7.5.5-2: UPDATE – EXPOSURE - LITERATURE SUMMARY

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

aFOR	adjusted fecundability odds ratio
$AUC_{0\text{-}60min}$	area under the curve from Time 0 to 60 minutes
BOE	biomarker of exposure
CI	confidence interval
Cr	creatinine
FeNO	fractional exhaled nitric oxide
FTND	Fagerström Test for Nicotine Dependence
GM	geometric mean
IL	interleukin
Iqmik	a home-made chewing tobacco made with tobacco leaves and ash
MHBMA	monohydroxy-3-butenyl mercapturic acid
MRTP	Modified Risk Tobacco Product
MRTPA	Modified Risk Tobacco Product Application
NHANES	National Health and Nutrition Examination Survey
NMR	nicotine metabolite ratio
NNAL	4-methylnitrosamino-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N´-nitrosonornicotine
sICAM-1	soluble intracellular adhesion molecule 1
ST	smokeless tobacco
TDS	Tobacco Dependence Screener
TNE	total nicotine equivalents
total NNAL	4-methylnitrosamino-1-(3-pyridyl)-1-butanol and its glucuronides
TSNA	tobacco-specific nitrosamine
TTP	time-to-pregnancy
U.S.	United States

7.5.5-2 EXPOSURE LITERATURE SUMMARY

7.5.5-2.1 Literature Summary on Exposure

The United States (U.S.) Food and Drug Administration's Modified Risk Tobacco Product Application (MRTPA) Draft Guidance (2012) Section V (A) requires that applicants provide "…information both favorable and unfavorable to the ability of the product to reduce risk or exposure and relating to human health."

The intent of this section is to summarize information regarding biomarkers of exposure (BOEs) associated with smokeless tobacco (ST) use.

7.5.5-2.2 Literature Search and Review Process

A comprehensive literature review was conducted through December 2014 that reviewed the health and behavioral effects of ST (Section 7.5.1), and literature summaries were drafted in areas that are important in the assessment of a Modified Risk Tobacco Product (MRTP) candidate. A second literature review was conducted for the period of December 08, 2014, to February 06, 2017, to update the original search. During the new search, 1,029 citations were identified, and, after applying predetermined inclusion and exclusion criteria, 234 articles were deemed to be in-scope. In general, the in-scope articles were peer-reviewed and studied ST products commercially available in the U.S.

A keyword assignment exercise was performed, and 19 articles that provide information regarding exposure to ST BOEs were identified. However, as new references became available after December 2014, they were, initially, included in the original narratives if they added new information. Of the 19 articles that provide information on exposure to ST BOEs in the updated search, four had already been included in the original literature summary in Section 7.5.5-1, even though they were published after the cutoff date. A summary of the remaining 15 articles identified is provided in Table 7.5.5-2-1, and this section is intended to supplement the previous literature review (Section 7.5.5-1) to provide a current, updated literature review of exposure to ST BOEs.

7.5.5-2.3 Biomarkers of Exposure

Studies evaluating BOEs associated with ST use generally measure one or more of the following constituents:

- nicotine and its metabolites in blood, urine, or saliva, including:
  - cotinine; and
  - total nicotine equivalents (TNE).
- tobacco-specific nitrosamines (TSNAs) in urine, including:
  - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL); and
  - N´-nitrosonornicotine (NNN).

- other BOEs, including:
  - monohydroxy-3-butenyl mercapturic acid (MHBMA), a marker of exposure to 1,3-butadiene;
  - heavy metals, including cadmium and lead; and
  - markers of inflammation and oxidative stress.

The following sections are organized to present data regarding these BOEs.

7.5.5-2.4 Nicotine and Its Metabolites

7.5.5-2.4.1. Nicotine in the Blood

Two studies identified in the literature search measured nicotine in the blood of subjects to assess exposure levels under modified or alternative product use conditions (Krautter, Chen, & Borgerding, 2015; Pickworth, Rosenberry, Koszowski, & Gold, 2014).

Pickworth et al. (2014) performed a study among seven adult ST users in Baltimore, Maryland, that had been using ST for at least one year and had not sought treatment for tobacco dependence. Subjects were required to participate in three study visits separated by at least 24 hours, during which two grams of one of three ST product conditions was used by mouth. To assess the effects of pH and flavor on absorption of nicotine from ST, nicotine absorption was measured in subjects exposed to modified ST products, including modified low (5.4) pH with wintergreen flavoring (Condition 1), modified high (8.3) pH with wintergreen flavoring (Condition 2), or unmodified pH (7.7) and unflavored ST (Condition 3). Each visit was about 75 minutes long: 15-minute baseline period, 30 minutes with ST product in the mouth, and 30 minute period without ST product in the mouth. Blood specimens were collected 10 minutes before and at 5, 10, 15, 20, 30, 35, 45, and 60 minutes after the product was placed in the mouth. The product was retained in the mouth for about 30 minutes. The average plasma nicotine levels ranged from 12.5 ±8.1 ng/mL in Condition 1 to 21.4 ±12.3 ng/mL for Condition 3. Peak plasma nicotine levels occurred at 20 to 35 minutes after initiation of exposure, and, after adjusting for baseline nicotine concentrations, the average nicotine boost at maximal concentration for Conditions 1, 2, and 3, respectively, were 6.6 ±3.9, 20.0 ±4.2, and 19.5 ±6.5 ng/mL.

In a study examining a panel of BOEs in smokers who were randomized to switch to an alternative tobacco product, Krautter et al. (2015) conducted a study among 167 adult smokers at three clinical sites (Daytona Beach [Florida], Evansville [Indiana], and Madison [Wisconsin]) from 2010 to 2011, in which subjects were randomized into one of six groups: dual use group, exclusive snus group, exclusive sticks group, exclusive strips group, exclusive orbs group, and tobacco abstinent group. Each group consist of 25 to 30 subjects, and each subject was assigned to a product for 5 days. Whole blood samples were collected on Days 1 to 5, and urine samples were collected on Days 1, 3, and 5. Krautter et al. observed a reduction in the mean plasma nicotine levels in subjects from 27.25 ng/mL at baseline to 10.37 ng/mL at 5 days after switching to exclusive snus use ($p \leq 0.05$), and from 28.43 ng/mL at baseline to 15.34 ng/mL at 5 days after switching to dual cigarette/snus use ($p \leq$

0.05). In comparison, nicotine levels in smokers who were randomized to abstain from tobacco decreased from 29.90 ng/mL at baseline to 0.78 ng/mL after 5 days ($p \leq 0.05$).

7.5.5-2.4.2. Cotinine in the Blood

Cotinine is the predominant metabolite of nicotine after consumption, and two studies measured levels of this metabolite in the blood of subjects as a marker of exposure (Krautter et al., 2015; Sapra, Sundaram, Buck, Barr, & Maisog, 2016).

Krautter et al. (2015) examined (Section 7.5.5-2.4.1) cotinine in the plasma of smokers at baseline (296.65 ±108.06 ng/mL) and at 5 days after switching to exclusive snus use (143.09 ±126.69 ng/mL) ($p \leq 0.05$). Switching to dual use was associated with a more modest reduction in plasma cotinine levels, from 300.63 ±135.61 ng/mL to 191.91 ±103.83 ng/mL ($p \leq 0.05$), and tobacco abstinence resulted in a decrease from 317.39 ±149.40 ng/mL to 3.85 ±5.88 ng/mL after 5 days ($p \leq 0.05$).

Sapra et al. (2016) investigated a relationship between exposure to tobacco product constituents in ST users, cigarette smokers, and nonusers and time-to-pregnancy (TTP) among 501 couples who discontinued contraception for purposes of becoming pregnant in 16 counties in Michigan and Texas from 2005 to 2009. Upon enrollment, couples were followed daily until a positive home pregnancy test or 12 months of trying. Additionally, at enrollment, each partner provided a blood specimen for measurements of blood metals and serum cotinine. Serum cotinine levels in male smokers, ST users, and never users were 82.78 ng/mL ($p < 0.05$, compared with never users), 60.00 ng/mL ($p < 0.05$, compared with never users), and 0.02 ng/mL, respectively, and were 69.16 ng/mL and 0.02 ng/mL in female smokers and never users, respectively ($p < 0.05$). There were no female ST users included in the study. ST use among males was not associated with a change in time-to-pregnancy (aFOR [adjusted fecundability odds ratio] = 1.17, 95 percent confidence interval [CI]: 0.70, 1.95).

7.5.5-2.4.3. Cotinine in Urine

Among the studies identified in the literature search, three evaluated ST exposure by measuring cotinine in the saliva of subjects (Allen et al., 2016; Flanagan et al., 2016; Hatsukami et al., 2016).

To assess the effects of product switching, urine samples from 391 cigarette smokers randomized to snus (n = 196) or 4 mg nicotine gum (n = 195) were analyzed for nicotine metabolites and TSNAs at 4 weeks after intervention (Hatsukami et al., 2016). Compared with baseline, reductions in cotinine were observed in subjects who exclusively used snus (3,385 ±1,672 to 2,152 ±2,005 ng/mL; $p < 0.0001$) or nicotine gum (3,481 ±1,839 ng/mL to 2,052 ±2,342 ng/mL; $p < 0.0001$). Dual cigarette/snus use was associated with a more modest change in urinary cotinine from 3,359 ng/mL at baseline to 3,079 ng/mL at Week 4. In a similar study evaluating sex differences with respect to cigarette avoidance in daily smokers who agreed to switch to snus or nicotine gum, Allen et al. (2016) found that randomization to snus was associated with a change in total urinary cotinine in men and women, respectively, from baseline levels of 3,291 and 3,506 nmol/mL to 2,686 and 2,849 nmol/mL at 4 weeks after intervention.

To determine if there is a correlation between tobacco use during pregnancy in Alaska Native women and fetal exposure to tobacco-specific carcinogens, Flanagan et al. (2016) collected demographic data and maternal and neonatal urine samples at delivery. Confirming prenatal self-reported tobacco use, baseline urine cotinine levels, expressed as the geometric mean (GM) (95 percent CI), in nonusers were 9.1 (8.1-10.2) ng/mL, in smokers were 307.5 (222.1-425.7) ng/mL, in Iqmik (a home-made chewing tobacco made with tobacco leaves and ash) users were 587.3 (270.4-1,275.7) ng/mL, and in commercial ST users were 987.6 (618.8-1,576.4) ng/mL. In smokers, there was a moderate-to-strong correlation between maternal cotinine and infant NNAL levels; however, no significant correlation was observed in any other group.

7.5.5-2.4.4. Cotinine in Saliva

Among the studies identified in the literature search, two evaluated ST exposure by measuring cotinine in the saliva of subjects (Ebbert, Schroeder, Severson, Danaher, & Benowitz, 2016; Mushtaq & Beebe, 2016).

Ebbert et al. (2016) studied nicotine lozenge intervention in ST users treated for tobacco dependence and whether nicotine metabolite ratio (NMR), the ratio of 3´-hydroxycotinine to cotinine in saliva, could predict self-reported nicotine lozenge use and tobacco abstinence. At baseline, mean ±SD levels of salivary cotinine and 3´-hydroxycotinine, respectively, were 410 ±288 ng/mL and 120 ±121 ng/mL for a NMR of 0.30 ±0.19. Mean NMR was positively correlated with self-reported lozenge use, but did not correlate with baseline amount of ST use, years of use, or level of dependence as measured by the Severson Smokeless Tobacco Dependency Scale.

The Tobacco Dependence Screener (TDS) is a self-administered questionnaire commonly used in cigarette smoking studies to measure tobacco dependence in ST users. In a study evaluating the TDS, exposure and dependence data were collected at a single time point from 95 male exclusive ST users living in Oklahoma (Mushtaq & Beebe, 2016). The majority (92 percent) of subjects were every day ST users, and the median (range) salivary cotinine level was 350.53 (15.5-1,772.07) ng/mL. Correlation analysis found that the TDS has a positive association with salivary cotinine concentrations ($r = 0.24$; $p = 0.018$).

7.5.5-2.4.5. Total Nicotine Equivalents in Urine

Several studies identified in the literature search measured TNE in the urine of subjects (Allen et al., 2016; Hatsukami et al., 2016; Krautter et al., 2015; Ogden, Marano, Jones, Morgan, & Stiles, 2015a). The studies were primarily assessing exposure to tobacco constituents in subjects after switching from their usual product of choice (generally cigarette smoking) to an alternative product, such as ST or nicotine gum.

Evaluating a large panel of BOEs in cigarette smokers who switched to one of several alternative tobacco products or abstinence, Krautter et al. (2015) found that TNE decreased in the urine of exclusive snus users from 21.46 mg/24 h at baseline to 11.25 ±9.83 mg/24 h at 5 days after intervention. In the urine of dual users, TNE decreased from 22.59 ±9.70 mg/24 h at baseline to 15.40 ±7.17 mg/24 h at 5 days after intervention. Tobacco abstinence

was associated with a change in TNEs from 22.89 ±9.53 mg/24 h at baseline to 0.56 ±0.47 mg/24 h after 5 days.

In a study assessing sex differences in daily smokers who were randomized to switch to either snus or nicotine gum, Allen et al. (2016) found that there were no gender differences in TNE at baseline (p = 0.78), but at Week 4, there was a significant interaction between gender and randomization (p = 0.03). Men randomized to snus had higher TNE compared to those randomized to nicotine gum, whereas women did not vary by randomization. Hatsukami et al. (2016) observed similar results in smokers randomized to switch to either snus or nicotine gum. In subjects from the snus group, exclusive snus use was associated with a change in urinary TNE from 59.5 nmol/mL at baseline to 35.6 nmol/mL at 4 weeks (an approximately 40% decrease), and dual cigarette/snus use was associated with a reduction in urinary TNE from 66.2 nmol/mL at baseline to 55.7 nmol/mL at 4 weeks (an approximately 16% decrease). Ogden et al. (2015a) observed a more modest reduction in urinary TNE in smokers who changed from their usual brand of cigarettes to snus, with a 9% decrease in TNE, compared with baseline, at both 12 and 24 weeks after intervention.

7.5.5-2.5 Exposure to Tobacco Specific Nitrosamines and Metabolites

7.5.5-2.5.1. N´-Nitrosonornicotine in Urine

Three studies compared exposure to NNN based on tobacco product use or the effects of switching from cigarette smoking to ST or another alternative by measuring urinary total NNN levels of tobacco users (Hatsukami et al., 2016; Krautter et al., 2015; Yang, Carmella, & Hecht, 2017).

Total NNN and NNN enantiomers were measured in the urine of cigarette smokers (n = 20) and ST users (n = 12) (2017). Total urinary NNN was significantly higher in ST users (67.1 ±56.7 fmol/mL) compared with cigarette smokers (20.5 ±27.1 fmol/mL) (p = 0.005); however, the highly carcinogenic (S)-NNN enantiomer accounted for a significantly higher (p < 0.001) proportion of the total NNN in cigarette smokers (67 ±5 percent) compared with ST users (56 ±3 percent). The results demonstrate that (S)-NNN is the major enantiomer in human urine and that the enantiomeric composition of NNN in human urine is similar in cigarette smokers and ST users.

Krautter et al. (2015) assessed changes in the levels of 32 BOEs in subjects after switching from cigarette smoking to one of five alternative products, including exclusive use of snus or dual use of cigarettes and snus, or to tobacco abstinence. Exclusive snus use was associated with a reduction in total urinary NNN from 42.70 ng/24 h at baseline to 22.62 ng/24 h at 5 days after intervention (47.0% reduction), and dual cigarette/snus use resulted in a change from 24.08 ng/24 h at baseline to 28.43 ng/24 h at 5 days (18.1% increase). Tobacco abstinence was associated with a 93.7% reduction in total NNN (28.29 ng/24 h at baseline to 1.79 ng/24 h at 5 days).

Hatsukami et al. (2016) conducted a study to measure total NNN levels in adult cigarette smokers randomized to either snus or nicotine gum for 12 weeks. Total NNN levels were significantly reduced in nicotine gum–only users (0.01 ±0.01 pmol/mg creatinine [Cr]) compared with either nicotine gum dual users (0.05 ±0.05 pmol/mg Cr; p = 0.008) or snus-

only users (0.06 ±0.07 pmol/mg Cr; $p < 0.0001$); however, no differences were observed across dual users or between snus-only users compared with snus dual users.

7.5.5-2.5.2. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in Urine

Several studies examined levels of NNAL in the urine of tobacco users, who were differentially exposed to TSNAs based on product use or consumption habits (daily versus nondaily) (Berg, Schauer, Ahluwalia, & Benowitz, 2012; Wei, Blount, Xia, & Wang, 2016). Other studies assessed the effects of switching products (from cigarette smoking to ST or another alternative) on exposure to TSNAs by measuring tobacco users' urinary NNAL levels (Allen et al., 2016; Hatsukami et al., 2016; Krautter et al., 2015; Ogden et al., 2015a).

To quantitatively estimate exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in the U.S. general population, Wei et al. (2016) examined data collected from the Centers for Disease Control and Prevention's 2011-2012 National Health and Nutrition Examination Survey (NHANES) to estimate urinary NNAL levels among smokers, ST and other tobacco product users, and nonusers. The investigators estimated absorbed daily dose of NNK using a probabilistic method based on a two-compartment model. The daily dose of NNK was calculated by multiplying the sampled creatinine-corrected urinary NNAL concentration, the sampled creatinine excretion rate normalized by bodyweight, and the ratio of the molecular weight of NNK to the molecular weight of NNAL. The GM (95 percent CI) for urinary total NNAL (NNAL and its glucuronides) levels in the ST group was 369 (236-578) pg/mL or 313 (196-502) pg/mg Cr. In comparison, in nonusers and smokers, mean total NNAL levels in urine was 1.08 and 200 pg/mL (1.19 and 216 pg/mg Cr), respectively. The mean estimated absorbed daily dose of NNK in the 2011 to 2012 U.S. population for cigarette smokers (80.7 ng/kg-body weight/day) was higher than that of ST users (13.0 ng/kg-body weight/day) and nonusers (0.12 ng/kg-body weight/day).

To investigate the correlation of levels of TSNA exposure with frequency of tobacco consumption, urine samples from 64 current cigarette smokers (37 nondaily smokers; 27 daily smokers), which included 14 concurrent users of other combustible tobacco products (11 nondaily smokers; 3 daily smokers) and 8 concurrent ST users (7 nondaily smokers; 1 daily smoker), were analyzed for NNAL levels (Berg et al., 2012). Among all subjects, mean NNAL in urine was 144.6 pg/mL. The mean urinary NNAL among daily smokers (238.7 pg/mL) was greater than that of nondaily smokers (71.9 pg/mL). Among nondaily smokers, the average number of cigarettes per day on smoking days and the number of days of ST use were associated with urine NNAL levels (all ps < 0.001), whereas, among daily smokers, the number of cigarettes per day was the only significant correlate of NNAL levels ($p = 0.02$).

Urine samples from 391 cigarette smokers randomized to snus (n = 196) or 4 mg nicotine gum (n = 195) for 12 weeks were analyzed for TSNA and nicotine metabolites (Hatsukami et al., 2016). Compared with baseline, urinary total NNAL was reduced only in nicotine gum users (1.39 pmol/mg Cr at baseline to 0.30 pmol/mg Cr at 4 weeks), but not snus users (1.28 pmol/mg Cr at baseline to 1.34 pmol/mg Cr at 4 weeks). At Week 4, total NNAL levels in urine were significantly reduced in nicotine gum–only users compared with either nicotine gum dual users (1.11 ±1.00 pmol/mg Cr; $p = 0.001$) or snus-only users (1.34 ±1.42 pmol/mg

Cr; $p < 0.001$), and significantly lower NNAL levels were observed in nicotine gum dual users (1.11 ±1.11 pmol/mg Cr) compared with snus dual users (1.55 ±1.67 pmol/mg Cr; $p < 0.005$). Similarly, in a study evaluating changes in BOEs in smokers who switched to snus, tobacco-heating cigarettes, or ultralow machine yield tobacco-burning cigarettes, Ogden et al. (Ogden et al., 2015a) observed statistically significant reductions in urine total NNAL ranging from 30 percent to 39 percent in all three groups at 12 and 24 weeks as compared with those at baseline levels.

Krautter et al. (2015) found that randomization of daily cigarette smokers to exclusive snus and dual use, respectively, was associated with a change in urinary NNAL from 596.37 ±282.61 ng/24 h at baseline to 496.67 ±330.54 ng/24 h at Day 5 and from 717.73 ±326.22 ng/24 h at baseline to 572.90 ±304.62 ng/24 h at Day 5. Tobacco abstinence was associated with a change from 672.00 ±299.84 ng/24 h at baseline to 267.89 ±146.77 ng/24 h at Day 5.

In a study assessing sex differences with respect to cigarette avoidance in daily smokers who switched products, exposure levels as measured by urinary total NNAL levels in men and women randomized to snus were 1.37 and 1.46 pmol/mg Cr, respectively, at baseline and 1.37 and 1.60 pmol/mg Cr 4 weeks after intervention (Allen et al., 2016). In comparison, randomization to nicotine gum was associated with a change in urinary total NNAL levels in men and women, respectively, from baseline levels of 1.31 and 1.68 pmol/mg CR to 0.67 and 1.04 pmol/mg Cr 4 weeks after intervention. Compared with men, women had significantly higher total NNAL levels at baseline ($p = 0.004$) and at Week 4 ($p = 0.05$). Whereas fewer men in the snus group completely avoided cigarettes than men in the gum group, there were no group differences in cigarette avoidance among women.

7.5.5-2.6 Other Biomarkers of Exposure

In addition to the common BOEs discussed in the previous sections, several studies examined less common biomarkers that are typically associated with a specific disease or condition, including markers of inflammation (cardiovascular disease), MHBMA (cancer), heavy metal exposure (time-to-pregnancy), or fractional exhaled nitric oxide (FeNO) (eosinophilic airway inflammation) (Krautter et al., 2015; Mehari, Hines, & Gillum, 2016; Nordskog et al., 2015; Ogden et al., 2015a; 2015b; Sapra et al., 2016).

Product switching (from cigarettes to exclusive ST, dual smoking/ST, or abstinence) was associated with a change in urinary MHBMA, a metabolite of the known human carcinogen 1,3-butadiene, from 8,911.75 ng/24 h at baseline to 707.07 ng/24 h (92.1% reduction) at Day 5 in exclusive snus users and from 7,688.81 ng/24 h at baseline to 3,833.51 ng/24 h (50.1% reduction) at Day 5 in dual users (Krautter et al., 2015). Tobacco abstinence was associated with a change from 10,702.67 ng/24 h at baseline to 738.32 ng/24 h (93.1% reduction) at Day 5 (Krautter et al., 2015). Similarly, in smokers who switched to snus, Ogden et al. (Ogden et al., 2015a) observed statistically significant reductions in urinary MHBMA levels of 67% and 54% at Weeks 12 and 24, respectively, compared with baseline levels.

In a study evaluating biomarkers of biological effect related to cardiovascular disease, Nordskog et al. (2015) found that inflammatory markers interleukin (IL)-12, IL-8, and soluble intracellular adhesion molecule 1 (sICAM-1) were elevated in the serum of smokers

compared with either nonconsumers of tobacco or ST consumers. Similarly, Ogden et al. (2015b) demonstrated that switching from cigarette smoking to one of the alternative tobacco products most consistently resulted in reductions in markers of inflammation at 12 and 24 weeks, including sICAM1 and white blood cell counts. Switching to tobacco-heating cigarettes had the greatest number of consistent reductions in markers of inflammation and oxidative stress; however, statistically significant differences in pairwise comparisons between product groups was not observed.

Sapra et al. (2016) examined heavy metal presence in the blood of ST users and cigarette smokers, and never users in a study assessing the relationship between tobacco use and TTP. In the study, cadmium (ng/mL) and lead (µg/dL) levels were higher in smokers (0.62 and 1.49, respectively) than in ST users (0.18 and 1.23, respectively) or never users (0.15 and 0.95, respectively). Cadmium levels were higher in smokers than in ST and never users, and adjusting for cadmium attenuated the cigarette-TTP association (especially among women). TTP was shorter among ST users when compared with cigarette smokers (FOR = 2.86, 95 percent CI: 1.47, 5.57).

Mehari et al. (2016) analyzed NHANES 2007-2012 data to assess the effects of ST on FeNO, a noninvasive marker of eosinophilic airway inflammation. In exposed men (use of ST within the past 5 days) and unexposed men, respectively, the GM FeNO was 14.30 and 16.59 ppb. Use of ST was associated with significantly lower natural logarithm FeNO after controlling for age and race (black versus nonblack).

7.5.5-2.7 Updated Findings

Information on BOEs associated with ST use in the updated literature review is consistent with that seen in the initial literature review. There is, however, information on BOEs, such as MHBMA and inflammatory markers, in the updated literature review that were not addressed in the initial review. The conclusions from the initial literature review (Section 7.5.5-1) have not changed based on the updated literature review.

A tabular summary of the literature informing BOEs associated with ST use is presented in Table 7.5.5-2-1.

Table 7.5.5-2-1: Literature Review for Exposure

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Yang et al., 2017)	Analysis of N'-nitrosonornicotine enantiomers in human urine by chiral stationary phase liquid chromatography–nanoelectrospray ionization–high resolution tandem mass spectrometry	Cross-sectional exposure study in 32 participants (20 cigarette smokers [an average of 5/d] and 12 ST users). 10 ST users used snuff (an average of 11g/d) and 2 used snus (an average of 4g/d); pooled urine from 6 nonsmokers was used as a negative control. Objective: to examine trace levels of enantiomeric metabolites of the carcinogen NNN in human urine.	NNN and (S)-NNN were assessed in the urine of participants.	Total NNN was significantly ($p = 0.005$) higher in ST users (snuff, 67.1 ±56.7 fmol/mL) than in cigarette smokers (20.5 ±27.1 fmol/mL); the highly carcinogenic (S)-NNN enantiomer accounted for significantly more of the total NNN in cigarette smokers (67% ±5%) than in conventional (snuff) ST users (56% ±3%) ($p < 0.001$).	Strengths: Quantified trace levels of enantiomeric metabolites in human urine; compared both total NNN and the more carcinogenic (S)-NNN in the urine of participants. Limitations: Small sample size; sex and age of participants are not reported.
(Allen et al., 2016)	Gender differences in snus versus nicotine gum for cigarette avoidance among a sample of U.S. smokers	A randomized, interventional study in daily smokers for the past year who were willing to switch from cigarettes to snus or nicotine gum; mean age = 43.9 ±12.5 years; n = 391(snus group [n = 196, 45% women]; nicotine gum group [n = 195, 49% women]). Objective: "To examine gender differences in response to snus versus nicotine gum for cigarette avoidance, as a means of harm reduction."	Cotinine, TNE, and total NNAL were assessed in the urine of participants.	Women had significantly higher total NNAL values at baseline and at Week 4 than men; at Week 4, men and women assigned to snus had higher total NNAL than those assigned to gum; baseline TNE and cotinine for men and women did not differ; assigned treatment did not affect TNE or cotinine at Week 4 for women, but men assigned to snus had greater TNE and cotinine at Week 4 than men assigned to gum. "Overall, fewer men in the snus group completely avoided cigarettes compared to men in the gum group (e.g., continuous abstinence at Week 12: OR = 0.43, 95% CI: 0.20, 0.93). Among women, there were no differences by randomization in cigarette avoidance."	Limitations: Men were more likely than women to use snus if they were assigned to that group. Women also smoked more cigarettes per week than men during the treatment period. Therefore, the differences in snus and cigarette usage between males of females likely affect the sex and intervention differences in the reported biomarkers.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Ebbert et al., 2016)	Nicotine metabolite ratio is associated with lozenge use but not quitting in smokeless tobacco users	A randomized interventional trial; daily users of primarily ST for ≥1 year; ST users were randomized to Assisted Self-Help, Lozenge-Assisted Self-Help, or Lozenge Self-Help cohorts; n = 152, 148 (97%) male, 4 (3%) female; 96% white/non-Hispanic; age ≥18 years. Objectives: to examine whether the NMR can be used to predict self-reported nicotine lozenge use and tobacco abstinence among ST users treated for tobacco dependence.	Saliva was analyzed for cotinine and 3'-hydroxycotinine.	Mean ±SD baseline exposure of nicotine metabolites were 410 ±288 ng/mL for cotinine, 120 ±121 ng/mL for 3'-hydroxycotinine, and 0.30 ±0.19 for NMR; mean NMR was not significantly correlated with baseline amount of ST used, years of use, or level of dependence; NMR was positively correlated with self-reported lozenge use.	Limitations: Data for BOEs were not reported for subjects at any time except baseline; participants were highly skewed toward males.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Flanagan et al., 2016)	Fetal exposure to carcinogens with tobacco use in pregnancy: phase 1 MAW study findings	A cross-sectional noninterventional study; pregnant women of an Alaska Native ethnicity; n = 148 enrolled; 54 smokers, 30 ST users (10 commercial ST users and 20 homemade Iqmik users), and 64 nonusers; nonusers had not used tobacco for at least 6 months; tobacco users had to have used tobacco in the last 7 days for inclusion; multiproduct users were categorized based on the product most frequently used; age ≥18 year. Objectives: "To correlate maternal cotinine levels with fetal exposure to a tobacco-specific carcinogen to incorporate in a biomarker feedback intervention to motivate tobacco cessation during pregnancy."	Urine biomarkers (cotinine and NNAL) were measured.	98% of nonusers had cotinine levels <50 ng/mL, indicative of nonuse; 93% of smokers, 85% of Iqmik users, and 100% of commercial ST users had cotinine levels ≥50 ng/mL, confirming them as active tobacco users. The correlation between maternal cotinine and newborn NNAL levels was not significant for commercial ST users (n = 9; r = 0.60; 95% CI: –0.11, 0.90; p = 0.088).	Strengths: Measured biochemical exposure to tobacco products to confirm use/nonuse. Limitations: Small sample size limits the statistical power of the study, particularly for ST users; study population was limited to women of an Alaska Native ethnicity, thus results may not be broadly applicable across ethnicities.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Hatsukami et al., 2016)	Randomised clinical trial of snus versus medicinal nicotine among smokers interested in product switching	Randomized, interventional study; cigarette smokers (≥10/d for ≥1 year) recruited in Minnesota and Oregon were randomized to either snus or 4 mg nicotine gum for 12 weeks; n = 391, 47.1% female, 81.8% non-Hispanic whites; age = 18-70 years, mean ±SD = 43.9 ±12.5 years. Objective: to examine the effects on biomarkers of tobacco exposure and product use of switching from cigarettes to snus or nicotine gum.	Urine samples were used to analyze levels of total NNAL and TNE.	Significant reductions in cotinine and TNE were observed from baseline to Week 4 for both nicotine gum and snus (both: $p < 0.0001$). A significant reduction in total NNAL was only observed at Week 4 for nicotine gum ($p < 0.0001$), but not for snus. "Comparisons of week 4 values were adjusted for baseline biomarker values and site, and corrected for multiple comparisons. Among product only and dual users, total NNAL was significantly higher among snus versus nicotine gum users ($p < 0.001$ and 0.005, respectively). While no significant differences were observed for the snus only and snus dual users, significantly lower levels of total NNAL were observed in the nicotine gum only versus gum dual users ($p = 0.001$)."	Strengths: Large sample size; measured biochemical levels of exposure along with reported levels of product use. Limitations: "(1) potential lack of generalizability to a general population of smokers because we examined smokers interested in trying an alternative product in a clinic setting, (2) testing only one snus product, which has lower levels of nicotine and higher TSNA than some of the Swedish snus products, (3) encouragement to use a specified number of pieces of each of the products; (4) implementation of a tapering period, which might have constrained substitution behavior; and (5) not examining the data by gender…"

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Mehari et al., 2016)	Smokeless tobacco use and fractional exhaled nitric oxide in men in a national survey	Data was generated by an analysis of NHANES 2007-2012; "Participants were categorized by smoking status in the past 30 days and the use of snuff or chewing tobacco in the past 5 days (yes/no)." Analysis was restricted to nonasthmatic men who used ST and nonsmokers (n = 3,791); 135 used ST, 3,656 were nonsmokers; age ≥18 years. Objective: "To estimate the association between use of ST and FeNO among U.S. men."	Exhaled FeNO was measured.	"In weighted linear regression analyses, use of [ST] was associated with significantly lower natural logarithm FeNO after controlling for age and race (black vs nonblack) (coefficient, –0.124; SE, 0.056; p = 0.03; 95% C:, –0.237, –0.011)." "Results were unchanged after additionally controlling for recent nitric oxide–rich vegetable consumption and upper respiratory tract infection, height, and body mass index (coefficient, –0.119; SE, 0.055; p = 0.04; 95% CI, –0.229 to –0.009)."	Strengths: Large, representative sample of the U.S. population and standardized measurement protocols with quality control checks. Limitations: Lack of data on women and on some possible confounders, such as recent exposure to electronic cigarettes, ozone air pollution, and use of inhaled corticosteroids 2 to 5 days before testing.
(Mushtaq & Beebe, 2016)	Assessment of the tobacco dependence screener among smokeless tobacco users	Community-based sample of exclusive ST users living in Oklahoma; participants were not currently smokers who had used ST (≥1 pouch or can per week); 92% of participants were everyday users of ST; n = 95; 100% of participants were male; aged 18-64 years, mean = 18.8 years. Objective: to evaluate the TDS, a self-administered 10-item screener, as a measure of tobacco dependence among ST users.	Salivary cotinine concentration was used as a criterion variable to assess the accuracy of the TDS.	Correlation analysis found that the TDS has a positive association with salivary cotinine concentration (r = 0.24, p = 0.018). "TDS demonstrated acceptable reliability and concurrent validity among" ST users.	Strength: Correlated between the TDS and cotinine concentrations, a biomarker of nicotine dependence. Limitations: Sample only consisted of males; limited data on exposure is presented because the study was designed to evaluate the TDS on tobacco dependence.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Sapra et al., 2016)	Time-to-pregnancy associated with couples' use of tobacco products	Multisite, longitudinal study; interview answers and serum samples were collected from 501 couples trying to become pregnant. Among males (age ≥18 years), 208 had never used tobacco, 48 were exclusive smokers, 28 were exclusive snuff/chew users, and 12 were smoking/ST dual users. Among females (aged 18-40 years), 357 had never used tobacco and 55 were exclusive smokers. Objective: to determine if couples' preconception tobacco use or specific chemicals may contribute to changes in TTP.	Blood samples were collected for quantification of heavy metals and cotinine. TTP was assessed.	Male ST users had GM (95% CI) levels of: cadmium (ng/mL): 0.18 (0.14-0.22) lead (µg/dL): 1.23 (1.06-1.43) cotinine (ng/mL): 60.00 (16.05-224.34) Lead and cotinine levels were significantly higher ($p < 0.05$) for ST users and smokers than for never users. Cadmium levels were comparable between never users and ST users, which both had levels significantly less than those for smokers ($p < 0.05$). "Compared with never users, [ST] did not alter TTP in our cohort; however, TTP was shorter compared with smokers… cadmium may partially contribute."	Strengths: First study to evaluate ST use in relation to TTP, recall biased was minimized by interviewing couples at the start of the pregnancy attempt. Limitations: Since there were small number of ST users, the authors may be underpowered to detect a difference and cannot conclude that ST has no effect on TTP. Furthermore, the authors could not evaluate female use of ST on TPP since women in the sample did not use ST. Another limitation is that about 20% of study population (mostly smokers) were lost to follow-up and therefore the association between smoking and TTP may be underestimated.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Wei et al., 2016)	Assessing exposure to tobacco-specific carcinogen NNK using its urinary metabolite NNAL measured in US population: 2011-2012	A cross sectional survey in a representative sample of U.S. residents collected by the 2011-2012 NHANES survey; n = 4,831 nonusers of tobacco; n = 961 cigarette users; n = 94 pipe or cigar users; n = 55 snuff or chewing tobacco users; age ≥6 years Objective: to quantitatively estimate exposure to NNK in the U.S. general population using urinary concentration of NNAL.	The daily absorption of NNK was estimated using urinary total NNAL concentration.	The GM (95% CI) for urinary total NNAL in the snuff or chewing tobacco group was 369 (236, 578) volume based (pg/mL) and 313 (196, 502) creatinine corrected (pg/mg creatinine). The GM of the estimated absorbed daily dose of NNK (ng/kg-body weight/d) in the U.S. population for 2011-2012 was 13.0 for cigarette smokers, 0.120 for nonusers of tobacco, 5.32 for pipe or cigar users, and 80.7 for snuff or chewing tobacco users. "[B]oth volume-based and creatinine-adjusted geometric means of total urinary NNAL for snuff or chewing tobacco users are >50% higher than those for all cigarette users." "Exclusive snuff or chewing product users had significantly higher daily dose of NNK than did cigarette smokers."	Strengths: Large cohort generated from multiple locations and representative of the U.S. general population; assessed exposure levels across a wide demographic range. Limitation: Exposure was not correlated to any health outcomes, such as biomarkers of effect.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Krautter et al., 2015)	Consumption patterns and biomarkers of exposure in cigarette smokers switched to Snus, various dissolvable tobacco products, Dual use, or tobacco abstinence	Randomized, open-label, parallel-group, intervention study; healthy U.S. smokers (≥10 cigarettes per day for ≥12 months, high or medium machine-measured "tar" yield, an FTND score ≥3) were recruited and randomized to the six groups. "Subjects smoked their usual brand of cigarette for 1 day prior to switching to their designated intervention condition"; n=167 (25-30/group); 46.1% female, 94.6% not Hispanic or Latino; aged 21-64 years, mean = 40.60 years. Objective: "To evaluate changes in levels of selected BOEs for smokers who switched to one of six conditions during clinical confinement: exclusive use of; Camel Snus, Sticks, Strips or Orbs, controlled Dual use of cigarettes and Camel Snus, or tobacco abstinence."	Thirty-two BOEs were analyzed from plasma, whole blood, urine, and feces.	Snus use produced statistically significantly greater reductions than dual use in 24 of the 32 measured BOEs, including total NNN. "After 5 days, substantial reductions of most biomarkers, including nicotine, were observed in all groups. Toxicant exposures were similar to being tobacco abstinent after switching exclusively to Camel Snus, Sticks, Strips or Orbs. Dual use reductions were more modest."	Strengths: Compared six different interventions on 32 BOEs. Limitations: The mandated 60% reduction in cigarettes smoked per day (from baseline) in the dual use group during intervention may or may not accurately reflect the way consumers actually dually use cigarettes and snus; the limited sample size and duration of the intervention period.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Nordskog et al., 2015)	Study of cardiovascular disease biomarkers among tobacco consumers, part 2: biomarkers of biological effect	An age-stratified, cross-sectional, single-site study; healthy U.S. adult males (aged 26-49 years) who were exclusive cigarette smokers (n = 60), moist snuff consumers (n = 48), and nonconsumers of tobacco (n = 60). Subjects were confined overnight and discharged the following day (Day 2). On Day 1, subjects observed a 45-minute tobacco abstention period followed by use of a single tobacco product, referred to as a challenge. Effects were measured at15 minutes and 30 minutes post-challenge; and.on the morning of Day 2 following an overnight tobacco abstention and fast. Objective: to evaluate biomarkers of biologic effect related to cardiovascular disease among cigarette smokers, moist snuff consumers, and nonconsumers of tobacco.	Biomarkers were analyzed from serum and urine samples.	Principal component analysis identified a separation between cigarette smokers and both moist snuff consumers and nonconsumers of tobacco, suggesting that IL-12(p70) (both $p < 0.0001$), sICAM-1 (both $p < 0.0001$), and IL-8 ($p = 0.0924$ and $p < 0.0001$), which are biomarkers for inflammation and immunity, are elevated in smokers compared with moist snuff consumers and nonconsumers of tobacco. "[T]his study has identified several [biomarkers of biological effect] that are dissimilar between consumers of combustible and non-combustible tobacco products and [non–tobacco consumers]." "[F]or the biomarkers measured, the risk profile of [moist snuff consumers] is skewed towards that of [non–tobacco consumers], with several biomarkers overlapping."	Strengths: Analyzed a wide range of biomarkers of effect. Limitations: Since male participants were included, the effect of products on females was not determined; biomarkers were measured after controlled abstention and usage periods, therefore they may not be representative of routine product usage.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Ogden et al., 2015a)	Switching from usual brand cigarettes to a tobacco-heating cigarette or snus: Part 2. Biomarkers of exposure	Randomized, multicenter, longitudinal, interventional study in 163 participants (50.9% female; 73% non-Hispanic white; 131 smokers, 32 never smokers). Adult smokers were randomly switched to tobacco-heating cigarettes (n = 44), snus (n = 43), or ultralow machine yield tobacco-burning cigarettes (n = 44), with a comparison group of never smokers at baseline only; aged 28-55 years. Objective: to assess differences in biomarkers of tobacco exposure between smokers and never smokers at baseline and among groups relative to each other and over time.	Biomarkers were determined from blood and urine samples.	The NNK exposure (i.e., as measured by total NNAL) was statistically significantly decreased (30%-39%) in all three product groups at Weeks 12 and 24 as compared with baseline (no significant pairwise group comparisons). In the snus group, no statistically significant difference at Week 12 compared with baseline was observed in serum cotinine, but a statistically significant increase was observed at Week 24 (approximately 32%). Results indicated that adult cigarette smokers switched from their usual brand of cigarettes to alternate tobacco products, including snus, had significantly reduced exposure to many potentially harmful constituents found in cigarette smoke.	Strengths: The long duration of the study (24 weeks), the extensive number of biomarkers evaluated, and the inclusion of the ultralow machine yield tobacco-burning cigarette group as a control and for comparison. Limitations: The predominantly white subject sample in general and the predominantly male sample in the per-protocol sample of smokers switched to snus, limiting generalizability of the findings.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Ogden et al., 2015b)	Switching from usual brand cigarettes to a tobacco-heating cigarette or snus: Part 3. Biomarkers of biological effect	Randomized, multicenter, longitudinal, interventional study in 163 participants (50.9% female; 73% non-Hispanic white; 131 smokers, 32 never smokers). Adult smokers were randomly switched to tobacco-heating cigarettes (n = 44), snus (n = 43), or ultralow machine yield tobacco-burning cigarettes (n = 44), with a comparison group of never smokers at baseline only; aged 28-55 years. Objective: to assess differences in biomarkers of tobacco exposure between smokers and never smokers at baseline and among groups relative to each other and over time.	Biomarkers were determined from blood and urine samples.	Results demonstrated that there were "decreases in markers of inflammation and oxidative stress in smokers switched to tobacco-heating cigarettes, snus, and ultralow machine yield tobacco-burning cigarettes." Switching to tobacco-heating cigarettes resulted in the greatest number of consistent reductions for markers of inflammation and oxidative stress.	Strengths: The 24-week duration of the study; comparisons between the effects of three different types of alternative tobacco products on a large number of health-related biomarkers. Limitations: Compliance with study product differed among participations; significant changes in the biomarkers assessed may not be predicative of clinical significance.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Pickworth et al., 2014)	Nicotine absorption from smokeless tobacco modified to adjust pH	Double-blind, within-subjects study; study participants had used ST for an average of 15 years and used an average of 1.1 tins per day; "compared nicotine absorption from a single unflavored referent ST product (pH 7.7) [Condition 3] that was flavored with a low level of wintergreen (2 mg/g) and the pH was amended to either high (8.3) [Condition 2] or low (5.4) [Condition 1] pH"; 43% of participants also smoked conventional cigarettes; n = 7 males; mean age = 45 years. Objective: to compare how pH and flavor influences absorption of nicotine from ST.	Plasma nicotine levels were measured.	Peak plasma nicotine levels occurred 20-35 minutes after product placed in participant's mouth for all three conditions. $AUC_{0\text{-}60min}$ (ng/mL/min) (mean ±SD): Condition 1: 736 ±461 Condition 2: 1,251 ±223 Condition 3: 1,195 ±706 Maximal average nicotine boost (ng/mL), corrected for baseline value (mean ±SD): Condition 1: 6.6 ±3.9 Condition 2: 20.0 ±4.2 Condition 3: 19.5 ±6.5 "This study shows that the unamended referent product and the high pH flavored product delivered significantly more nicotine than the low pH flavored product."	Strengths: Each participant was exposed to all three ST products; thus, the effects of pH could be compared among individual participants. Limitations: Small sample size; about half of study participants used cigarettes and ST, overrepresenting dual product use in the general population; effects of pH on nicotine absorption were not determined in women.

Author	Title	Study Methods	Primary Study Measurements and Endpoints	Author's Findings Related to Exposure	Author's Strengths/Limitations
(Berg et al., 2012)	Correlates of NNAL levels among nondaily and daily smokers in the college student population	Cross-sectional survey of 62 subjects (35 nondaily cigarette smokers and 27 daily cigarette smokers; 14 concurrently used other combustible tobacco products, and 8 concurrently used ST; 59.7% female, 72.6% non-Hispanic white; mean age = 26.3 years). Objective: to examine correlates of urine NNAL levels among nondaily and daily smoker.	Biomarkers were determined from urine samples.	"In multivariate analysis, average cigarettes per day on smoking days (B = 23.00, 95% CI: 13.81, 32.20; $p < 0.001$) and number of days of [ST] use (B = 17.11, 95% CI: 13.53, 20.70; $p < 0.001$) were associated with NNAL levels among nondaily smokers ($R^2 = 0.234$)." "Multivariate analysis indicated that average cigarettes per day (B = 15.83, 95% CI: 2.89, 28.76; $p = 0.02$) was the only significant correlate of NNAL levels among daily smokers."	Strengths: Compared BOEs between daily smokers and nondaily smokers; evaluated dual product use on levels of exposure. Limitations: Small sample size and limited generalizability of the cohort; self-reporting of tobacco use; dichotomy daily vs. nondaily smokers.

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