in urine as pg/mg creatinine.
- 2 reported NNAL in urine as ng/24 h.
- 1 reported NNAL in urine as µg/g creatinine.

Of the 13 study populations that reported NNAL excretion as pmol/mg creatinine, the range of average values was 0.98 pmol/mg creatinine (Mendoza-Baumgart et al., 2007) to 4.14 pmol/mg creatinine (Benowitz et al., 2012). These values appear to be representative of typical levels as reflected by a study by Lemmonds et al (2005), in which ST topography and toxin exposure was studied in 54 male ST consumers. In this study, the mean (SD) NNAL level was reported to be 3.3 (2.1) pmol/mg creatinine.

Our literature search identified five study populations that reported N'-nitrosonornicotine (NNN) levels in the urine of ST consumers. Three reported urinary NNN levels as pmol/mg creatinine, two reported it as ng/24 h. Average urinary levels in the three studies expressing NNN as pmol/mg creatinine were as follows: 0.00070 (Benowitz et al., 2012), 0.284 (Borgida et al., 2015), and 0.25 (Stepanov & Hecht, 2005). Average urinary levels in the two

studies expressing NNN as ng/24 h were : 48.9 (Prasad et al., 2016) and 4.52 (snus) (Sarkar et al., 2010). Palladino (Palladino, Adams, Brunnemann, Haley, & Hoffmann, 1986) reported average NNN and N'-nitrosoanatabine levels in saliva of college students who were snuff consumers to be 99.2 and 204.0 ng/g, respectively.

7.5.5-1.2.8. Other Biomarkers of Exposure

In addition to the most commonly used biomarkers of nicotine, cotinine, and TSNAs, investigators have measured biomarkers of exposure of other ST constituents. Naufal et al. (Naufal, Marano, Kathman, & Wilson, 2011) used data from the NHANES Mobile Examination Centers from 1999 to 2008 to evaluate exposure to snuff and chewing tobacco constituents among 368 ST consumers. A total of 33 analytes were measured. Findings for the analytes for constituents on the FDA HPHC list for ST products are presented in Table 7.5.5-1-3.

Table 7.5.5-1-3: Biomarkers of Exposure Reported to Harmful and Potentially Harmful Constituents in Smokeless Tobacco by Naufal, 2011^a

Biomarker	Unadjusted Geometric Mean (95% CI)	Units
Cadmium (blood)	0.28 (0.25-0.30)	µg/L
Cadmium (urine)	0.16 (0.13-0.20)	µg/g creatinine
1-hydroxypyrene (urine)	67.4 (55.7-81.5)	ng/g creatinine
Arsenic (urine)	6.17 (5.05-7.46)	µg/g creatinine
NNAL (urine)	0.99 (0.72-1.38)	µg/g creatinine
Cotinine (blood)	188.7 (152.9-235.1)	ng/mL

CI = confidence interval; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

^aAdapted from Naufal et al. (2011).

Campbell et al. (Campbell et al., 2015) conducted a cross-sectional study of biomarkers of exposure and biological effects among consumers of cigarettes and ST products. The participants were aged 26 to 49 years and included 48 moist snuff consumers. Over a dozen biomarkers of exposure were measured. Findings for the analytes for constituents on the FDA HPHC list for ST products are included in Table 7.5.5-1-4.

Table 7.5.5-1-4: Biomarkers of Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Reported by Campbell, 2015^a

Biomarker	LS Means (SE)	Units
NNAL (urine)	1594 (85.6)	pg/mg creatinine
1-hydroxypyrene (urine)	0.08 (0.02)	ng/mg creatinine

Biomarker	LS Means (SE)	Units
Crotonaldehyde (urine)	598 (309)	ng/mg creatinine
Nicotine (blood)	28.7 (1.15)	ng/mL
Cotinine (blood)	466 (46.9)	ng/mL

LS = least squares; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

^a Adapted from [Campbell et al. \(2015\)](#).

[Prasad et al. \(2016\)](#) conducted a cross-sectional study of biomarkers of exposure and effect in smokers and moist snuff consumers. ST consumers included 41 moist snuff consumers aged 35 to 60 years. In this study, 40 biomarkers of exposure were measured. Findings for the analytes for constituents on the FDA HPHC list for ST products are provided in Table 7.5.5-1-5.

Table 7.5.5-1-5: Biomarkers of Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Reported by Prasad, 2016^a

Biomarker	Group Mean (SD)	Units
NNAL (urine)	2,310.8 (2415.0)	ng/24 h
1-hydroxypyrene (urine)	181.4 (238.0)	ng/24 h
Cadmium (urine)	0.2 (0.1)	µg/g creatinine
Nicotine (blood)	2.7 (1.9)	ng/mL
TNE (urine)	29.4 (20.9)	mg/24 h
Cotinine (blood)	309.7 (166.2)	ng/mL

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TNE = total nicotine equivalents.

^a Adapted from [Prasad et al. \(2016\)](#).

Cadmium and arsenic levels in the urine of ST consumers were reported to be 0.25 µg/g creatinine and 6.14 µg/mg creatinine, respectively ([Marano, Naufal, Kathman, Bodnar, Borgerding, Garner, et al., 2012](#); [Marano, Naufal, Kathman, Bodnar, Borgerding, & Wilson, 2012](#)).

The percentage of nicotine, TSNAs, and benzo[a]pyrene extracted from snus pouch products by consumers after ad libitum use was measured in a 7-day ambulatory study ([Caraway & Chen, 2013](#)). Each day after using the product, consumers collected used pouches in a jar and froze them until the pouches were returned to the investigators for analysis. Based on the difference in constituent content before compared with after use, the investigators concluded that 60% to 90 % of nicotine, TSNAs, and benzo[a]pyrene remained in the pouches after use, suggesting that 10% to 40% of these constituents were removed during use of the product.

In an earlier study, investigators estimated that 59 percent of NNK was extracted from experimental moist snuff tobacco products under laboratory conditions ([Hecht, Carmella, Edmonds, et al., 2008](#)). In this study, subjects were instructed to use an experimental product

after the end of a 3-week abstinence period. The experimental product was fabricated from two grams of loose moist snuff tobacco that was “put into a tea bag–like sealed pouch.” It is likely that the way the study participants used the product was influenced by the experimental nature of the product and the 3-week abstinence period before use. Findings from this latter study, therefore, are unlikely to be generalizable to consumers using ST under natural conditions.

7.5.5-1.3.General Exposure Studies

ST is used orally and not combusted. Therefore, the use of ST involves no inhalation exposure and minimal oral exposure to constituents arising from tobacco smoke combustion. Nonetheless, studies have reported that exposure to nicotine or TSNA in ST consumers can be comparable, or even higher, than in smokers (Carmella, Han, Fristad, Yang, & Hecht, 2003; Rostron et al., 2015; Stepanov & Hecht, 2005). In contrast, biomarker studies of ST consumers indicate that exposures to most of the constituents associated with tobacco combustion are lower than in smokers and are similar to those of non–tobacco users (Campbell et al., 2015; Naufal et al., 2011; Prasad et al., 2016).

Consumer characteristics, such as use patterns, can influence exposure to ST constituents. Hatsukami et al. (1992) reported that total dip duration plus time from onset to offset of ST use during the day may be the best predictors of nicotine exposure. Likewise, Lemmonds et al. (2005) reported that the duration that ST is kept in the mouth is positively associated with exposure. Swallowing tobacco juices has been associated with greater nicotine uptake (J. O. Ebbert, Dale, Nirelli, et al., 2004; Mushtaq, Beebe, & Vesely, 2012). Increasing years of ST use has also been associated with higher exposures to cotinine and TSNA (Hecht, Carmella, Stepanov, et al., 2008; Palladino et al., 1986). Neither the half-life of nicotine (Kyerematen, Dvorchik, & Vesell, 1983) nor NNAL (Hecht et al., 2002) appears to be different between ST consumers and non–tobacco users.

In addition to consumer characteristics, exposures can be influenced by product characteristics. Hatsukami et al. (2004) reported urinary NNAL levels were significantly lower in ST consumers of “brands of ST marketed in the United States” after switching to snus. Likewise, in a study of switching ST consumers from products with higher to lower nicotine contents, Hatsukami et al. (2007) reported that switching led to a significant reduction in cotinine and NNAL exposures.

The effect of flavors on exposures from ST use has been investigated. Oliver et al. (2013) examined data from five previously completed treatment or switching studies to evaluate whether the use of flavored ST products compared with non-flavored ST products is associated with differences in behavioral characteristics and biomarkers of exposure. The investigators reported that the use of flavored ST products was not associated with greater dependence or exposures to nicotine or carcinogens.

7.5.5-1.4.Literature Review Summary

A summary of results from studies in ST consumers of some of the most commonly used biomarkers of exposure to HPHC in ST is presented in Table 7.5.5-1-6. A more extensive review of all 62 publications related to ST exposure is presented [Table 7.5.5-1-7](#).

Table 7.5.5-1-6: Results from Commonly Used Biomarkers of Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers

Biomarker	Units	Level Reported in ST Consumers	Reference
Nicotine in blood ^a	Mean (SD), ng/mL	32.4 (16.2)	(Benowitz et al., 1989)
Nicotine in urine	Mean (SD), µg/24 h	3,219.6 (2,916.4)	(Prasad et al., 2016)
Total nicotine equivalents in urine	Mean (SD), mg/24 h	5.53 (5.21)	(Sarkar et al., 2010)
Cotinine in blood	Mean (95% CI), ng/mL	179.6 (145.8–221.1)	(Rostron et al., 2015)
Cotinine in urine	Mean (95% CI), ng/mL	4,157 (3,510–4,922)	(Oliver et al., 2013)
Cotinine in saliva	Median (range), ng/mL	460 (17–2469)	(Ferketich et al., 2007b)
NNAL in urine	Mean (SD), pmol/mg creatinine	3.3 (2.1)	(Lemmonds et al., 2005)
NNN in urine	Mean (SD), pmol/mg creatinine	0.284 (3.374)	(Borgida et al., 2015)
1-hydroxypyrene in urine	LS mean (SE), ng/mg creatinine	0.08 (0.02)	(Campbell et al., 2015)

CI = confidence interval; LS = least squares; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN = N'-nitrosonornicotine.

^a In the original manuscript by Benowitz, blood nicotine levels were reported as µmol/L. For the sake of presentation into more commonly used units of expression, we converted the original units of µmol/L to ng/mL.

Based on the published research, the following can be concluded about constituent exposures associated with ST use:

- Biomarkers can be useful for characterizing exposure to select constituents associated with the use of ST products.
- The most commonly used biomarkers for evaluating exposures associated with ST use are measurements of nicotine and its metabolites in blood and urine,; measurements of cotinine in blood, urine, and saliva,; and measurements of NNAL in urine.
- Exposure to most cigarette smoke constituents associated with combustion is lower in ST consumers than in cigarette smokers, and is generally similar to non-tobacco users.

- The use of ST can result in exposures to nicotine and TSNA's at levels comparable to, or greater than, cigarette smokers.
- Constituent exposures in ST consumers can be significantly influenced by the way the consumer uses the product and by product characteristics.
- The use of flavors in ST products appears to have no effect on dependence or exposure to nicotine or carcinogens.
- Consumers remove approximately 10 to 40 percent of select constituents as a result of *ad libitum* use of ST.

Table 7.5.5-1-7: Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Prasad et al., 2016)	A cross-sectional study of biomarkers of exposure and effect in smokers and moist snuff consumers	Cross-sectional clinical study; 120 healthy male subjects (ST users = 41); aged 35–60 y.	Blood: Nicotine, ng/mL, mean (SD): 2.7 (1.9) Urine: TNE, mg/24 h, mean (SD): 29.4 (20.9) Urine: Nicotine µg/24 h, mean (SD): 3,219.6 (2,916.4)	Blood: Cotinine, ng/mL, mean (SD): 309.7 (166.2) Urine: Cotinine µg/24 h, mean (SD): 3,916.0 (2,678.4)	Not included in article	Urine: Total NNAL ng/24 h, mean (SD): 2,310.8 (2,415.0) Total NNN, ng/24 h, mean (SD): 48.9 (34.7)	Urinary biomarkers: 1-hydroxypyrene ng/24 h, mean (SD): 181.4 (238.0) Cadmium, µg/g creatinine, mean (SD): 0.2 (0.1) Others measures presented in report are not presented here.	Strengths: Moderate study size and list of measured biomarkers. Limitations: No statement of products used, product use topography nor amount used. Also inclusion criteria of ≥2 cans/week for at least 3 years may limit extrapolation of results.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Campbell et al., 2015)	Study of cardiovascular disease biomarkers among tobacco consumers, part 1: biomarkers of exposure	Exposure study; male participants; 48 moist snuff consumers; aged 26–49 y.	Blood: Nicotine, ng/mL, LS means (SE): 28.7 (1.15)	Blood: Cotinine, ng/mL, LS means (SE): 466 (46.9)	Not included in article	Urine: Total NNAL, pg/mg creatinine, LS mean (SE): 1594 (85.6)	Urinary biomarkers: 1-hydroxypyrene, ng/mg creatinine, LS mean (SE): 0.08 (0.02) Crotonaldehyde, ng/mg creatinine, LS mean (SE): 598 (309) Others measures presented in report are not presented here. ²	Limitation: Data presented as LS means may not be comparable to data expressed as arithmetic means.
(Borgida et al., 2015)	Evidence supporting product standards for carcinogens in smokeless tobacco products	Study of the relationships between ST product characteristics, use patterns and constituent exposures; 359 subjects, 96.6% male; ages mean (SD): 37.1 (12.6) y.	Urine: TNE, nmol/mg creatinine, mean (SD): 72.8 (70.9)	Not included in article	Not included in article	Urine: Total NNAL, pmol/mg creatinine, mean (SD) = 3.29 (3.77) Total NNN, pmol/mg creatinine, mean (SD) = 0.284 (3.374)	Not included in article	Limitations: "...the sample was comprised predominantly of white males; thus, the extent to which these findings can be generalized to other populations is unknown."

² Some manuscripts reported results for dozens of biomarkers of exposure. For the sake of brevity, we presented only those biomarkers for constituents on the FDA HPHC list for ST products.

Table 7.5.5-1-7.Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Marano et al., 2015)	Study of cardiovascular disease biomarkers among tobacco consumers. Part 3: evaluation and comparison with the US National Health and Nutrition Examination Survey	Study of cardiovascular disease biomarkers among tobacco consumers. 368 ST consumers; Ages (y): <ul style="list-style-type: none"> • 20-29 = 15.8% • 30-39 = 28.2% • 40-49 = 23.8% • 50-59 = 15.75% • 60-69 = 7.4% • ≥70 = 9.1% 	Not included in article	Blood: Cotinine, ng/mL, median (25th-75th percentiles): 292 (146-465)	Not included in article	Not included in article	Report presents markers of physiological effect not presented here.	Strength: Nationally representative data sample.
(Rostron et al., 2015)	Nicotine and toxicant exposure among U.S. smokeless tobacco users: results from 1999 to 2012 National Health and Nutrition Examination Survey data	Biomarker data from the 1999 to 2012 NHANES; 488 exclusive ST users; mean age (95% CI): 44.2 (42.7–45.7) y.	Not included in article	Blood: Cotinine, ng/mL, mean (95% CI): 179.6 (145.8–221.1)	Not included in article	Urine: NNAL, pg/mg creatinine, mean (95% CI): 583.0 (445.2–763.5)	Blood: Cadmium, µg/L, mean (95% CI): 0.220 (0.201-0.240) Urine: Arsenic, ng/mg creatinine, mean (95% CI): 6.43 (5.36–7.71) Others measures presented in report are not presented here.	Strength: Nationally representative survey. Limitations: No statement of products used or product use topography. Self-reported data.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Agaku & King, 2014)	Validation of self-reported smokeless tobacco use by measurement of serum cotinine concentration among US adults	Data from surveys spanning 2003 – 2010 from the NHANES; U.S.; adults aged ≥18 y; 418 exclusive ST users.	Not included in article	Blood: Cotinine, ng/mL, median (range): 293 (0.1–1,438) among self-reported exclusive SLT users.	Not included in article	Not included in article	Not included in article	Limitations: Sample included only U.S. adults and over indexed with non-Hispanic white males, some subpopulations had few ST users, the definition for SLT did not exclude the use of NRT, e-cigarettes, snus, or other non-smoked nicotine containing products.
(Mushtaq et al., 2014)	A multiple motive/multi-dimensional approach to measure smokeless tobacco dependence	Study of ST dependence methods; 100 male ST consumers; mean (SD) age = 31.85 (12.08) y.	Not included in article	Not included in article	Salivary cotinine, ng/mL, mean (SD): 480.30 (415.30)	Not included in article	Not included in article	Limitations: Not an exposure study per se, salivary cotinine measured to confirm tobacco use. Data appear the same data reported in Mushtaq, 2012.
(Agaku, Ayo-Yusuf, Vardavas, Alpert, & Connolly, 2013)	Proposed cutoff for identifying adult smokeless tobacco users with urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol: an aggregated analysis of NHANES 2007-2010 data	Data were aggregated from the 2007–2008 and 2009–2010 waves of the NHANES to determine an optimal threshold for identifying ST users with NNAL; 264 exclusive ST users; ages 18-24 = 27; 25-44 = 104; 45-64 = 86; ≥56 = 47.	Not included in article	Not included in article	Not included in article	Urine: NNAL, pg/mL, median (range): 880.0 (2.0-33,000)	Not included in article	Limitations: No statement of products used or influence of product use topography. Did not include snus users in calculation.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Caraway & Chen, 2013)	Assessment of mouth-level exposure to tobacco constituents in U.S. snus consumers	Mouth level exposure study (proportion of constituent removed from pouch product after <i>ad libitum</i> use); 53 subjects (46 males/7 females); 25 (47.2%) of the subjects used the Frost variety, 16 (30.2%) used Spice, and 12 (22.6%) used Original; Mean (SD) age of the subjects was 31.9 (7.6), and the ages ranged from 21-47 y.	Not included in article	Not included in article	Not included in article	Not included in article	Percent of constituent removed from pouch, mean (SD): Nicotine = 39.2 (23.0); NNN = 23.1 (22.1); NNK = 29.9 (20.6); Total TSNAs = 21.6 (21.4); B[a]P = 29.0 (13.8)	Strength: Moderate sample size. Limitations: Mouth level exposure to trace metals and B[a]P could not be estimated for some participants because these subjects collected an inadequate amount of used pouches to complete all constituent determinations.
(Oliver et al., 2013)	Flavored and non-flavored smokeless tobacco products: rate, pattern of use, and effects	Comparison of BOEs associated with consumers of flavored ST products; N = 275; mean (SD) age: 32.5 (7.8) y vs. consumers of nonflavored ST products, N = 193, mean (SD) age: 37.3 (7.7) y. Exposure data for mix of Copenhagen® and Kodiak Wintergreen brands. Sample sizes for Study 1 was 66, Study 2 was 106, Study 3 was 102, Study 4 was 199, and Study 5 was 41.	Not included in article	Urine: Cotinine, ng/mL, mean (95% CI): Nonflavored = 4,157 (3,510–4,922); Flavored = 3,838 (3,427–4,297)	Not included in article	Urine: NNAL, pmol/mL, mean (95% CI): Nonflavored = 3.90 (3.36–4.52); Flavored = 3.20 (2.82–3.63)	Not included in article	Limitations: Convenience sample, different inclusion/exclusion criteria between the studies.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Benowitz et al., 2012)	Exposure to nicotine and carcinogens among Southwestern Alaskan Native cigarette smokers and smokeless tobacco users	Exposure study, participants included 76 ST users; 35 men and 41 women; mean (SD) age: 39.2 (12.8) y.	Urine: Nicotine equivalents, pmol/mg creatinine, mean (95% CI): 72.8 (59.6-86.0)	Blood: Cotinine ng/mL, mean (95% CI): 221.0 (190.7-251.2)	Not included in article	Urine: NNAL, pmol/mg creatinine, mean (95% CI): 4.14 (3.40-5.04) Urine: NNN, pmol/mg creatinine, mean (95% CI): 0.00070 (0.00053-0.00092)	Urinary biomarkers: 1-hydroxypyrene, pmol/mg creatinine, mean (95% CI): 1.05 (0.75-1.46) Others measures presented in report are not presented here	Limitations: Although samples of smokeless products were collected they were not reported in the publication. Lack of ethnic diversity may preclude extrapolation of results.
(J. O. Ebbert et al., 2012)	A comparison of three smokeless tobacco dependence measures	Comparison of 3 measures of ST dependence; 225 all male study participants, chewing tobacco or snuff consumers (113 were assigned to bupropion SR and 112 were assigned to placebo); mean age (range): 38.1 (19-72) y.	Baseline blood: Nicotine, ng/mL, mean (SD): 31.4 (14.2)	Baseline blood: Cotinine, ng/mL, mean (SD): 431 (224)	Not included in article	Not included in article	Not included in article	Limitations: Cessation study, subjects; results may not be generalizable to entire ST users population. Units not provided for serum nicotine and cotinine levels, they appear to be ng/mL.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Marano, Naufal, Kathman, Bodnar, Borgerding, & Wilson, 2012)	Arsenic exposure and tobacco consumption: Biomarkers and risk assessment	Exposure study; 90 ST consumers; all participants aged ≥20 y.	Not included in article	Not included in article	Not included in article	Not included in article	Urine: Arsenic, geometric mean (95% CI) among ST (mix Skoal, Skoal Bandit, or Copenhagen®) consumers: as µg/L = 7.55 (6.12, 9.31); as µg/mg creatinine = 6.14 (4.86, 7.74)	Limitations: Specific products used not well described. Strength: Data obtained from NHANES 2003–2008.
(Marano, Naufal, Kathman, Bodnar, Borgerding, Garner, et al., 2012)	Cadmium exposure and tobacco consumption: Biomarkers and risk assessment	Exposure study; 90 ST (Skoal, Skoal Bandit, or Copenhagen®) consumers; all participants aged ≥20 y.	Not included in article	Not included in article	Not included in article	Not included in article	Urine: Cadmium, µg/g creatinine, (n = 87), geometric mean = 0.25 Blood: Cadmium (ng/mL) (n = 272), geometric mean = 0.39	Limitations: Specific products used not well described. Variance estimate not provided with mean estimate. Strength: Data collected in NHANES Mobile Examination Centers from 1999 to 2006.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Mushtaq et al., 2012)	Determinants of salivary cotinine concentrations among smokeless tobacco users	Exposure study; 100 ST consumers (41% of subjects were using Copenhagen®); mean (SD) age: 31.9 (12.2) y.	Not included in article	Not included in article	Salivary Cotinine, ng/mL, median (range): 350.5 (15.5-1,772.1) “Fine-cut ST products led to higher salivary cotinine concentrations as compared with long cut ($p = .0286$) when adjusted for age, years of ST use, number of cans used per week, and swallowing of tobacco juices.”	Not included in article	Not included in article	Limitation: Although product specific data was collected it was not presented.
(Schiller et al., 2012)	Comparing an immediate cessation versus reduction approach to smokeless tobacco cessation	ST-cessation study; 199 ST consumers interested in reducing, but not quitting ST use; mean (SD) age = 34.8 (8.5) y. Group 1 = immediate cessation, Group 2 = brand switching, Group 3 = nicotine lozenge	Not included in article	Urine: Baseline cotinine, ng/mL, mean (SD): Group 1 = 2,522 (2,319); Group 2 = 2,420 (1,848); Group 3 = 2,354 (1,861)	Not included in article	Not included in article	Not included in article	Strengths: biomarker confirmation of abstinence. Limitations: No biomarker data linked to specific product use, no use topography data reported.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Naufal et al., 2011)	Differential exposure biomarker levels among cigarette smokers and smokeless tobacco consumers in the National Health and Nutrition Examination Survey 1999–2008	Comparison of BOEs associated with use of different tobacco products using data from NHANES; 368 ST consumers; age ≥20 y.	Not included in article	Blood: Serum cotinine, ng/mL, geometric mean (95% CI): 188.7 (152.9-235.1)	Not included in article	Urine: NNAL, µg/g creatinine, mean (95% CI): 0.99 (0.72-1.38)	Urine: Cadmium, µg/g creatinine, mean (95% CI): 0.16 (0.13-0.20) Urine: Arsenic, µg/g creatinine, mean (95% CI): 6.17 (5.05-7.46) Urine: 1-hydroxypyrene, ng/g creatinine, mean (95% CI): 67.4 (55.7-81.5) Others measures presented in report are not presented here	Strength: Comprehensive array of biomarkers, large sample size.
(Jon O. Ebbert, Edmonds, Luo, Jensen, & Hatsukami, 2010)	Smokeless tobacco reduction with the nicotine lozenge and behavioral intervention	Exposure after ST reduction; 57 ST consumers; mean (SD) age: 35.0 (7.5) y.	Not included in article	Urine: Baseline cotinine, ng/mL urine, mean (SD): 8,835 (6,652)	Not included in article	Urine: NNAL, pmol/mL, mean (SD): 6.4 (5.7) NNAL, pmol/mg creatinine, mean (SD): 4.0 (3.3)	Not included in article	Strength: Identification of brands used by subjects. Limitations: No placebo lozenge.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Sarkar et al., 2010)	Evaluation of biomarkers of exposure in adult cigarette smokers using Marlboro snus	Exposure study switching smokers to an ST product; 15 cigarette smokers switched to ST only for eight days; age (SD) (range) = 35.7 (8.3) (23–53) y.	Urine: TNE, mg/24 h, mean (SD): 5.53 (5.21) mg/24 h at post-baseline	Not included in article	Not included in article	Urine: Total NNAL, ng/24 h, mean (SD): 278.25 (156.99 ng/24 h at post-baseline Urine: Total NNN, ng/24 h, mean (SD): 4.52 (7.14) ng/24 h at post-baseline	Urinary biomarkers: 3-OHBP, pg/24 h, mean (SD): 78.89 (51.15 pg/24 h at post-baseline Others measures presented in report are not presented here.	Limitations: Small number of snus users. All subjects were cigarette only consumers, therefore baseline findings not relevant to ST, only post-baseline findings are presented here. ST group may not be generalizable because use conditions were controlled and atypical.
(Blank, Sams, Weaver, & Eissenberg, 2008)	Nicotine delivery, cardiovascular profile, and subjective effects of an oral tobacco product for smokers	Exposure after use of novel ST product; participants were cigarette smokers; 5 men and 5 women; mean (SD) age = 32.8 (8.5) y; test product was Ariva tablets.	1-tablet dose, 2-tablet dose, and 3-tablet dose were given subsequently to each subject. Blood: For 1 tablet, mean plasma nicotine increased in 45 min from 2.4 (0.9)-3.4(1.4) ng/mL, for 2 tablets, 2.7 (0.9)-7.3 (4.0) ng/mL, for 3 tablets, 6.0 (3.7)-9.7 (4.4) ng/mL	Not included in article	Not included in article	Not included in article	Not included in article	Strengths: Product and use topography specified. Limitations: Small sample size. No positive control. Subjects were all smokers who used an ST product under controlled conditions, data may not be generalizable.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Gray et al., 2008)	Potential reduced exposure products (PREPs) for smokeless tobacco users: clinical evaluation methodology	Exposures associated with several novel ST products. Study 1: (nicotine exposure) N = 13, mean (SD) age: 29.2 (9.8) y Study 2: (NNAL excretion) N = 19, mean (SD) age: 24.0(12.2) y.	Blood (Study 1): Own Brand nicotine increased from mean (SEM): 2.6(0.6) to 16.1(4.6) ng/mL General snus nicotine increased from mean (SEM): 2.0 (0.0) to 8.7 (1.1) ng/mL	Urine cotinine (Study 2): Day 1, collapsed across condition: mean (SEM) = 104.5 (217.0) ng/mL	Not included in article	Urine (Study 2): NNAL levels were similar on day 1 (baseline) between different product groups; (mean (SEM), collapsed across condition = 770.6 (156.1) pg/mL	Not included in article	Strength: Brand name identification of ST product used by subjects. Limitations: No statement of subbrands of products used, limited number of measurements in subjects using a single brand of ST. Data presentation somewhat unclear and confusing.
(Hatsukami et al., 2008)	Smokeless tobacco reduction: preliminary study of tobacco-free snuff versus no snuff	Exposure after reduction in ST product use, N = 106; 52 in the tobacco-free snuff group, 54 in the control group; age, mean (SD) = 33.7 (7.3) y in the tobacco-free snuff group, 31.5 (6.6) y in the control group.	Not included in article	Urine: Baseline cotinine, ng/mL: Tobacco-free snuff group; mean (SD): 10,602 (6,340) Control group: mean (SD): 10,250 (5,750)	Not included in article	Urine: NNAL, pmol/mL urine, mean (SD): Tobacco-free snuff group = 3.0 (2.3) Control group = 2.6 (1.5)	Not included in article	Limitations: Preliminary study, dropout rate; limited number of measurements.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Hecht, Carmella, Edmonds, et al., 2008)	Exposure to nicotine and a tobacco-specific carcinogen increase with duration of use of smokeless tobacco	Exposure after different durations of ST use (31% of the users in this study used Copenhagen® fine and long cuts); 212 male ST consumers; mean (95% CI) age = 33.8 (32.8–34.8) y.	Not included in article	Urine: Cotinine, nmol/mL, geometric mean (95% CI), Years of use: <ul style="list-style-type: none"> • 0-5: 23.6 (13.5-41.2) • 6-10: 37.3 (30.9-45.1) • 11-15: 43.9 (37.4-51.6) • 16-20: 44.3 (35.2-55.7) • 21+: 55.9 (40.4-77.3) 	Not included in article	Urine: Total NNAL, pmol/mL, geometric mean (95% CI), Years of use: Years of use: <ul style="list-style-type: none"> • 0-5: 2.47 (1.42-4.31) • 6-10: 3.21 (2.62-3.92) • 11-15: 4.26 (3.60-5.03) • 16-20: 4.07 (3.34-4.94) • 21+: 5.21 (4.06-6.70) 	Not included in article	Strength: Study size. Limitations: Product measurements were obtained from investigator purchased product not from subjects product. Only enrolled subjects interested in quitting.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Hecht, Carmella, Stepanov, et al., 2008)	Metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to its biomarker total NNAL in smokeless tobacco users	Metabolism study of 15 male subjects after a single use of a 2-g portion of Copenhagen® for 30 min; mean (SD) age = 32.2 (6.1) y.	Not included in article	Not included in article	Not included in article	Mouth level extraction from product: mean (SD) percentage of NNK extracted from 2 g tobacco during chewing = 59 (23)%	Not included in article	Limitations: The experimental product was a 2-g portion of loose moist snuff product that was put into a “tea bag–like sealed pouch.” Since this product was unlikely be representative of either machine-made pouch products or natural use of loose moist snuff, it may have altered how the product was used compared with use under natural conditions. Background urinary NNAL from noncompliance may have been a confounding factor in the dose analyses.
(Kauffman et al., 2008)	Factors associated with smokeless tobacco cessation in an Appalachian population	ST-cessation study; 116 men, ages = <30 = 25%; 30-39 = 46.55%; 40-49 = 15.5%; ≥50 = 12.9%.	Not included in article	Not included in article	Salivary baseline cotinine, ng/mL: Highest tertile (681–1,596): 33.04%; Middle tertile (383–680): 33.91%; Lowest tertile (17–382): 33.04%	Not included in article	Not included in article	Limitations: No statement of products used or product use topography.

Table 7.5.5-1-7.Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Ferketich, Wee, Shultz, & Wewers, 2007a)	A measure of nicotine dependence for smokeless tobacco users	Nicotine dependence methods study; 216 ST consumers; mean (SD) age = 34.8 (10.9) y.	Not included in article	Not included in article	Salivary cotinine ng/mL, mean (SD): 560 (369), range: 17-2,469	Not included in article	Not included in article	Strength: Brands used were specified. Limitations: No statement of products used, and uniqueness of subject recruitment may limit generalization of results.
(Ferketich et al., 2007b)	Smokeless tobacco use and salivary cotinine concentration	Development of baseline salivary cotinine levels in ST consumers; 256 male ST consumers; mean (SD) age = 34 (11.1) y.	Not included in article	Not included in article	Salivary cotinine, ng/mL, mean (SD): Copenhagen®: 644 (381) Skoal: 464 (339) Timberwolf: 435 (241) Red Seal: 675 (273) Other: 570 (377)	Not included in article	Not included in article	Strength: Brands used were specified. Limitations: Uniqueness of subject recruitment may limit generalization of results.
(Hatsukami, Ebbert, et al., 2007)	Smokeless tobacco brand switching: a means to reduce toxicant exposure?	Exposure after ST brand switching; 66 subjects seeking treatment to reduce their use; age, mean (SE) = 31.8 (5.05) y. Subjects had to initially be users of Copenhagen® or Kodiak.	Not included in article	Urine cotinine was approximately 9000 ng/mL at baseline.	Not included in article	Urine: Total NNAL reduced by brand switching from a mean of 3.2 to 1.8 pmol/mg creatinine.	Not included in article	Strengths: Moderate size (n = 66); subjects interested in reducing ST exposure. Limitations: Short use duration, specific brand of ST use during switching.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Hecht et al., 2007)	Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers	Exposure study comparing use of ST to smoking; 182 ST consumers; mean age (95% CI) = 32.9 (31.9-33.9) y.	Not included in article	Urine: Cotinine, nmol/mg creatinine, geometric mean (95%CI): 28.1 (25.4-31.2)	Not included in article	Urine: Urinary T- NNAL, pmol/mg creatinine, geometric mean (95% CI) = 2.54 (2.28- 2.82)	Not included in article	Strength: Study size. Limitations: Demographic differences between smokers and ST subjects; not a complete statement of products used or complete use topography.
(Kotlyar et al., 2007)	Nicotine pharmacokinetics and subjective effects of three potential reduced exposure products, moist snuff and nicotine lozenge	Pharmacokinetic study on consumers of Ariva Revel, and Stonewall; 10 subjects; mean age (range) = 30.9 (20–49) y.	Blood: C _{max} (ng/mL), geometric mean (95% CI): Copenhagen® moist snuff = 16.1 (12.1-21.5); Commit = 7.3 (5.5-9.8); Stonewall = 4.1 (3.1-5.4); Ariva = 2.7 (2.0-3.6); Revel = 2.6 (2.0-3.5)	Not included in article	Not included in article	Not included in article	Nicotine AUC ₀₋₉₀ (ng x min/mL), geometric mean (95% CI): Copenhagen® = 1,038 (806-1,336); Commit = 467 (361-604); Stonewall = 292 (226-376); Ariva = 192 (149-248); Revel = 189 (146 -244)	Limitations: Small number of subjects; no statement of product use topography; controlled product use may not be representative of natural conditions.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Mendoza- Baumgart et al., 2007)	Pilot study on lower nitrosamine smokeless tobacco products compared with medicinal nicotine	Study 1: Total: n = 39; age = mean (SD) 38.7 (12.0) y; Group 1: MNL-Exalt: n = 19; age = mean (SD) 37.9 (12.1) y; Group 2: Exalt-MNL: n = 20; age = mean (SD) 39.5 (12.1) y Study 2: Total: n = 26; age = mean (SD) 35.7 (11.2) y; Group 1: MNL-Ariva: n = 12; age = mean (SD) 36.4 (10.5) y; Group 2: Ariva-MNL: n = 14; age = mean (SD) 35.1 (12.1) y	Not included in article	Urine: Baseline cotinine, nmol/mg creatinine, geometric mean (95% CI): Study 1: Period 1: Group 1 = 21.7 (8.4, 56.0); Group 2 = 6.1 (3.4, 11.0) Period 2: Group 1 = 25.4 (4.6, 139.7); Group 2 = 25.2 (4.3, 147.7) Study 2: Period 1: Group 1 = 15.5 (6.8, 35.5); Group 2 = 11.2 (8.0, 15.8) Period 2: Group 1 = 15.0 (8.7, 25.9); Group 2 = 11.2 (7.6, 20.0)	Not included in article	Urine: Baseline NNAL, pmol/mg creatinine, geometric mean (95% CI): Study 1: Period 1: Group 1 = 0.35 (0.2, 0.6); Group 2 = 0.88 (0.7, 1.2) Period 2: Group 1 = 0.79 (0.4, 1.7); Group 2 = 0.38 (0.2, 0.7) Study 2: Period 1: Group 1 = 0.29 (0.2, 0.6); Group 2 = 0.32 (0.2, 0.4) Period 2: Group 1 = 0.36 (0.2, 0.8); Group 2 = 0.34 (0.2, 0.8)	Not included in article	Limitations: Small study groups.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(J. O. Ebbert et al., 2006)	The Fagerstrom Test for Nicotine Dependence- Smokeless Tobacco (FTND-ST)	Nicotine dependence measure method development; 42 ST consumers; mean (SD) age = 35.7 (7.5), range = 20–56 y.	Not included in article	Blood: Baseline serum cotinine ng/mL, mean (SD): 517 (309), range: 152-1,892	Not included in article	Not included in article	Not included in article	Strength: Product use topography measured. Limitations: No statement of products used; small sample size; subjects were part of cessation trial and selected for high product use.
(Thomas et al., 2006)	Measuring nicotine dependence among smokeless tobacco users	Nicotine dependence study; 68 ST consumers; age, mean (SD) = 36.5 (12.5) y.	Blood: Nicotine, ng/mL, mean (SD): 23 (11); median (range): 22 (7-48)	Blood: Cotinine, ng/mL mean (SD): 454 (256); median (range): 402 (102-1,230)	Not included in article	Not included in article	Not included in article	Limitations: No statement of products used or product use topography, and only individuals interested in quitting were recruited.
(Lemmonds et al., 2005)	Smokeless tobacco topography and toxin exposure	Exposure study among 54 male ST consumers; mean (SD) age = 32.1 ± 7.5 y.	Urine: Total nicotine, nmol/mg creatinine, mean (SD): 10.5 (5.6)	Urine: Total cotinine, nmol/mg creatinine, mean (SD): 23.5 (14.3)	Not included in article	Urine: Total NNAL, pmol/mg creatinine, mean (SD): 3.3 (2.1)	Not included in article	Strength: Moderate study size.
(Stepanov & Hecht, 2005)	Tobacco-specific nitrosamines and their pyridine-N-glucuronides in the urine of smokers and smokeless tobacco users	Biomarker methods development study; 11 ST consumers.	Not included in article	Urine: Cotinine, nmol/mg creatinine, mean (SD): 4.67 (3.93)	Not included in article	Urine: NNAL, pmol/mg creatinine, mean (SD): 2.73 (1.76) NNN, pmol/mg creatinine, mean (SD): 0.25 (0.19)	Others measures presented in report are not presented here	Limitations: Small sample size; tobacco use level of subjects not provided; no details of urine collection (spot vs. 24-hour collection) or subject general demographics.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Wolk et al., 2005)	Hemodynamic and autonomic effects of smokeless tobacco in healthy young men	A study of hemodynamic and autonomic effects of ST in 16 healthy young men; habitual snuff tobacco users; aged 21 ± 1 y.	Blood: Nicotine, ng/mL, mean (SEM): 10.4 (1.1), increased from 2.8 (0.5) after 30 min of use of Copenhagen® moist tobacco snuff.	Blood: Cotinine, ng/mL, mean (SEM): 61 (19) before chewing to 64 (18) after chewing	Not included in article	Not included in article	Not included in article	Strengths: Double-blinded and placebo-controlled with product use topography reported. Limitations: Small size of study and geographically limited; most measurements taken in supine position, which does not reflect typical product use behavior.
(J. O. Ebbert, Dale, Vickers, et al., 2004)	Residential treatment for smokeless tobacco use: a case series	Exposure study among ST consumers treated for cessation; 24 ST consumers, 21 were snuff consumers, two were chewing tobacco consumers, and one used pipe tobacco as ST.	Blood: Nicotine, baseline, peak, ng/mL, mean (SD): 30.5 (10.8), range: 13-55	Blood: Cotinine, baseline, ng/mL, mean (SD): 468.6 (158), range: 147-796	Not included in article	Not included in article	Not included in article	Strengths: Products use topography was measured, and brand of products used was specified. Limitations: Limited number of measurements.
(J. O. Ebbert, Dale, Nirelli, et al., 2004)	Cotinine as a biomarker of systemic nicotine exposure in spit tobacco users	Exposure study; correlation of nicotine vs. cotinine; 68 daily ST consumers; mean (SD) age = 36.5 (12.5) y, range: 24–79 y.	Blood: Nicotine, ng/mL, mean (SD) Overall: 23 (11), range = 7–48; Chewing tobacco only: 23 (10), range = 9–42; Snuff only: 24 (11), range = 7–48	Blood: Cotinine, ng/mL, mean (SD) Overall: 454 (256), range = 102–1230; Chewing tobacco only: 450 (244), range = 102–1183; Snuff only: 471 (265), range = 167–1230	Not included in article	Not included in article	Not included in article	Strengths: Moderate study size, and product use topography was quantified. Limitation: Specific products used not specified.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Hatsukami et al., 2004)	Evaluation of carcinogen exposure in people who used 'reduced exposure' tobacco products	Exposure after use of various ST products; 54 subjects were recruited and data from 41 subjects were analyzed (19 were assigned to snus and 22 were assigned to nicotine patch); mean (SD) age = 31.4 (5.9) y, range: 22–47 y. Some subjects (13/41) used Copenhagen®.	Not included in article	Urine: Baseline, ng/mL, mean (95% CI), snus group = 6193 (4579-7807), nicotine patch group = 5759 (4310-7208)	Not included in article	Urine: NNAL, baseline, pmol/mg creatinine, mean (95% CI): 2.8 (2.0-3.5)	Not included in article	Limitations: Compliance with use of only the prescribed product; small sample size.
(Carmella et al., 2003)	Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine	Study of TSNA urinary metabolite quantification; 55 snuff dippers.	Not included in article	Not included in article	Not included in article	Urine: The mean amount of total NNAL in the urine of 55 snuff dippers was 3.25 ± 1.77 pmol/mg creatinine	Not included in article	Limitations: Poor descriptions of products used, subject characteristics, or product use topography.
(Granberry, Smith, Troillett, & Eidt, 2003)	Forearm endothelial response in smokeless tobacco users compared with cigarette smokers and nonusers of tobacco	Comparisons of ST (Skoal and Copenhagen®) vs. cigarette consumers; 5 ST consumers; mean (SD) age = 28.8 (3.3) y.	Not included in article	Blood: Serum cotinine, mg/dL = 226 ± 124	Not included in article	Not included in article	Not included in article	Strength: Brand name identification of ST product used by subjects. Limitations: Small number of subjects; limited number of measurements; not clear about whether variance estimate is SD or not.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Carmella, Le, Upadhyaya, & Hecht, 2002)	Analysis of N- and O-glucuronides of 4- (methylnitrosamino)- 1-(3-pyridyl)-1- butanol (NNAL) in human urine	Study of TSNA urinary metabolite quantification; 10 snuff consumers.	Not included in article	Not included in article	Not included in article	Urine: "Total NNAL amounted to 1.22 pmol/mL in smokers in this study compared to 1.95 pmol/mL in a previous one while the corresponding figures for snuff-dippers were 4.30 and 4.22 pmol/mL."	Not included in article	Limitations: Poor descriptions of products, study participants, or product use topography.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Hecht et al., 2002)	Quantitation of metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use	Exposure study after cessation of ST; 11 snuff consumers and 2 chewing tobacco consumers; age = 27-61 y.	Not included in article	Not included in article	Not included in article	<p>Blood: NNAL, mean (SD), pmol/mL = 0.209 (0.063); NNAL-gluc, pmol/mL = 0.127 (0.043);</p> <p>Urine: NNAL, mean (SD) pmol/mL urine = 1.11 (0.92); pmol/mg creatinine = 0.937 (0.81); pmol/24 h urine = 1,710 (1,810)</p> <p>Urine: NNAL-Gluc, mean (SD) pmol/mL urine = 3.11 (2.61); pmol/mg creatinine = 2.61 (2.21); pmol/24 h urine = 4,890 (4,980)</p>	<p>NNAL distribution half-lives, $t_{1/2\alpha}$ (days), mean (SD): ST consumers = 1.32 (0.85); Smokers = 3.35 (1.86);</p> <p>NNAL Terminal half-lives $t_{1/2\beta}$ (days), mean (SD): ST consumers = 26.3 (16.7); Smokers = 45.2 (26.9)</p>	Limitations: Small number of subjects; no statement of products used or product use topography.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Fant et al., 1999)	Pharmacokinetics and pharmacodynamics of moist snuff in humans	Measurement of the time course of plasma nicotine levels after use of a single portion of 4 brands of moist snuff; Participants = 10 ST users; mean (range) age = 32.2 (26–45) y.	Blood: Nicotine, maximum increase, ng/mL, mean (SEM): Copenhagen® = 19.5 (4.1); Skoal long cut cherry = 14.9 (3.0); Skoal original wintergreen = 14.9 (2.4); Skoal Bandits = 4.2 (1.4)	Not included in article	Not included in article	Not included in article	Blood: Nicotine AUC ₀₋₃₀ (ng/mL min ⁻¹), mean (SEM): Copenhagen® = 530.4(120.8); <u>Skoal</u> long cut cherry = 333.9 (60.7); Skoal original wintergreen = 376.3 (61.6); Skoal Bandits = 208.0 (33.0)	Limitations: Amount of nicotine in each product was not measured. Not clear if differences in amount of tobacco between MST and pouched product was factored into conclusions.
(Escher et al., 1998)	Smokeless tobacco, reaction time, and strength in athletes	Effect of ST on some exercise performance measures; 20 athletes, ST consumers; mean (range) age = 20.6 (18–23) y.	Blood: Nicotine, baseline, ng/mL, mean (SD): 22(14)	Blood: Cotinine, baseline, ng/mL, mean (SD): 144(169)	Not included in article	Not included in article	Not included in article	Limitations: No statement of products used; and limited number of measurements.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Carmella, Borukhova, Akerkar, & Hecht, 1997)	Analysis of human urine for pyridine-N-oxide metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific lung carcinogen	Methods development study for TSNA biomarker; 11 ST consumers.	Not included in article	Not included in article	Not included in article	Urine: “Levels of NNAL-N-oxide in urine of ST users ranged from 0.02-1.2 pmol/mg creatinine, mean \pm SD 0.41 \pm 0.35 pmol/ mg creatinine.” Free NNAL range: 0.28 - 2.1 pmol/mg creatinine; NNAL-Gluc range: 0.75 - 6.77 pmol/mg creatinine	Not included in article	Limitations :Poor descriptions of products, study participants, or product use topography.
(Hatsukami et al., 1996)	Effects of behavioral and pharmacological treatment on smokeless tobacco users	ST cessation study; 210 male ST consumers; mean (SD) age = 30.5 (9.3) y.	Not included in article	Not included in article	Salivary cotinine, baseline levels, ng/mL, mean (SD): 441.1 (320.3)	Not included in article	Not included in article	Strength: Product use topography reported. Limitations: No statement of products used. Data may not be generalizable due to subject selection criteria.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Kresty et al., 1996)	Metabolites of a tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in the urine of smokeless tobacco users: relationship between urinary biomarkers and oral leukoplakia	Comparison of BOEs associated with prevalence of oral leukoplakia; 23 snuff dippers, 13 tobacco chewers, 3 users of both products; the average ages of the snuff dippers and chewers were 27 and 25 years, respectively.	Not included in article	Urine: Cotinine, nmol/mg creatinine, mean (SD): Snuff dippers: 13.7 (11.9); Chewers: 7.21 (10.8)	Not included in article	Urine: NNAL-gluc, pmol/mg creatinine, mean (SD): Snuff dippers = 4.67 (7.28); Chewers = 1.57 (2.00) Urine: NNAL, pmol/mg creatinine, mean (SD): Snuff dippers = 1.20 (2.00) Chewers = 0.49 (0.58)	Not included in article	Limitations: No statement of products used or product use topography.
(Allen, Hatsukami, Jensen, Grillo, & Bliss, 1995)	Effects of treatment on cardiovascular risk among smokeless tobacco users	Exposure study of effects of NRT treatment of ST consumers; 56 male ST consumers; age = 18-65 y.	Not included in article	Not included in article	Baseline salivary cotinine, ng/mL, mean (SD): 447.7 (295.0)	Not included in article	Not included in article	Limitations: No statement of products used or product use topography for non-abstinent subjects. Did not monitor diet during study. Study excluded those with high blood pressure.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Jacob et al., 1993)	Gas chromatographic-mass spectrometric method for determination of anabasine, anatabine and other tobacco alkaloids in urine of smokers and smokeless tobacco users	Characterization of nicotine metabolites in urine of 8 chewing tobacco and 6 snuff consumers,	Urine: Nicotine, ng/mL, mean (SD): Chewing tobacco = 1047 (754); Snuff = 732 (521) Nicotine, µg/24 h, mean (SD): Chewing tobacco = 1321 (698); Snuff = 1562 (901)	Urine: Cotinine, ng/mL, mean (SD): Chewing tobacco = 1730 (1598); Snuff = 1269 (632) Cotinine, µg/24 h, mean (SD): Chewing tobacco = 2311 (1572); Snuff = 2933 (1694)	Not included in article	Not included in article	Others measures presented in the article are not included here	Limitations: Poor descriptions of products used, study participants, or product use topography.
(Hatsukami et al., 1992)	Smokeless tobacco abstinence effects and nicotine gum dose	Exposure following ST abstinence; 23 Copenhagen® ST consumers; mean (SD) age = 20.9 (1.6) y; Experiment 1: abstinence effects from smokeless Tobacco; Experiment 2: effects of different doses of nicotine gum on smokeless tobacco abstinence effects.	Not included in article	Not included in article	Baseline salivary cotinine levels, ng/mL, mean (SE): Experiment 1 Group 1 (ST) = 233.1 (28.6); Group 2 = (no ST) 346.9 (69.4) Experiment 2: 0-mg gum = 200.5 (27.8); 2-mg gum = 301.7 (35.3); 4-mg gum = 323.8 (52.9)	Not included in article	Not included in article	Strengths: Brand name identification of ST product used by subjects. Limitations: Limited number of measurements in subjects using a single brand of ST; 4 individuals included in 0-mg group with cotinine levels greater than 20 ng/mL.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Siegel, Benowitz, Ernster, Grady, & Hauck, 1992)	Smokeless tobacco, cardiovascular risk factors, and nicotine and cotinine levels in professional baseball players	Exposure study (70% of snuff consumers used Copenhagen®); biomarker data obtained in 230 professional baseball players who are ST consumers; ages = <20 = 5.7%; 20-24 = 56.9%; 25-29 = 26.0%; 30-34 = 7.0%; 35-39 = 1.9%; >40 = 2.5%	Not included in article	Blood: Cotinine, ng/mL, mean (SE): Snuff consumers: 143.9 (7.4); Chewing tobacco consumers: 82.1 (15.1)	Not included in article	Not included in article	Not included in article	Limitations: Although collected, specific smokeless products and their use topography data were not linked to biomarker data. Unique population that may not be representative.
(Hatsukami, Anton, Callies, & Keenan, 1991)	Situational factors and patterns associated with smokeless tobacco use	Exposures after various patterns of ST use; 30 male Copenhagen® ST consumers; mean (SD) age = 20.5 (2.3) y.	Not included in article	Blood: Cotinine, ng/mL, mean (SD): 198.3 (109.2); range: 26.0-509.0	Salivary cotinine, ng/mL, mean (SD): 264.2 (110.9), range: 64.0-591.0	Not included in article	Not included in article	Strengths: Brand name identification of ST product used. Limitations: No statement of sub-brands of products used.
(Carmella et al., 1990)	Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers, and nonsmokers	Exposure study among 22 male snuff consumers from an Eastern Military Academy.	Not included in article	Blood: Cotinine, ng/mL, mean (SD): 59.2 (64.4)	Salivary Cotinine, ng/mL, mean (SD): 174 (124)	Not included in article	Not included in article	Limitations: Poor descriptions of products, study participants, or product use topography.
(Livingston, Reed, Olson, & Lockey, 1990)	Induction of nuclear aberrations by smokeless tobacco in epithelial cells of human oral mucosa	Oral pathology study; 24 ST consumers; mean age = 21 y.	Not included in article	Not included in article	Salivary Cotinine, ng/mL, mean (SD): 214 (212), range: 30-705	Not included in article	Not included in article	Limitations: Products used and amount of product used not well described.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Bauman et al., 1989)	On the measurement of tobacco use by adolescents. Validity of self-reports of smokeless tobacco use and validity of cotinine as an indicator of cigarette smoking	Assessment of validity of self-reports of tobacco use; 1,854 persons aged 12-14 y.	Not included in article	Not included in article	Salivary Cotinine ng/mL, mean (SD): 205.9 (440.8), range: 10.0-2,908.9	Not included in article	Salivary Thiocyanate $\mu\text{mol/L}$, mean (SD) = 784.1 (512.3), range = 0.0-1,780.0	Limitations: No statement of products used or product use topography.
(Benowitz et al., 1989)	Daily use of smokeless tobacco: Systemic effects	Exposure study of daily use of ST (either Copenhagen®, Skoal Bandits-Wintergreen, or Hawken-Wintergreen); 8 men who smoked cigarettes and had previously used ST; mean (range) age = 49 (27-61) y.	Blood: Nicotine, maximum concentration, $\mu\text{mol/L}$, mean (SD) (range): Oral snuff = 0.20 (0.10) (0.07-0.38); Chewing tobacco = 0.17 (0.07) (0.07-0.29) Urine: Nicotine concentration, $\mu\text{mol/24 h}$, mean (SD) (range): Oral snuff = 5.58 (5.06) (1.04-15.84); Chewing tobacco = 6.45 (4.65) (1.29-13.68)	Not included in article	Not included in article	Not included in article	Nicotine AUC, $\mu\text{mol L}^{-1} \text{h}^{-1}$ mean (SD) (range): Oral snuff = 2.48 (1.13) (0.97-4.67); Chewing tobacco = 2.06 (0.84) (0.83-3.69)	Strength: Products are reported. Limitation: Does not reflect ad libitum product use. Only heavy cigarette smokers recruited.
(Cummings, Michalek, Carl, Wood, & Haley, 1989)	Use of smokeless tobacco in a group of professional baseball players	Nicotine exposure in ST consumers; Minor league professional baseball team members; 17 were ST consumers; mean (range) age = 29 (22-44) y.	Not included in article	Not included in article	Salivary Cotinine, ng/mL, mean (range): 178 (7-838)	Not included in article	Not included in article	Strength: Specific brands of product were reported. Limitations: Small sample size; cross-sectional study design.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Benowitz et al., 1988)	Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum	Time course of nicotine absorption after single 2.5 - g portion of ST (either Copenhagen® or Hawken-Wintergreen); 10 smokers with prior experience using oral snuff; ages = 24 -61 y.	Blood: Average incremental increases above baseline: ST = 14.3 ng/mL	Not included in article	Not included in article	Not included in article	Estimated nicotine doses resulting from use: oral snuff = 3.6 mg; chewing tobacco = 4.5 mg	Strength: Products are reported. Limitation: Does not reflect <i>ad libitum</i> product use.
(Palladino et al., 1986)	Snuff-dipping in college students: a clinical profile	Use patterns and exposure study of ST consumers; 30 male ST consumers from an Eastern Military College.	Urine: Nicotine, ng/mg creatinine, by minutes/day dipping, mean (SD): 110 = 424 (390) 235 = 569 (364) 254 = 1,154 (578)	Urine: Cotinine, ng/mg creatinine, by minutes/day dipping, mean (SD): 110 = 587 (632) 235 = 705 (530) 254 = 934 (529)	Salivary Cotinine, ng/g, by minutes/day dipping, mean (SD): 110 = 523 (236) 235 = 776 (363) 254 = 687 (305)	Not included in article	Salivary NNN, ng/g, mean (SD): 99.2 (41.7) Salivary NAT, ng/g, mean (SD): 204.0 (98.8)	Limitations: Specific brands of snuff analyzed not specified, and no product use topography reported. Poor description of subject characteristics.
(Kyerematen et al., 1983)	Influence of different forms of tobacco intake on nicotine elimination in man	Study is to determine the Influence of different forms of tobacco intake on nicotine elimination in man; 6 ST consumers; age = 21-26 y.	Blood: No difference in mean plasma nicotine half-lives between never user of tobacco and ST consumers.	Not included in article	Not included in article	Not included in article	Nicotine, plasma $t_{1/2\beta}$, mean (SD): <u>Nonconsumer</u> = 1.7 (0.1) Chronic consumer = 1.9 (0.2)	Strength: Limit of detection by use of radiolabeled material. Limitations: Lack of specific product use characterization and small sample size.

Table 7.5.5-1-7. Summary of Methods, Participant Characteristics, and Findings From Studies Evaluating the Exposure to Harmful and Potentially Harmful Constituents in Smokeless Tobacco Consumers (continued)

Author/ Year	Title	Study Type and Participant Characteristics	Nicotine in Blood or Urine	Cotinine in Blood or Urine	Cotinine in Saliva	TSNAs in Blood or Urine	Other BOE Measures	Comments
(Gritz et al., 1981)	Plasma nicotine and cotinine concentrations in habitual smokeless tobacco users	Exposure study in ST consumers; 12 male college students; ST consumers; mean (range) age = 19.8 (18-22) y.	Blood: Plasma nicotine after overnight abstinence: 2.9 ng/mL; After 6-8 h of <i>ad libitum</i> consumption: 21.6 ng/mL	Plasma Mean (SEM): 137.3 (42.1) ng/mL after overnight abstinence to 197.2 (48.0) ng/mL after 6 - 8 h <i>ad libitum</i> consumption	Not included in article	Not included in article	Not included in article	Strength: Tested different defined nicotine levels of ST. Limitations: No statement of products used. Poor presentation of findings.

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