6.1.2 Clinical Studies

The epidemiological data presented in Section 6.1.1 provide evidence that use of Camel Snus presents a substantial reduction in health risks when compared to cigarette smoking. In addition to epidemiology, clinical studies of human subjects also support a conclusion that Camel Snus presents less risk than cigarette smoking. Clinical studies encompass a wide range of study designs and endpoints, and can include metrics such as nicotine pharmacokinetics and product preferences, subjective and physiological responses, and biomarkers of exposure and effect. Clinical studies that can be applied to MRTP evaluations were reviewed extensively in Hatsukami *et al.* 2009. Hatsukami *et al.* named five study types that are relevant to an MRTP evaluation: in-laboratory clinical trials; short-term clinical trials; intermediate-term clinical trials; abuse liability assessment studies; and cross-sectional studies. In-laboratory clinical trials involve product use by study subjects once or a few times, but only in a laboratory setting; short-term clinical trials involve use of a particular product or products at home or in a residential facility, and the products are used throughout the day for a period of less than 2 weeks; intermediate-term clinical trials are more extensive, lasting between 2 weeks and 12 months; abuse liability assessment studies involve studies of levels and rates of nicotine delivery, pharmacokinetics, methods of use, etc.; and cross-sectional studies can assess effects of longer periods of use and larger numbers of subjects, determined at a single point in time.

This section reviews the available clinical study data reported both in the published scientific literature and in clinical studies of Camel Snus sponsored by RJRT in support of this Application. The results of the clinical biomarker studies comparing toxicant exposure and early indicators of potential harm are consistent with the results of epidemiological studies. As compared with cigarette smoking, smokeless tobacco (ST) use presents less risk of lung cancer, oral cancer, respiratory disease and heart disease. Clinical studies provide evidence that cigarette smokers who switch completely to products such as Camel Snus will significantly reduce their harm and risk of these tobacco-related diseases compared to continued use of cigarettes.

6.1.2.1 Rationale for the use of human clinical studies in comparative evaluations of tobacco products

A fundamental premise of toxicology is that risk and exposure dose are correlated. As such, human clinical studies play a critical role in assessing MRTPs by providing information about how a candidate MRTP's composition and manner of use translate into exposure to toxicants and related effects in users (IOM 2012). Given the long latency period between tobacco or tobacco smoke exposure and the development of overt disease, validated biomarkers provide important information over a much shorter time frame. Therefore, the primary purpose for evaluating a candidate MRTP in human clinical studies is to provide an understanding of changes in tobacco-related toxicant exposure and biological effects in users of the candidate product compared with other tobacco products (*e.g.,* Camel Snus vs. cigarettes). Clinical study results provide a sound foundation for science-based assessment of a candidate MRTP and its potential to reduce health risks relative to other tobacco products.

Human clinical studies frequently involve determining how using a candidate MRTP affects changes in or leads to differences in biomarkers of exposure and effect (also referred to as biomarkers of potential harm), which serve as proxies for harm (Hatsukami *et al.* 2009). The importance of including research on biomarkers as part of the scientific evidence to support an MRTP is emphasized in the FDA Draft Guidance document for MRTPs: "FDA recommends that applicants conduct human studies to assess the full range of the human health risks related to the use of the tobacco product, including exposure to tobacco-related compounds (*e.g.*, biomarkers of exposure) and health outcomes (*e.g.*, disease incidence or mortality)..." (FDA 2012a, p. 25). Similarly, the Institute of Medicine outlined recommendations for studies to be used in the evaluation of MRTPs, including the statement that "validated biomarkers and other surrogates of tobacco-related disease outcomes that can provide information over a shorter time frame, therefore, will play a critical role in the evaluation of MRTPs" (IOM 2012, p. 80).

Available biomarkers can be separated into two broad categories—biomarkers of exposure and biomarkers of effect (also referred to as biomarkers of risk or biomarkers of potential harm) (IOM 2012; USDHHS 2010; LSRO 2007b). Some investigators have further classified them into four groups: a) measures of chemical exposure; b) measures of toxicity; c) measures of early injury or potential harm; and d) direct measures of health outcomes (Hatsukami *et al.* 2006). Since most of the serious health outcomes caused by smoking arise from cumulative effects that develop over many years, few, if any, viable biomarkers of this latter category are available. This section of the Application uses the broader two-category classification (exposure and effect/potential harm) to discuss biomarkers results for smokers and ST users reported in the published literature.[1]

Biomarkers of exposure refer generally to any chemical, or its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in a compartment in an organism (IOM 2012). Biomarkers may be the constituents themselves, metabolites of the constituents in urine, blood, breath, saliva, nails, or hair; or protein- or DNA-binding products (adducts) of the constituents or their metabolites (IOM 2012, p. 81). Biomarkers of biologically effective dose (*see* Figure 6.1.2-1 below), a concept related to exposure, is defined as the amount of a tobacco constituent or metabolite that actually binds to or alters a macromolecule (USDHHS 2010, p. 52). A key advantage of human exposure biomarkers over measurements of either product composition or product yield assessed via machine smoking methods is that biomarkers provide a realistic and direct assessment of toxicant dose for an individual, and are considered reliable metrics of the levels of exposure that consumers actually experience when using tobacco products (Hecht *et al.* 2010; Chang *et al.* 2016). Biomarkers of exposure offer the advantage of integrating product differences and product use behaviors to measure an individual's constituent or toxicant exposure over hours, days, or weeks, depending on the specific compound's clearance rate in an individual user (Ashley *et al.* 2010; Gregg *et al.* 2013). A limitation of using biomarkers of exposure as part of an MRTPA assessment arises from differences in the manner and extent of toxicant metabolism

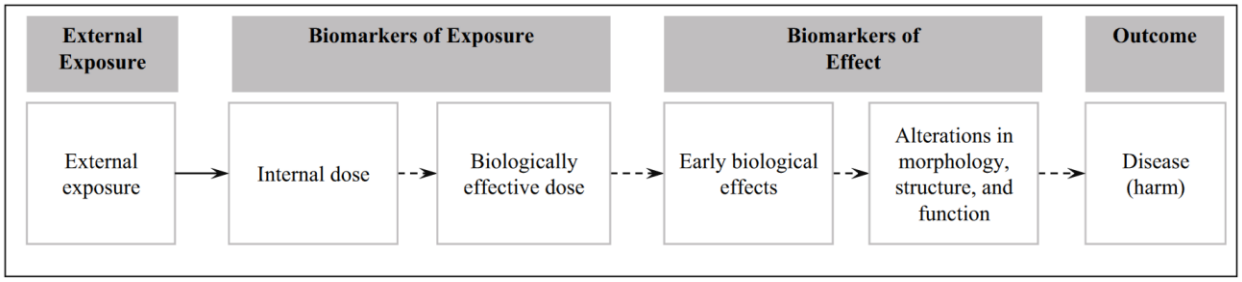
[1] Additional discussion is presented in several recent reviews (*e.g.*, ENVIRON 2013; USDHHS 2010; IARC 2007b; and RJRT's Citizen Petition Appendix A) included as part of this application.

influenced by an individual's genetic makeup. An equally important consideration in the context of cross-category comparisons of tobacco products such as cigarettes and smokeless tobacco is the influence of the route of exposure on toxicant metabolism and of subsequent interpretation of biomarker data from those tobacco users.

A biomarker of effect, sometimes referred to as a biomarker of potential harm, reflects biological changes that may result from toxicant exposure and is selected to indicate potential changes along a disease pathway prior to the manifestation of disease (USDHHS 2010; IOM 2012). Quantification of effects biomarkers in surrogate tissues is also performed in instances where sampling of primary target tissues is impractical. A number of biomarkers of exposure and effect relevant to tobacco use have been identified and can be tested in biological samples such as saliva, blood, urine, and exhaled breath. However, there is currently no single accepted biomarker that predicts the risk of disease in people who use tobacco products (IOM 2012, p. 82), but rather, it has been recommended that a panel of biomarkers be employed to most accurately estimate exposure (IOM 2012, p. 92), and possibly, risk[2]. Extensive reviews of biomarkers in both cigarette smokers and ST users, including snus users, have been published (*e.g.,* USDHHS 2010; ENVIRON 2013; IARC 2004; IARC 2007b; Frost-Pineda *et al.* 2011).

A graphical representation of the relationship among biomarkers of exposure and effect and disease outcomes is presented below (from LSRO 2007b, p. 47):

Figure 6.1.2-1: Relationships among biomarkers of exposure and effect and disease outcome



Note: Broken lines indicate that the biomarkers used may or may not be directly related to the final disease or condition.

6.1.2.2 Published clinical studies of tobacco product exposure, effect, and use

The following sections present information from a representative selection of published clinical studies of biomarkers of tobacco exposure and effect identified using the National Library of Medicine's PubMed database. These studies demonstrate that users of smokeless tobacco products are exposed to far fewer toxicants and substantially lower levels of most, but not all, cigarette smoke-related toxicants, particularly toxicants related to tobacco combustion. These studies also demonstrate reduced levels of biomarkers of pathological effect in smokeless

[2] Church *et al.* 2009 and Yuan *et al.* 2011a reported that urinary NNAL may predict lung cancer risk in smokers; Yuan *et al.* 2011b reported that urinary total NNN may predict esophageal cancer risk in smokers.

tobacco users, compared to cigarette smokers, even though smokeless tobacco users are exposed to similar or higher levels of nicotine. This section reviews published biomarker studies of smokers and smokeless tobacco users, including studies of Camel Snus. Results from studies of traditional U.S. ST products, many of which contain toxicant levels often higher than are found in Camel Snus, are relevant for discussion, as they comprise a relatively large data set, and inform as to comparative levels of biomarkers between cigarette smokers and ST users. Importantly, it is these traditional ST products whose health effects have been evaluated in epidemiological studies, and which have been demonstrated to present lower risk for most smoking-related diseases.

6.1.2.2.1 Biomarkers of exposure in smokers and smokeless tobacco users

A wide range of exposure biomarkers, obtained from a variety of biological matrices, has been evaluated in cigarette smokers. Historically, a smaller subset of biomarkers has been examined in smokeless tobacco users, including snus users. More recently, however, studies have focused greater attention on exposure biomarkers for snus products, including Camel Snus. The most extensively studied biomarkers of tobacco exposure across all forms of tobacco use are nicotine and its metabolites, primarily the long-lived nicotine metabolite cotinine. Types of studies range from blood nicotine measurements of single-use effects, typically to obtain pharmacokinetic data, to measures of urinary cotinine and urinary total nicotine equivalents (the sum of nicotine, cotinine, 3-hydroxycotinine and their glucuronides) as indicators of daily nicotine intake. Nicotine biomarkers will be summarized in a later section in the context of smokers and ST users together, since once nicotine enters circulation, its distribution, metabolism and excretion are independent of the route of administration (IARC 2007b, p. 252). Results of nicotine biomarker studies indicate that daily nicotine exposures are similar or higher in ST users compared with smokers. Toxicant exposures and their corresponding biomarkers will be considered individually for smokers and ST users, and then comparatively to each other, since the levels of most toxicants differ considerably between smokers and ST users.

6.1.2.2.1.1 Biomarkers of toxicant exposure in smokers

Cigarette smoke contains approximately 80 chemicals that have been identified as known, probable, or possible carcinogens by the International Agency for Research on Cancer (IARC), the U.S. National Toxicology Program (NTP), and EPA (Smith *et al.* 1997; Smith *et al.* 2000; Smith *et al.* 2001; Smith *et al.* 2003; NTP 2014). More recently, FDA has created a list of 93, and an abbreviated list of harmful and potentially harmful constituents (HPHCs) that are variously found in cigarette smoke (18), smokeless tobacco (9) and in other tobacco products (*see* Table 6.1.2-1 below from FDA 2012b, p. 4). FDA selected the constituents on the abbreviated list based on the availability of established analytical methods, and to represent the different chemical classes of tobacco toxicants.

Table 6.1.2-1: Abbreviated list of harmful and potentially harmful constituents

HPHCs in Cigarette Smoke	HPHCs in Smokeless Tobacco	HPHCs in Roll-your-own Tobacco and Cigarette Filler
Acetaldehyde	Acetaldehyde	Ammonia
Acrolein	Arsenic	Arsenic
Acrylonitrile	Benzo[a]pyrene	Cadmium
4-aminobiphenyl	Cadmium	Nicotine (total)
1-aminonaphthalene	Crotonaldehyde	NNK*
2-aminonaphthalene	Formaldehyde	NNN**
Ammonia	Nicotine (total and free)	
Benzene	NNK*	
Benzo[a]pyrene	NNN**	
1,3-butadiene		
Carbon monoxide		
Crotonaldehyde		
Formaldehyde		
Isoprene		
Nicotine (total)		
NNK*		
NNN**		
Toluene		

*4-(methynitrosamino)-1-(3-pyridy1)-1-butanone
** N-nitrosonornicotine

An extensive body of literature published over the last decades has examined biomarkers of exposure to many cigarette smoke toxicants, including HPHCs, as well as other constituents (*e.g.*, IARC 2004; Scherer 2006; Roethig *et al.* 2009; USDHHS 2010). Extensive literature has likewise examined various biomarkers of cigarette smoke exposure that have been used to assess potential reduced-exposure products (PREPs), including biomarkers of tobacco-related carcinogen exposure (*see* Gregg *et al.* 2013; Hecht *et al.* 2010; USDHHS 2010, p. 230; Hatsukami *et al.* 2006). Of particular importance are those biomarkers that have been analytically validated, and for which there is a noticeable difference in concentrations between tobacco users and non-users, whose levels decrease as cigarette use decreases (confirmed in product switching or cessation studies), and whose presence and concentrations are not usually confounded by exposure to dietary or environmental sources. These biomarkers include nicotine equivalents, carboxyhemoglobin (COHb), urinary NNAL and NNAL-glucuronide, and

carbon monoxide, which are all significantly elevated in cigarette smokers compared with nonsmokers (Hatsukami *et al.* 2006; Theophilus *et al.* 2015).

Just as there is a range of cigarette smoke exposure biomarkers, there are also a number of different matrices that may be examined for the presence of these biomarkers, including exhaled breath, saliva, urine, and blood or serum. Urinary biomarkers are the most widely applied biomarkers of carcinogen exposure in smokers, in part because sample collection is less invasive than obtaining blood samples (reviewed in Hecht 2002; USDHHS 2010, p. 230; Gregg *et al.* 2013). Biomarkers of cigarette smoke exposure detected in blood or serum include COHb (a measure of carbon monoxide exposure), nicotine, cotinine and NNAL, all of which are elevated in smokers (IARC 2004, pp. 1060-1068). Salivary biomarkers of exposure such as the nicotine metabolite, cotinine (IARC 2004), and the hydrogen cyanide detoxification product, thiocyanate (Scherer 2006), are elevated in cigarette smokers and can be used to distinguish smokers from nonsmokers.

Toxicants found in cigarette smoke may be divided between products of combustion, which are formed during the burning of cigarettes, and direct transfer of tobacco constituents, which are aerosolized during smoking and transferred to the smoker with minimal alteration.

Compared with never-users of tobacco, smokers are exposed to elevated levels of a wide spectrum of combustion products. Carbon monoxide (CO) is a product of incomplete combustion of organic materials, including tobacco, and is thought to contribute to cardiovascular risk and adverse reproductive effects (USDHHS 2010). It effectively competes with oxygen for binding to hemoglobin, resulting in the formation of COHb, a well-established biomarker that may be measured in blood samples. Exhaled CO levels correlate well with COHb levels, and provide a less invasive method for measuring CO exposures. Smokers show significantly elevated COHb and exhaled CO levels compared with nonsmokers (Scherer 2006).

Aromatic amines and heterocyclic amines are combustion products found in the particulate phase of tobacco smoke (USDHHS 2010). The aromatic amine 4-aminobiphenyl (4-ABP) is an IARC Group 1 human bladder carcinogen and is known to form covalently bonded protein adducts in the body. Human exposure to 4-ABP can be assessed by either measuring urinary 4-ABP or by measuring levels of 4-ABP-hemoglobin adducts (4-ABP-Hb) in blood (discussed in Roethig *et al.* 2009; Hecht 2003; Riedel *et al.* 2006). Elevated levels of 4-ABP-hemoglobin adducts in serum can clearly distinguish smokers from non-smokers (Perera *et al.* 1987).

Thiocyanate, the chief metabolite of the combustion product hydrogen cyanide, is substantially elevated in the serum of smokers but is not elevated in either ST users or in non-users of tobacco (Holiday *et al.* 1995). However, the value of thiocyanate as a biomarker of cigarette smoke exposure has been questioned by some due to numerous dietary sources of both thiocyanate and its precursor, hydrogen cyanide (Scherer 2006).

Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion of organic materials, including tobacco, and include the IARC Group 1 carcinogen benzo[a]pyrene, which is widely considered to be a potential causative agent for lung cancer. Multiple PAHs, including 14

rated as having sufficient evidence for carcinogenicity in laboratory animals, are present in cigarette smoke (Hecht 2012). 1-hydroxypyrene (1-HOP) (a non-carcinogenic pyrene metabolite often used an indicator of PAH exposure) is a widely used but non-specific urinary biomarker for PAHs (USDHHS 2010, p.230; Hatsukami *et al.* 2006). 1-HOP is elevated in the urine of smokers and declines with smoking cessation or reduced cigarette use (Lowe *et al.* 2013; Heudorf and Angerer 2001). However, results for individuals may be confounded by the multiple environmental sources of exposure, including diet (USDHHS 2010, p. 53). Biomarkers of other PAHs have also been studied; 1-hydroxyfluorene, 2-hydroxynapthalene, and 1-/ 9-hydroxyphenanthrene exposures are all significantly elevated in smokers compared with non-smokers (Hagedorn *et al.* 2009; St. Helen *et al.* 2012).

In addition to PAHs, a number of volatile organic compounds (VOCs) are found in mainstream cigarette smoke. Ethylene oxide, benzene and 1,3-butadiene are IARC Group 1 carcinogens (IARC 2012, p. 44), and acrolein-DNA adducts are found in lung tissues of smokers (Yuan *et al.* 2012). Some VOCs, such as benzene, 1,3-butadiene, and 2,5-dimethylfuran, may be detected in exhaled breath (IARC 2004, p. 1060; Gordon *et al.* 2002). However, VOCs are most frequently assessed as mercapturic acid derivatives measured in urine. Urinary mercapturic acids are well-established and validated biomarkers for uptake of acrolein, benzene, 1,3-butadiene, crotonaldehyde, and ethylene oxide, and all are found at higher levels in the urine of smokers than in non-smokers (Yuan *et al.* 2012; Hecht *et al.* 2010). Mercapturic acid biomarkers for acrylamide and acrylonitrile have been identified and can also distinguish smokers from non-smokers (Yuan *et al.* 2012; Minet *et al.* 2011; Hecht *et al.* 2010; Urban *et al.* 2006).

Acrolein (2-propenal) is formed naturally in the body as a result of lipid peroxidation and amino acid metabolism, but is also a toxicant in mainstream cigarette smoke. The biological effects of acrolein are a consequence of its reactivity towards biological nucleophiles such as guanine in DNA and cysteine, lysine, histidine, and arginine residues in critical regions of nuclear factors, proteases, and other proteins. Studies have shown that urinary levels of 3-(hydroxypropyl)mercapturic acid (3-HPMA), a biomarker of acrolein exposure, are typically higher in smokers compared with nonsmokers, and are reduced upon smoking cessation (Carmella *et al.* 2007; Roethig *et al.* 2009; Carmella *et al.* 2009).

Benzene, an IARC Group 1 carcinogen, has been described by the U.S. Surgeon General as another very prevalent potent carcinogen in cigarette smoke and the most probable cause of leukemia in smokers (USDHHS 2010, pp. 227, 300). S-phenylmercapturic acid (SPMA) is a validated biomarker for exposure to benzene, and has been used in many recent studies to monitor benzene exposure in cessation and switching studies (Hatsukami *et al.* 2006; Carmella *et al.* 2009; Hatsukami *et al.* 2010).

1,3-butadiene has been described by the U.S. Surgeon General as one of the most prevalent potent carcinogens in cigarette smoke (USDHHS 2010, p. 227). Two metabolites have historically been used to indicate exposure to 1,3-butadiene, although recent publications have concluded that monohydroxybutenylmercapturic acid (MHBMA), rather than 1,2-

dihydroxybutylmercapturic acid (DHBMA), is the preferred biomarker for 1,3-butadiene exposure from cigarette smoking (LSRO 2007b; van Sittert *et al.* 2000; Carmella *et al.* 2009).

Crotonaldehyde is a volatile unsaturated aldehyde (like acrolein) in tobacco smoke, which is mutagenic in various systems and causes liver tumors in rats (Carmella *et al.* 2009; Scherer *et al.* 2006). It is a possible human carcinogen based on its genotoxic activity (U.S. EPA Integrated Risk Information System). A metabolite of crotonaldehyde, 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), is measurable in urine of both smokers and nonsmokers, and is decreased upon smoking cessation (Scherer *et al.* 2007; Carmella *et al.* 2009).

Ethylene oxide is a volatile combustion product present in cigarette smoke and is considered carcinogenic to humans by IARC, based on a combination of epidemiological evidence for associations between occupational exposure to ethylene oxide and lymphatic and hemoatopoietic malignancies and consistent mechanistic data demonstrating its alkylating and mutagenic effects in various test systems and humans. Inhalation studies demonstrate that ethylene oxide causes alveolar/bronchiolar adenomas and carcinomas of the lung in mice but not in rats (IARC 2008). 2-hydroxyethyl mercapturic acid (HEMA) is a urinary biomarker that allows for quantitation of ethylene oxide exposure and has been validated to distinguish differences in exposure not only between smokers and nonsmokers but also between smoking of conventional and test cigarettes with a highly activated carbon granule filter (Scherer *et al.* 2010).

Acrylamide is a neurotoxin and an IARC Group 2A probable human carcinogen based on evidence of carcinogenicity in experimental animals (Smith *et al.* 2000; NTP 2014). The mercapturic acids of acrylamide, AAMA (N-acetyl-S-(2-carbamoylethyl)-L-cysteine) and its metabolite glycidamide, GAMA (N-(R/S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine), are used to determine acrylamide exposure in smokers and nonsmokers (Urban *et al.* 2006).

Acrylonitrile is an IARC Group 2B carcinogen present in cigarette smoke. Urinary 2-cyanoethylmercapturic acid (CEMA) is an acrylonitrile metabolite and a biomarker for acrylonitrile exposure. Smokers excrete >100-fold higher amounts of urinary CEMA than non-smokers, and CEMA levels in smokers are significantly correlated with ISO tar yield, daily cigarette consumption, and urinary biomarkers of smoke exposure (Minet *et al.* 2011)

Besides the tobacco combustion products described above, a number of tobacco constituents are transferred during the process of smoking directly to the smoker without significant alteration. Tobacco-specific nitrosamines (TSNAs) are formed in the tobacco plant, mainly during the curing of tobacco. Their levels vary widely depending on tobacco processing and the types of tobacco used in product formulation. While the largest fraction of TSNA in mainstream tobacco smoke is the result of transfer, there is evidence that between 5% and 25% is pyrosynthesized during the smoking process (Moldoveanu and Borgerding 2008). The most well-studied TSNAs, NNK and NNN, are IARC Group 1 carcinogens and are considered by some researchers to be important drivers of cancer risk associated with tobacco use (*e.g.*, Hecht *et al.* 2015). Total NNAL and total NNN (the sum of free and glucuronidated NNK and NNN, respectively) measured in urine are especially useful biomarkers of tobacco exposure and

possibly cancer risk, since they are present in all tobacco products, are tobacco-specific, and are considered strong carcinogens (Hecht *et al.* 2015). NNK is a strong systemic lung carcinogen in rodents, and its metabolites (NNAL plus glucuronides), often analyzed in urine samples, may provide the most useful discriminatory carcinogen biomarker for tobacco use (USDHHS 2010, p. 234). There is also animal-based evidence indicating that NNN may be a cause of esophageal cancer (Hecht 2003), and the development of a urinary biomarker of NNN exposure in smokers and smokeless users has been reported (Stepanov and Hecht 2005). In smokers, urinary levels of nicotine, cotinine and related metabolites, and NNAL and its glucuronides (metabolites of NNK), correlate generally with the number of cigarettes smoked per day (Joseph *et al.* 2005). When smokers switch to lower TSNA cigarettes or to a nicotine patch, total NNAL levels are significantly decreased (Hatsukami *et al.* 2004; Ashley *et al.* 2010). More recent studies suggest that urinary levels of total NNAL in smokers may also be statistically associated with lung cancer risk in a dose-dependent manner (Yuan *et al.* 2009; Yuan *et al.* 2011a). Urinary total NNN levels have also been epidemiologically associated with increased risk for esophageal cancer in a large cohort of male Chinese smokers (Yuan *et al.* 2011b).

Other biomarkers assessed in smokers indicate elevated exposures to certain trace metals such as lead, cadmium and arsenic, but these substances are widespread in the environment, and thus difficult to ascribe to smoking exposures exclusively. While reports indicate that urinary and blood levels of some metals in smokers may exceed those of nonsmokers, the levels may not be indicative of an increased risk for disease (Marano *et al.* 2012a; Marano *et al.* 2012b).

A commonly assessed serum biomarker of cigarette smoke exposure is the presence and level of carcinogen-DNA and protein adducts in both target and surrogate tissues. DNA adducts are useful markers of carcinogen exposure, providing an integrated measurement of carcinogen intake, metabolic activation, and delivery to the target macromolecule in target tissues (Phillips 2005) as well as being indicators of tobacco-induced DNA damage (Hecht 2003; Hecht *et al.* 2015). DNA adducts are sometimes referred to as biomarkers of biologically effective dose since they reflect exposure to an agent at a specific target site. According to the 2010 report of the U.S. Surgeon General, overwhelming evidence indicates that DNA adducts are higher in most tissues of smokers compared with nonsmokers (USDHHS 2010, p. 232). Tobacco carcinogens may also form adducts with proteins. Hemoglobin adducts of TSNAs, 4-ABP and other toxicants are present at higher levels in smokers compared with nonsmokers (Hecht 2003).

Hecht 2006 provided a review and summary of the levels of carcinogenic substances found in mainstream cigarette smoke. He suggested that "the most important, based on their carcinogenic potency and levels in cigarette smoke are probably PAH, N-nitrosamines, aromatic amines, 1,3-butadiene, benzene, aldehydes, and ethylene oxide."

A list of representative exposure biomarkers related to tobacco carcinogens and toxicants, including ranges of values found for smokers and nonsmokers, was published by Hecht and co-workers (Hecht *et al.* 2010), and reproduced in a report from the Institute of Medicine (IOM 2012, pp. 84-87). Only tobacco users and individuals exposed to secondhand smoke exhibited elevated levels of biomarkers of exposure of tobacco-specific compounds such as TSNAs and

nicotine, while biomarkers of combustion products were detected in smokers and nonsmokers alike due to the nearly ubiquitous presence of combustion products in the environment. As a consequence, there is sometimes overlap between the value ranges for smokers and nonsmokers. The list presented below (adapted from IOM 2012, Table 3-1, pp. 84-87) contains analytically validated biomarkers for many of the HPHCs in cigarette smoke identified by the FDA (FDA 2012b).

Table 6.1.2-2: Representative exposure biomarkers related to tobacco carcinogens and toxicants (from Hecht *et al.* 2010; IOM 2012)

Urinary biomarkers[a]	Source	Range of mean biomarker values (nmol/24 h unless otherwise noted)	
		smokers	nonsmokers
nicotine equivalents[b]	nicotine	70.4 – 154 µmol/24 h	NA[c]
total NNAL	NNK	1.1 – 2.9	NA
total NNN	NNN	0.049 – 0.24	NA
1-HOP	pyrene	0.50 – 1.45	0.18 – 0.50
MHBMA	1,3-butadiene	15.5 – 322	0.65 – 7.5
SPMA	benzene	3.2 – 32.1	0.17 – 3.14
HPMA	acrolein	5,869 – 11,190	1,131 – 1,847
HBMA	crotonaldehyde	9,800 – 26,000	1,200 – 3,200
HEMA	ethylene oxide	19.1 – 102	6.51 – 38.8
Cd	cadmium	2.3 – 12.8	1.34 – 8.04
Hemoglobin adducts[d]	**Source**	**(pmol/g globin; mean ± S.D.)**	
		smokers	**nonsmokers**
cyanoethylvaline	acrylonitrile	112 ± 81	6.5 ± 6.4
carbamoylethylvaline	acrylamide	84.1 ± 41.8	27.8 ± 7.1
hydroxyethylvaline	ethylene oxide	132 ± 92	21.1 ± 12.7
4-aminobiphenyl-globin	4-aminobiphenyl	0.26 ± 0.006[e]	0.067 ± 0.009[e]
Other	**Source**	**smokers**	**nonsmokers**
exhaled CO	carbon monoxide	17.4 – 34.4 ppm	2.6 – 6.5 ppm
carboxyhemoglobin	carbon monoxide	3.4 – 7.1%	0.35 – 1.45%

[a]Measured in nmol/24 h unless noted otherwise (based on 1.3 g creatinine per 24 h in smokers and 1.5 g creatinine per 24 h in nonsmokers, or 1.5 L urine per 24 h).

[b]Abbreviations: nicotine equivalents, the sum of nicotine, cotinine, 3′-hydroxycotinine, and their glucuronides; total NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; total NNN, N′-nitrosonornicotine and its glucuronides; 1-HOP, 1-hydroxypyrene and its glucuronides/sulfates; MHBMA, the sum of 1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene; SPMA, S-phenyl mercapturic acid; HPMA, 3-hydroxypropyl mercapturic acid; HBMA, 4-hydroxybut-2-yl mercapturic acid; HEMA, 2-hydroxyethyl mercapturic acid.

[c]N/A = Not applicable because these are not detected in the urine of nonsmokers unless they use other tobacco products, nicotine replacement products (for nicotine equivalents, and sometimes NNN (Stepanov *et al.* 2009)), or are exposed to secondhand smoke, in which case levels are usually less than 5 percent of smoker levels (IARC 1999b; IARC 2006).

[d]Measured in pmol/g globin; mean ± S.D.
[e]Weighted mean ± S.D.

Finally, urinary mutagenicity in smokers has been shown to be an effective and reliable method of quantifying human exposure to mutagens created by combusted tobacco (IOM 2012, p. 114) and generally correlates with the number of cigarettes smoked (reviewed in DeMarini 2004). DeMarini's review suggested that the chemicals responsible for smoking-related urine mutagenicity determined in bacterial mutagenesis assays are primarily aromatic amines and/or heterocyclic amines (DeMarini 2004), carcinogenic combustion products found in relatively high levels in cigarette smoke (Hecht 2006). A more recent study, however, found that heterocyclic aromatic amines contribute only modestly (~2-15%) to the total mutagenic activity of mainstream tobacco smoke (Liu *et al.* 2013). Nevertheless, urine mutagenicity as a biomarker is highly relevant because: (1) it reflects exposure to both known and potentially unknown mutagens; (2) it goes beyond measurement of individual chemicals to capture an integrative toxicological response in an integrative biological matrix (24-h urine); and (3) it displays a clear dose–response relationship to reductions in cigarettes smoked per day, even over short experimental time-frames (Theophilus *et al.* 2015, p. 233).

6.1.2.2.1.2 Biomarkers of toxicant exposure in smokeless tobacco users

Clinical studies of tobacco exposure biomarkers in ST users have focused primarily on traditional smoking-related constituents, including TSNAs and other tobacco toxicants and their metabolites, and of nicotine and its primary metabolite cotinine in serum, urine, and saliva. This section presents representative published data for biomarkers of exposure from use of U.S. and Swedish ST products, many of which contain toxicant levels often higher than are found in Camel Snus, but which inform as to comparative levels of biomarkers between cigarette smokers and users of non-combusted tobacco products, and provide context when evaluating biomarkers of exposure in Camel Snus users discussed in later sections.

***Tobacco combustion products*:** Compared with smokers, ST users are exposed to fewer toxicants and HPHCs, especially those toxicants related to combustion. The chemical profiles of cigarette smoke and ST are substantially different. ST lacks or has considerably lower concentrations of many of the carcinogens and other toxicants formed during the combustion of tobacco, including CO, polycyclic aromatic hydrocarbons, aldehydes, and other VOCs such as ethylene oxide, benzene, and acrolein (Hecht *et al.* 2007). Because ST is not combusted, and does not produce mainstream smoke or environmental tobacco smoke, it eliminates exposure in both users and non-users to the combustion products found in cigarette smoke, except for exposures among ST users to the much smaller amounts of some combustion products that remain in those ST products that contain fire-cured tobacco. The differences in chemistry, combined with the route of exposure in ST users, allow ST users to reduce or eliminate exposures to many combustion products and eliminate direct exposure of lung tissues to the harmful effects of those compounds. Presented below are the specific results from comparisons of biomarkers of tobacco combustion products in ST users and smokers.

The FDA's abbreviated list of HPHCs includes 18 found in tobacco smoke, but only 9 found in smokeless tobacco (FDA 2012b, p. 4). Compared with smokers, ST users have lower levels of the biomarkers for most combustion products. A 1995 study investigating possible biomarkers that could distinguish among non-users, ST users, and smokers found that serum levels of thiocyanate (a biomarker for hydrogen cyanide uptake) were 4.5-fold higher in smokers than in ST users (Holiday *et al.* 1995). Urinary and blood biomarkers of acrolein, benzene, pyrene, carbon monoxide and 1,3-butadiene are also significantly lower among moist snuff users compared to smokers, and not significantly different from levels in non-users of tobacco (Campbell *et al.* 2015).

Users of traditional U.S. moist snuff have higher median levels of NNAL and its glucuronides per milliliter of urine (Hecht *et al.* 2007), and possibly higher blood levels of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), a marker for hemoglobin adducts of NNK and NNN (Schaffler *et al.* 1993). Despite these findings, the urine of ST users is less mutagenic than that of smokers (Curvall *et al.* 1987; Benowitz *et al.* 1989; Sarkar *et al.* 2010).

Trace metals can be detected in tobacco, depending on the tobacco variety and local crop growing conditions. Levels of cadmium are higher in smokers compared to ST users and non-users, while some reports indicate that lead levels are elevated in both smokers and ST users.

To date, relatively few studies have been conducted that directly compare levels and types of biomarkers of exposure among smokers, smokeless tobacco users, and non-users of tobacco. However, two relatively recent, large surveys of U.S. tobacco users utilized NHANES data to report on comparative levels of tobacco exposure biomarkers (Naufal *et al.* 2011; Rostron *et al.* 2015). These studies offer the advantages of analyses based upon a very large data set developed from well-characterized, representative U.S. populations.

Naufal *et al.* 2011 analyzed NHANES data on 33 blood and urine biomarkers in cigarette smokers, ST (combined moist snuff and chewing tobacco) users, and non-users of tobacco. The cigarette smokers had significantly higher levels of biomarkers for 18 tobacco constituents (*e.g.,* benzene, styrene, naphthalene) compared with ST users, reflecting high levels of exposure to combustion products. In contrast, the biomarker levels in ST users generally resembled those observed in non-users of tobacco – showing no significant differences between ST users and non-users in 21 of the 33 analytes tested. Smokers, by contrast, differed significantly from non-users in levels of 28 biomarkers. Higher levels of urinary NNAL (discussed further below) and urinary biomarkers of halogenated aromatic hydrocarbons were found in ST users compared with either smokers or non-tobacco users. Data as described by Naufal *et al.* 2011 are included in Table 6.1.2-3 and Table 6.1.2-4.

Table 6.1.2-3: Levels of urinary metabolites by tobacco use category (creatinine-corrected); NHANES data 1999-2008 (from Naufal *et al.* 2011)

Chemical Class	Analyte	Unit of measure	Non-tobacco users	Smokeless tobacco users	Smokers	% difference between smokeless tobacco users and smokers
PAH[a]	1-hydroxypyrene (1-HOP)	ng/L	43.8	67.4	122.7	-45.1[b]
PAH	2-hydroxyfluorene (2-OH-Fluor)	ng/L	196.4	301.9	962.9	-68.6[b]
PAH	3-hydroxyfluorene (3-OH-Fluor)	ng/L	71.5	135.6	555.6	-75.6[b]
PAH	9-hydroxyfluorene (9-OH-Fluor)	ng/L	214.9	387.6	411.6	-5.8[c]
PAH	1-hydroxyphenanthrene (1-OH-Phen)	ng/L	129.0	148.4	190.6	-22.1[c]
PAH	2-hydroxyphenanthrene (2-OH-Phen)	ng/L	47.0	60.9	85.6	-28.9[b]
PAH	3-hydroxyphenanthrene (3-OH-Phen)	ng/L	83.1	108.9	177.7	-38.7[b]
PAH	4-hydroxyphenanthrene (4-OH-Phen)	ng/L	19.3	19.1	39.6	-51.8[b]
PAH	1-hydroxynaphthalene (1-OH-Naph)	ng/L	1636	1339	7187	-81.d[b]
PAH	2-hydroxynaphthalene (2-OH-Naph)	ng/L	1808	1881	8955	-79.0[b]
TSNA[a]	4-(methynitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	µg/L	0.0010	0.99	0.21	+371.4[d]

[a]PAH: polycyclic aromatic hydrocarbon; TSNA: tobacco-specific nitrosamine
[b]Value is statistically significantly lower in smokeless users compared with smokers
[c]Value is not statistically significantly different between smokeless users and smokers
[d]Value is statistically significantly higher in smokeless users compared with smokers

Table 6.1.2-4: Levels of blood/serum metabolites by tobacco use category; NHANES data 1999-2008 (from Naufal *et al.* 2011)

Chemical Class	Analyte	Unit of measure	Non-tobacco users	Smokeless tobacco users	Smokers	% difference between smokeless tobacco users and smokers
VOC[a]	Benzene	ng/mL	0.031	0.031	0.12	-74.2[b]
VOC	Toluene	ng/mL	0.10	0.12	0.34	-64.7[b]
VOC	Styrene	ng/mL	0.031	0.033	0.067	-50.7[b]
VOC	m/p-Xylene	ng/mL	0.13	0.15	0.22	-31.8[b]
VOC	Ethylbenzene	ng/mL	0.028	0.029	0.063	-54.0[b]
Other	Acrylamide (AA) (Hb adducts)	pmoL/g Hb	47.9	53.5	122.7	-56.4[b]
Other	Glycidamide (GA) (Hb adducts)	pmoL/g Hb	47.5	50.4	101.5	-50.3[b]
HAH[a]	1,2,3,4,5,6,7,8-octa-chlorodibenzo-p-dioxin (OCDD)	pg/g lipid	298.9	308.0	200.3	+53.8[d*]
HAH	1,2,3,4,6,7,8-hepta-chlorodibenzo-p-dioxin (HpCDD)	pg/g lipid	42.1	45.6	18.5	+146.5[d*]
HAH	1,2,3,6,7,8-hexa-chlorodibenzo-p-dioxin (HxCDD)	pg/g lipid	22.4	24.0	17.5	+37.1[d*]
HAH	1,2,3,4,6,7,8-hepta-chlorodibenzofuran (HpCDF)	pg/g lipid	7.39	10.6	7.17	+47.8[d*]
HAH	2,3,4,7,8-penta-chlorodibenzofuran (PeCDF)	pg/g lipid	5.21	5.47	4.18	+30.9[c*]
HAH	1,2,3,4,7,8-hexa-chlorodibenzofuran (HxCDF)	pg/g lipid	4.71	5.00	4.18	+19.6[c*]

[a]VOC: volatile organic compound; HAH: halogenated aromatic hydrocarbon
[b]Value is statistically significantly lower in smokeless users compared with smokers
[c]Value is not statistically significantly different between smokeless users and smokers
[d]Value is statistically significantly higher in smokeless users compared with smokers

**Note that values of these analytes are lower in smokers than in non-users of tobacco. Of these analytes, there was no significant difference between levels in ST users and non-users with the exception of OCDD (+3.0%) and HpCDF (+43.4%).*

Building on the study of Naufal *et al.* 2011, Rostron *et al.* 2015 used results from over 23,000 NHANES participants, updated to include data available through 2012, to compare levels of seven different biomarkers of tobacco exposure (serum cotinine, urinary total NNAL, blood cadmium, lead and mercury, urinary total arsenic, and urinary CYMA, a biomarker of acrylonitrile) among four different tobacco user groups: nontobacco users, exclusive ST users, cigarette smokers, and dual users of cigarettes and ST (Rostron *et al.* 2015). As Naufal *et al.* 2011 had reported, mean serum cotinine and urinary NNAL levels were significantly higher in ST users compared with smokers (Table 6.1.2–5), blood lead levels were comparable to those of smokers, and mean concentrations of blood cadmium, blood mercury, and urinary arsenic (Table 6.1.2–10) were not elevated in ST users and comparable to nontobacco users. CYMA levels in ST users were likewise no different than in non-tobacco users. Reported values from Rostron *et al.* 2015 are presented in Table 6.1.2-5 below.

Table 6.1.2-5: Geometric mean concentrations of exposure biomarkers by tobacco use status; NHANES data 1999 to 2012 (Mean (95% CI)) (from Rostron *et al.* 2015)

Biomarker of exposure	Non-tobacco users	Exclusive smokeless tobacco users	Exclusive smokers	Dual smokers and smokeless tobacco users
Serum cotinine (ng/mL)	0.043 (0.041 – 0.046)	179.6 (145.8 – 221.1)	130.6 (122.3 – 139.6)	184.1 (132.4 – 256.0)
Urinary NNAL (pg/mg creatinine)	0.98 (0.92 – 1.04)	583.0 (445.2 – 763.5)	217.6 (193.0 – 245.2)	430.3 (284.8 – 650.1)
Urinary CYMA (ng/mg creatinine)	1.47 (1.37 – 1.58)	2.21 (1.11 – 4.39)	117.3 (103.1 – 133.4)	35.4 (2.1 – 606.8)

NOTE: Urinary NNAL and CYMA concentrations were adjusted for creatinine. NNAL data were available for 2007 to 2012 NHANES participants, and CYMA data were available for 2005 to 2006 and 2011 to 2012 NHANES participants.

In addition to determining mean biomarker levels for each tobacco use category, Rostron *et al.* 2015 also conducted an analysis of time-dependent trends. Cotinine concentrations for smokers and smokeless tobacco users were relatively constant over 1999 through 2012. In smokers, urinary NNAL levels were likewise generally unchanged over the years 2007 to 2012 (the period over which these data were available). In contrast, mean urinary NNAL levels for smokeless tobacco users declined dramatically between 2007 and 2012, from a 2007-2008 geometric mean of 1013.7 pg/mg creatinine to a 2011-2012 mean of 328.6 pg/mg creatinine. The 2007 levels were approximately 3-fold higher than those found in smokers, and by 2012, the levels were similar to those found for smokers. Given that serum cotinine levels did not change over this period, it is very likely that the reduction seen in urinary NNAL in ST users reflects a reduction in TSNAs in smokeless products overall, a transition among ST users toward products with lower levels of these constituents, such as snus, or some combination.

Besides these large national surveys, smaller cross-sectional studies have reported similar results for smokers, moist snuff consumers and non-tobacco users using large panels of exposure biomarkers. Campbell and co-workers conducted a cross-sectional study of adult male cigarette smokers (n=60), moist snuff consumers (n=48), and non-consumers of tobacco (n=60) (Campbell *et al.* 2015). Urinary and blood biomarkers of acrolein, benzene, pyrene, carbon monoxide and 1,3-butadiene were significantly lower among moist snuff users compared to smokers, but were not significantly different from levels in non-users of tobacco. As has been reported in other studies (*e.g.,* Hecht *et al.* 2007; Naufal *et al.* 2011; Rostron *et al.* 2015), urinary NNAL was higher in smokeless tobacco users compared with smokers. As discussed below and in Section 2, this finding may reflect differences in NNK metabolism from inhaled vs. oral sources, and not necessarily greater exposure to NNK in smokeless tobacco users. The results of this study demonstrate that smokeless tobacco users have lower systemic exposures to many harmful and potentially harmful constituents, particularly combustion products, compared with smokers.

Prasad *et al.* 2016 conducted a similar cross-sectional study of adult males in North Carolina, including smokers (n=40), moist snuff consumers (n=40) and non-tobacco users (n=40). Consistent with findings from the large population-based studies by Naufal *et al.* 2011 and Rostron *et al.* 2015, levels of tobacco combustion product biomarkers such as COHb, thiocyanate, 1-hydroxypyrene, and 4-aminobiphenyl were significantly higher in smokers than in either moist snuff consumers or non-tobacco consumers (*see* Table 6.1.2-6 below). The magnitude of difference between exposure groups varied depending on the particular class of compound under investigation; smaller differences between smokers and moist snuff users were observed for urinary PAHs (approximately 2- to 4-fold higher in smokers), and greater differences for urinary aromatic amines and mercapturic acid metabolites (3- to 7-fold and 2- to 10-fold higher in smokers respectively). Data as described by Prasad *et al.* 2016 are included in Table 6.1.2-6 and Table 6.1.2-7.

Table 6.1.2-6: Blood and urine biomarkers of tobacco combustion product exposure in smokers (SMK), moist snuff consumers (MSC) and non-tobacco consumers (NTC) (from Prasad *et al.* 2016)

Biomarker	Unit	Group Mean ± SD		
		SMK	MSC	NTC
Blood biomarkers				
COHb (CO exposure)	%	4.0 ± 1.7[a,b]	0.9 ± 1.3	1.0 ± 1.4
Plasma thiocyanate (hydrogen cyanide exposure)	µmol/L	155.6 ± 51.5[a,b]	16.7 ± 7.9	39.4 ± 26.6
Urine PAHs				
1-hydroxypyrene	ng/24 h	369.3 ± 345.2[a,b]	181.4 ± 238.0	113.4 ± 113.8
2-hydroxyfluorene	ng/24 h	2208.3 ± 1611.3[a,b]	811 ± 1185.3	560.9 ± 623.3

Biomarker	Unit	Group Mean ± SD		
		SMK	MSC	NTC
Urine aromatic amine				
4-aminobiphenyl	pg/24 h	23 ± 11.3[a,b]	4.6 ± 2.4	5.5 ± 2.8
o-toluidine	pg/24 h	245.1 ± 115.5[a,b]	84.3 ± 48.8	65.5 ± 35.8
Urine mercapturic acid metabolites				
HMPMA (crotonaldehyde exposure)	ng/24 h	1782.9 ± 894.7[a,b]	333.0 ± 212.5	346.0 ± 192.3
SPMA (benzene exposure)	ng/24 h	6043.2 ± 4998.5[a,b]	603.3 ± 890.7	746.8 ± 716.1
3-HPMA (acrolein exposure)	µg/24 h	3747.2 ± 1663.9[a,b]	746.0 ± 648.1	632.5 ± 436.9
MHBMA (1,3-butadiene exposure)	ng/24 h	195.6 ± 106.4[a,b]	70.0 ± 99.1	31.5 ± 45.4

Abbreviations used: COHb, carboxyhemoglobin; 3-HPMA, 3-hydroxypropyl-mercapturic acid; HMPMA, hydroxy-methyl-propyl-mercapturic acid; MHBMA, monohydroxy-butenyl-mercapturic acid; PAHs, polycyclic aromatic hydrocarbons; SPMA, S-phenyl-mercapturic acid

[a] Difference compared with NTC is statistically significant

[b] Difference compared with MSC is statistically significant

Biomarkers of tobacco specific nitrosamine (TSNA) exposures were higher in MSC relative to SMK (*see* Table 6.1.2-7 below).

Table 6.1.2-7: Urinary biomarkers of TSNA exposure in smokers (SMK), moist snuff consumers (MSC) and non-tobacco consumers (NTC) (from Prasad *et al.* 2016)

Urine nitrosamines	Unit	Group Mean ± SD		
		SMK	MSC	NTC
Total NNAL (NNK exposure)	ng/24 h	578.3 ± 366.3[a,b]	2310.8 ± 2415.0[a]	55.8 ± 53.3
Total NNN	ng/24 h	17.2 ± 14.2[a,b]	48.9 ± 34.7[a]	2.1 ± 1.9

NOTES: All values are rounded to one decimal place; all measurements were made in 24-h urine collections and are expressed as mass per 24-h excretion.

[a] Difference compared with NTC is statistically significant

[b] Difference compared with MSC is statistically significant

Tobacco-specific nitrosamines: Some researchers consider TSNAs the most important carcinogens in ST as well as in cigarette smoke (Hecht *et al.* 2008a). Particular attention has been paid to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN) because of their levels in unburned tobacco products as well as in cigarette smoke, and their potency in rodent carcinogenicity studies. NNK and NNN have been listed by IARC as Group 1 carcinogens (carcinogenic to humans) (Hecht *et al.* 2008a), and based primarily on animal studies, are widely considered major causative factors for tobacco-induced cancers of the lung (NNK) and of the esophagus and oral cavity (NNN) (Hecht *et al.* 2015). After absorption by tissues, NNK is metabolized to NNAL and glucuronide conjugates of this metabolite. NNAL

and its glucuronide conjugates may be detected in urine, and have been used to estimate NNK exposure in ST users. NNN is also extensively metabolized by several pathways, with only small amounts of NNN excreted in urine. As with NNK, pyridine-N-glucuronidation is an important pathway, and measurement of urinary total NNN, comprising NNN and its glucuronide, provides a specific biomarker of NNN exposure (Stepanov and Hecht 2005).

As noted above, the finding of higher median levels of urinary total NNAL in ST users compared with smokers has been reported in most studies (*e.g.,* Stepanov and Hecht 2005; Hecht *et al.* 2007; Hatsukami *et al.* 2007; Naufal *et al.* 2011; Rostron *et al.* 2015; Campbell *et al.* 2015; Prasad *et al.* 2016). It has also been shown that levels of urinary NNAL, as well as urinary cotinine, increase as a function of years of daily ST use, but not as a function of ST brand or even levels of NNK or nicotine in different ST brands (Hecht *et al.* 2008b). However, this finding does not necessarily indicate higher NNK exposures for ST users than for cigarette smokers. Pharmacokinetic data on NNK and NNAL in smokers and ST users is limited, and differences in routes of administration could affect urinary NNAL levels (Hecht *et al.* 2007). Research has also shown that metabolic conversion of NNK to NNAL occurs to a greater extent (3 – 4 fold) in ST users (Hecht *et al.* 2008a) than in cigarette smokers (Stepanov *et al.* 2008b), possibly reflecting more efficient delivery of orally absorbed NNK to hepatic metabolic processes via enterohepatic circulation.

In a comparative study of smokers and ST users, Hecht *et al.* 2007 concluded that both groups have "similar" exposures to NNK. Regardless, the biological significance of either NNK exposures or urinary total NNAL levels in ST users is not clear. Although urinary NNAL levels have been demonstrated to be significantly associated with lung cancer risk among smokers (Church *et al.* 2009; Yuan *et al.* 2009; Yuan *et al.* 2011a), ST use is not associated with lung cancer risk in most studies[3].

Other health outcomes assessed in epidemiological studies of ST users, discussed in Section 6.1.1 of this Application (*e.g.,* Lee and Hamling 2009a), likewise demonstrate that the higher urinary total NNAL levels reported for ST users do not translate into elevated cancer risks. The lack of consistently elevated cancer risks in ST users in spite of higher urinary total NNAL levels suggests that NNK is not a principal driver of cancers in ST users, or possibly that urinary NNAL levels do not accurately reflect any such risks that may exist for ST users.

The role of NNK in disease induction has likewise been questioned for cigarette smokers. Watanabe *et al.* conducted a statistical review of the relationship of NNK in cigarette smoke and incremental lifetime risk for lung cancer. Conclusions from that study were that NNK could only account for a small proportion (approximately less than 2%) of the overall risk, and that if NNK and other potential carcinogens such as NNN and B[a]P were completely removed from cigarette smoke, it likely would bring little to no reduction in cancer risks due to smoking

[3] A finding of elevated mortality risk from lung cancer associated with ST use in CPS-II may reflect chance or confounding by unreported smoking; no dose response was observed for either duration or amounts of ST use (Henley *et al.* 2005). *See* Section 6.1.1 for additional discussion.

(Watanabe *et al.* 2009). Given the importance that continues to be placed on NNK levels in tobacco products by many public health agencies, additional clarification of its role in tobacco-related disease induction is needed.

Higher levels of urinary total NNN in ST users compared with smokers have also been reported in a small 2005 study (Stepanov and Hecht 2005). However, since the difference between the ratio of total NNN between ST users compared to smokers (3.5-fold) was greater than the corresponding difference in the total NNAL ratio (1.8-fold), the authors, speculated that at least part of this could be due to endogenous formation of NNN in ST users but not smokers.

Other biomarkers of TSNA exposure have been considered, some reflecting exposure as well as metabolic activation and interaction with cellular macromolecules. Compared with smokers, ST users display higher blood levels of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), a marker for hemoglobin adducts of NNK and NNN (Hecht *et al.* 1991; Schäffler *et al.* 1993; Carmella *et al.* 1990; Hecht *et al.* 1993). However, the lack of correlation between HPB and 1) levels of TSNAs in smokeless products (Schäffler *et al.* 1993), 2) amount of product use (Schäffler *et al.* 1993; Carmella *et al.* 1990), and 3) levels of salivary or plasma cotinine (Carmella *et al.* 1990; Hecht *et al.* 1994) raises questions as to the utility of HPB as a biomarker of relative TSNA exposures between smokers and ST users.

Trace metals: Exposures to trace metals have been assessed for smokers and ST users in several studies, including three of those discussed above. Naufal *et al.* 2011 found that with the exceptions of urinary cadmium and urinary lead, which were significantly higher in smokers compared with either moist snuff consumers or non-tobacco users (Table 6.1.2-8 and Table 6.1.2-9), no statistically significant differences were seen between any groups in this study for biomarkers of these trace metals, consistent with other published reports (*e.g.*, Marano *et al.* 2012a; Marano *et al.* 2012b).

Table 6.1.2-8: Levels of trace metals measured in urine by tobacco use category (creatinine-corrected); NHANES data 1999-2008 (from Naufal *et al.* 2011)

Chemical Class	Analyte	Unit of measure	Non-tobacco users	ST users	Smokers	% difference in ST users vs smokers
Metal	Arsenic (Total) (As)	µg/L	9.58	6.17	8.08	-23.6[b]
Metal	Cadmium (Cd)	µg/L	0.24	0.16	0.34	-52.9[a]
Metal	Cobalt (Co)	µg/L	0.34	0.26	0.33	-21.2[b]
Metal	Lead (Pb)	µg/L	0.59	0.58	0.72	-19.4[a]
Metal	Mercury (Total) (Hg)	µg/L	0.54	0.36	0.42	-14.3[b]

[a]Value is statistically significantly lower in smokeless users compared with smokers
[b]Value is not statistically significantly different between smokeless users and smokers

Table 6.1.2-9: Levels of trace metals measured in blood/serum by tobacco use category; NHANES data 1999-2008 (from Naufal *et al.* 2011)

Chemical Class	Analyte	Unit of measure	Non-tobacco users	ST users	Smokers	% difference in ST users vs smokers
Metal	Cadmium (Cd)	µg/L	0.30	0.28	0.90	-68.9[a]
Metal	Lead (Pb)	µg/L	1.39	1.92	1.86	+3.2[b]
Metal	Mercury (Total) (Hg)	µg/L	1.06	0.78	0.81	-3.7[b]
Metal	Selenium (Se)	µg/L	137.0	137.0	130.3	+5.1[b*]

[a]Value is statistically significantly lower in smokeless users compared with smokers
[b]Value is not statistically significantly different between smokeless users and smokers
**Value was lower in smokers than in non-users of tobacco. There was no significant difference between levels in ST users and non-users.*

Rostron *et al.* 2015 found higher levels of blood lead among smokers, ST users and dual users compared with non-tobacco users. Blood cadmium was substantially elevated among smokers, elevated to a lesser degree among dual users, and as with both blood mercury and urinary arsenic, was not elevated among ST users (Table 6.1.2-10).

Table 6.1.2-10: Geometric mean concentrations of exposure biomarkers by tobacco use status; NHANES data 1999 to 2012 (Mean (95% CI)) (from Rostron *et al.* 2015)

Biomarker of exposure	Non-tobacco users	Exclusive smokeless tobacco users	Exclusive smokers	Dual smokers and smokeless tobacco users
Blood cadmium (mg/L)	0.268 (0.262 – 0.273)	0.220 (0.201 – 0.240)	0.941 (0.916 – 0.968)	0.644 (0.515 – 0.806)
Blood lead (mg/L)	1.18 (1.16 – 1.21)	1.76 (1.62 – 1.91)	1.76 (1.71 – 1.81)	1.76 (1.55 – 2.00)
Blood mercury (mg/L)	1.02 (0.97 – 1.06)	0.82 (0.73 – 0.93)	0.77 (0.73 – 0.81)	0.63 (0.49 – 0.80)
Urinary arsenic (ng/mg creatinine)	9.53 (8.98 – 10.11)	6.43 (5.36 – 7.71)	7.65 (7.05 – 8.30)	6.73 (4.84 – 9.37)

NOTE: Urinary NNAL, arsenic, and CYMA concentrations were adjusted for creatinine. NNAL data were available for 2007 to 2012 NHANES participants, arsenic data were available for 2003 to 2012 NHANES participants, and CYMA data were available for 2005 to 2006 and 2011 to 2012 NHANES participants. Former cigarette smokers were excluded from smokeless tobacco users for the analysis for cadmium.

Prasad *et al.* 2016 found higher levels of urinary cadmium among smokers, but not ST users. (Table 6.1.2-11).

Table 6.1.2-11: Urinary biomarkers of trace metals exposure in smokers (SMK), moist snuff consumers (MSC) and non-tobacco consumers (NTC) (from Prasad *et al.* 2016)

Trace metals	Unit	Group Mean ± SD		
		SMK	MSC	NTC
Cadmium	µg/g creatinine	0.5 ± 0.4[a,b]	0.2 ± 0.1	0.3 ± 0.2
Chromium	µg/g creatinine	0.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.1
Selenium	µg/g creatinine	33.9 ± 13.8	37.2 ± 14.4	34.4 ± 11.2

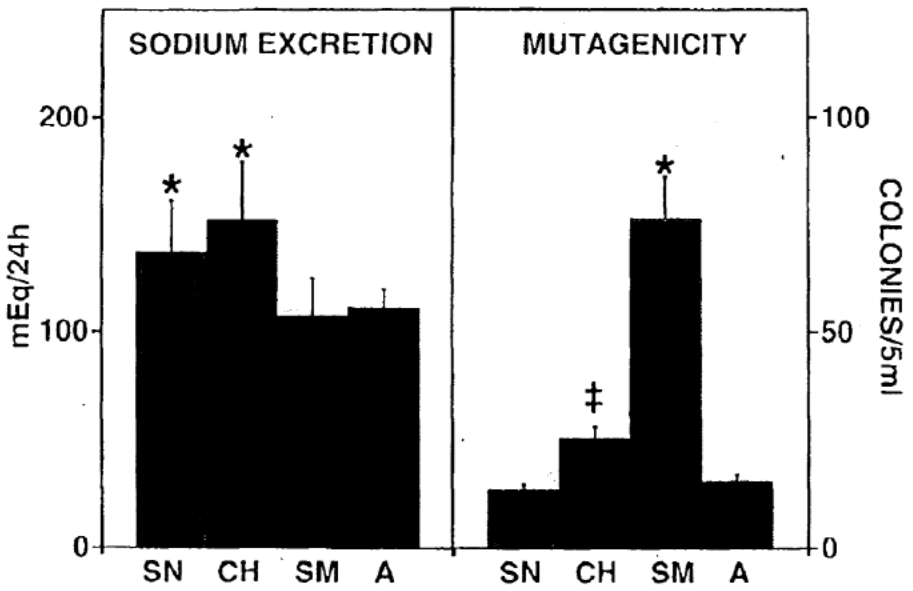
NOTES: All values are rounded to one decimal place; trace metals were measured in the first void and are corrected for creatinine excretion.

[a] Difference compared with NTC is statistically significant

[b] Difference compared with MSC is statistically significant

Urine mutagenicity: Urine mutagenicity testing has been used as a biomarker for exposure to environmental agents, including cigarette smoke, and, to a lesser extent to smokeless tobacco. Data reported by Benowitz *et al.* 1989 indicated that users of chewing tobacco and moist snuff as well as non-users had significantly lower urine mutagenicity than smokers. Moist snuff users did not have increased urine mutagenicity compared to non-users; urinary mutagenicity in users of chewing tobacco "tended to be increased" when compared to that of non-users, but this increase was not statistically significant at the $p < 0.05$ confidence level (Benowitz *et al.* 1989). Benowitz later presented a graphic illustration of the low level of urine mutagenicity among users of American chewing tobacco and moist snuff, compared with smokers (Figure 6.1.2-2 below; from Benowitz 1997).

Figure 6.1.2-2: Urinary sodium and mutagenic activity with daily cigarette smoking and smokeless use



Urinary sodium and mutagenic activity with daily cigarette smoking and smokeless use. SN = snuff; CH = chewing tobacco; SM = cigarette smoking; A = tobacco abstinence. * = $p < 0.05$, compared with abstinence; ‡ = $p < 0.10$, compared with abstinence. (from Benowitz 1997)

Similarly, Curvall *et al.* 1987 examined urine mutagenicity in a small sample of Swedish moist snuff users (n=8), smokers (n=8), and never-users (n=6). In spite of no significant difference in the 24-hour urinary levels of nicotine and cotinine between moist snuff users and smokers, urine samples from smokers were far more mutagenic than samples from either current snuff users, snuff users who had abstained from snuff use for one week, or non-users of tobacco. There were no significant differences in samples from snuff users compared with never-smokers (Table 6.1.2-12 from Curvall *et al.* 1987).

Table 6.1.2-12: Mutagenic activity of urine concentrates from smokers, snuff users and non-tobacco users towards salmonella strain TA98 with the addition of S9

Subjects	Revertants per mL of urine			Revertants per 24 h (10^3)		
	Mean	S.D.	Range	Mean	S.D.	Range
Smokers (*n* = 8)	6.5	2.7	4.2 – 12.8	8.6	4.5	4.2 – 17.6
Snuff users (*n* = 8)	0.9	0.4	0.3 – 1.5	1.3	0.8	0.3 – 2.5
Abstinent snuff users (*n* = 6)	0.8	0.3	0.4 – 1.1	1.3	0.7	0.5 – 2.4
Non-tobacco users (*n* = 6)	0.5	0.2	0.2 – 0.9	0.9	0.7	0.4 – 2.2

The study authors concluded that, in the case of smoking, the levels of nicotine exposure in normal consumers are accompanied by elevated urinary levels of mutagens, while no such increase is observed among users of Swedish snuff at the same nicotine levels. It should also be noted that the smokeless tobacco products that would have been consumed at the time of this study and the study described by Benowitz *et al.* 1989 likely contained higher levels of toxicants compared with contemporary smokeless products including snus (Nilsson 2011; Hatsukami *et al.* 2007).

6.1.2.2.1.3 Nicotine and nicotine metabolites in smokers and smokeless tobacco users

The following section reviews published literature regarding nicotine biomarkers in smokers and smokeless tobacco users, with emphasis on relative levels of daily exposures between these groups. A discussion of nicotine in the context of abuse liability is presented in section 6.1.5, and in a separate report, *An Assessment of Camel Snus Abuse Liability* (Henningfield *et al.* 2017), an extensive review and analysis of published and unpublished scientific data pertinent to the abuse liability of Camel Snus prepared at the request of RJRT by PinneyAssociates. The report contains additional information regarding clinical studies of nicotine pharmacology, product usage topography, and subjective assessments of Camel Snus and other ST products.

Nicotine exposures in tobacco users as well as non-users have been assessed using a variety of different biomarkers. In the absence of NRT use, and with the exception of some dietary

sources, nicotine measurement is specific for tobacco exposure. However, nicotine's short half-life (2 h) limits its general utility as a biomarker. Nicotine is extensively metabolized to six primary metabolites by the liver. Quantitatively, the most important metabolite of nicotine in most mammalian species is cotinine. In humans, about 70–80% of nicotine is converted to cotinine. Like nicotine, cotinine is a highly specific and sensitive marker for tobacco exposure, and the above caveats for contributions from non-tobacco sources notwithstanding, offers the advantage of a half-life of approximately 16 hours (Benowitz 2009). Although a high percentage of nicotine is metabolized via the cotinine pathway in humans, only 10–15% of nicotine absorbed by smokers appears in the urine as unchanged cotinine (Benowitz *et al.* 1994). 3-hydroxy-cotinine and its glucuronide conjugate account for 40 – 60% of the nicotine dose in urine. The extent of cotinine metabolism varies widely among individuals. Therefore, cotinine levels are only approximately correlated with the daily intake of nicotine (Stratton *et al.* 2001). More recently, the reporting of "nicotine equivalents," the combination of nicotine, cotinine, 3'-hydroxycotinine, and their glucuronides, which together represent 73–96 percent of internalized nicotine in a user of tobacco products (Hukkanen *et al.* 2005), has been utilized. This combination is a widely accepted biomarker of nicotine uptake that directly measures, to a high percentage, the total nicotine dose (IOM 2012).

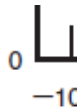
Typical U.S. cigarettes contain about 15 – 18 mg of nicotine per gram of unburned tobacco, or approximately 8 – 14 mg of nicotine per cigarette, depending on the weight of tobacco in the cigarette. The amount of nicotine absorbed from a cigarette depends to a large degree on individual smoking behaviors, such as puff frequency, puff volume, inhalation and other tobacco use behaviors (Benowitz *et al.* 2009; Benowitz *et al.* 2014). Delivery of nicotine from different styles of cigarettes, and as affected by individual smoking behaviors, may vary between 0.4 mg and over 3 mg or even higher (Benowitz *et al.* 1991; Benowitz *et al.* 2014; Benowitz 1996; RCP 2007, p. 92). Typically, between 1 mg and 2 mg of nicotine enter the systemic circulation from smoking a single cigarette.

A detailed review of nicotine chemistry, metabolism and biomarkers of nicotine exposure was recently published (Benowitz *et al.* 2009). According to Benowitz *et al.*, inhaled nicotine results in blood concentrations of nicotine that rise rapidly during smoking, and high levels reach the brain in 10 – 20 seconds, although the actual dynamics of nicotine accumulation in the brain are complex and not completely defined. For example, using radio-labeled nicotine and PET scanning, Rose *et al.* 2010 found that puff-associated spikes in brain nicotine do *not* occur during habitual cigarette smoking. Rather, brain nicotine concentrations gradually increase during smoking. Rose *et al.* 2010 also found that dependent smokers have a lower brain nicotine accumulation rate compared with non-dependent smokers. In contrast, absorption of nicotine by smokeless tobacco users occurs primarily via the oral mucosa and secondarily via the gastrointestinal tract (Benowitz *et al.* 1989). It has been reported that oral absorption of nicotine is influenced by the pH of the product, and to a lesser degree, the pH of the oral cavity (Benowitz 2009). Although absorption through oral tissues is rapid, the rise in brain nicotine levels is much slower than absorption through the lungs, reaching a plateau at about 30 minutes following use. Oral absorption of nicotine is slower to the brain than if absorbed through the lungs because nicotine diffuses into the tissues of the oral cavity, then enters the

venous circulation draining from those tissues, which subsequently returns first to the heart and lungs before entering the systemic venous circulation.

A small study conducted earlier by Benowitz *et al.* 1988 compared the systemic doses of nicotine delivered by moist snuff, chewing tobacco, nicotine gum, and cigarette smoke. ST users were asked to hold 2.5 g of moist snuff or 7.9 g (average) of chewing tobacco in their mouths for 30 minutes; cigarette smokers were asked to take one puff on their usual brand of cigarette every 45 seconds for 9 minutes (12 puffs); other participants were asked to use two pieces of 2 mg nicotine gum chewed slowly over 30 minutes. Blood nicotine concentration was then measured at regular intervals over the course of 2 hours. Measurements of blood nicotine levels showed that smokers rapidly absorbed nicotine through pulmonary circulation and peak blood nicotine concentration was reached in about 10 minutes; the blood nicotine levels subsequently dropped off quickly as nicotine was distributed from vascular space to tissues. Initial absorption of nicotine from ST use across oral mucous membranes also took place rapidly, and then slowed until peak venous blood nicotine concentrations were reached in about 30 minutes, also dropping off gradually thereafter. Although peak nicotine levels seen with cigarette smoking and ST use were similar, ST use resulted in the absorption of more total nicotine due to the prolonged period of exposure. Increases in blood nicotine with nicotine gum use were significantly less than with either cigarettes or ST use, with a slower absorption rate followed by a gradual decline over 90 minutes (Figure 6.1.2-3 below; from Benowitz *et al.* 1988).

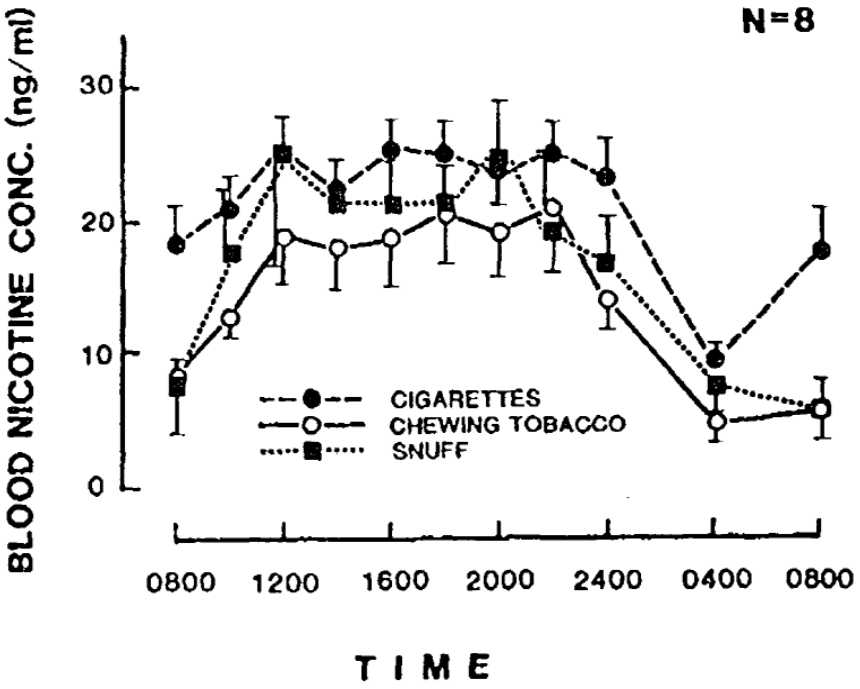
Figure 6.1.2-3: Blood nicotine concentrations with cigarette smoking and the use of smokeless tobacco in single doses



Error bars represent standard error of the mean for 10 subjects (from Benowitz *et al.* 1988).

A 1997 review by Benowitz summarized data indicating that, although the nicotine content of ST and cigarettes differ, and in spite of very different nicotine absorption kinetics for ST products and cigarettes, the overall systemic absorption and levels of nicotine are similar in users of smokeless tobacco and cigarettes. Benowitz also concluded that both the levels and patterns of nicotine exposure during day-long usage by cigarette smokers and users of moist snuff, as determined by blood analysis, were very similar (Benowitz *et al.* 1989; Figure 6.1.2-4; adapted from Benowitz 1997). As a consequence, Benowitz concluded that the presence or absence of any health effects from smoking attributed to nicotine would be similar for ST users as well.

Figure 6.1.2-4: Blood nicotine concentrations with daily cigarette smoking and use of smokeless tobacco



Error bars represent standard error of the mean of eight subjects. (from Benowitz *et al.* 1989).

A number of studies of nicotine intake from cigarettes have used cotinine as a biomarker for daily nicotine intake (Benowitz *et al.* 2009, p. 50). There is a high correlation among cotinine concentrations measured in plasma, saliva, and urine. Measurements of cotinine in any of these biological matrices may be used to estimate nicotine exposures in smokers. An advantage of cotinine as a nicotine biomarker is its longer half-life (~16h vs. ~2h for nicotine), as well as it being present in higher serum concentrations. A limitation of using cotinine as a nicotine biomarker is that variation among individuals in the activity of CYP2A6, a key enzyme involved in nicotine metabolism, may result in substantial differences in cotinine levels, even in the presence of the same tobacco exposure (Zhu *et al.* 2013).

Higher levels of serum (Naufal *et al.* 2011; Rostron *et al.* 2015) and urinary (Hecht *et al.* 2007) cotinine have been reported for ST users, compared with smokers. Two studies using U.S. data from the National Health and Nutrition Examination Survey (NHANES) reported similar nicotine exposure results for tobacco users, showing that levels of serum cotinine (measured as ng/mL) were higher in ST users than in smokers (Table 6.1.2-13; Table 6.1.2-14; adapted from Naufal *et al.* 2011; Rostron *et al.* 2015 respectively). The higher levels of cotinine observed for ST users is the result of significant first-pass metabolism of nicotine that is ingested by swallowing. Swallowed nicotine is metabolized to cotinine by the liver prior to entering systemic circulation, thus contributing to the higher serum cotinine concentrations observed in ST users compared with smokers (Ebbert *et al.* 2004). Because the level of cotinine is also influenced by the amount of swallowing by an individual user, it is a less accurate general measure of nicotine

exposure in ST users. Higher cotinine levels in ST users therefore do not necessarily imply higher nicotine exposure.

Table 6.1.2-13: Serum cotinine concentrations (NHANES 1999-2008) by tobacco-consumption category (from Naufal *et al.* 2011, p. 225)

	Cigarette smokers	Smokeless tobacco consumers	Non-consumers of tobacco/NRT
Serum cotinine (ng/mL) Geometric mean (95% CI)	127.7 (119.1 – 135.6)	188.7 (152.9 – 235.1)	0.050 (0.047 – 0.054)

Table 6.1.2-14: Geometric mean serum cotinine concentrations by tobacco use status, NHANES 1999 to 2012 (from Rostron *et al.* 2015, p. 1832)

	Non-tobacco users	Exclusive ST users	Exclusive cigarette smokers	Dual cigarette and ST users
Serum cotinine (ng/mL) Geometric mean (95% CI)	0.043 (0.041 – 0.046)	179.6 (145.8 – 221.1)	130.6 (122.3 – 139.6)	184.1 (132.3 – 256.0)

While no usage data were presented in Naufal *et al.* 2011, Rostron *et al.* 2015 reported limited usage data from NHANES for smokers and ST users. Exclusive smokers smoked less than 1 pack per day, while dual users smoked less, although the difference was not statistically significant. Exclusive ST users used their products on most, but not all days. No additional data on dips[4]/day, duration of dips, etc. were provided (Table 6.1.2-15).

Table 6.1.2-15: Tobacco usage data (mean values (95% CI)), NHANES 1999 to 2012 (from Rostron *et al.* 2015, p. 1831)

Past 5-day cigarette/ smokeless tobacco use	Exclusive ST users	Exclusive cigarette smokers	Dual cigarette and ST users
# of days cigarettes smoked	na	4.4 (4.4 — 4.5)	3.2 (2.7 — 3.7)
# cigarettes smoked per day smoking	na	14.8 (14.4 — 15.3)	11.9 (8.6 — 15.2)
# days chewing tobacco used	4.2 (4.1 — 4.4)	na	3.7 (3.3 – 4.2)
# days snuff used	4.3 (4.1 – 4.5)	na	3.5 (3.0 – 4.0)

[4] A user-selected pinch of tobacco placed in the mouth is often referred to as a "dip".

The relationship between manner of ST use and nicotine exposure has been investigated in several other studies. A small clinical study (n=56) conducted by Hatsukami *et al.* 1988 recorded detailed information on how ST (Copenhagen moist snuff) was used by a group of younger (ages 18 to 30) male ST users. Nicotine exposure was determined by measures of salivary cotinine, where the mean level was 280.22 ng/ml (S.D. ± 178.57).

Table 6.1.2-16: Smokeless tobacco product use topography in college-age males (data from Hatsukami *et al.* 1988)

Product use parameter	Mean (SD)	Range
# of tins used / week	2.8 (1.5)	NR*
Duration of ST use (years)	5.2 (2.4)	NR
Age of ST initiation (age)	16.2 (2.3)	NR
# dips / day	6.3 (2.2)	2.5 – 12.5
Time between dips (min)	102.6 (42.1)	41.1 – 240.5
Duration of a dip (min)	39.9 (16.5)	13.9 – 83.9
Total dip duration/day (min)	254.6 (129.3)	41 – 588
Amount of tobacco/dip (g)	1.97 (0.96)	0.62 – 5.91
Total tobacco used/day (g)	12.0 (6.8)	5.1 – 42.5

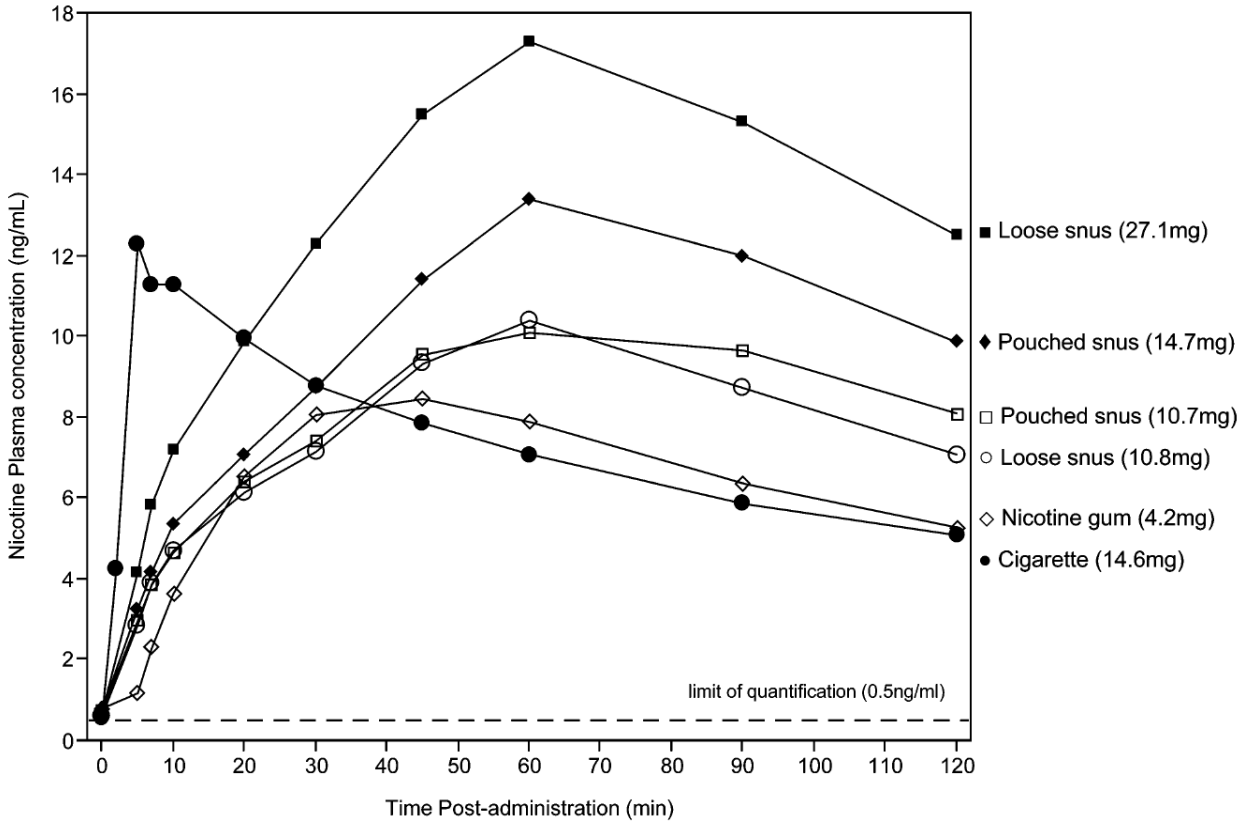
*NR: not reported

The authors noted that as with cigarette smoking, there was great variability in the pattern of smokeless tobacco use among individuals, as evidenced by the wide range of recorded values (Table 6.1.2-16). The ST usage metrics that showed the best correlation with nicotine exposure were the number of dips per day and duration of dips per day. This study as well as an earlier study (Hatsukami *et al.* 1987) confirmed that these metrics were better predictors of nicotine exposure than the amount of tobacco used. This finding may be interpreted as the amount of nicotine absorption depends on how long the product is used and the frequency of dipping, rather than the weight or amount of tobacco that is used (Hatsukami *et al.* 1988).

A similar study was conducted by Lemonds *et al.* 2005, who examined the relationship between self-reported measures of ST use and urinary levels of nicotine, cotinine, total nicotine (nicotine and its glucuronides) and total cotinine (cotinine and its glucuronides) among 54 male ST users. The study added a further dimension by including measurements of urinary total NNAL. As was reported earlier by Hatsukami and co-workers, the frequency and duration of ST use, but not the amount of ST used, were the most important correlates of nicotine exposure. These were also the strongest correlates of urinary total NNAL. Two metrics of ST use (dips/day = 6.1; tins/week = 2.8; were remarkably similar to those recorded by Hatsukami *et al.* 17 years earlier. However, in this study, the average duration of ST exposure per day was 423 minutes (SD±224.4), considerably longer than the 255 minutes reported by Hatsukami *et al.* 1988.

Nicotine and cotinine exposure levels have also been studied specifically in snus users. A 20-person study conducted by Digard and co-workers provided comparative data on nicotine absorption (nicotine plasma concentration) over the course of 2 hours from smoking a single cigarette, from using two different amounts of loose snus (the predominant form of snus prior to the 1970s) and from using two different sizes of pouched snus (Digard *et al.* 2012). Systemic nicotine exposures, measured as the plasma concentration-time curve, and peak plasma concentrations of nicotine, ranged from 26.9 to 14.8 ng.h/ml and 17.9 to 10.8 ng/ml, respectively, among the cigarette and snus products. The study reported that while nicotine was absorbed more rapidly from cigarettes, systemic exposure (14.8 ng.h/ml) and maximum plasma concentration (12.8 ng.h/ml) from smoking was within the range seen with the different snus products. These data are consistent with findings from the 1988 study and 1997 review of smokeless tobacco use reported by Benowitz (*see* above). Among the various sizes and types of snus products, differences in systemic exposure to nicotine after 60 minutes of use was dependent on the total nicotine content of the portion (quantity by weight of tobacco x nicotine content of the blend), and not on the form of snus (loose vs pouched) (*see* Figure 6.1.2-5 below).

Figure 6.1.2-5: Mean plasma nicotine concentrations at each time point following single use of the different tobacco products and nicotine gum (from Digard *et al.* 2012)



The dashed line represents the limit of quantification (0.5 ng/ml). Products (nicotine content): • Cigarette (14.6 mg); □ Pouched snus (10.7 mg); ○ Loose snus (10.8 mg); ♦ Pouched snus (14.7 mg); ▪ Loose snus (27.1 mg); ◊ Nicotine gum (4.2 mg).

A 2011 study by Lunell and Curvall reported similar results (Lunell and Curvall 2011). Fourteen smokers who had never used smokeless tobacco abstained from smoking overnight, then used two different pouched snus products (sizes not reported) for 30 minutes. Maximum plasma nicotine concentrations were 14.8 (range: 6.8 – 19.9 ng/ml) and 13.7 (range: 6.8 – 19.9 ng/ml) for the two products. The mean time to maximum plasma nicotine concentration was 37 minutes (note that snus use was for 30 minutes in this study compared with 60 minutes in the study by Digard *et al.* 2012 reviewed above). Similarly, Curvall *et al.* 1987 examined mean 24-hour urinary nicotine and cotinine levels in smokers (n=8; 15 – 38 cigarettes/day) and Swedish users of snuff (n=8; 15 – 40 g/day). The authors found no significant differences in urinary nicotine or cotinine levels between snuff users and smokers.

In summary, multiple studies have found that although the kinetics and routes of nicotine absorption are different for smokeless tobacco users compared with cigarette smokers, and that there is considerable variability in usage patterns for both smokers and ST users, regular smokers and regular ST users experience generally similar daily levels of nicotine exposure, with ST users sometimes exhibiting higher levels.

Clinical studies of biomarkers of nicotine exposure in tobacco users show that, despite different routes of exposure, different doses per use, and different pharmacokinetic patterns, smokers and ST users, including snus users, maintain similar daily plasma nicotine levels (Foulds *et al.* 2003; Naufal *et al.* 2011; Bolinder *et al.* 1997; LSRO 2008, p. 55). ST users generally have higher plasma cotinine levels compared with smokers, but this likely reflects rapid first-pass liver metabolism of orally ingested nicotine that occurs in ST users to a much greater extent than it does in smokers (Ebbert *et al.* 2004).

6.1.2.2.1.4 Summary and conclusions: Biomarkers of exposure in smokers and smokeless tobacco users

Taken together, published clinical studies that have examined biomarkers of exposure in cigarette smokers indicate significant exposure to a wide range of toxic tobacco combustion products, as well as exposure to tobacco constituents directly transferred to smoke. Published clinical studies reporting biomarkers of exposure in ST users show that ST users generally are exposed to fewer and to lower levels of many toxicants compared with cigarette smokers. The fundamental differences in chemistry between ST products and cigarette smoke, along with different routes of exposure and manners of use, are consistent with the differences observed in biomarker studies. Two recent clinical studies (Campbell *et al.* 2015; Prasad *et al.* 2016) and two studies based on large national survey data (Naufal *et al.* 2011; Rostron *et al.* 2015) found that ST users are exposed to substantially lower levels of combustion toxicants found in cigarette smoke; in most cases, biomarker levels for these toxicants in ST users do not differ significantly from the corresponding levels in non-users of tobacco.

Biomarker studies of moist snuff users have shown higher levels of urinary NNAL and possibly total NNN compared with smokers, however substantial epidemiological evidence indicates that higher urinary TSNA metabolite excretion by ST users is not reflected in concomitant elevations in cancer risks, as discussed in Section 6.1.1 of this Application. The differences in

routes of exposure (*i.e.,* inhalation exposure vs. oral exposure) and/or subsequent differences in metabolism in ST users compared with smokers (*i.e.*, direct inhalation exposures to respiratory target tissues, as opposed to the extensive first-pass metabolic detoxification that follows oral exposures) may provide a biological basis for the observation that relatively higher TSNA biomarker levels in ST users do not translate into higher levels of cancer risk that are evident in epidemiological studies.

Although the relationship between the level of any given constituent in cigarette smoke or smokeless tobacco and its corresponding biomarker level may be confounded by other exposures and by individual genetic modifying factors, the body of published clinical studies containing biomarker data shows that carcinogen and toxicant exposures in smokers are consistent with the substantially higher adverse health risks observed in the epidemiological literature.

All tobacco products contain nicotine, and biomarker studies show that ST users are exposed to daily nicotine levels similar to those of cigarette smokers. Urine mutagenicity has been used as an informative biomarker of internal exposure to environmental and food mutagens. Urine from users of moist snuff is no more mutagenic than urine from non-users of tobacco, whereas urine from cigarette smokers is significantly more mutagenic than urine from either ST users or non-users of tobacco. In summary, the differences between exposures that have been demonstrated in published biomarker studies are consistent with the body of epidemiological evidence showing reduced chronic disease risk in ST users compared to smokers.

6.1.2.2.2 Biomarkers of effect in smokers and smokeless tobacco users

Biomarkers of effect, sometimes referred to as biomarkers of potential for harm, are measures of early biological alterations due to exposure (Stratton *et al.* 2001). As such, the 2010 report of the U.S. Surgeon General has collectively referred to these alterations as "biomarkers of biologic events with the potential to lead to harm" (USDHHS 2010, p. 54). Examples of biomarkers of effect in both smokers and ST users have been provided in a number of publications (*e.g.,* Stratton *et al.* 2001; IOM 2012; USDHHS 2010; IARC 2004; IARC 2007b; Frost-Pineda *et al.* 2011). A review of effect biomarker studies relevant for illustrating the disparity in health risks between cigarette smoking and ST use is presented below.

6.1.2.2.2.1 Biomarkers of effect in smokers

Biomarkers of effect described for cigarette smokers that indicate the potential for, or presence of, adverse events, include gross physiological markers such as cough, osteoporosis or periodontal disease, histological markers such as hyperplasia or dysplasia in bronchial or other tissues, and markers of cellular function such as vascular endothelial dysregulation or abnormalities in blood (USDHHS 2010, p. 54). Molecular and cellular biomarkers of effect have also been examined in smokers, including elevated levels of fibrinogen (Bazzano *et al.* 2003), increased white blood cell count (Roethig *et al.* 2010), changes in the expression patterns of mRNA or proteins, DNA damage such as mutations in certain genes and epigenetic DNA modification such as promoter hypermethylation.

Frost-Pineda and co-workers presented data from a large, U.S. cross-sectional study that examined "biomarkers of potential harm" in adult smokers (n=3585) and nonsmokers (n=1077) (Frost-Pineda *et al.* 2011). The 29 different surveyed biomarkers obtained from blood or serum encompassed a range of endpoints commonly used in the evaluation of oxidative stress, inflammation, platelet activation, endothelial function, lipid metabolism, lung function and others, representing processes associated to varying degrees with increased risk for diseases ascribed to cigarette smoking, including cancers, non-malignant respiratory diseases, cardiovascular diseases and other adverse conditions. Statistically significant differences were found between smokers and nonsmokers for 21 of the 29 biomarkers. The largest percent differences between smokers and nonsmokers were observed for markers of oxidative stress (8-epi-$PGF_{2\alpha}$ (+42%)), coagulation/platelet activation (11-DHTB (+29%)), and inflammation (white blood cell count (+19%)). Other biomarkers differences between smokers and nonsmokers included average serum hs-CRP level (+14.5%) and average plasma fibrinogen level (+5.7%), both markers of systemic inflammation; von Willebrand Factor (+7.3%), a marker of endothelial dysfunction; average HDL cholesterol (-6.4%), average triglycerides (+16.4%); mean hemoglobin (+3.5%) and average hematocrit (+4.4%).

In addition to molecular biomarkers, physiological biomarkers indicated the increased potential for harm from cigarette smoking. Average heart rate was significantly higher, and lung function (mean FEV_1, mean FVC) was lower in smokers compared with nonsmokers. Nicotine exposure (as measured by nicotine equivalents [mg/24 h]) was statistically significant for 18 of the biomarkers and was the most important factor in algorithms that modeled differences in biomarker levels only for the two biomarkers (WBC and 11-DHTB), whereas BMI was the most important factor for 12 measures. This finding underscores a cautionary statement in the 2010 U.S. Surgeon General's Report that "despite the large number of biomarkers of biologic events with the potential to lead to harm, most are not specific to exposure to cigarette smoke and require additional testing to establish their specificity, sensitivity, and reliability when smoking behaviors or product characteristics vary" (USDHHS 2010, p. 56).

6.1.2.2.2.2 Biomarkers of effect in smokeless tobacco users

Investigations of effects from ST products indicative of potential harm have historically concentrated on the limited number of endpoints relevant to conditions suspected of being associated with ST use — primarily oral cancer and cardiovascular diseases. With respect to potential biomarkers for oral cancer, some researchers have suggested the occurrence of leukoplakia (white patches of keratosis) in the oral cavity, considered a biomarker of damage to oral tissues, might be a biomarker for tissue damage leading to oral cancer. However, the very low rate at which lesions progress to serious disease and the complete regression following ST cessation suggests that leukoplakia occurrence in ST users predicts a benign outcome that is in marked contrast to the substantially greater risk of oral cancer among smokers than ST users (reviewed in Rodu and Godshall 2006). With respect to cardiovascular disease, biomarkers of cardiovascular effects in ST users are thought to be mediated primarily by nicotine, and cardiovascular effects associated with smoking are thought to be mediated by tobacco combustion products as well as nicotine.

Recent studies have extended the scope of effect biomarker analyses to include markers that reflect changes in certain biochemical pathways, such as those involved in inflammation or oxidative stress and known to be altered in cigarette smokers. Two cross-sectional studies comparing long-term smokers, moist snuff users, and non-users of tobacco have demonstrated that some biomarkers of effect can discriminate between smokers and ST (moist snuff) users, and show a more adverse spectrum of effects in smokers compared with ST users and non-users of tobacco (Prasad *et al.* 2016; Prasad *et al.* 2015).

In the first cross-sectional study (Prasad *et al.* 2016), differences were observed consistent with increased systemic inflammation (leukocyte counts, total white blood cells, lymphocytes, monocytes and neutrophils) in smokers, while moist snuff users and non-users of tobacco exhibited low levels of these biomarkers. Likewise, biomarkers related to lipid metabolism (apolipoprotein B100 and oxidized low-density lipoprotein) and thrombogenic potential (thromboxane metabolites) revealed evidence of greater dysfunction among smokers compared with moist snuff users and non-users of tobacco. A biomarker of effect related to oxidative DNA damage (urinary 8-hydroxy-2'-deoxyguanosine) was not significantly different among smokers, moist snuff users, and non-users of tobacco. Overall, Prasad *et al.* 2016 revealed that moist snuff users possess a biomarker of effect profile more similar to non-users of tobacco than to cigarette smokers.

The second cross-sectional study (Prasad *et al.* 2015) was unique in that untargeted metabolomic plasma, urine and saliva profiles were evaluated rather than focusing on a specified set of target diseases or adverse conditions. For each of three study groups, (smokers, moist snuff users, and non-tobacco users), metabolomic profiles were obtained using both GC/MS and LC/MS/MS platforms. The identities of metabolites in plasma, urine and saliva were established by comparing the chromatographic patterns of the test samples against a library of standards, and, where novel patterns were found, additional library entries were created. Although the authors considered this primarily a "discovery" exercise to investigate possible new metabolomic biomarkers of effect, the study revealed key differences among the profiles for smokers, moist snuff users and non-users of tobacco. Overall, smokers exhibited distinct metabolomic profiles, indicative of several adverse conditions, relative to metabolomic profiles from moist snuff users and non-tobacco users. For example, smokers exhibited lower levels of several antioxidants (threonate, ascorbate, glutathione) as well as patterns indicative of increased oxidative stress and inflammation compared with patterns from moist snuff users or non-users. While use of metabolomics in routine biomarker analysis awaits additional qualification and validation, this nontargeted approach to the study of biomarkers in tobacco users provides further demonstration of the more benign effects of smokeless tobacco use compared with smoking.

6.1.2.2.2.3 Cardiovascular disease biomarkers of effect in smokers and ST users

A number of pathophysiological mechanisms are believed to underlie smoking-related adverse cardiovascular health effects. Such mechanisms include oxidative stress, inflammation, induction of endothelial dysfunction, platelet activation, and abnormal lipid metabolism, all of

which are elevated in smokers and are likely contributors to atherosclerosis and cardiovascular disease (Frost-Pineda *et al.* 2011; USDHHS 2010; Benowitz *et al.* 2002). Additional cardiovascular biomarkers found elevated in smokers include von Willebrand factor and plasma fibrinogen levels.

Cardiovascular biomarkers of effect examined in ST users include blood pressure levels, serum lipid levels, fibrinolytic variables, and antioxidant vitamin levels. A 2003 review considered the effect of ST use on these and related endpoints (Asplund 2003). The Asplund 2003 review cited early studies that indicated increased blood pressure among oral snuff ST users during the period of time that the smokeless tobacco product was held in the mouth with a suggestion that an elevated blood pressure was still present for at least a few minutes after removal of the product from the mouth (Squires *et al.* 1984). In addition, compared to non-users of tobacco, an increase of diastolic blood pressure was reported in consumers of smokeless tobacco products, although no details were provided regarding the period between most recent use and blood pressure observation (Schroeder and Chen 1985). Systolic blood pressure and integrated heart rate "tended to be greater" for users of moist snuff and chewing tobacco compared with smokers. An older study reported that smoking and ST use resulted in similar maximal increases in heart rate (Benowitz *et al.* 1988). However, more recent studies (*e.g.,* Ernster *et al.* 1990; Siegel *et al.* 1992) have failed to confirm this finding. Asplund 2003 speculated that changes in the sodium content of snuff could at least partly explain the discrepant observations in early and some later studies. The absence of an effect on blood pressure during nonexposure to tobacco is consistent with the reported absence of sustained hypertension in smokers (Green *et al.* 1986). Transient increases in both blood pressure and heart rate have also been reported after ST use (Benowitz *et al.* 1988). Lunell and Curvall 2011 reported an initial increase in heart rate after beginning snus use, reaching a maximum increase of 9 – 10 beats/min after 20 minutes. However, as with resting blood pressure, resting heart rate appears largely unaffected by ST use (Asplund 2003). A study examining the effects of reduced cigarette smoking exposures through progressive reductions in the number of cigarettes smoked per day (Theophilus *et al.* 2015) showed no major effects on vital signs except for the observation that smoking reduction led to consistent reductions in heart rate with 5 and 0 cigarettes/day (compared with 19-25 cigarettes/day), with a transient decrease in a 10 cigarettes/day group. Based on these data, and consistent with other reports (Swan *et al.* 2007), heart rate was considered a useful biomarker of smoking exposure despite the inherently high variability in the measure, because it is almost completely non-invasive and is well-known to be linked to nicotine (Theophilus *et al.* 2015 citing Benowitz *et al.* 2002), but not to carbon monoxide exposure (Zevin *et al.* 2001).

ST does not cause elevation in values for hemoglobin or hematocrit, an increase in either leukocyte counts or high sensitivity C-reactive protein (two important markers of systemic inflammation that are elevated in smokers), impairment of the fibrinolytic system, or reduction in circulating antioxidant vitamins (Asplund 2003). Smokers often have a less favorable lipid profile than non-users of tobacco, in part due to diets that tend to be high in saturated fats (Asplund 2003). The lipid profiles of snuff users resemble those of non-users of tobacco rather than those of smokers (Asplund 2003; Siegel *et al.* 1992). Consistent with these findings, a

recent commentary by Benowitz opined that CVD from tobacco use appears to be related primarily to combustion products, including particulates and other oxidant chemicals that would be absent from ST products (Benowitz 2011). Likewise, the 2010 U.S. Surgeon General's Report cited a study (Axelsson *et al.* 2001) indicating that ST produces neither the inflammatory reaction found in smokers nor endothelial dysfunction, activation of platelets, or evidence of oxidative stress, with markers of these conditions similar in ST users and non-users of tobacco (USDHHS 2010, p. 381-382).

More recent studies have also found many of these and other differences in cardiovascular biomarkers of effect among smokers, moist snuff users, and non-users of tobacco. Nordskog and co-workers used data from a cross-sectional study of U.S. adult male representatives of these three groups to investigate a variety of responses using a large panel of serum, blood, and urinary biomarkers of effect (Nordskog *et al.* 2015). Results indicated that smokers exhibited significantly higher values for hemoglobin, mean corpuscular volume, white blood cell count, and a higher fraction of neutrophils compared to non-users of tobacco. Additionally, it was possible to discriminate among the three exposure groups, using principal component analysis, based on three biomarkers: IL-12(p70), an interleukin signaling molecule involved in innate and adaptive immune responses; sICAM-1, a signaling molecule involved in the recruitment of inflammatory cells to sites of inflammation or injury; and IL-8, a chemokine responsible for the recruitment of neutrophils to sites of inflammation. The analysis demonstrated that molecules involved in inflammation and immune response are elevated in smokers, but not in moist snuff users or non-users of tobacco. These findings are consistent with results from epidemiological studies that indicate use of smokeless tobacco presents lower risk for diseases caused by smoking.

A related analysis of cardiovascular biomarkers of effect (Marano *et al.* 2015) compared results among three different cohorts. The first, obtained from the National Health and Examination Survey (NHANES (1999 – 2008)), included adult males age 20 and above who were smokers, smokeless tobacco users, or non-users of tobacco. The second, also obtained from the larger NHANES cohort, was limited to males age 26 – 49 years. The third, comprising 168 adult males age 26 – 49, was the same adult cohort described in the studies published by Campbell *et al.* 2015 and Nordskog *et al.* 2015 reviewed above. In all three cohorts, smokers exhibited elevated white blood cell counts, hematocrit, fibrinogen levels and C-reactive protein compared with non-users of tobacco, consistent with prior study reports (*e.g.,* Frost-Pineda *et al.* 2011). In contrast, there were no significant differences in biomarker values between smokeless tobacco users and non-users of tobacco with the exception of lower serum folate levels, found in both smokers and smokeless tobacco users. While some studies suggest that low folate among smokers may be related to CVD risk (Okumura and Tsukamoto 2011), the link is tenuous, and any association between lower folate and tobacco use may be confounded by dietary differences between tobacco and non-tobacco users. The finding of similar results across the three exposure cohorts examined in this study adds strength to the data presented in Nordskog *et al.* 2015 and Campbell *et al.* 2015, and provides a basis of biological plausibility for the findings of lower cardiovascular risk for smokeless tobacco users compared to smokers as reported in epidemiological studies. The consistency in differences in cardiovascular findings for

smoking and ST use that is seen across published studies of different design further suggests that such findings may be broadly applied across other population groups.

In a weight-of-evidence approach taken in the Life Science Research Office (LSRO) evaluation of comparative risks for CVD associated with different categories of tobacco products, changes in lipids, biomarkers of inflammation, and measures of atherosclerosis were weighted more heavily than were changes in blood pressure or heart rate. Blood pressure and heart rate findings for smokeless tobacco products to date are inconsistent, as discussed above. The LSRO concluded that ST users appear to have a lower degree of CVD risk than smokers (LSRO 2008, p. 6).

6.1.2.2.2.4 Summary and conclusions: Biomarkers of effect in smokers and ST users

In summary, clinical studies that have reported biomarkers of effect show that smokers experience measurable and sometimes significant changes in molecular, cellular, and physiological systems that reflect damage or potential damage from cigarette smoke. These changes can be related to increases in risks for cancers, non-neoplastic pulmonary disease, cardiovascular disease and a variety of other adverse health effects in smokers. While few if any of the changes are specific to cigarette smoking, smoking's effects on oxidative stress, inflammation, lipid metabolism, and other processes are all evident in various biomarkers of effect. ST users, in contrast, exhibit generally fewer or less severe changes in most biomarkers used to evaluate smoking-related effects. In particular, effect biomarkers for the two most frequently considered potential adverse effects of ST use, oral cancer and cardiovascular disease, demonstrate considerably lower potential for disease development compared with cigarette smoking. In conclusion, biomarkers of effect in smokers and ST users support the findings of epidemiological studies that show substantially lower health risks associated with ST use compared with cigarette smoking.

6.1.2.2.3 Biomarkers of exposure and effect in switchers and dual users

It is anticipated that some individuals who switch to the exclusive use of Camel Snus may experience an initial, transient period of concurrent (dual) use with their current tobacco product, while others may maintain a more stable pattern of dual tobacco product use. Current epidemiological data suggests that there are no additional or unique health risks associated with the dual use of cigarettes and smokeless tobacco products that are not observed for exclusive use of either one or the other product (reviewed by Lee 2014; Frost-Pineda *et al.* 2010). Further support for this conclusion comes from analysis of biomarkers in dual users and switchers to determine if differences exist in exposures to nicotine and to tobacco-related toxicants that may suggest an increase or decrease in tobacco-associated harm. A review of several representative studies that report biomarkers of exposure and effect in switchers and dual users of cigarettes and smokeless tobacco is presented below. For a more comprehensive review of this subject, a recent publication by ENVIRON 2013, Appendix III, p. 9-16, is submitted with this Application. As with the biomarker studies reviewed in prior sections, although these studies are not specific to Camel Snus, the similarities in composition and usage between Camel Snus and these older ST products informs as to reductions in exposure and effects that should

be anticipated when switching from cigarettes to non-combustible tobacco products, reductions that are likely equal or even greater for switchers to Camel Snus.

Published studies of dual ST and cigarette use: A 2004 study by Hatsukami and co-workers examined a number of biomarkers of exposure for a group of typical U.S. smokeless tobacco users who were switched to either a low-nitrosamine snus product (1 g portion packs of General Snus) or to a nicotine patch for a period of 4 weeks (Hatsukami *et al.* 2004). Among switchers to snus, the amount of smokeless tobacco used did not differ significantly from prior use, and nicotine exposure remained similar, verified by no change in the levels of urinary cotinine. However, urinary NNAL levels in switchers to snus had fallen approximately 50%, reflecting the lower levels of TSNAs in snus products compared with traditional U.S. moist snuff. It is also noteworthy that although urinary NNAL levels of switchers to snus remained higher than the group that switched to the nicotine patch, the mean level was lower (1.4 pmol/mg creatinine) than the level determined at baseline for a group of cigarette smokers (2.2 – 2.4 pmol/mg creatinine).

Sarkar and co-workers studied biomarkers of tobacco exposure in groups of adult smokers who continued to smoke (n=60) or were switched to exclusive snus (Marlboro Snus) use (n=15), to dual use of cigarettes and snus (n=60), or to no tobacco use (n=15) for 8 days (Sarkar *et al.* 2010). Biomarkers of smoke exposure measured at baseline and after 8 days included urinary biomarkers (total NNAL, total NNN, nicotine equivalents, others) plasma biomarkers (nicotine, cotinine, COHb), and urinary mutagenicity. After correcting for residual levels of biomarkers determined from those who abstained from tobacco entirely as referent, statistically significant reductions of ~50% in the levels of all the biomarkers were observed among the dual users and exclusive snus users compared with those who continued to smoke. Greater reductions in the levels of all measured biomarkers were seen in the group that abstained from tobacco use. Since dual users reduced their cigarette consumption by 50% and used on average 2.2 snus pouches per day, it would appear that snus use in these dual users contributed little, if any, to the toxicants measured in this study. Dual users, who were permitted to use higher levels of snus product, did not compensate for a reduced amount of smoking by either changes in smoking behaviors or by an equivalent increase in snus use in order to maintain the same nicotine exposure as recorded at baseline. Exclusive snus users experienced dramatic reductions in the levels of all biomarkers after switching from smoking cigarettes. However, they also used on average only 3.5 pouches per day, and consequently experienced substantially lower levels of nicotine exposure. The study authors speculated that in this small, short-term study, at least some of the switchers experienced an adequately satisfying tobacco experience, even at this reduced level of consumption.

Published studies that have included use of Camel Snus: Several recently published clinical studies have examined biomarker changes among smokers who were switched to or also used Camel Snus. Summaries of those published studies are provided in this section. However, more extensive and detailed discussions of the studies that were sponsored and published by RJRT researchers (Krautter *et al.* 2015; Round *et al.* 2015; Ogden *et al.* 2015b; Ogden *et al.* 2015c) as

well as unpublished studies sponsored by RJRT on Camel Snus use are provided in Section 6.1.2.3 below.

A cross-over study conducted by Blank and Eissenberg examined changes in tobacco toxicant exposures as well as subjective measures of smoking abstinence symptom suppression in a test of new products for potential harm reduction (Blank and Eissenberg 2010). Among the conditions tested, subjects switched to Camel Snus (Camel Snus Frost, 0.4 g pouch), stopped all tobacco use, or continued to smoke. The trial involved four 5-day periods of use. Smokers (of approximately 1 pack/day) who switched to Camel Snus (using 11 – 12 pouches/day) had urinary cotinine levels on day 5 that were slightly lower than cotinine levels measured in urine of smokers who continued smoking. Use of Camel Snus resulted in rapid and substantial lowering of CO exposure, slightly lower levels of urinary cotinine, but no significant change in the level of urinary NNAL. Subjective measures of tobacco use satisfaction and smoking abstinence symptom suppression indicated the smokeless product tested in this study was less enjoyable than smoking, and failed to suppress all cigarette cravings.

Smokers interested in cessation were enrolled in a 4-week study that examined the use of smokeless products, including Camel Snus (Original, Frost, and Spice flavors; sizes not specified) as well as medicinal nicotine, as possible cessation aids (Kotlyar *et al.* 2011). For all participants who completed the study (Camel Snus, n = 30; medicinal nicotine, n = 18), concentrations of exhaled CO, urinary cotinine, and urinary total NNAL were significantly lower at week 4 compared with baseline. For Camel Snus users, NNAL reductions were less than subjects using medicinal nicotine, and NNN levels were not significantly lower than levels determined at baseline. Nicotine craving and withdrawal symptom scores indicated that neither oral product was completely effective in providing a substitute tobacco use experience for these smoking subjects.

A small study of current Camel Snus users (Caraway and Chen 2013) employed a multi-center design to determine mouth-level exposures (MLE) to selected tobacco constituents of Camel Snus in regular adult consumers. Fifty-three adult subjects, recruited in September 2008, used their usual brand styles (Original, Frost, or Spice 600 mg pouches, purchased at retail) *ad libitum* for 7 days, collecting their snus pouches after use. The collected pouches and unused product were then analyzed for arsenic, cadmium, chromium, lead, nickel, nicotine, TSNAs, and B[a]P. MLE was estimated by calculating the difference between constituent amounts in unused Camel Snus pouches and in pouches after use. Most subjects (86.6%) reported concurrent use of other tobacco products. Seven subjects (13.2%) used Camel Snus exclusively, while 26 subjects (49.1%) reported dual use of Camel Snus and cigarettes. Most of the subjects (88.7%) reported using only one pouch of Camel Snus at a time, while six (11.3%) used two or three pouches concurrently. Overall Camel Snus consumption averaged 3.3 pouches/day or approximately 1.98 g/day, with exclusive users of Camel Snus consuming more pouches/day (5.4) than dual users (2.8).

Mean mouth-level exposure to nicotine was 2.8 mg/pouch and 9.4 mg/day, corresponding to 39% of the nicotine originally in the product prior to use. Similar values were obtained for

exclusive users and for dual users of Camel Snus and cigarettes. Mean mouth-level exposure to TSNAs was 171.5 ng/pouch (22% extracted) or 527 ng/day, and B[a]P exposure averaged 0.2 ng/pouch (29% extracted) or 0.68 ng/day. Trace metal results were inconclusive due to variability in baseline constituent level determinations. This is the first study to report MLE from snus consumption under "normal" use conditions. A key finding was that on average, approximately 60 – 90% of the original amounts of nicotine, TSNA, and B[a]P remained in the snus pouch after use. This finding underscores the limitations on using solely product chemistry to infer human exposures to tobacco toxicants in Camel Snus. Additional discussion of this published study is provided in Section 6.1.2.3 below.

Changes in the levels of a large set of exposure biomarkers were determined in a short-term, confinement cross-sectional clinical trial of smokers (n = 29), mandated to reduce their cigarette consumption by 60%, but with Camel Snus (600 mg pouches; users' choice of Frost or Mellow flavors) added to their daily use of tobacco (dual users) (Krautter *et al.* 2015). Biomarker values after 5 days of dual use were compared with baseline values (representative of the levels in smokers) and with values obtained from separate groups of smokers who had either switched to exclusive use of Camel Snus (n = 30) or who had quit tobacco use entirely (n = 25). On the first of 5 days, dual users reduced cigarette consumption from a mean of 19.24 (±7.40) cigarettes per day to 7.62 (± 2.99) cigarettes per day, and maintained that level of reduced smoking through the end of the trial on day 5. Camel Snus use among the dual users was approximately 3 pouches per day, compared with approximately 6 pouches per day used by the group using Camel Snus exclusively. The levels of snus usage likewise did not change over the course of the trial.

In contrast to the study of Sarkar *et al.* 2010, Krautter *et al.* 2015 studied changes in smoking behavior among the dual users after mandatory 60% reductions in cigarette usage and measured mouth level exposure to tar and nicotine. Data revealed that both tar and nicotine exposures from smoking were reduced by ~50%, indicating that even though cigarettes were smoked with somewhat greater intensity compared to baseline, substantial reductions in exposure to smoke toxicants still occurred. Of the 32 studied biomarkers of exposure representing toxicants commonly associated with tobacco-related morbidity and mortality, substantial reductions in most were experienced by dual users of cigarettes and Camel Snus (Table 6.1.2-17). Smokers who switched to exclusive use of Camel Snus experienced even greater reductions, usually to the same levels as those who had quit tobacco use entirely.

Table 6.1.2-17: Biomarker of exposure determinations among quitters (abstinent), switchers (snus) and dual users of Camel Snus. Measurements of 24-h urine, whole blood, or plasma, and percent changes on Day 5 compared to baseline (group mean ± S.D.) (from Krautter *et al.* 2015)

Biomarker of exposure	Usage	Baseline	Day 5	% Change
Total nicotine equivalents (mg/24-h Urine)	Dual Use	22.59 ± 9.70	15.40 ± 7.17	-31.8%[a,b]
	Snus	21.46 ± 7.49	11.25 ± 9.83	-47.6%[a,b]
	Abstinent	22.89 ± 9.53	0.56 ± 0.47	-97.6%[b,d]
Total TSNAs (ng/24-h Urine)	Dual Use	1222.20 ± 540.79	907.90 ± 477.59	-25.7%[a,b]
	Snus	1115.05 ± 503.76	712.32 ± 588.06	-36.1%[a,b]
	Abstinent	1217.17 ± 548.40	272.86 ± 148.44	-77.6%[b,d]
Total NNAL (ng/24-h Urine)	Dual Use	717.73 ± 326.22	572.90 ± 304.62	-20.2%[a,b]
	Snus	596.37 ± 282.61	496.67 ± 330.54	-16.7%[a]
	Abstinent	672.00 ± 299.84	267.89 ± 146.77	-60.1%[b,d]
Total NNN (ng/24-h Urine)	Dual Use	24.08 ± 21.93[*]	28.43 ± 41.40	18.1%[a]
	Snus	42.70 ± 69.46[*]	22.62 ± 50.55	-47.0%[b,d]
	Abstinent	28.29 ± 19.77	1.79 ± 2.06	-93.7%[b,d]
Total NAB (ng/24-h Urine)	Dual Use	68.30 ± 35.44	42.57 ± 26.88	-37.7%[a,b]
	Snus	68.26 ± 37.74	29.73 ± 41.51	-56.4%[a,b]
	Abstinent	74.85 ± 43.16	1.25 ± 0.87	-98.3%[b,d]
Total NAT (ng/24-h Urine)	Dual Use	412.92 ± 238.52	264.00 ± 184.62	-36.1%[a,b]
	Snus	407.72 ± 250.76	163.30 ± 252.32	-59.9%[a,b]
	Abstinent	442.03 ± 236.39	1.93 ± 2.29	-99.6%[b,d]
COHb (% Saturation in Whole Blood)	Dual Use	5.58 ± 2.27	2.90 ± 0.90	-48.1%[a,b]
	Snus	5.24 ± 1.62	0.98 ± 0.27	-81.3%[b,d]
	Abstinent	6.19 ± 2.55	1.08 ± 0.22	-82.5%[b,d]
Thiocyanate (µmol/24-h Urine)	Dual Use	201.49 ± 100.07	157.65 ± 79.35	-21.8%[b]
	Snus	224.24 ± 122.60	119.29 ± 46.74	-46.8%[b,d]
	Abstinent	222.38 ± 144.84	135.91 ± 92.48	-38.9%[b]
Thiocyanate (µmol/L Plasma)	Dual Use	126.76 ± 45.11	99.78 ± 32.21	-21.3%[a,b]
	Snus	121.12 ± 39.48	80.55 ± 26.74	-33.5%[b,d]
	Abstinent	122.36 ± 51.37	79.50 ± 28.04	-35.0%[b,d]
YG1024 Mutagenicity (Revertants/10^3/24-h Urine)	Dual Use	172.62 ± 118.10	100.44 ± 61.22	-41.8%[a,b]
	Snus	164.51 ± 85.17	49.74 ± 81.45	-69.8%[b]
	Abstinent	187.78 ± 107.49	34.36 ± 43.41	-81.7%[b,d]
3-aminobiphenyl (ng/24-h Urine)	Dual Use	11.75 ± 6.34	6.46 ± 3.88	-45.0%[a,b]
	Snus	12.22 ± 5.49	1.41 ± 0.75	-88.5%[b,d]
	Abstinent	14.10 ± 6.15	1.33 ± 0.87	-90.6%[b,d]
4-aminobiphenyl (ng/24-h Urine)	Dual Use	26.42 ± 11.39	14.64 ± 6.24	-44.6%[a,b]
	Snus	26.09 ± 11.36	3.74 ± 1.41	-85.6%[b,d]
	Abstinent	27.44 ± 10.79	4.31 ± 1.67	-84.3%[b,d]

Biomarker of exposure	Usage	Baseline	Day 5	% Change
2-aminonaphthalene (ng/24-h Urine)	Dual Use	37.92 ± 17.13	20.35 ± 9.27	-46.3%[a,b]
	Snus	37.65 ± 14.77	3.28 ± 1.43	-91.3%[b,d]
	Abstinent	39.34 ± 16.97	3.53 ± 1.35	-91.0%[b,d]
o-Toluidine (ng/24-h Urine)	Dual Use	228.39 ± 86.02*	159.04 ± 62.47	-30.4%[a,b]
	Snus	223.18 ± 75.07*	89.82 ± 36.41	-59.8%[b,d]
	Abstinent	255.76 ± 84.22	102.82 ± 43.38	-59.8%[b,d]
1-OH-Pyrene (ng/24-h Urine)	Dual Use	392.62 ± 173.07	404.56 ± 386.46	3.0%[nsd]
	Snus	410.85 ± 172.96	377.07 ± 520.67	-8.2%[nsd]
	Abstinent	408.13 ± 218.78	241.13 ± 259.45	-40.9%[nsd]
1-OH-Naphthalene (µg/24-h Urine)	Dual Use	12.08 ± 5.72	7.60 ± 3.68	-37.1%[a,b]
	Snus	12.80 ± 5.95	2.59 ± 4.03	-79.7%[b,d]
	Abstinent	12.60 ± 6.29	2.60 ± 4.59	-79.4%[b,d]
2-OH-Naphthalene (µg/24-h Urine)	Dual Use	17.75 ± 7.24	11.33 ± 5.27	-36.2%[a,b]
	Snus	18.76 ± 6.63	4.08 ± 3.08	-78.2%[b,d]
	Abstinent	18.01 ± 6.22	3.30 ± 1.69	-81.7%[b,d]
2-OH-Flourene (ng/24-h Urine)	Dual Use	2048.77 ± 813.21	1406.72 ± 565.19	-31.3%[a,b]
	Snus	2166.66 ± 780.94	574.39 ± 217.58	-73.5%[b,d]
	Abstinent	2141.86 ± 860.33	597.85 ± 260.32	-72.1%[b,d]
1-OH-Phenanthrene (ng/24-h Urine)	Dual Use	263.02 ± 88.87	204.90 ± 63.79	-22.1% [a,b]
	Snus	284.52 ± 91.67	139.17 ± 62.90	-51.1% [b,d]
	Abstinent	284.93 ± 111.25	131.74 ± 57.74	-53.8% [b,d]
2-OH-Phenanthrene (ng/24-h Urine)	Dual Use	149.49 ± 48.21	120.28 ± 38.69	-19.5% [a,b]
	Snus	153.17 ± 59.28	80.57 ± 54.85	-47.4% [b,d]
	Abstinent	153.75 ± 62.31	74.60 ± 51.04	-51.5% [b,d]
3-OH-Phenanthrene (ng/24-h Urine)	Dual Use	316.06 ± 102.81	242.37 ± 85.47	-23.3% [a,b]
	Snus	332.80 ± 94.68	129.17 ± 73.03	-61.2% [b,d]
	Abstinent	336.08 ± 130.20	118.55 ± 55.34	-64.7% [b,d]
4-OH-Phenanthrene (ng/24-h Urine)	Dual Use	65.91 ± 25.71	49.71 ± 18.43	-24.6%[a,b]
	Snus	69.69 ± 24.21	25.48 ± 17.12	-63.4%[b,d]
	Abstinent	75.48 ± 35.44	23.08 ± 16.83	-69.4%[b,d]
9-OH-Phenanthrene (ng/24-h Urine)	Dual Use	270.68 ± 140.19	194.75 ± 85.07	-28.1%[a,b]
	Snus	289.94 ± 116.74	71.71 ± 64.85	-75.3%[b,d]
	Abstinent	317.14 ± 155.69	68.33 ± 37.21	78.5%[b,d]
CEMA (µg/24-h Urine)	Dual Use	274.56 ± 137.25	150.79 ± 74.27	-45.1%[a,b]
	Snus	278.63 ± 122.74	37.69 ± 17.42	-86.5%[b,d]
	Abstinent	287.24 ± 117.63	40.99 ± 22.94	-85.7%[b,d]
HEMA (µg/24-h Urine)	Dual Use	12.33 ± 6.91	8.73 ± 5.50	-29.2%[a,b]
	Snus	16.07 ± 8.40*	6.73 ± 2.99	-58.1%[b,d]
	Abstinent	15.64 ± 12.86	7.27 ± 4.49	-53.5%[b,d]
HPMA (µg/24-h Urine)	Dual Use	2820.28 ± 1352.86	1415.78 ± 558.73	-49.8%[a,b]
	Snus	2678.81 ± 978.80	445.39 ± 288.51	-83.4%[b,d]
	Abstinent	2865.21 ± 1210.80	473.70 ± 212.59	-83.5%[b,d]

Biomarker of exposure	Usage	Baseline	Day 5	% Change
AAMA (µg/24-h Urine)	Dual Use	359.58 ± 115.27*	265.04 ± 95.57	-26.3%[a,b]
	Snus	428.89 ± 140.62*	152.92 ± 73.80	-64.3%[b,d]
	Abstinent	405.57 ± 124.00	146.11 ± 37.85	-64.0%[b,d]
GAMA (µg/24-h Urine)	Dual Use	49.79 ± 20.00	39.18 ± 15.33	-21.3%[a,b]
	Snus	57.31 ± 22.00	27.64 ± 10.53	-51.8%[b,d]
	Abstinent	49.06 ± 20.82	24.81 ± 7.72	-49.4%[b,d]
HMPMA (µg/24-h Urine)	Dual Use	642.88 ± 287.21	363.11 ± 164.18	-43.5%[a,b]
	Snus	617.87 ± 242.53	125.78 ± 57.97	-79.6%[b,d]
	Abstinent	614.94 ± 231.47	125.49 ± 62.93	-79.6%[b,d]
MHBMA (ng/24-h Urine)	Dual Use	7688.81 ± 4716.43	3833.51 ± 2285.38	-50.1%[a,b]
	Snus	8911.75 ± 5896.10	707.07 ± 247.61	-92.1%[b,d]
	Abstinent	10702.67 ± 8487.79*	738.32 ± 328.26	-93.1%[b,d]
SPMA (ng/24-h Urine)	Dual Use	6349.75 ± 4247.47	3411.91 ± 2350.75	-46.3%[a,b]
	Snus	6302.54 ± 3168.36	523.27 ± 301.82	-91.7%[b,d]
	Abstinent	7288.75 ± 4947.08*	612.93 ± 408.37	-91.6%[b,d]
Nicotine (ng/mL Plasma @ 22:00)	Dual Use	28.43 ± 14.34	15.34 ± 6.84	-46.0%[a,b]
	Snus	27.25 ± 9.21	10.37 ± 9.18	-61.9%[a,b]
	Abstinent	29.90 ± 14.91	0.78 ± 0.90	-97.4%[b,d]
Cotinine (ng/mL Plasma @ 22:00)	Dual Use	300.63 ± 135.61	191.91 ± 103.83	-36.2%[a,b]
	Snus	296.65 ± 108.06	143.09 ± 126.69	-51.8%[a,b]
	Abstinent	317.39 ± 149.40	3.85 ± 5.88	-98.8%[b,d]

*Statistically ($p \leq 0.05$) different for between group pair-wise comparisons at baseline
[nsd] Not statistically different in either between group pair-wise, or within group baseline comparisons
[a] Statistically ($p \leq 0.05$) different compared to Abstinent group
[b] Statistically ($p \leq 0.05$) different compared to within group baseline comparison
[d] Statistically ($p \leq 0.05$) different compared to the Dual use group

Two apparent exceptions to the general trend of biomarkers indicating reduced toxicant exposure were 1-OH-pyrene (1-HOP) and total NNN. Since eight other PAH metabolites displayed consistent and substantial reductions in both dual users and exclusive Camel Snus users, the results suggest, as other publications have suggested (*e.g.*, Hecht *et al.* 2004; St. Helen *et al.* 2012; USDHHS 2010), that 1-HOP is not a reliable biomarker of PAH exposure in tobacco studies. Likewise, the 18% increase in total urinary NNN for dual users was not significantly different from baseline, and biomarkers for total TSNAs and all other individual TSNAs were significantly reduced in dual users and exclusive Camel Snus users. Although the wider applicability of the study's findings may be limited by restrictions inherent in this type of trial, they nonetheless point to meaningful reductions in tobacco-related toxicants for dual users and those who switch completely to Camel Snus. A more detailed examination of this published study is provided in the review of RJRT clinical study CSD0901 in Section 6.1.2.3 below.

In addition to the Krautter *et al.* 2015 confinement study described above, a series of ambulatory clinical studies were conducted to evaluate biomarkers in adult smokers who

progressively reduced the use of cigarettes over a period of 4 weeks (reduce by 25% the first week, 50% the second, 75% the third), while substituting several types of smokeless tobacco products, including Camel Snus Frost and Camel Snus Mellow (600 mg pouches) (Round *et al.* 2015). By week 4, mean self-reported cigarette consumption reductions of 59% were achieved in the Camel Snus group (n=32) relative to week 1. Over the three weeks of dual use, subjects used between 2.7 and 3.5 Camel Snus pouches on average per day, and pouch use increased, but not with statistical significance. Comparing week 1 and week 4, statistically significant reductions were observed in mouth level exposures to daily tar (-60.2%; Table 4), daily nicotine (-45.1%; Table 6), and daily NNK (-65.4%; Table 6) as a result of the combination of reduced cigarette consumption and dual use of Camel Snus. Reductions were also seen in serum cotinine (-9.5%), urinary nicotine equivalents (-16.0%), urinary 3-HPMA (a biomarker of acrolein exposure; -23.4%) and urinary total NNAL (-8.6%), although only urinary 3-HPMA reductions reached statistical significance (Table 7). Statistically significant reductions in 16 additional biomarkers were also observed, with decreases ranging from 12.4% to 35.7% (Table 8). No statistically significant increases in any measured biomarkers were observed in dual users. According to questionnaire data collected as part of the study, acceptability of cigarettes decreased and acceptability of Camel Snus increased over time; however, ratings of cigarettes remained higher throughout the study. By week 4, subjects' overall opinions of Camel Snus were only slightly less than their opinion of cigarettes. The results indicate that smokers who switch to dual use of cigarettes experience statistically significant decreases in exposures to many important tobacco toxicants. A more detailed examination of this published study is provided in the review of RJRT clinical study CSD0905 in Section 6.1.2.3.

In a randomized, multi-center study of adult cigarette smokers randomly switched to Camel Snus Frost (400 mg), Camel Snus Spice (400 mg) and Camel Snus Original (400 mg) for 24 weeks, biomarkers of exposure and effect were compared to study subject baseline measurements (representing biomarker levels associated with current smoking) and to measurements from never-smokers (Ogden *et al.* 2015a; Ogden *et al.* 2015b; Ogden *et al.* 2015c). Products other than Camel Snus (a tobacco heating cigarette and a very low-yielding combustible cigarette) were also assessed in this study, but since they are not relevant to this MRTP, they will not be further discussed.

With respect to biomarkers of exposure, samples for 24-h urine, spot urine and fasting blood were collected and analyzed for nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), aromatic amines, polycyclic aromatic hydrocarbons (PAHs), acrylamide, 1,3-butadiene, crotonaldehyde, acrolein, benzene and carbon monoxide. In addition, urine mutagenicity was evaluated using the Ames assay with bacterial strains TA98 and YG1024. Measurements were taken from samples obtained at baseline (week 0) and at weeks 12 and 24 (Ogden *et al.* 2015b). Forty-three smokers were initially enrolled in the study and switched to Camel Snus, but only 20 completed the full 24-week study duration, and subjects switched to Camel Snus continued to smoke some cigarettes each day.

Urinary biomarkers of nicotine exposure revealed no significant change from baseline at either 12 or 24 weeks for switchers to Camel Snus. Serum cotinine levels were likewise unchanged at

week 12, but were significantly increased (32%) at week 24. Biomarkers of most other measured tobacco toxicants were significantly reduced compared to baseline as follows: NNK exposure (as measured by total NNAL) was decreased by 39% at week 12, and 35% at week 24; urinary biomarkers of 4 aromatic amines (3-ABP, 4-ABP, 2-AN, and o-T) were all significantly reduced (42-66%) at weeks 12 and 24; of the six PAH biomarkers, 2-naphthol, 2-OHF, 1-/9-OHPh, and 2-/3-OHPh were significantly reduced at weeks 12 and 24, while 1-naphthol and 1-OHP showed no significant changes at either time point; acrylamide exposure, assessed by AAMA and GAMA, were significantly reduced (21-39%) at both weeks 12 and 24; 1,3-butadiene exposure, assessed by the more accurate of two reported biomarkers (MHBMA) (Carmella *et al.* 2009), was significantly reduced by approximately 60% at both weeks 12 and 24; urinary biomarkers for crotonaldehyde (HMPMA), acrolein (HPMA) and benzene (SPMA) were all significantly reduced at weeks 12 and 24. Urinary mutagenicity was likewise reduced at weeks 12 (by 63%) and week 24 (by 53%).

Blood biomarkers of carbon monoxide exposure (COHb) were reduced in switchers to Camel Snus by 59% at week 12 and 37% at week 24; for 4-ABP-Hb adducts (a blood biomarker of aromatic amines), levels were reduced 12-23%, but the reductions did not reach statistical significance. Tables of values for levels of all measured biomarkers as well as percent reductions compared to baseline are presented in the two tables below (Table 6.1.2-18; Table 6.1.2-19).

Results from this 24-week study confirm that adult cigarette smokers who switch from their usual brand to Camel Snus significantly reduce their exposures to many tobacco toxicants compared to continued smoking. The observed reductions occurred despite study subjects' dual use of Camel Snus and cigarettes during the study period. Thus, the biomarker values after 12 and 24 weeks of "switching" should be interpreted as estimates of exposures due to dual or poly-tobacco use, rather than estimates of exposures due to complete switching. Greater improvements in these biomarkers of exposure should occur with complete switching (Ogden *et al.* 2015b).

Table 6.1.2-18: Select urine biomarkers of exposure over time: Switchers from usual brand cigarettes to Camel Snus (from Ogden *et al.* 2015b)

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
NICEq (mg/24 h)	26.7 (20.8 – 32.6)	12	19	24.3 (19.5 – 29.1)	-9
	26.7 (20.8 – 32.6)	24	19	24.3 (19.4 – 29.2)	-9
Total NNAL (ng/24 h)	747 (506 – 988)	12	19	459* (325 – 593)	-39
	747 (506 – 988)	24	19	489* (334 – 645)	-35
3-ABP (ng/24 h)	12.7 (9.45 – 15.9)	12	19	5.34* (3.76 – 6.93)	-58
	12.7 (9.45 – 15.9)	24	19	6.27* (4.14 – 8.40)	-51
4-ABP (ng/24 h)	28.1 (22.7 – 33.5)	12	19	13.3* (10.3 – 16.3)	-53
	28.1 (22.7 – 33.5)	24	19	15.5* (11.1 – 20.0)	-45
2-AN (ng/24 h)	41.8 (33.4 – 50.3)	12	19	14.4* (10.0 – 18.8)	-66
	41.8 (33.4 – 50.3)	24	19	18.8* (12.5 – 25.1)	-55
o-T (ng/24 h)	303 (243 – 363)	12	19	162* (125 – 200)	-46
	303 (243 – 363)	24	19	176* (130 – 221)	-42

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
1-Naphthol (µg/24 h)	39.3 (27.5 – 51.1)	12	19	29.8 (20.2 – 39.5)	-24
	39.3 (27.5 – 51.1)	24	19	44.3 (24.2 – 64.4)	13
2-Naphthol (µg/24 h)	31.1 (24.9 – 37.4)	12	19	17.9* (14.3 – 21.5)	-42
	31.1 (24.9 – 37.4)	24	19	21.2* (14.6 – 27.7)	-32
2-OHF (µg/24 h)	3.94 (3.09 – 4.78)	12	18	2.51* (1.65 – 3.36)	-36
	4.10 (3.23 – 4.96)	24	19	2.69* (1.62 – 3.77)	-34
1-/9-OHPh (µg/24 h)	1.23 (0.95 – 1.50)	12	19	0.72* (0.49 – 0.96)	-41
	1.23 (0.95 – 1.50)	24	19	0.68* (0.50 – 0.86)	-45
2-/3-OHPh (µg/24 h)	0.93 (0.71 – 1.14)	12	19	0.56* (0.32 – 0.81)	-39
	0.93 (0.71 – 1.14)	24	19	0.53* (0.38 – 0.68)	-43
1-OHP (ng/24 h)	633 (442 – 823)	12	19	626 (189 – 1063)	-1
	633 (442 – 823)	24	19	411 (306 – 516)	-35
AAMA (µg/24 h)	379 (295 – 463)	12	19	243* (184 – 303)	-36
	379 (295 – 463)	24	19	230* (180 – 281)	-39
GAMA (µg/24 h)	57.4 (41.5 – 73.3)	12	19	41.6* (31.2 – 51.9)	-28
	57.4 (41.5 – 73.3)	24	19	45.1* (35.8 – 54.3)	-21
DHBMA (µg/24 h)	812 (677 – 947)	12	19	713 (613 – 814)	-12
	812 (677 – 947)	24	19	741 (601 – 881)	-9
MHBMA (µg/24 h)	7.53 (4.43 – 10.6)	12	19	2.51* (1.68 – 3.35)	-67
	7.53 (4.43 – 10.6)	24	19	3.43* (2.11 – 4.75)	-55
HMPMA (mg/24 h)	9.65 (7.75 – 11.6)	12	19	4.35* (3.00 – 5.71)	-55
	9.65 (7.75 – 11.6)	24	19	5.02* (3.26 – 6.78)	-48
HPMA (mg/24 h)	2.26 (1.75 – 2.77)	12	19	1.25* (0.884 – 1.61)	-45
	2.26 (1.76 – 2.77)	24	19	1.32* (0.922 – 1.73)	-41
SPMA (µg/24 h)	14.1 (9.81 – 18.3)	12	19	5.98* (4.18 – 7.78)	-57
	14.1 (9.81 – 18.3)	24	19	7.03* (4.88 – 9.18)	-50
Urinary Mutagenesis (TA98) (revertants /24 h)	$1.86X10^7$ ($1.26X10^7$ – $2.48X10^7$)	12	18	$8.26X10^6$* ($5.49X10^6$ – $1.10X10^7$)	-56
	$1.87X10^7$ ($1.26X10^7$ – $2.47X10^7$)	24	18	$8.61X10^6$* ($5.64X10^6$ – $1.16X10^7$)	-54

*p < 0.05. Statistical model tested if adjusted least square mean is 0

‡Arithmetic mean based on matched subjects between Week 0 and Week 12 or 24. Values are rounded

¶Calculated using actual means. Values are rounded

Table 6.1.2-19: Select blood biomarkers of exposure over time: Switchers from usual brand cigarettes to Camel Snus (from Ogden *et al.* 2015b)

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
4-ABP-Hb (pg/g Hb)	63.7 (50.2 – 77.2)	12	15	49.2 (33.4 – 64.9)	-23
	63.7 (51.1 – 76.2)	24	16	55.8 (44.5 – 67.1)	-12
COHb (% saturation)	5.93 (4.98 – 6.87)	12	19	2.43* (1.81 – 3.05)	-59
	5.93 (4.98 – 6.87)	24	19	3.75* (2.51 – 4.99)	-37
Cotinine (nmol/L)	1.66 (1.42 – 1.91)	12	15	1.46 (1.21 – 1.71)	-12
	1.59 (1.35 – 1.82)	24	18	2.09* (1.66 – 2.53)	32

*p < 0.05. Statistical model tested if adjusted least square mean is 0

‡Arithmetic mean based on matched subjects between Week 0 and Week 12 or 24. Values are rounded

¶Calculated using actual means. Values are rounded

To complement the measurements of exposure biomarkers presented above, biomarkers of biological effect were also determined in this same group of switchers to Camel Snus (Ogden *et al.* 2015c). Various biomarkers of inflammation, oxidative damage, lipids, hypercoagulable state, insulin resistance, endothelial function and DNA were again compared at baseline, representing current smoking, and at 12 and 24 weeks after switching to Camel Snus. This study also included a never smoker group (n=32) for baseline comparisons. Notably, only half of the biomarkers of biological effect that were evaluated were statistically significantly different in the baseline comparisons between smokers and never smokers. Differences in CRP, HDL, LDL, HDL/LDL, triglycerides, fibrinogen, and platelets had been reported in some previous studies (Frost-Pineda *et al.* 2011; Hatsukami *et al.* 2006; Lowe *et al.* 2013) but not all (Calapai *et al.* 2009; Lowe *et al.* 2009). No differences in these markers were observed between baseline current smoking and following switching to Camel Snus in the current study.

Tables illustrating baseline biomarker values, values at 12 and 24 weeks, and percent changes from baseline are presented (Table 6.1.2-20; Table 6.1.2-21).

Table 6.1.2-20: Select urine biomarkers of biological effect over time: Switchers from usual brand cigarettes to Camel Snus (from Ogden *et al.* 2015c)

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
$iPF_{2\alpha}$-III (µg/24 h)	0.827 (0.585 – 1.07)	12	19	0.688 (0.523 – 0.852)	-17
	0.827 (0.585 – 1.07)	24	19	0.625 (0.410 – 0.840)	-24*
$PGF_{2\alpha}$ (µg/24 h)	2.01 (1.46 – 2.55)	12	19	1.96 (1.32 – 2.59)	-2
	2.01 (1.46 – 2.55)	24	19	1.72 (1.32 – 2.12)	-14
2,3-dinor- $iPF_{2\alpha}$-III (µg/24 h)	4.89 (3.83 – 5.95)	12	19	4.36 (3.60 – 5.13)	-11
	4.89 (3.83 – 5.95)	24	19	4.17 (3.24 – 5.10)	-15
(±)5-$iPF_{2\alpha}$-VI (µg/24 h)	2.82 (2.19 – 3.46)	12	19	2.57 (2.10 – 3.05)	-9
	2.83 (2.19 – 3.46)	24	19	2.55 (1.93 – 3.16)	-10
8,12-iso- $iPF_{2\alpha}$-VI (µg/24 h)	5.36 (4.16 – 6.56)	12	19	4.70 (3.73 – 5.67)	-12
	5.36 (4.16 – 6.56)	24	19	4.44 (3.47 – 5.42)	-17*

*$p < 0.05$. Statistical model tested if adjusted least square mean is 0.
‡Arithmetic mean based on matched subjects between Week 0 and Week 12 or 24. Values are rounded.
¶Calculated using actual means. Values are rounded.

Five of eight biomarkers of inflammation or oxidative stress ($iPF_{2\alpha}$-III, (±)5-$iPF_{2\alpha}$-IV, 8,12-iso-$iPF_{2\alpha}$-VI, sICAM1, and WBC counts), three of five hypercoagulable state biomarkers (HCT, HgB and homocysteine) and the marker of DNA damage (sister chromatid exchange (SCE) assay) were all higher in baseline measurements comparing smokers and never smokers. In the switchers to Camel Snus, the isoprostane biomarkers $iPF_{2\alpha}$-III and 8,12-iso- $iPF_{2\alpha}$-VI were significantly reduced at week 24, but not at week 12; sICAM1 was significantly reduced by approximately 13% at both time points; WBC counts were significantly reduced by 10% at week

12, but not at week 24. No changes were observed in the markers for hypercoagulable state. HgBA1c, a marker of insulin resistance, was not different between smokers and never smokers at baseline; however, a small (2%) but statistically significant increase was observed at week 12 but not week 24 in the switchers to Camel Snus. Levels of circulating endothelial precursor cells (CEPs), a biomarker of endothelial function, were not significantly different between smokers and never smokers at baseline, but were non-significantly elevated by 220% and 140% at weeks 12 and 24 respectively in switchers to Camel Snus. Finally, a biomarker of DNA damage, the SCE assay, was higher (14%) in snus users at week 12, but lower (4%) at week 24, suggesting that the increase was a spurious finding.

As was noted in the publication reporting biomarkers of exposure (Ogden *et al.* 2015b), switching from cigarette smoking to Camel Snus was less than 100%, and results should be interpreted as being associated with dual or poly-tobacco use in an ambulatory setting. In spite of this limitation, a number of biomarkers of biological effect changed among the switchers to Camel Snus in a favorable direction, and none significantly changed in an unfavorable direction. Whether changes in certain biomarkers of biological effect are directly relevant to changes in risk for smoking-related disease is not currently known. However, these results are consistent with epidemiology and other data that confirm that smokers who switch to smokeless tobacco products, including Camel Snus, lower their risk of disease compared with continued cigarette smoking.

Table 6.1.2-21: Select blood biomarkers of biological effect over time: Switchers from usual brand cigarettes to Camel Snus (from Ogden *et al.* 2015c)

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
OxLDL (U/L)	106 (90.9 – 122)	12	15	97.6 (82.8 – 112)	-8
	104 (90.4 – 118)	24	17	102 (87.4 – 117)	-2
CRP (mg/L)	3.20 (1.61 – 4.79)	12	17	3.08 (1.67 – 4.49)	-4
	3.15 (1.66 – 4.64)	24	18	3.13 (2.14 – 4.11)	-1
HDL (mmol/L)	1.09 (0.930 – 1.25)	12	18	1.10 (0.907 – 1.29)	1
	1.10 (0.928 – 1.26)	24	17	1.12 (0.944 – 1.29)	2
HDL/LDL (ratio)	0.322 (0.262 – 0.382)	12	17	0.359 (0.279 – 0.438)	11
	0.334 (0.268 – 0.400)	24	15	0.366 (0.285 – 0.447)	10
LDL (mmol/L)	3.56 (3.23 – 3.88)	12	17	3.30 (2.86 – 3.74)	-7
	3.53 (3.16 – 3.89)	24	15	3.42 (2.93 – 3.91)	-3
CEP (counts)	13.1 (0.965 – 25.3)	12	15	41.9 (10.5 – 73.2)	219
	22.7 (-0.608 – 45.9)	24	16	54.5 (22.4 – 86.8)	141
Fibrinogen (g/L)	3.22 (2.95 – 3.48)	12	16	3.22 (2.98 – 3.47)	0
	3.18 (2.90 – 3.46)	24	15	3.23 (2.96 – 3.50)	2
HCT (%)	0.461 (0.440 – 0.481)	12	17	0.457 (0.433 – 0.481)	-1
	0.460 (0.439 – 0.481)	24	14	0.456 (0.433 – 0.480)	-1
HgB (g/L)	148 (142 – 155)	12	19	148 (141 – 155)	0
	149 (142 – 156)	24	17	150 (142 – 157)	1

Biomarker	Baseline Mean‡ (95% CI)	Follow-up (Week #)	n	Follow-up Mean‡ (95% CI)	% change¶
HgBA1c (%)	5.57 (5.39 – 5.75)	12	19	5.69 (5.50 – 5.88)	2*
	5.59 (5.39 – 5.79)	24	17	5.62 (5.30 – 5.95)	1
Homocysteine (µmol/L)	8.86 (7.85 – 9.87)	12	19	8.55 (7.54 – 9.55)	-4
	8.86 (7.85 – 9.87)	24	19	8.99 (8.04 – 9.94)	1
Platelets (GI/L)	286 (266 – 306)	12	17	272 (250 – 295)	-5
	292 (270 – 314)	24	17	276 (248 – 304)	-6
SCE (Mean events)	7.23 (6.77 – 7.69)	12	19	8.21 (7.63 – 8.79)	14*
	7.24 (6.76 – 7.73)	24	18	6.92 (6.25 – 7.59)	-4
sICAM1 (ng/mL)	318 (280 – 356)	12	16	281 (248 – 314)	-12*
	310 (275 – 346)	24	18	272 (241 – 302)	-13*
Triglycerides (mmol/L)	2.22 (1.55 – 2.88)	12	18	2.24 (1.46 – 3.01)	1
	2.16 (1.46 – 2.85)	24	17	2.20 (1.45 – 2.96)	2
WBC (GI/L)	8.01 (7.07 – 8.96)	12	19	7.25 (6.49 – 8.01)	-10*
	8.03 (6.96 – 9.11)	24	17	7.58 (6.62 – 8.54)	-6

*p < 0.05. Statistical model tested if adjusted least square mean is 0
‡Arithmetic mean based on matched subjects between Week 0 and Week 12 or 24. Values are rounded
¶Calculated using actual means. Values are rounded

A more detailed examination of this published study is provided in the review of RJRT clinical study HSD0702 in Section 6.1.2.3.

Levels of seven biomarkers of tobacco exposure, obtained from NHANES data from 1999-2012, have also been recently determined from a representative sample of U.S. male dual users of cigarettes and smokeless tobacco, as well as from exclusive smokers and exclusive ST users (Rostron *et al.* 2015) [results for exclusive smokers and ST users are discussed in prior sections]. Dual users smoked fewer cigarettes than exclusive smokers (11.9 vs. 14.8), but the difference did not reach statistical significance. Dual users also smoked cigarettes on fewer of the past 5 days on average than exclusive smokers, and dual users who used smokeless tobacco (chewing tobacco or snuff) in the past 5 days used those products on fewer days than exclusive smokeless tobacco users. Levels of biomarkers were either similar to those found in exclusive ST users, or intermediate between exclusive ST users and exclusive smokers. Serum cotinine concentrations and urinary NNAL were higher for dual users than for exclusive smokers, and similar to levels found for exclusive ST users. These results indicate that dual users are not exposed to higher levels of these, or likely any other important tobacco toxicants, compared to either exclusive smokers or ST users, and may reduce the levels of several toxicants compared with cigarette smoking.

6.1.2.2.3.1 Summary and conclusions: Biomarker studies of exposure and effect in Switchers and Dual Users

In summary, clinical studies of individuals who switch to smokeless tobacco or engage in dual use of cigarettes and smokeless tobacco, including Camel Snus, indicate that such individuals are exposed to either the same or lower levels of most tobacco toxicants that have been

measured. It is also noteworthy that, at least in clinical settings, individuals who are switched to Camel Snus and engage in dual use smoke fewer cigarettes, and often experience a reduced total nicotine exposure. The observed reductions in the number of cigarettes smoked, which accounts for the overwhelming fraction of tobacco-related risk in dual users, is a clearly beneficial outcome.

6.1.2.2.4 Clinical studies of smokeless tobacco products and FDA-approved smoking cessation products: Smoking cessation/reduction and biomarker analysis

The focus of the messaging proposed in the MRTPA for Camel Snus is that exclusive use of Camel Snus presents specific lower health risks compared with cigarette smoking. However, both IOM 2012 and the FDA Draft Guidance note that health risks presented by the modified risk product compared with those associated with the use of FDA-approved smoking cessation products is also informative, since "these [latter] products pose very few, if any risks to health. These products provide an aspirational goal for risk and exposure from MRTPs. In principle, the closer the risks and exposures from the MRTP are to cessation products, the more confident a regulator can be in the chances for net public health benefit" (IOM 2012, p. 11-12). The guidance documents point out that the goal is to evaluate comparative risks or comparative toxicant exposures from these products, rather than their utility as aids in smoking cessation. Nonetheless, it would seem obvious that if use of a smokeless tobacco product results in the reduction or elimination of cigarette smoking, that exposure to many toxicants, and as a consequence, risk, would likely be lower as has been demonstrated in both biomarker and epidemiological studies. Clinical studies, in which both smokeless tobacco products and approved smoking cessation products are evaluated in terms of biomarker analysis, as well as product preferences and smoking reduction effects, are therefore relevant and are reviewed below.

A small 2007 pilot study examined the effects of smokeless tobacco products, including a low nitrosamine moist snuff (snus) product (Exalt™, manufactured by Swedish Match), as compared to medicinal nicotine (Commit medicinal nicotine lozenge) (Mendoza-Baumgart *et al.* 2007). The study design involved 39 adult smokers interested in quitting. After a series of baseline measurements conducted during the first week of the study, participants were asked to quit smoking, with half using the moist snuff product, and half using medicinal nicotine for 2 weeks (period 1); each group then crossed over to use the other product for 2 weeks (period 2). A final study week allowed participants to use either product. Over the course of the 6-week study, blood, urine, carbon monoxide (CO), heart rate and blood pressure were analyzed. No significant differences in the amounts of each product used were observed (use of either Exalt or Commit results in similar concentrations of plasma nicotine), but less product was used by both groups in period 2. Significant reductions in CO levels, total cotinine levels and total NNAL levels were observed from baseline measures during *ad lib* smoking to the end of periods 1 and 2 of product use within each group. While there were no significant differences in CO levels or cotinine levels between Exalt and Commit observed, significantly higher total NNAL levels were observed after Exalt use, although levels were decreased approximately 55% compared with baseline smoking levels. The two products showed equal effects in moderating cravings or

withdrawal symptoms, and produced no significant differences in vital signs (blood pressure, heart rate), white blood cell counts or hemoglobin levels. The authors noted that it is already evident that ST use is less hazardous than cigarette smoking, and that low nitrosamine products such as snus expose users to lower toxicant levels compared to most conventional U.S. moist snuff. Although the participants in this small study preferred medicinal nicotine over the snus product during the final study week, many smokers may prefer a tobacco product such as snus. This study has demonstrated that for many measures, snus and medicinal nicotine are equivalent and that while nitrosamine levels are elevated in association with snus use, they are substantially lower than from cigarette smoking.

Another small clinical study conducted in 2009 compared several ST products including Camel Snus (Original flavor; 400 mg size) to cigarettes and medicinal nicotine lozenges (Commit; 2 mg nicotine size) (Cobb *et al.* 2010). Twenty-eight regular smokers abstained from smoking overnight followed by a series of laboratory sessions where biomarker, physiological and subjective endpoints were measured over two periods of product use. Relative to baseline, smoking a usual brand cigarette resulted in significant increases in plasma nicotine as soon as 5 minutes after product administration, with peaks of 20.7 and 20.6 ng/ml during each of the two periods of use. In contrast, Camel Snus plasma nicotine levels were significantly greater than baseline 15 minutes after the second period of use, but reached a peak of only 7.6 ng/ml (SEM 1.1); no other conditions or time points were significantly different from baseline (the maximum level achieved following use of medicinal nicotine lozenges was 4.6 ng/ml (SEM 0.5)). Heart rate was significantly increased by cigarette use (from 67.8 bpm (SEM 1.9) to 82.3 bpm (SEM 2.3)) and by Camel Snus use (from 67.8 bpm (SEM 2.3) to 72.0 bpm (SEM 2.4), but not by use of medicinal nicotine lozenges. CO levels were significantly increased by smoking but not by either Camel Snus or medicinal nicotine. Subjective scores reflective of abstinence symptom suppression indicated that although both Camel Snus and medicinal nicotine were somewhat effective at suppressing abstinence symptoms, neither was as effective as smoking. The authors suggested that low levels of nicotine delivery coupled with only modestly effective abstinence symptom suppression raises questions regarding the ability of these particular versions of smokeless products to replace cigarette smoking in a harm reduction scenario. Regarding the relative acceptability of snus and nicotine replacement therapies, a small New Zealand study found that snus and the NRT product Zonnic (a small sachet of peppermint-flavored nicotine) were preferable to nicotine gum (Caldwell *et al.* 2010).

A 2007 study by Kotlyar *et al.* used a randomized cross-over clinical study design in which various effects of five different nicotine/tobacco products, including a loose moist snuff product (Copenhagen; 2 g per dip), medicinal nicotine lozenges (Commit, 4 mg size), and three other products not relevant to this review, were assessed (Kotlyar *et al.* 2007). The 10 male subjects were regular users of Copenhagen moist snuff (average use 2.4 tins/week, 8.1 dips/day) for at least one year. In a series of laboratory sessions, each participant used each product for 30 minutes, during which subjective measures of nicotine craving and withdrawal were obtained, and plasma nicotine concentrations determined during product use and for a subsequent period of 60 minutes. Nicotine exposure was significantly greater for moist snuff use compared with medicinal nicotine use as measured by either area under the concentration-time curve

(AUC $_{0\text{-}90}$ (ng x min/ml) = 1038 (806 – 1336) vs. 467 (361 – 604) respectively) or by maximal nicotine concentration (C_{max} (ng/ml) = 16.1 (12.1 – 21.5) vs. 7.3 (5.5 – 9.8) respectively). Time to maximal plasma nicotine concentration (T_{max}) did not differ significantly between products (an average of 27 – 33 minutes after initiating product use). Consistent with the different nicotine exposures, and the fact that Copenhagen was the usual brand of this small group of ST users, subjective measures of craving, withdrawal, and other measures of satisfaction were all significantly greater during use of moist snuff compared with medicinal nicotine.

Smokers interested in cessation were enrolled in a multi-week pilot study that examined the use of smokeless products, including Camel Snus (Original, Frost, and Spice flavors; sizes not specified) as possible cessation aids, medicinal nicotine (a choice of gum or lozenge), and other products not relevant to this review (Kotlyar *et al.* 2011). The study entailed an initial 2-week period for baseline smoking measurements, followed by a 1-week sampling period where smokers were given their randomly assigned product and asked to use it at least 1-2 times per day, followed by a 4-week intervention period where smokers quit smoking cigarettes and used only their assigned product at least every 2 hours, with additional use as necessary. Starting in the fifth week, subjects gradually reduced the use of their assigned product until complete cessation of nicotine use occurred by week's end. For the participants who completed the study (Camel Snus, n = 30; medicinal nicotine, n = 18), concentrations of exhaled CO, urinary cotinine, and urinary total NNAL were significantly lower at week 4 compared with baseline. NNAL reductions were not as great when using Camel Snus compared with medicinal nicotine. NNN levels were likewise significantly lower in users of medicinal nicotine, but not in Camel Snus users compared with baseline. Nicotine craving and withdrawal symptom scores were similar for Camel Snus and medicinal nicotine, but scores indicated that neither oral product was completely effective at providing a substitute tobacco use experience. Although the study was not sufficiently powered to detect differences in smoking cessation rates, no differences were noted between the study groups. The authors suggested that for smokeless products to be effective in smoking cessation, nicotine levels should exceed a certain threshold.

A randomized clinical trial, similar to but larger than that published by Kotlyar *et al.* 2011, was recently published (Hatsukami *et al.* 2016). The study recruited 391 smokers interested in completely switching to snus or nicotine gum. After 1 week of baseline smoking data collection, participants were randomized into one of the two product use groups: Camel Snus (Winterchill or Robust, 2.5 or 2.6 mg nicotine/pouch respectively; 1 g pouch size) or nicotine gum (Nicorette, 4 mg) for 12 weeks. Participants were encouraged but not required to use only the assigned product, at least 6-8 pieces per day for 30 minutes each. Follow-up sessions were conducted 13 and 26 weeks after the initiation of product use. Biomarkers were determined at baseline and at week 4 (to maximize data prior to possible relapse or drop-outs). At week 6, 56 of the 149 smokers in the Camel Snus group were using Camel Snus exclusively, 82 were cigarette and snus dual users, 5 were using only cigarettes and 6 had quit tobacco product use. By week 12, the respective numbers were 37, 73, 16 and 12. In the 153-member nicotine gum group, 56 were using gum exclusively, 79 were dual users, 9 were only smoking and 9 had quit tobacco use at week 6. At week 12, respective numbers were 40, 82, 6 and 13. For cotinine and total nicotine equivalents, significant reductions were observed from baseline to week 4 for both

groups. For urinary total NNAL, significant reductions were seen for nicotine gum but not for snus in the overall groups. When separated into product only and dual users, total NNAL was higher among snus versus nicotine gum users in both comparisons. And while no significant differences were observed between exclusive snus use and snus/cigarette dual users, significantly lower total NNAL levels were observed for exclusive nicotine gum use compared to gum/cigarette dual use. For NNN, significant reductions were observed among those who used gum only, but not for gum/cigarette dual users. No significant changes in NNN were observed for either exclusive or dual snus users. Usual brand cigarettes were more satisfying and psychologically rewarding than either smokeless product. With regard to continued product use at the final 26-week follow-up, 14.9% reported exclusive Camel Snus use compared with 6.0% exclusive gum use. Overall, the study results showed similar outcomes for Camel Snus and nicotine gum in regard to amount of product used, levels of cotinine attained, and the ability of each product to substitute for smoking. Nitrosamine biomarker levels were higher for snus users compared with medicinal nicotine as expected, given the relative levels of these constituents in each product, and as reported in other studies. In an online comment to this study from Peter Hajek (director of the Tobacco Dependence Research Unit at Barts and The London School of Medicine and Dentistry, Queen Mary), it was brought out that at 6 months, neither product led to substantial smoking cessation rates (5% and 3%), but 27% continued to use Camel Snus compared with 13% who still used gum (Hajek 2016). This use disparity suggested that longer term, snus is more attractive to smokers than gum, and that "even if the two products have the same effect, snus would have a better population impact."

6.1.2.2.4.1 Summary and conclusions: Biomarker studies of smokeless tobacco products and FDA-approved smoking cessation products

In summary, the IOM has stated that FDA-approved smoking cessation products pose very few, if any risks to health, and that the closer the risks and exposures from the MRTP are to cessation products, the more confident a regulator can be in the chances for net public health benefit. Smokeless tobacco products, including Camel Snus share important characteristics with cessation products, with each delivering nicotine to the user, but presenting no tobacco combustion-related toxicants. In both types of products, the levels of these toxicants are far below those delivered by cigarette smoking. Clinical studies that have compared characteristics of smokeless tobacco and medicinal nicotine have found higher levels of two TSNAs in smokeless products, including Camel Snus, but approximately equal performance regarding measures of tobacco use satisfaction. Larger and more representative studies are called for, but to date, clinical study results suggest a potentially important role for smokeless tobacco in reducing smoking-related toxicant exposures:

> "…an MRTP should be compared with one or more NRTs in RCTs (Kotlyar *et al.* 2007); however, note that the MRTP need not necessarily be "better" or even equivalent to the NRT in order to exert a public health benefit. An MRTP that is inferior to NRTs (more toxicants, less effective at boosting cessation of smoking conventional cigarettes) could still exert a net public health benefit if its modest effects were additive, meaning they occurred on top of those of NRTs. For example, while not being very effective at helping smokers quit when used as a sole product, it is possible that the combination of NRT

plus the MRTP yields additive (or even positive synergistic) effects on smoking cessation when in combination. This is entirely possible because combinations of NRT medications are more effective than single medications (Fiore *et al.* 2008; Piper *et al.* 2009; Smith *et al.* 2009). Another possibility is that dual use reduces the rate of cigarette use and exposure to toxicants and therefore results in a net benefit to both individual and public health" (IOM 2012, p. 184).

6.1.2.2.5 Published clinical studies - summary and conclusions

Results from published clinical studies that include biomarker measurements consistently present contrasting overall biomarker profiles for ST users, including Camel Snus, compared with cigarette smokers. In particular, it is clear that ST users in general, and Camel Snus users in particular, are exposed to substantially fewer and lower amounts of many tobacco combustion-related toxicants (*e.g.*, CO, VOCs, aromatic amines) than cigarette smokers. Camel Snus and ST users are exposed to some tobacco-related toxicants (*e.g.,* nicotine, tobacco specific nitrosamines and polyaromatic hydrocarbons), although those levels are generally comparable to or less than that of smokers.

In conclusion, the results of published clinical biomarker studies comparing toxicant exposure and early indicators of potential harm are consistent with the results of epidemiological studies. As compared with cigarette smoking, smokeless tobacco (ST) use presents less risk of lung cancer, oral cancer, respiratory disease and heart disease. Clinical studies provide evidence that cigarette smokers who switch completely to products such as Camel Snus will significantly reduce their harm and risk of these tobacco-related diseases compared to continued use of cigarettes.

6.1.2.3 RJRT Clinical Studies of Camel Snus

R.J. Reynolds Tobacco Company has sponsored a series of clinical studies involving Camel Snus which were conducted between 2007 and 2012. These clinical studies included two cross-sectional studies of Camel Snus adopters (CSD0804, CSD0904), five studies of smokers switched partially or completely to Camel Snus (CSD901, CSD0905, CSD0914, CSD1101, HSD0702) and a randomized clinical trial comparing smoking cessation rates between users of Camel Snus and users of nicotine-replacement therapy (NRT) (CSD1010). The RJRT-sponsored clinical studies submitted in support of this Application are listed in Table 6.1.2-22 and summarized in this Section. Study-related documents, including final study reports, study protocols and raw data are submitted with this Application in Section 7.

Table 6.1.2-22: Index of RJRT clinical studies

Reference	Title	Publication(s)
CSD0804	Assessment of Mouth-Level Exposure to Tobacco Constituents in U.S. Snus Consumers	Caraway and Chen 2013
CSD0901	Switching from Usual Brand Cigarettes to Camel "Snus," Camel Dissolvable Tobacco "Sticks," "Strips," or "Orbs," Dual Use of Usual Brand Cigarettes and Snus, or Tobacco Abstinence – A Multi-Center Evaluation of Select Modern Smoke-Free Tobacco Products	Krautter *et al.* 2015
CSD0904	Post-Market Surveillance of Tobacco Products: A Multicenter Clinical Trial of Natural Adopters of Cigarettes, Moist Snuff, Camel SNUS, and Dual Use	--
CSD0905	Ambulatory Study Comparing Ad Libitum use of Usual Brand Cigarettes to Dual Use of Camel Snus with Reduced Smoking	Round *et al.* 2015
CSD0914	Assessment of Serum Nicotine Exposure from Modern Smoke-Free Tobacco Products	--
CSD1010	A Randomized, Multicenter Clinical Trial to Compare Smoking Cessation Rates with Camel Snus, with and without Smokeless Tobacco Health-Related Background Information, and a Nicotine Lozenge	--
CSD1101	Assessment of Smokers' Nicotine Uptake and Urge to Smoke After Use of Smokeless Tobacco Products	--
HSD0702	Switching from Usual Brand Cigarettes to a Tobacco Heating Cigarette or Snus – A Multi-Center Evaluation of Heath-Related Quality of Life Assessments and Biomarkers of Exposure and Harm	Ogden *et al.* 2015a Ogden *et al.* 2015b Ogden *et al.* 2015c

6.1.2.3.1 Studies of natural adopters of Camel Snus, cigarettes and smokeless tobacco products

RJRT sponsored two clinical studies evaluating adopters of Camel Snus. The first study, Caraway and Chen 2013, evaluated mouth-level exposure to tobacco toxicants and nicotine. The second study, CSD0904, was a cross-sectional post-market surveillance study of adopters of several tobacco products (including Camel Snus) and non-users of tobacco that evaluated mouth-level exposure, biomarkers of exposure and effect, product use patterns and health status measures.

6.1.2.3.1.1 Caraway and Chen 2013 [CSD0804]: Assessment of Mouth-Level Exposure to Tobacco Constituents in U.S. Snus Consumers

The objective of this study was to characterize mouth-level exposure (MLE) to selected tobacco toxicants and nicotine in current adult users of Camel Snus.

Study Description and Methodology: This study was a multi-center design assessing MLE to nicotine, TSNAs, B[a]P and selected metals in adult consumers who regularly used Camel Snus Frost, Original or Spice. Subjects used their usual brand style (0.6 g pouches, purchased at retail) *ad libitum* for 7 days, collecting and freezing their used pouches at the end of each day. The subjects also completed questionnaires to record their usual Camel Snus brand style, consumption of other tobacco- or nicotine-containing products and tobacco use behaviors.

The study protocol was reviewed and approved by the RJRT Research and Development Human Research Review Committee prior to study initiation. Written informed consent was obtained from all subjects before enrollment in the study. All subjects were current Camel Snus users at least 21 years of age. Tobacco constituent analyses were performed according to Health Canada Official Test Methods T-301, T-306, T-307 and T-309, with minor modifications to accommodate the product format. Tobacco constituent analyses were conducted by an ISO/IEC 17015:2005 accredited testing laboratory.

Study Population and Eligibility Criteria: A total of 56 subjects (out of 60 planned) were enrolled in the study and randomized to one of three study groups (Table 6.1.2-23). A total of 54 subjects completed the study.

Table 6.1.2-23: Enrollment goals

Study Groups	Target Enrollment
Camel Snus Frost	20
Camel Snus Original	20
Camel Snus Spice	20

To be eligible for enrollment, Camel Snus consumers were required to be 21–55 years of age with self-reported, weekly consumption of at least 15 pouches of a single brand style of Camel Snus for at least the previous three months.

Subjects who met any of the following conditions were excluded from the study:

- interested in quitting the consumption of tobacco products.
- women who were pregnant, lactating or intending to get pregnant.
- self-reported heart disease, kidney disease, liver disease or any medical condition that might be affected by tobacco use.

Evaluation Criteria: Collected Camel Snus pouches (used and unused) were evaluated for levels of nicotine, tobacco-specific nitrosamines (TSNAs), trace metals and benzo[a]pyrene. Additional information regarding the bioanalytical laboratory and procedures is available in Caraway and Chen 2013.

Initial questionnaires following enrollment assessed tobacco use, NRT use and subject demographics.

Final questionnaires assessed pouch collection, tobacco use, NRT use, Camel Snus use (mouth location, movement, duration, etc.), the Fagerström Test for Nicotine Dependence - Smokeless Tobacco (FTND-ST) and the Fagerström Test for Nicotine Dependence (FTND).

Statistical Methods: Descriptive statistics were calculated for subject age, Camel Snus pouches consumed per day and each MLE endpoint. Descriptive statistics were also calculated for each constituent baseline level in unused Camel Snus pouches by brand style. A one-way analysis of variance (ANOVA) test was applied to test for differences in (a) the measured constituents among unused Camel Snus styles and (b) MLE endpoints among subjects grouped by usual Camel Snus styles and among subjects grouped by self-reported product use time. Tukey-Kramer honestly significant difference test ($\alpha = 0.05$) was used to determine which means were significantly different from each other.

Total TSNAs were calculated as the sum of the NNN, NNK, NAT and NAB observed values. When a value was reported as below the limit of detection, one-half of the limit of detection was imputed for the calculations. The midpoint between the limit of quantitation and limit of detection was used when a value below the limit of quantitation was reported.

Study Results: A summary of study data is provided below. For a full description of all study results, refer to Caraway and Chen 2013.

Subject Demographic Summary and Disposition: Subject demographics and disposition are described below in Table 6.1.2-24 and Table 6.1.2-25.

Table 6.1.2-24: Demographic summary

	Frost	Original	Spice	Overall
N	25	12	16	53
Gender				
Female	1	2	4	7
Male	21	10	16	46
Age (years)				
Mean ± SD	29.9 ± 7.2	36.7 ± 8.3	31.5 ± 6.7	31.8 ± 7.6
Min, Max	21, 45	24, 47	22, 47	21, 47
Duration of Camel Snus use				
3-6 months	11	4	9	23
7-12 months	6	5	7	17
>1 year	8	3	4	13
Number of tins purchased in the previous 30 days				
Mean ± SD	7.2 ± 4.2	5.4 ± 3.1	5.6 ± 2.9	6.3 ± 3.7
Min, Max	4, 20	4, 12	4, 12	4, 20

Note: One subject enrolled into the Spice study group used both the Spice and Original brand styles and was excluded from analysis, so effectively n = 53. Three other subjects were enrolled into the Spice study group, but actually used the Frost brand style.

Table 6.1.2-25: Disposition of subjects

	Frost	Original	Spice	Overall
Enrolled	23	13	20	56
Completed	22	12	20	54

Note: One subject enrolled into the Spice study group used both the Spice and Original brand styles and was excluded from analysis, so effectively n = 53. Three other subjects were enrolled into the Spice study group, but actually used the Frost brand style.

Tobacco Use Behaviors: Subjects were allowed to use other tobacco products during the study, and 46 subjects (86.8%) reported using other tobacco products concurrently with Camel Snus. Seven subjects (13.2%) consumed only Camel Snus, whereas 26 (49.1%) were dual users of both Camel Snus and cigarettes. Two subjects (3.8%) were dual users of both Camel Snus and moist snuff, and two (3.8%) were dual users of both Camel Snus and a tobacco product other than cigarettes or moist snuff. Moreover, 16 subjects (30.2%) were users of 3 or more types of tobacco products (Camel Snus, cigarettes and at least one other tobacco product).

Forty-seven subjects (88.7%) reported using one pouch at a time when consuming Camel Snus. Six subjects (11.3%) reported using two or more pouches simultaneously. Of the six subjects who reported using more than one pouch simultaneously, four subjects used two pouches and two subjects used three pouches simultaneously.

Subjects were asked how long they typically kept Camel Snus in their mouth during use: 14 subjects (26.4%) reported that they typically used the product for less than 10 minutes; 25

subjects (47.2%) reported that they usually kept their Camel Snus in their mouths for 10–30 minutes; and 14 subjects (26.4%) reported they used Camel Snus for more than 30 minutes. There were no significant differences in MLE among these groups.

Study subjects were also asked whether they moved or repositioned the Camel Snus pouches in their mouths while using the product. A similar number of subjects reported moving the pouches around during use (50.9%) as those who reported maintaining the pouches in the same mouth location (49.1%).

Overall, subjects used 3.3 ± 1.9 Camel Snus pouches/day. Subjects who used Camel Snus exclusively used 5.4 ± 3.7 pouches/day, and dual users of Camel Snus and cigarettes used 2.8 ± 1.2 pouches/day.

Table 6.1.2-26 shows the mean values for constituents in unused Camel Snus pouches.

Table 6.1.2-26: Baseline constituent levels in unused Camel Snus pouches (Mean ± SD)

Constituent	Frost (n = 6)	Original (n = 6)	Spice (n = 6)	ANOVA p value
Nicotine (mg/pouch)	7.3 ± 0.7[a]	6.9 ± 0.7[a]	6.5 ± 0.3[a]	0.1196
NNN (ng/pouch)	427.5 ± 47.5[a]	425.4 ± 39.1[a]	392.2 ± 31.0[a]	0.2584
NNK (ng/pouch)	142.7 ± 15.0[a]	137.7 ± 6.9[a]	78.7 ± 7.6[b]	<.0001
NAT (ng/pouch)	220.5 ± 34.1[a]	217.8 ± 15.8[a]	216.3 ± 19.3[a]	0.9544
NAB (ng/pouch)	29.3 ± 3.5[a]	29.0 ± 1.4[a]	28.0 ± 3.9[a]	0.7683
Total TSNAs (ng/pouch)	820.0 ± 96.5[a]	809.9 ± 48.7[a]	715.2 ± 56.9[a]	0.0396
B[a]P (ng/pouch)	0.59 ± 0.03[a]	0.53 ± 0.07[a]	0.85 ± 0.10[b]	<.0001
Arsenic (ng/pouch)	49.7 ± 4.4[a]	49.8 ± 3.5[a]	45.8 ± 6.1[a]	0.2934
Cadmium (ng/pouch)	198.8 ± 15.8[a]	196.5 ± 4.4[a]	195.5 ± 7.8[a]	0.9745
Chromium (ng/pouch)	261.8 ± 11.3[a]	257.8 ± 17.9[a]	251.9 ± 33.7[a]	0.7604
Lead (ng/pouch)	72.3 ± 2.4[a]	71.4 ± 3.9[a,b]	67.3 ± 2.9[b]	0.0331
Nickel (ng/pouch)	385.9 ± 28.1[a]	367.2 ± 25.8[a,b]	342.9 ± 20.1[b]	0.0293

Note: ANOVA = analysis of variance; NNN = N´-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NAT = N´-nitrosoanatabine; NAB = N´-nitrosoanabasine; TSNAs = tobacco-specific nitrosamines; B[a]P = benzo[a]pyrene. For a constituent, mean brand style baseline levels that share the same letter (a or b) were not found to be significantly different using the Tukey–Kramer honestly significant difference test (α = 0.05).

There were no statistically significant (p > .05) differences in the mean baseline levels of nicotine, NNN, NAT, NAB, arsenic, cadmium and chromium between the Camel Snus Frost, Original and Spice brand styles (Table 6.1.2-26).

The mean baseline NNK level for Camel Snus Spice was approximately 60 ng/pouch lower than for the Frost and Original styles. The mean baseline B[a]P levels for the Frost and Original styles were approximately 0.3 ng/pouch lower than Spice. The mean baseline level of lead for Spice was 5 ng/pouch lower than Frost. The mean baseline level of nickel for Spice was 43 ng/pouch lower than Frost.

Mouth-Level Exposure (MLE): The average amounts of nicotine, TSNAs and B[a]P extracted by the subjects are summarized in Table 6.1.2-27. The average per pouch extraction levels for nicotine, TSNAs and B[a]P were all less than 40%, meaning that at least 60% of those constituents remained in the pouch after use. The mean values for the nicotine MLE end points, NNK MLE per pouch and NNK MLE per day were significantly ($p < .05$) lower among the subjects who used the Spice variety than among those who used Frost. Some of these differences may be attributable to differences between the brand styles in baseline values shown in Table 6.1.2-26.

Trace metal results were inconclusive due to variability in baseline determinations.

Table 6.1.2-27: Tobacco constituent extraction from Camel Snus during use

Constituent	Extracted per Pouch				Extracted per Day				Percent Extracted			
	Frost	Original	Spice	All	Frost	Original	Spice	All	Frost	Original	Spice	All (mean ± SD)
Nicotine (mg)	3.4	2.7	1.8	2.8	12.2	7.7	6.4	9.4	47%	39%	28%	39% ± 23%
NNN (ng)	123.7	83.3	65.8	97.1	412.9	254.1	166.1	302.4	29%	20%	17%	23% ± 22%
NAT (ng)	43	NSD	32.4	34.1	123.6	NSD	78.2	94.1	19%	NSD	15%	16% ± 26%
NNK (ng)	50	35.6	19.5	37.5	173	114.8	55.6	124.4	35%	26%	25%	30% ± 21%
B[a]P (ng)	0.15	0.13	0.33	0.2	0.57	0.35	1.08	0.68	25%	24%	38%	29% ± 14%

NSD = No significant difference between amount measured in used and unused pouches.

Questionnaire Responses: Subject responses to both the FTND-ST and FTND questionnaires are listed in Table 6.1.2-28. Subjects who used Camel Snus and cigarettes concurrently completed both the FTND-ST and the FTND.

Table 6.1.2-28: Fagerström Test for Nicotine Dependence (FTND)

Variable	Frost	Original	Spice	Overall
FTND-ST				
Mean ± SD	3.8 ± 1.5	3.1 ± 1.9	3.9 ± 1.9	3.6 ± 1.7
(Min, Max)	(2, 7)	(0, 6)	(1, 8)	(0, 8)
N	22	12	20	54
FTND				
Mean ± SD	3.5 ± 2.6	3.6 ± 2.5	3.2 ± 2.6	3.4 ± 2.5
(Min, Max)	(0, 7)	(0, 6)	(0, 7)	(0, 7)
N	18	10	17	45

Note: One subject assigned to the Camel Snus Spice group and excluded from MLE analysis due to concurrent use of Camel Snus Spice and Original is included in this table.
Scoring for Level of Nicotine Dependence.
0-2 Very low dependence
3-4 Low dependence
5 Medium dependence
6-7 High dependence
8-10 Very high dependence

6.1.2.3.1.2 CSD0904: Post-Market Surveillance of Tobacco Products: A Multicenter Clinical Trial of Natural Adopters of Cigarettes, Moist Snuff, Camel SNUS and Dual Use

The primary objective of CSD0904 was to establish baseline values for tobacco constituent/toxicant exposure levels, tobacco effect biomarker levels, tobacco user behaviors and health status of natural adopters of several tobacco product classes (*i.e.*, cigarettes, moist snuff, Camel Snus, dual use of cigarettes and Camel Snus, dual use of cigarettes and moist snuff) and of non-tobacco users.

The secondary objective of CSD0904 was to evaluate current differences in tobacco constituent/toxicant exposure, effect measures and health status among balanced subsets of subjects in the 5 groups of natural adopters of tobacco products and the non-tobacco users.

Study Description and Methodology: CSD0904 was a cross-sectional, multicenter, post-market surveillance study. Duration of subject participation from the Initial Screening Visit through final visit was approximately 5.5 weeks or less (depending upon study group) and included up to 7 consecutive days of pre-clinic procedures before 1 overnight clinical confinement period.

Up to 14 days prior to clinic admission, eligible subjects continued to use their usual brand (UB) tobacco products at their normal rates and collected their used cigarette butts (1-day collection) and/or used Camel Snus pouches (7-day collection) and retained moist snuff containers (1-day

usage). Subjects reported to the clinical research unit in the morning on Day 1 for baseline testing, confirmation of continued study eligibility, check-in of their UB tobacco product(s) and used tobacco product collections. Camel Snus brand styles in this study included Frost (0.6 g), Mellow (0.6 g) and Winterchill (1.0 g).

Subjects were confined to the clinical research unit for a period of approximately 24 hours (Days 1 and 2) and were permitted to use their UB tobacco products *ad libitum* until starting an 8-10 hour fasting (from all food and drink, except water) and tobacco product abstention period. Urine collections (24-hour) were initiated, carboxyhemoglobin (COHb) blood samples and expired carbon monoxide (ECO) measurements were obtained, and subjects completed health status and tobacco product use questionnaires and performed spirometry and Six-Minute Walk Test (6MWT) procedures. Subjects completed Day 2 study procedures during the fasting and tobacco product abstention period, including collection of urine, blood, buccal cell and saliva samples for analysis of biomarkers of tobacco exposure and biomarkers of effect. Clinical site staff maintained a "dispensary" to distribute tobacco products, document tobacco usage, collect and store samples of cigarettes and Camel Snus and monitor moist snuff usage. A description of tobacco product dispensing, collection and processing procedures is provided in the study protocol (CSD0904 CSR, Appendix 16.1.1).

CSD0904 was conducted in accordance with the U.S. Code of Federal Regulations (CFR) governing Protection of Human Subjects (21 CFR 50) and IRBs (21 CFR 56). In addition to the Federal Regulation, the study followed the guidelines of the ICH for designing, conducting, recording and reporting clinical studies, commonly known as GCP, which are consistent with the Declaration of Helsinki. The Principal Investigators also were required to disclose any financial equity interests in R.J. Reynolds Tobacco Company (RJRT) and any conflicts of interest, as defined by RJRT.

The bioanalytical work was performed under "Fit-for-Purpose" principles. RJRT has developed a Good Laboratory Practice (GLP)-like guidance (as described below), under which the bioanalytical work was performed. Any exploratory biomarker work was performed under the contracted bioanalytical laboratory's Standard Operating Procedures. Study site personnel followed urine and blood biomarker sample collection methods provided in a Laboratory Specifications Manual. This study was performed according to applicable GLP requirements and in compliance with SOPs in effect at the contracted bioanalytical laboratories. The SOPs are based on the principles and requirements described in the U.S. Code of Federal Regulations 21 CFR 58. To ensure the integrity of the reported data, the bioanalytical laboratories verified all results. Bioanalytical reports (inclusive of data summaries, results and conclusions; and if applicable, deviations from sample analysis plan or SOPs) can be found in the CSD0904 CSR, Appendix 16.5.

A list of all laboratories performing bioanalytical work is provided in the study protocol (CSD0904 CSR, Section 6).

Study Population and Eligibility Criteria: A total of 320 subjects were enrolled into one of the following 6 study groups based on previous tobacco usage:

Table 6.1.2-29: Enrollment goals

Study Groups	Target Enrollment
Moist Snuff Users	50
Camel Snus Users	50
Dual Users of Camel Snus and Cigarettes	50
Dual Users of Moist Snuff and Cigarettes	50
Cigarette Smokers	40 male; 20 female
Non-Tobacco Users	40 male; 20 female

The study eligibility criteria outlined below are abbreviated. For a full description of eligibility criteria, including group-specific criteria, definitions and verification procedures, refer to CSD0904 CSR, Section 9.2.

Inclusion Criteria:

- generally healthy male or female, at least 19 years of age; and

Exclusion Criteria:

- use of any type of non-tobacco nicotine-containing product/device or any nicotine replacement therapy within 6 months prior to study entry or during the study.

Evaluation Criteria: Biomarkers of tobacco exposure and effect were assessed from blood, urine, buccal cells and saliva samples. A complete list of the 42 exposure biomarkers measured in the study is provided in the study report (CSD0904 CSR, Section 9.3.1).

Other assessments of tobacco exposure included expired CO and mouth-level exposure (MLE) measures to smoke and tobacco constituents.

Functional capacity assessments included spirometry (to quantify FEV1, FEV1 % predicted, forced vital capacity [FVC], FVC % predicted and the FEV1/FVC ratio) and the Six-Minute Walk Test (6MWT).

Tobacco product use was determined by collection of used cigarette butts, used Camel Snus pouches and by recording use by weight of moist snuff remaining in used snuff containers. Questionnaires to assess nicotine dependence, self-reported health status and tobacco product usage included the following:

- Tobacco Product Usage Questionnaire; administered to all subjects in all groups.

- Fagerström Test for Nicotine Dependence (FTND); administered to all subjects in the cigarette smoker and dual user groups.

- Fagerström Test for Nicotine Dependence-Smokeless Tobacco (FTND-ST); administered to all subjects in the moist snuff, Camel Snus, and dual user groups.

- Smoking Cessation Quality of Life Questionnaire (SCQoL), inclusive of the Short Form Health Survey (SF-36v2®); administered to all subjects in all groups.

- American Thoracic Society Division of Lung Disease Questionnaire ATS-DLD-78-A (ATS); administered to all subjects in all groups.

Safety monitoring included assessment of adverse event (AE) reports, clinical laboratory tests, vital signs, 12-lead electrocardiograms (ECGs) and a complete physical examination with a brief oral assessment.

Statistical Methods: For the primary objective of establishing baseline values for tobacco constituent/toxicant exposure levels, tobacco effect biomarker levels, tobacco user behaviors and health status in natural adopters of several tobacco product classes and of non-tobacco users, all endpoints were summarized using descriptive statistics. Summaries of smoker and non-smoker data (specifically means and standard deviations) were adjusted (weighted) by taking the proportion of smoking/non-smoking males in the U.S.[5] and dividing by the observed proportion of males in the study groups. Tables with weighted summary data are footnoted in the final study report to indicate that such adjustments were performed.

For the secondary objective, evaluating current differences in tobacco constituent/toxicant exposure, effect measures and health status among balanced subsets of subjects in the 5 groups of natural adopters of tobacco products and the non-tobacco users, an analysis of variance (ANOVA) model was used to compare all study groups for a given endpoint. If the analysis showed differences among any study groups ($p \leq 0.05$), then all pairwise comparisons were analyzed as well.

Complete details on the statistical methodology are provided in the Statistical Analysis Plan (SAP) in the study report (CSD0904, Appendix 16.1.6).

A post-hoc analysis also was performed, combining individual biomarkers of polycyclic aromatic hydrocarbon (PAH) exposure (*e.g.*, 1-OH-naphthalene and 2-OH-naphthalene into "naphthalene equivalents") (RDM PC 2016 274-a). Unless otherwise specified, the results of this post-hoc analysis are presented below in lieu of a discussion of the individual PAH biomarker results available in the study report (CSD0904 CSR, Section 11.2).

Study Results: A summary of study data is provided below. For a full description of all study results, refer to CSD0904 CSR, Section 11.

Subject Demographic Summary and Disposition: Subject demographics and disposition are described below in Table 6.1.2-30 and Table 6.1.2-31.

[5] Based on data from the 2008 Behavioral Risk Factor Surveillance System (*see* CSD0904 CSR, p.9), the percentage of smokers in the population who are male is 54.8%, and 38.2% of all non-smokers are male.

Table 6.1.2-30: Demographic summary at screening by study group

	Moist Snuff	Camel Snus	Camel Snus & Cigarettes	Moist Snuff & Cigarettes	Cigarettes	Non-Tobacco
N	50	50	50	50	60	60
Gender						
Female	2 (4.0%)	3 (6.0%)	8 (16.0%)	0	20 (33.3%)	20 (33.3%)
Male	48 (96.0%)	47 (94.0%)	42 (84.0%)	50 (100.0%)	40 (66.7%)	40 (66.7%)
Age (years)						
Mean (SD)	37 (14)	30 (10)	35 (13)	31 (11)	41 (13)	36 (11)
Min, Max	19, 73	19, 63	19, 62	19, 59	19, 71	20, 64
Race						
White	47 (94.0%)	48 (96.0%)	44 (88.0%)	44 (88.0%)	46 (76.7%)	48 (80.0%)
Black	3 (6.0%)	0	6 (12.0%)	5 (10.0%)	14 (23.3%)	12 (20.0%)
Other	0 (0%)	2 (4%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)

Table 6.1.2-31: Disposition of subjects

	Moist Snuff	Camel Snus	Camel Snus & Cigarettes	Moist Snuff & Cigarettes	Cigarettes	Non-Tobacco
Enrolled	50	50	50	50	60	60
Completed	49	50	50	49	60	59
Withdrew Consent	1	0	0	1	0	1

Biomarkers of Exposure: Unweighted biomarker results for all subject groups are summarized in Table 6.1.2-32 and brief descriptive summaries of biomarker results for Camel Snus use groups are provided below.

Table 6.1.2-32: CSD0904 unweighted biomarker results

Biomarker of Exposure (units)	Mean (Standard Deviation)					
	Moist Snuff (N=50)	Camel Snus (N=50)	Dual Users (Camel Snus & Cigarettes) (N=50)	Dual Users (Moist Snuff & Cigarettes) (N=50)	Cigarettes (N=60)	Non-Tobacco (N=60)
NIC_{Eq}-T (µg/24 hr)	n=49 31969.9 (24096.7) [1,2,4,5]	n=50 12645.1 (11175.6) [2,3,5]	n=50 19218.7 (15403.8) [5]	n=50 23256.2 (13154.7) [4,5]	n=60 17353.1 (8750.1) [5]	n=59 87.8 (347.1)
Total NAB (ng/24 hr)	n=49 144.83 (130.17) [1,2,4,5]	n=50 34.00 (50.86) [3,5]	n=50 47.82 (43.16) [3,5]	n=50 112.18 (97.05) [4,5]	n=60 53.44 (41.20) [5]	n=59 1.39 (1.13)
Total NAT (ng/24 hr)	n=49 1610.55 (1512.85) [1,2,4,5]	n=50 360.55 (736.73) [3,5]	n=50 298.75 (294.69) [3,5]	n=50 1033.15 (1034.10) [4,5]	n=60 348.17 (278.82) [5]	n=59 3.46 (10.35)
NNAL-T (ng/24 hr)	n=49 2134.24 (1836.30) [1,2,3,4,5]	n=50 651.22 (1036.81) [5]	n=50 554.27 (517.07) [3,5]	n=50 1024.13 (1062.81) [4,5]	n=60 541.35 (367.75) [5]	n=59 8.00 (19.85)
Total NNN (ng/24 hr)	n=49 59.63 (54.09) [1,2,4,5]	n=50 13.43 (17.70) [3,5]	n=50 13.36 (10.99) [3,5]	n=50 40.34 (35.86) [4,5]	n=60 15.44 (13.29) [5]	n=59 1.31 (1.50)
3-ABP (ng/24 hr)	n=49 0.60 (0.25) [2,3,4]	n=50 0.78 (1.14) [2,3,4]	n=50 6.51 (5.92) [5]	n=50 5.84 (5.31) [5]	n=60 7.74 (5.73) [5]	n=59 0.58 (0.29)
4-ABP (ng/24 hr)	n=49 3.74 (2.31) [3,4]	n=50 4.87 (4.06) [3,4]	n=50 629.47 (4318.52)	n=50 17.38 (11.08) [5]	n=60 21.09 (13.37) [5]	n=59 3.65 (2.90)
4-ABP (ng/24 hr) Excluding Extreme Values	n=49 3.74 (2.31) [2,3,4]	n=50 4.87 (4.06) [2,3,4]	n=49 18.74 (12.68) [5]	n=50 17.38 (11.08) [5]	n=60 21.09 (13.37) [5]	n=59 3.65 (2.90)
2-AN (ng/24 hr)	n=49 3.24 (2.41) [2,3,4]	n=50 3.63 (2.75) [2,3,4]	n=50 22.28 (15.14) [5]	n=50 22.10 (13.35) [5]	n=60 27.39 (15.89) [5]	n=59 2.56 (2.06)
o-T (ng/24 hr)	n=49 132.15 (56.67) [2,3,4]	n=50 158.48 (91.43) [2,3,4]	n=50 235.29 (124.61) [5]	n=50 231.43 (145.04) [5]	n=60 235.96 (155.04) [5]	n=59 128.88 (67.04)
Naphthalene equivalents (ng/24 hr)	n=49 5983.1 (5735.5) [2,3,4]	n=50 14818.6 (52692.3)	n=50 37101.8 (112862.9)	n=50 18879.5 (9637.6) [5]	n=60 24405.2 (19681.2) [5]	n=59 5457.2 (3141.2)
Naphthalene equivalents excluding extreme values (ng/24 hr)	n=49 5983.1 (5735.5) [2,3,4]	n=50 14818.6 (52692.3)	n=49 21240.3 (12727.1) [5]	n=50 18879.5 (9637.6) [5]	n=60 24405.2 (19681.2) [5]	n=59 5457.2 (3141.2)
1-OHP (ng/24 hr)	n=49 172.4 (119.8) [2,3,4]	n=50 227.3 (368.5)	n=50 313.4 (311.5) [5]	n=50 314.1 (223.8) [5]	n=60 341.9 (449.3) [5]	n=59 155.2 (152.3)

Biomarker of Exposure (units)	Mean (Standard Deviation)					
	Moist Snuff (N=50)	Camel Snus (N=50)	Dual Users (Camel Snus & Cigarettes) (N=50)	Dual Users (Moist Snuff & Cigarettes) (N=50)	Cigarettes (N=60)	Non-Tobacco (N=60)
1-OHP excluding extreme values (ng/24 hr)	n=49 172.4 (119.8) [2,3,4]	n=50 227.3 (368.5)	n=50 313.4 (311.5) [5]	n=50 314.1 (223.8) [5]	n=59 288.1 (169.8) [5]	n=59 155.2 (152.3)
2-OH-Fluorene (ng/24 hr)	n=49 513.6 (319.9) [2,3,4,5]	n=50 457.7 (336.1) [2,3,4]	n=50 1597.0 (1119.3) [5]	n=50 1417.7 (773.9) [5]	n=60 1620.3 (831.1) [5]	n=59 321.9 (227.5)
Phenanthrene equivalents (ng/24 hr)	n=46 685.9 (535.7) [3]	n=47 573.1 (655.5) [3,4]	n=50 894 (888.6) [5]	n=50 892.8 (470.8) [5]	n=58 897.6 (563.7) [5]	n=57 473 (449.9)
Phenanthrene equivalents excluding extreme values (ng/24 hr)	n=46 685.9 (535.7) [3]	n=46 488.8 (313.2) [2,3,4]	n=49 787.0 (470.6) [5]	n=50 892.8 (470.8) [5]	n=58 897.6 (563.7) [5]	n=57 473.0 (449.9)
Acrylamide Equivalents (µg/24 hr)	n=49 55.2 (39.9) [2,3,4]	n=50 63.9 (39.6) [2,3,4]	n=50 135.8 (92.1) [5]	n=50 121.1 (56.0) [5]	n=60 111.0 (57.1) [5]	n=59 52.3 (23.0)
HMPMA (µg/24 hr)	n=49 152.55 (92.43) [2,3,4]	n=50 160.59 (96.48) [2,3,4]	n=50 467.29 (350.86) [5]	n=50 419.48 (261.13) [5]	n=60 520.97 (335.48) [5]	n=59 156.76 (109.46)
HPMA (µg/24 hr)	n=49 610.92 (398.56) [1,2,3,4]	n=50 813.34 (634.12) [2,3,4]	n=50 2128.99 (1722.79) [5]	n=50 1762.44 (980.73) [5]	n=60 2160.05 (1496.94) [5]	n=59 585.98 (388.45)
MHBMA (ng/24 hr)	n=49 427 (317) [1,2,3,4]	n=50 621 (461) [2,3,4,5]	n=50 4929 (6862) [5]	n=50 5178 (4511) [5]	n=60 4932 (4187) [5]	n=59 399 (281)
SPMA (ng/24 hr)	n=49 319.3 (190.5) [2,3,4]	n=50 564.6 (775.0) [2,3,4]	n=50 3588.0 (3839.8) [5]	n=50 3663.9 (3103.8) [5]	n=60 3998.1 (2945.7) [5]	n=59 400.4 (497.9)
Urine Creatinine (mg/24 hr)	n=49 1992.25 (719.44)	n=50 2011.16 (674.33)	n=50 1848.72 (1451.74)	n=50 1914.53 (650.70)	n=60 1611.38 (883.43)	n=59 1729.69 (647.04)
SCN (µg/24 hr)	n=49 1368.58 (1293.08) [1,2,3,4,5]	n=50 2671.87 (2813.89) [2,3,4]	n=50 8587.25 (6053.93) [5]	n=50 6665.48 (5440.13) [4,5]	n=60 10595.88 (7393.54) [5]	n=59 2255.52 (1582.64)
Urine Mutagenicity (revertants per 24 hr)	n=47 20881.5 (25922.9) [2,3,4]	n=50 17491 (13227.4) [2,3,4,5]	n=50 99746.2 (83380.7) [3,4,5]	n=49 134171.9 (135402.1) [5]	n=58 187436.5 (131426.6) [5]	n=55 26384 (47687.9)
Urine Mutagenicity excluding extreme values (revertants per 24 hr)	n=47 20881.5 (25922.9) [2,3,4]	n=50 17491 (13227.4) [2,3,4,5]	n=50 99746.2 (83380.7) [3,4,5]	n=49 134171.9 (135402.1) [5]	n=57 183960 (129876.5) [5]	n=55 26384 (47687.9)
COHb (%)	n=46 0.97 (0.30) [2,3,4]	n=45 1.13 (0.38) [2,3,4]	n=48 4.18 (2.12) [4,5]	n=47 4.24 (1.66) [4,5]	n=56 5.89 (2.04) [5]	n=55 1.03 (0.38)
NIC-U (ng/mL)	n=49 6.32 (4.37) [1,2,5]	n=50 3.57 (3.09) [3,5]	n=50 3.54 (2.19) [3,5]	n=50 5.16 (3.71) [5]	n=59 4.67 (4.57) [5]	n=59 1.87 (1.80)

Biomarker of Exposure (units)	Mean (Standard Deviation)					
	Moist Snuff (N=50)	Camel Snus (N=50)	Dual Users (Camel Snus & Cigarettes) (N=50)	Dual Users (Moist Snuff & Cigarettes) (N=50)	Cigarettes (N=60)	Non-Tobacco (N=60)
COT-U (ng/mL)	n=49 384.17 (260.69) [1,2,3,4,5]	n=50 159.83 (134.84) [3,5]	n=50 215.96 (115.41) [3,5]	n=50 282.41 (151.17) [4,5]	n=59 225.39 (114.93) [5]	n=59 1.28 (7.41)
SCN (µmol/L)	n=49 25.56 (23.99) [1,2,3,4,5]	n=50 44.26 (32.22) [2,3,4]	n=50 122.09 (56.70) [5]	n=50 95.86 (60.89) [4,5]	n=59 152.31 (53.28) [5]	n=59 39.16 (21.49)
4-ABP hemoglobin adducts (pg/g Hb)	n=38 8.31 (4.11) [2,3,4]	n=35 18.00 (21.55) [2,3,4]	n=36 47.94 (35.04) [5]	n=35 50.65 (33.44) [5]	n=52 54.55 (32.27) [5]	n=50 9.37 (4.50)
ECO (ppm): Day 1/prior to spirometry and 6MWT	n=50 2 (1) [2,3,4]	n=50 3 (1) [2,3,4]	n=50 13 (8) [4,5]	n=50 13 (7) [4,5]	n=60 18 (8) [5]	n=59 2 (1)
Derived COHb (%): Day 1/prior to spirometry and 6MWT	n=50 1.0 (0.2) [2,3,4]	n=50 1.1 (0.2) [2,3,4]	n=50 2.7 (1.3) [4,5]	n=50 2.7 (1.1) [4,5]	n=60 3.6 (1.3) [5]	n=59 1.0 (0.2)

Statistically significant pairwise comparisons: [1] vs. Camel Snus Users; [2] vs. Dual Users of Camel Snus and Cigarettes; [3] vs. Dual Users of Moist Snuff and Cigarettes; [4] vs. Cigarette Smokers; [5] vs. Non-Tobacco Users.

Source of table data is the CSD0904 CSR for all constituents except phenanthrene equivalents, naphthalene equivalents, acrylamide equivalents and urine mutagenicity, which were calculated in a post-hoc analysis (RDM PC 2016 274-a).

Camel Snus vs. Cigarettes: The majority of the exposure biomarkers measured were statistically significantly reduced in Camel Snus users compared to exclusive cigarette smokers (biomarker measures of acrylamide, aromatic amines, carbon monoxide, carbonyls, hydrogen cyanide, low molecular weight hydrocarbons, urine mutagenicity and select PAHs). No statistically significant differences in biomarker levels were observed for Camel Snus users compared to exclusive cigarette smokers for nicotine, TSNAs, naphthalene equivalents and 1-OH-pyrene. No exposure biomarkers were statistically significantly increased in Camel Snus users compared with exclusive cigarette smokers.

Dual Use (Camel Snus + Cigarettes) vs. Cigarettes: Levels of blood thiocyanate (weighted values only), blood %COHb and urine mutagenicity were significantly lower in dual users of Camel Snus and cigarettes compared to exclusive cigarette smokers. No exposure biomarkers were statistically significantly increased in dual users of Camel Snus and cigarettes compared with exclusive cigarette smokers.

Camel Snus vs. Non-Tobacco: Urine mutagenicity was statistically significantly lower in Camel Snus users compared to non-tobacco users. The majority of the exposure biomarkers measured were not statistically significantly different in Camel Snus users compared to non-tobacco users (biomarker measures of acrylamide, aromatic amines, carbon monoxide, carbonyls, hydrogen cyanide, selected low molecular weight hydrocarbons and PAHs). Levels of nicotine, TSNAs and MHBMA (a marker of 1,3-butadiene) were significantly higher in Camel Snus users compared to non-tobacco users.

Biomarkers of Effect:

Camel Snus vs. Cigarettes: Out of 80 total biomarkers of effect evaluated, 8 were lower, 2 were higher and 70 were not significantly different in Camel Snus users compared to cigarette smokers. Lower biomarkers were Cit (nitric oxide); BDNF, haptoglobin, IL12P70 (with and without extreme values), ICAM-1 and MMP-9 (inflammation mediators); mean cell volume levels (hematology); and $iPF_{2\alpha}$-III (oxidative stress). Higher biomarkers were TNFβ (inflammation) and hematocrit (hematology).

Dual Use (Camel Snus + Cigarettes) vs. Cigarettes: Out of 80 total biomarkers of effect evaluated, 6 were lower, 1 was higher and 73 were not significantly different in dual users of Camel Snus and cigarettes compared to exclusive cigarette smokers. Lower biomarkers were CRP, ferritin, haptoglobin and VDBP (inflammation); Apo B (lipids); and RBCs (hematology). The higher biomarker was SDMA (nitric oxide pathway).

Camel Snus vs. Non-Tobacco: Out of 80 total biomarkers of effect evaluated, 74 were not significantly different and 6 were higher in Camel Snus users compared to non-users of tobacco. These biomarkers were Hcy (nitric oxide pathway); ferritin, ICAM-1, stem cell factor and VEGF (inflammation); and $iPF_{2\alpha}$-VI (oxidative stress).

Cigarettes vs. Non-Tobacco: Out of 80 total biomarkers of effect evaluated, 22 were higher in smokers and 6 were lower in smokers when compared to non-users of tobacco. Fifty-two biomarkers of effect were not significantly different between the two groups. For nitric oxide biomarkers measured, levels of Hcy, ADMA, Cit and SDMA were significantly higher in cigarette smokers than in non-tobacco users. For inflammation mediators measured, 11 biomarkers were significantly higher and 3 were significantly lower in cigarette smokers. For lipid biomarkers, Lp(a) was significantly lower and VLDL was significantly higher in cigarette smokers. For hematology parameters, 7 biomarkers were significantly higher in cigarette smokers. Hemoglobin A1c levels were significantly lower in cigarette smokers. Two biomarkers of oxidative stress, $iPF_{2\alpha}$-VI and $iPF_{2\alpha}$-III, were significantly higher in cigarette smokers.

Mouth-Level Exposure: Two measures of mouth-level exposure (cigarette yield-in-use and snus-after-use) were conducted to compare exposures between dual users and cigarette smokers.

Mouth-Level Exposure (Cigarette Yield-in-Use): Analysis of used cigarette butts showed that dual users of Camel Snus and cigarettes were exposed to significantly less tar and nicotine per day than exclusive cigarette smokers. MLE to tar and nicotine was not statistically significantly different between dual users of Camel Snus and cigarettes and exclusive cigarette smokers on a per cigarette basis, suggesting the reduction in per day exposure to tar and nicotine was the result of dual users smoking fewer cigarettes per day than exclusive cigarette smokers.

Mouth-Level Exposure (Snus-after-Use): Mouth-level exposures to nicotine per Camel Snus pouch and per day were lower in dual users of Camel Snus and cigarettes compared to exclusive Camel Snus users. Dual users of Camel Snus and cigarettes extracted less of the nicotine from Camel Snus pouches than exclusive Camel Snus users and used fewer pouches per day.

Mouth-level exposures to NNN and NNK per day were lower in dual users of Camel Snus and cigarettes compared to exclusive Camel Snus users. The mouth-level exposure to NNK per pouch was lower for the dual users of Camel Snus and cigarettes compared to the exclusive Camel Snus users. NNN per pouch was not different. Dual users of Camel Snus and cigarettes used fewer pouches per day and extracted less of the NNK contained in the pouches.

Tobacco Usage: Subjects in the exclusive Camel Snus group used significantly more pouches per day compared to dual users of Camel Snus and cigarettes (*see* Table 6.1.2-33). Daily smoking rates changed during the in-clinic portion of the study, with both dual users and exclusive smokers smoking 5-6 fewer cigarettes per day while in clinical confinement. This decrease in cigarettes smoked per day was likely due to more limited opportunities to smoke because of study procedures and requirements to smoke inside a designated area. However, during both pre-clinic and in-clinic portions of the study, dual users of Camel Snus and cigarettes smoked fewer cigarettes per day compared with exclusive cigarette smokers, showing differences of up to 25% (*see* CSD0904, Table 18; Table 6.1.2-33).

Table 6.1.2-33: Study CSD0904 product use rates of natural tobacco product adopters

User Group	Time Point	Pouches/Day		Cigarettes/Day	
		(Mean ± SD)	Min, Max	(Mean ± SD)	Min, Max
Exclusive Cigarettes	Pre-Clinic Use	--	--	18 ± 7 cigarettes	(6, 47)
	In-Clinic Use	--	--	12 ± 4 cigarettes	(4, 27)
Dual Use (Camel Snus + Cigarettes)	Pre-Clinic Use	2 ± 2 pouches	(1, 12)	15 ± 8 cigarettes	(4, 43)
	In-Clinic Use	2 ± 2 pouches	(0, 12)	9 ± 4 cigarettes	(0, 21)
Exclusive Camel Snus	Pre-Clinic Use	4 ± 2 pouches	(1, 8)	--	--
	In-Clinic Use	5 ± 3 pouches	(1, 15)	--	--

Functional Capacity: Spirometry results obtained in this study confirmed a population of subjects who had generally healthy lung function. Very minimal changes were observed in standard spirometry indices from pre- to post-6MWT. Thus, no compromised lung function was evidenced by subjects performing the 6MWT. In general, the 6MWT results suggest that the 6MWT may not be a sensitive measure for differentiating between generally healthy consumers of different types of tobacco products.

Questionnaires: All study groups assessed themselves as healthier than the population norm for physical and mental health/functioning, as measured by the SF-36v2®. Among all six groups, the Non-Tobacco Users scored the highest in their self-assessment of physical and mental health, suggesting they viewed themselves as healthier than did the tobacco-user groups. The respiratory symptoms self-reported in the Respiratory Questionnaire suggest that smokers experience more respiratory effects from smoking cigarettes compared to consumers using smokeless tobacco products and non-tobacco users.

Safety Evaluation: There were no deaths or serious adverse events (SAEs) reported during the study. Less than 3% (9/320) of the subjects reported an AE, and no AEs resulted in cessation of tobacco usage or study discontinuation. All AEs were mild in intensity and not related to the study products. The most commonly reported AEs were headache (3 subjects). There were no significant differences in the number or type of AEs reported with tobacco users compared to non-tobacco users. No trends were noted in vital sign data.

6.1.2.3.2 Studies of smokers switched to Camel Snus

RJRT sponsored five clinical studies of adult smokers switched to Camel Snus (CSD0901, CSD0905, CSD0914, CSD1101, HSD0702). The endpoints evaluated varied by study and included mouth-level exposure, biomarkers of exposure, biomarkers of effect, nicotine pharmacokinetics, safety profiles and questionnaires. A summary of each study is provided below.

6.1.2.3.2.1 CSD0901: Switching from Usual Brand Cigarettes to Camel "Snus," Camel Dissolvable Tobacco "Sticks," "Strips," or "Orbs," Dual Use of Usual Brand Cigarettes and Snus, or Tobacco Abstinence – A Multi-Center Evaluation of Select Modern Smoke-Free Tobacco Products

The objectives of CSD0901 were to: (1) assess product usage rates, subjective responses and mouth-level exposure (MLE) in subjects switched from usual brand (UB) cigarettes to Camel Snus, dual use of Camel Snus and UB cigarettes, or tobacco abstinence; (2) measure nicotine and carbon monoxide (CO) exposures in expired air, blood and/or urine specimens and compare between study groups; (3) measure select biomarkers of exposure in blood and urine specimens and compare between study groups; and (4) determine the potential of the different assigned study products to influence subjective responses, as measured by questionnaires on smoking urges, nicotine withdrawal and product preferences.

Study Description and Methodology: CSD0901 was a multi-center, open-label, randomized, forced-switching, parallel-group design to measure daily product usage rates, quantify daily MLE to tar and/or nicotine, quantify biomarkers of exposure and survey for nicotine dependence, smoking urges, abstinence symptoms and product preference when switching from UB cigarettes to one of three intervention conditions (dual use of UB cigarettes and Camel Snus, exclusive use of Camel Snus or abstinence from tobacco).

Once qualified and selected into the study, subjects were randomized into interventions (groups) and kept blinded to their product assignment until the first day of their designated intervention. Subjects smoked their UB cigarette for 1 day (Baseline or D -1) prior to switching to their designated intervention for Days 1-5.

Camel Snus styles used in this study were Frost (0.6 g) and Mellow (0.6 g). Subjects preselected the style they preferred during screening, and if assigned to a Camel Snus group used their selected style exclusively throughout the intervention period.

All subjects assigned to a dual use or exclusive Camel Snus group were encouraged to use as much Camel Snus as desired without exceeding their daily maximum use level (MUL) (calculated individually based on 130% of UB cigarette consumption at Baseline, *see* CSD0901 CSR, Appendix 16.1.1). Product use was permitted between the hours of 07:00 to 23:00.

Subjects assigned to the dual use group had their daily UB cigarette consumption restricted to 40% of their Baseline level during their intervention period. While it was anticipated that smokers would smoke their entire 40% allotment, it was not mandatory. The dual use group was also instructed to not smoke a cigarette and use Camel Snus at the same time, but rather to use products separately at different times.

Levels of 32 biomarkers of exposure were determined in samples of plasma, whole blood and urine (collected on Days -1, 1, 3 and 5) and feces (collected on Days -1 and 5). Questionnaires that scored nicotine dependence, withdrawal discomfort and product preferences were also administered. The Fagerström Test for Nicotine Dependence (FTND) was administered during

Screening. The Brief Questionnaire of Smoking Urges (B-QSU) and the Minnesota Nicotine Withdrawal Scale (MNWS) were given on Days -1, 1, 3 and 5. A sponsor-provided questionnaire and exit interview was given on Day 5.

The trial procedures outlined in the protocol and protocol amendment were conducted in accordance with the United States (US) Code of Federal Regulations (CFR) governing Protection of Human Participants (21 CFR 50), Financial Disclosure by Clinical Investigators (21 CFR 54) and IRBs (21 CFR 56). As such, these sections of US Title 21 CFR, along with the applicable International Conference on Harmonisation Guidelines, are commonly known as Good Clinical Practices, which are consistent with the Declaration of Helsinki.

A list of all laboratories performing bioanalytical work is provided in the study protocol (CSD0901 CSR, Section 6).

Study Population and Eligibility Criteria: Up to 32 subjects per study group were planned to be enrolled to complete a minimum of 25 subjects per group. Generally healthy adult men and women who self-reported smoking 10 or more menthol or non-menthol cigarettes per day were enrolled into one of the following study groups:

Table 6.1.2-34: Enrollment goals

Study Groups	Target Enrollment
Camel Snus	32
Dual Use (Camel Snus + UB Cigarettes)	32
Abstinence	32
Total	96

The study eligibility criteria outlined below are abbreviated. For a full description of eligibility criteria, including definitions and verification procedures, refer to CSD0901 CSR, Section 9.3.

Inclusion Criteria:

- generally healthy male or female, age 21 to 65 years (inclusive);
- self-reported daily use ≥10 cigarettes/day for ≥12 months;
- screening Fagerström Test for Nicotine Dependence (FTND) score of "low" or greater (*i.e.*, 3 to 10);
- screening urinary cotinine ≥200 ng/mL;
- screening expired-air CO (ECO) level ≥15 parts per million (measure taken 30 to 60 minutes after smoking a single UB cigarette);

- no intent to quit smoking during the study period, but were willing to either switch to Camel Snus or completely abstain from smoking and use of any other tobacco product for a period of 5 consecutive days plus the next morning; and

Exclusion Criteria:

- use of any type of smokeless tobacco or nicotine-containing product(s), or smoked marijuana-based materials within 30 days prior to study start.

Evaluation Criteria:

Questionnaires/Surveys: The following questionnaires were administered at both Screening Visits and at specified times and days during the study: FTND questionnaire, Brief Questionnaire of Smoking Urges (B-QSU) and Minnesota Nicotine Withdrawal Scale (MNWS). A Sponsor-provided questionnaire was also administered near the end of the study that included questions regarding product satisfaction.

Biomarkers: Expired CO (ECO), carboxyhemoglobin (%COHb) and other select biomarker concentrations in blood, plasma, fecal and urine specimens were evaluated to determine toxicant exposure at specified times and days during the trial.

Mutagenicity: Urine mutagenicity (UM) in 24-hour urine samples was evaluated for mutagen exposure on specified days during the trial.

Smoking Behavior: Smoking behavior was evaluated by measures of cigarettes per day and smoked cigarette butt length.

Mouth-Level Exposure: MLE to nicotine from Camel Snus was evaluated by "snus-after-use" (SAU). Each subject's used pouches were counted and analyzed for residual nicotine content. The MLE to nicotine was calculated based on the difference in nicotine content for used and unused Camel Snus pouches. MLE to nicotine and tar was evaluated by "yield-in-use" (YIU), a measurement based upon evaluation of cigarette butts after smoking.

Safety Evaluation: Basic safety was monitored via evaluation of adverse events (AEs), clinical laboratory evaluations, 12-lead ECGs, vital signs, physical examinations and "How Do You Feel?" inquiries.

Statistical Methods: Descriptive statistics were calculated for all study endpoints, including product usage and MLE, biomarkers, UM, subject-reported outcomes and surveys, as well as safety parameters. Pairwise comparisons assessed differences between and within all groups over time using statistical approaches specified in the Statistical Analysis Plan (SAP). The SAP is provided in CSD0901 CSR, Appendix 16.1.9.

Study Results: A summary of study data is provided below. For a full description of all study results, refer to CSD0901 CSR, Section 11.

Subject Demographic Summary and Disposition:

Table 6.1.2-35: Demographic summary for the per protocol population

	Dual Use	Camel Snus	Abstinence
N	**29**	**30**	**25**
Age (years)			
Mean ± SD (Min, Max)	42.45 ± 12.28 (23, 63)	37.60 ± 11.42 (21, 59)	43.32 ± 11.20 (21, 58)
Gender, n (%)			
Male	14 (48.3)	18 (60.0)	14 (56.0)
Female	15 (51.7)	12 (40.0)	11 (44.0)
Race, n (%)			
White	25 (86.2)	23 (76.7)	21 (84.0)
Black	4 (13.8)	6 (20.0)	3 (12.0)
Other	---	1 (3.3)	1 (4.0)

Table 6.1.2-36: Disposition of subjects

	Dual Use n (%)	Camel Snus n (%)	Abstinence n (%)
Enrolled/Randomized	30 (100.0)	31 (100.0)	32 (100.0)
Early Withdrawal Reason	1 (3.3)	1 (3.2)	7 (21.9)
Adverse Event	---	1 (3.2)	---
Consent Withdrawn	1 (3.3)	---	6 (18.8)
Other	---	---	1 (3.1)
Completed Study	29 (96.7)	30 (96.8)	25 (78.1)

Tobacco Product Use: Among smokers switched to exclusive Camel Snus use, mean product use rates were relatively stable across all 5 days of the study (pouches/day ranged from 5.71 to 6.43). Smokers switched to dual use of Camel Snus and cigarettes experienced a decrease in mean cigarettes smoked per day between Baseline (19.24 CPD) and Day 5 (7.62 CPD), which was expected due to the mandatory reduction of cigarette consumption by 60%.

Butt Length: Mean butt lengths for dual users were slightly shorter on Day 5 (34.75 mm) than at Baseline (37.29 mm), suggesting the possibility of increased smoking intensity by dual users in this study.

Mouth-Level Exposure (Yield-in-Use): For smokers switched to dual use of Camel Snus and cigarettes, daily MLE to tar and nicotine were reduced by approximately 50%. Tar MLE went from an average of 415.3 mg/day at Baseline to 207.4 mg/day on Day 5, while nicotine MLE went from an average of 34.26 mg/day at baseline to 17.21 mg/day on Day 5. These reductions occurred despite per-cigarette MLE increases of approximately 27% for tar and 28% for nicotine.

Mouth-Level Exposure (Snus-after-Use): There were no statistically significant differences in MLE to nicotine from Camel Snus (mg/pouch or mg/day) from Day 1 through Day 5 in either the dual use or Camel Snus groups.

Urine Biomarkers: Twenty-seven out of 29 urine biomarkers decreased significantly from Baseline when subjects switched completely from UB cigarettes to Camel Snus, with many of those decreasing by up to 80% or more on Day 5 (*see* Figure 6.1.2-6, Figure 6.1.2-7 and Figure 6.1.2-8). The decreases in Camel Snus users were similar to those observed in the tobacco abstinence group for all biomarkers except total nicotine equivalents, TSNAs and 1-OH-pyrene. Levels of total NNAL and 1-OH-pyrene did not decrease significantly in Camel Snus users.

Twenty-seven out of 29 urine biomarkers decreased significantly in dual users but the extent of those decreases was not as great as observed for the exclusive Camel Snus and tobacco abstinence groups. For dual users, total NNAL was significantly decreased while total NNN was not.

Figure 6.1.2-6: Changes in 24-hr urine biomarker means from baseline to Day 5 (1 of 3)

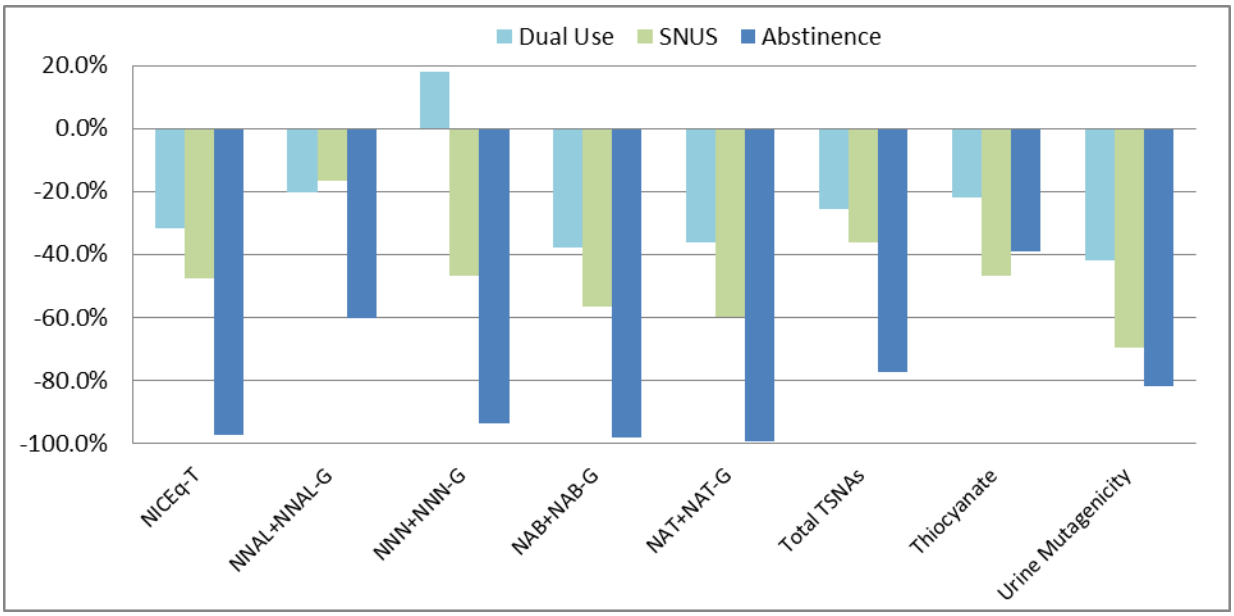


Figure 6.1.2-7: Changes in 24-hr urine biomarker means from baseline to Day 5 (2 of 3)

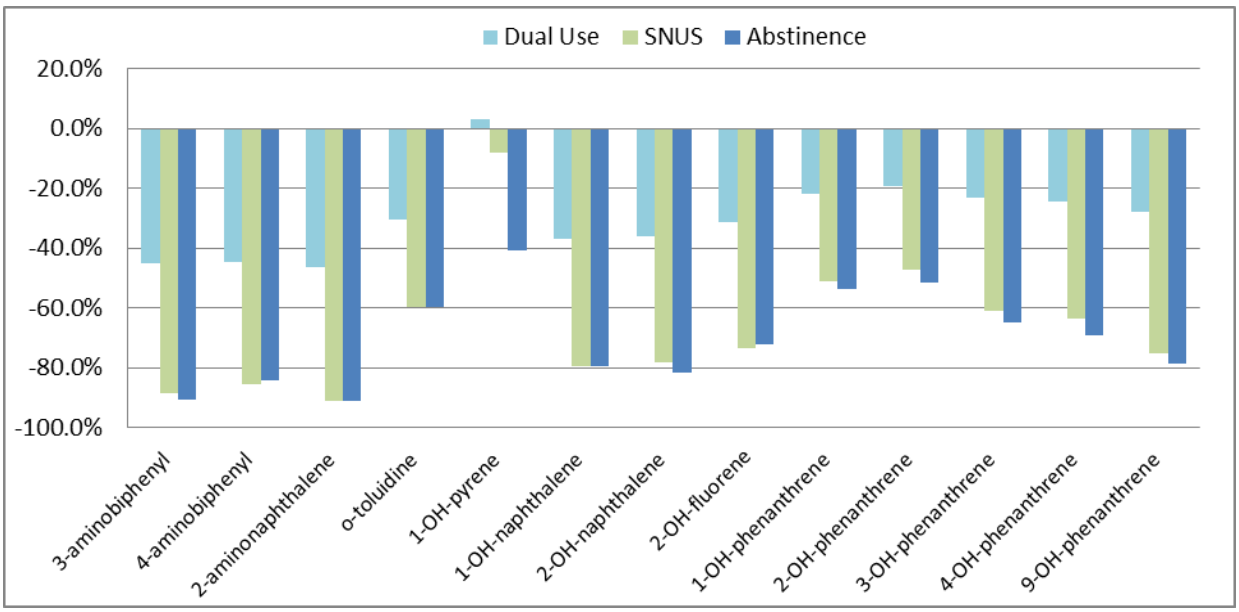
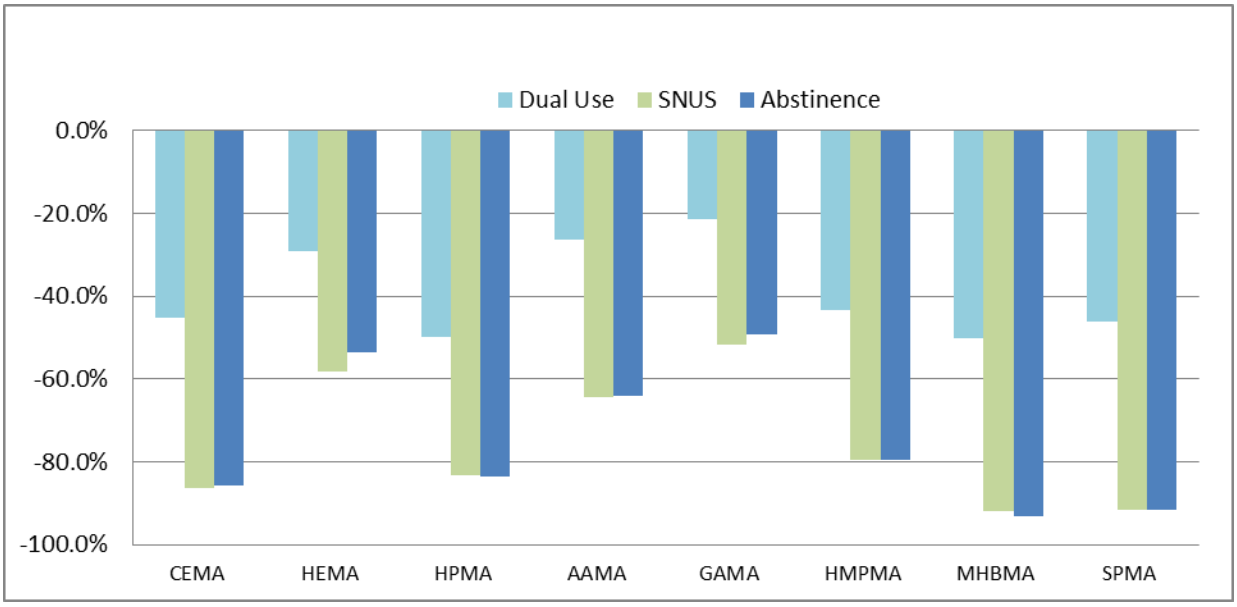


Figure 6.1.2-8: Changes in 24-hr urine biomarker means from baseline to Day 5 (3 of 3)



Expired Air Biomarkers: %COHb was significantly decreased from Baseline for exclusive Camel Snus users, dual users and tobacco abstainers for all measured time points (07:00, 12:00 and 22:00 on Days 1, 3 and 5) except for 07:00 on Day 1. Reductions from Baseline were similar in exclusive Camel Snus users and tobacco abstainers at 22:00 on Day 5 (-81.3% and -82.5%, respectively), while lesser reductions were observed in dual users of Camel Snus and cigarettes (-48.1%) at that time point.

Similar decreases in ECO were observed for all three groups, where significant decreases were again recorded for all time points except 07:00 on Day 1. Reductions from Baseline were similar

in exclusive Camel Snus users and tobacco abstainers at 22:00 on Day 5 (-92.5% and -91.9%, respectively), while lesser reductions were observed in dual users of Camel Snus and cigarettes (-54.7%) at that time point.

Plasma and Whole Blood Biomarkers: Levels of plasma cotinine, nicotine and thiocyanate, as well as whole blood %COHb, decreased significantly from Baseline to Day 5 in exclusive users of Camel Snus, dual users of Camel Snus and cigarettes and tobacco abstainers. Levels of whole blood %COHb and plasma thiocyanate were comparable between Camel Snus users and tobacco abstainers (-81.3% vs. -82.5% and -33.5% vs. -35.0%, respectively), while dual user reductions were of lesser magnitude (-48.1% for %COHb and -21.3% for thiocyanate).

Greater decreases in levels of plasma nicotine and cotinine were observed in exclusive Camel Snus users compared to dual users of Camel Snus and cigarettes, but these differences did not reach statistical significance (-61.9% vs. -46.0% and -51.8% vs. -36.2%). The tobacco abstinent group experienced declines in plasma nicotine (-97.4%) and cotinine (-98.8%) between Baseline and Day 5 that were statistically significantly greater than those observed in exclusive Camel Snus users or dual users.

Fecal Nicotine Biomarkers: There were no statistically significant reductions from Baseline to Day 5 in exclusive users of Camel Snus, dual users or tobacco abstainers.

Questionnaires/Surveys: Mean FTND scores at Baseline were highest in the tobacco abstinence group (6.32), which was significantly different from the Camel Snus group (5.13) but not from the dual use group (5.52).

The mean B-QSU desire to smoke and expected relief scores increased significantly by approximately 30% on Day 1 for the Camel Snus and tobacco abstinence groups, but returned to levels similar to Baseline values on Days 3 and 5. Desire to smoke and expected relief scores did not change on Day 1 for the dual use group, but were significantly decreased by approximately 30% on Day 5.

The MNWS 9- and 15-item assessments showed significant increases in mean withdrawal scores for tobacco abstainers on Day 5 (105.47% and 73.56%, respectively). Camel Snus user scores increased by approximately 30% on Day 1 but returned to Baseline levels by Day 5. Dual user scores decreased by approximately 25% by Day 5, but did not reach statistical significance.

Safety Evaluation: Adverse events (AEs) that were considered possibly, probably or definitely related to study product are summarized in Table 6.1.2-37. No deaths or serious adverse events (SAEs) occurred during this study.

Table 6.1.2-37: Summary of adverse events (per-protocol population)

	Incidences (Number of Subjects)		
	Dual Use	Camel Snus	Abstinence
	N = 29	N = 30	N = 25
Any Adverse Events	58 (n=20)	43 (n=16)	41 (n=16)
Mild	58 (n=20)	40 (n=16)	40 (n=16)
Moderate	--	3 (n=2)	1 (n=1)
Severe	--	--	--
Possibly, Probably, or Definitely Related	26 (n=14)	27 (n=10)	5 (n=4)
Possibly Related	6 (n=5)	5 (n=2)	3 (n=3)
Probably Related	16 (n=9)	16 (n=6)	2 (n=1)
Definitely Related	4 (n=3)	6 (n=6)	--

Complete safety evaluation data is available in CSD0901 CSR, Section 12.

6.1.2.3.2.2 CSD0905: Ambulatory Study Comparing Ad Libitum use of Usual Brand Cigarettes to Dual Use of Camel Snus with Reduced Smoking

The objective of CSD0905 was to evaluate changes in (1) product use patterns, (2) biomarkers of tobacco exposure and (3) subjective responses in smokers as they changed their daily tobacco usage to include Camel Snus and reduce smoking.

Study Description and Methodology: Usual brand smokers were examined throughout a three-week tobacco product transition from exclusive, *ad libitum* use of their usual brand (UB) cigarettes to dual use with Camel Snus Frost (0.6 g) or Mellow (0.6 g). During the dual use periods, Camel Snus was used *ad libitum* in conjunction with instructions to reduce cigarettes smoked per day.

Subjects smoked their UB cigarettes without restriction during Week 1 of the study. Based on self-reported averages of daily cigarettes smoked during Week 1, subjects were given individualized goals for reducing their daily cigarette consumption over the course of the study. Subjects were instructed to reduce their average cigarette consumption by 25% during Week 2, 50% during Week 3 and 75% during Week 4. Subjects were also given Camel Snus to use *ad libitum* during Weeks 2, 3 and 4 of the study as they reduced their daily cigarette consumption.

Study procedures were conducted over four weeks, with each seven-day period ending in a study visit. Subjects reported to a testing facility for one study visit per week, the same day and time each week. Subjects were asked to refrain from tobacco use for 30 minutes prior to each test session.

Changes in a number of 24-hour urine biomarkers were evaluated. Carbon monoxide (CO) levels in expired breath samples and carboxyhemoglobin (COHb) levels in whole blood were measured as additional measures of cigarette smoke exposure. During the testing facility visit following Week 1, subjects were asked to smoke one cigarette. During facility visits following

Weeks 2, 3 and 4, subjects were asked to use one Camel Snus pouch. Serum nicotine and cotinine levels were measured following product use at the testing facility (cigarettes on Week 1, Camel Snus on Week 4) to evaluate nicotine absorption.

Used cigarette filters were collected by subjects the day before each study visit and analyzed to estimate mouth-level exposure (MLE) to mainstream smoke tar and nicotine from the subjects' UB cigarettes. Used Camel Snus pouches were collected each day for the last two weeks of the study for determination of MLE to tobacco constituents from using Camel Snus ("snus-after-use").

Subjects' overall opinions of UB cigarettes and Camel Snus, sensory and cigarette withdrawal experiences, as well as individual patterns of UB cigarette and Camel Snus use were evaluated.

Recruits were asked to attend an orientation session that provided an overview of all study requirements and gave those interested the option to sample Camel Snus before consenting to participate. Smokers who agreed to follow the study protocol provided written informed consent for study participation before beginning any study procedures. The RJRT R&D Human Research Review Committee (HRRC) approved this study after a review of the experimental protocol.

Study Population and Eligibility Criteria: A total of 43 subjects provided informed consent to participate in the study following an orientation session. Thirty-six subjects satisfied the inclusion/exclusion criteria and were enrolled in the study.

To be eligible for inclusion, current daily smokers were required to be 21-55 years of age, be in generally good health with no active oral lesions and have no history of major health conditions. In addition, subjects needed to report current smoking at least 7 UB cigarettes per day with Cambridge Filter Method (CFM) tar levels of 8.0-14.0 mg/cigarette (*see* CSD0905, p.4). If smokers reported they were in the process of quitting, they were excluded from the study.

Evaluation Criteria: Complete descriptions of all analytical methods used in this study are available in the CSD0905 CSR.

Product Use: Subjects recorded daily cigarette and Camel Snus consumption throughout the study.

Mouth-Level Exposure (*Yield-in-Use):* Filters collected from all cigarettes smoked the day before each study visit were analyzed to assess tar and nicotine mouth-level exposure.

Mouth-Level Exposure (Snus-after-Use): Used Camel Snus pouches collected each day during the third and fourth weeks of the study were analyzed to measure mouth-level exposure to nicotine, TSNAs, trace metals and B[a]P after using Camel Snus.

Expired CO: Subjects provided breath samples for determination of expired carbon monoxide concentrations just prior to and 25 minutes after the start of product use at Visits 1 (cigarettes) and Visits 2, 3 and 4 (Camel Snus).

Nicotine Uptake: At Visits 1 and 4, serum samples for nicotine and cotinine analyses were drawn at 15 time points from -2 until 90 minutes after the start of product use. To determine the nicotine uptake from cigarette smoking (Visit 1) or Camel Snus use (Visit 4), it was necessary to estimate and subtract the levels of baseline nicotine that were expected to be cleared from the blood at each time point over the collection period. The procedure for serum nicotine concentration corrections is described in the CSD0905 CSR.

Carboxyhemoglobin: At Visits 1 and 4, whole blood samples were drawn at -2 and 25 minutes with respect to the start of product use for determination of carboxyhemoglobin saturation (%COHb). At Visits 2 and 3, one whole blood sample was drawn within 10 minutes prior to the start of product use.

Urinary Biomarkers of Exposure: 24-hour urine samples were collected at the end of Weeks 1 and 4 for assessment of 28 biomarkers of exposure (*see* CSD0905, Table 11 for a list of all biomarkers evaluated).

Questionnaires: The Fagerström Test for Nicotine Dependence (FTND) was administered to all enrollees during the Orientation session. The FTND for smokeless tobacco products was administered at Visit 4.

The Minnesota Nicotine Withdrawal Scale (MNWS) was administered at Visits 2-4. The first 9 questions were summed to create an overall nicotine withdrawal discomfort score.

An exit questionnaire was administered at the end of Visit 4 and included questions about subjects' final opinions of Camel Snus as well as protocol compliance.

Safety Evaluation: AEs were recorded by study staff and were assessed for relationship to study product by a contracted physician who served as the medical advisor for the study.

Statistical Methods: Descriptive statistical analyses were performed for all study endpoints. Arithmetic means were reported for variables with data points that were normally distributed, which included cigarettes per day (CPD), Camel Snus pouches per day, ECO, %COHb and questionnaire responses. Medians were reported for urinary biomarkers because data points showed evidence of skewed distribution.

A mixed model with repeated measures (MMRM) was used to assess changes across time in product use (cigarettes and Camel Snus), ECO, %COHb and FTND. A Wilcoxon Sign-Rank test was performed to assess within-subject changes in urinary biomarkers from Week 1 to Week 4. Changes in questionnaire responses (thermometer, attributes, impact and MNWS) were analyzed using Kendall's test for trend. Questionnaire endpoints were normalized to account for subject scale-usage differences prior to testing.

Statistical significance was specified as $p \leq 0.05$, and all references to significance were made with regard to this criterion. Nominal significance was specified as $0.05 < p \leq 0.10$. Final analyses were based on data from the 32 subjects who completed the study.

Study Results: A summary of study data is provided below. For a full description of all study results, refer to CSD0905.

Subject Demographic Summary and Disposition:

Table 6.1.2-38: Demographic and disposition summary

N (Enrolled / Completed)	36/32
Gender	
Female	18
Male	14
% (female/male)	56/44%
Age (years)	
Mean (SD)	41 (9)
Min, Max	21, 55
Smoking History (years)	
Mean (SD)	21 (9)
Min, Max	3, 39

Tobacco Product Use: During Weeks 2, 3 and 4, average reported CPD significantly decreased and average reported Camel Snus per day significantly increased (*see* CSD0905 CSR, Table 5 and Table 6). Average cigarettes smoked per day decreased by 24% at Week 2, 41% at Week 3 and 59% by Week 4. Subjects began using Camel Snus in Week 2 and, based on their reports, increased Camel Snus pouches per day by 39% from Week 2 to Week 4.

Mouth-Level Exposure (Yield-in-Use): On a per cigarette basis, small but statistically significant reductions in mean MLE to nicotine and tar were seen from Visit 1 to Visit 2 (p=0.0151 and p=0.0029, respectively). These changes were no longer significant at Week 4. MLE to nicotine per cigarette decreased by 8.7% from Visit 1 to Visit 4 (p=0.0697), while MLE to tar per cigarette did not change from Visit 1 to Visit 4 (p=0.2367).

On a per day basis, statistically significant reductions in nicotine and tar were seen over the study, likely as a result of the reduction in CPD (*see* CSD0905 CSR, Table 12).

Mouth-Level Exposure (Snus-after-Use): Mouth-level exposure (MLE) was calculated for nicotine, nornicotine, anatabine, B[a]P, Cd, Cr, Ni, Pb, As, Se, NNN, NAT, NAB and NNK. Nicotine, nornicotine, anatabine, B[a]P, NNN, NAT, NAB and NNK MLEs ranged from 10.1% to 30.5% of the quantities initially present in the Camel Snus pouch. The trace metals data, however, frequently resulted in negative MLE values due to variability of the analytical method. MLE values for nicotine, B[a]P and TSNAs did not differ between the Frost and Mellow styles. An average of 9.7 µg more nornicotine (p=0.0424) and an average of 3.8 µg more anatabine (p=0.0978) were extracted from the Mellow style compared to the Frost style.

Urinary Biomarkers of Exposure: All seven biomarkers of exposure to mainstream cigarette smoke vapor phase compounds measured in this study, HPMA (-23.4%), SPMA (-35.5%),

HMPMA (-23.4%), MHBMA (-30.8%), CEMA (-21.4%), HEMA (-25.2%) and thiocyanate (-39.3%), showed statistically significant decreases from Visit 1 (exclusive cigarette smoking) to Visit 4 (dual use of Camel Snus and cigarettes). These results show that the study subjects significantly decreased their smoke exposure.

Of the 21 cigarette smoke particulate phase biomarkers examined, significant decreases were observed from Visit 1 to Visit 4 for NAB (-20.9%); the aromatic amines 3-aminobiphenyl (-28.3%), 4-aminobiphenyl (-13.7%), 2-aminonaphthalene (-26.1%) and *o*-toluidine (-16.3%); PAHs 1-OH-naphthalene (-27.4%), 2-OH-naphthalene (-21.6%) and 2-OH-fluorene (-25.6%); and acrylamide markers AAMA (-22.0%) and GAMA (-21.7%). Levels of nicotine, NNN, NAT, NNK, phenanthrene and pyrene biomarkers were not statistically significantly different between Visit 1 and Visit 4. These results are also consistent with the study subjects significantly decreasing their smoke exposure.

Carbon Monoxide Exposure (%COHb, ECO): Average reductions in ECO and %COHb 25 minutes after product use from Visit 1 (cigarettes) to Visit 4 (Camel Snus) were 27.8% and 21.1%, respectively. In contrast, average reductions in ECO and %COHb when the values were measured just prior to product use were 11.2% and 8.8%, respectively.

Nicotine Uptake: Average serum nicotine AUC and peak nicotine concentrations following smoking of one UB cigarette (Visit 1) were significantly higher than concentrations following use of one Camel Snus pouch (Visit 4). This was true for observed and corrected values. Averages of observed and corrected results are reported in CSD0905 CSR, Table 14. Baseline-corrected AUC values for nicotine were 546.6 (UB cigarettes) and 299.3 (Camel Snus), and baseline-corrected peak nicotine concentrations were 23.2 ng/mL (UB cigarettes) and 9.2 ng/mL (Camel Snus).

Questionnaires (Sensory Perceptions, MNWS, FTND): Questionnaires captured data relating to subjects' acceptance of Camel Snus, their experiences of using Camel Snus and cigarettes, sensory perceptions of Camel Snus and cigarettes, cigarette withdrawal symptoms and FTND-cigarettes and FTND-smokeless tobacco scores over the course of the study. The Cigarette Thermometer (CSD0905, Attachment 1) and Camel Snus Thermometer (CSD0905, Attachment 4) questionnaires captured overall product ratings each week. The UB cigarette and Camel Snus Evaluation Questionnaires (CSD0905, Attachments 2 and 5) captured subjects' sensory opinions about product attributes. The Cigarette and Snus Impact Questionnaires (CSD0905, Attachments 3 and 6) captured perceived physical impact in different regions of the body during product use. The Minnesota Nicotine Withdrawal Scale (CSD0905, Attachment 9) captured withdrawal symptoms reported by subjects. The Fagerström Test for Nicotine Dependence was administered for cigarettes at the beginning and end of the study, and for smokeless tobacco at the end of the study.

Initially, subjects rated Camel Snus as "Quite Good" on the thermometer scale and, by the end of the study, rated it closer to "Very Good," with a significant upward trend in rating over time. Subjects rated the sweetness, tobacco taste and texture of Camel Snus to be "Just Right" throughout the study. They rated Camel Snus as having slightly too much flavor at Visit 1, but flavor ratings trended toward "Just Right" in Visits 2, 3 and 4.

Subjects also reported changes in their UB cigarette perceptions over the course of the study. Differences included a significant downward trend in thermometer rating, satisfaction, smoothness, strength of taste and tobacco taste for their UB cigarettes. Subjects reported significant increases in harshness and aftertaste of their UB cigarettes. They also reported experiencing increased impact in the nose and chest while smoking.

Snus and cigarette questionnaire responses are summarized in CSD0905 CSR, Table 15 and Table 17, respectively.

Changes in tobacco product use over the course of the study resulted in a minimal change in nicotine withdrawal symptoms. Of the nine MNWS symptoms validated as accurate measures of nicotine withdrawal, only weight gain/appetite showed a small, nominally significant increase in rating as the study progressed. In contrast, small but significant reductions were seen in anxiety, desire to smoke, insomnia, restlessness and coughing. Average withdrawal symptom ratings and overall withdrawal symptom scores as measured using the MNWS are found in CSD0905 CSR, Table 18.

FTND for cigarettes was administered at the start and end of the study, and the FTND for smokeless tobacco (modified for Camel Snus) was administered at the end of the study. FTND response means by question and overall scores for subjects who completed all visits are available in CSD0905 CSR, Table 19.

Safety Evaluation: No serious adverse events (SAEs) were reported during this study. Adverse events (AEs) that were determined by the medical advisor to be possibly, probably or definitely related to the use of Camel Snus included: nausea, throat irritation/burn, mouth burn, indigestion/heartburn/stomach discomfort, hiccups, headache and worsening of acid reflux. The numbers of subjects reporting these events at each visit are reported in CSD0905 CSR, Table 12.

6.1.2.3.2.3 CSD0914: Assessment of Serum Nicotine Exposure from Modern Smoke-Free Tobacco Products

The primary objective of CSD0914 was to determine serum nicotine uptake over a three-hour period following Camel Snus use. Secondary objectives of this study were (1) to assess tobacco abstinence symptoms prior to and at designated intervals following use of Camel Snus and (2) to assess carboxyhemoglobin levels prior to and for one hour following use of Camel Snus after overnight abstention from all tobacco.

Study Description and Methodology: All subjects were usual brand (UB) smokers. They provided and smoked one UB cigarette at Test Visit 1. Subjects were then given six pouches of Camel Snus Frost (0.6 g) or Mellow (0.6 g) for use at home over the next six days in order to provide familiarization with the product. Subjects used the same Camel Snus product during a subsequent Test Visit. Subjects recorded daily cigarette and Camel Snus consumption during the week on written log sheets that were returned at the later Test Visit.

During the subsequent Test Visit, subjects used one pouch of the Camel Snus product they tested throughout the previous week. Subjects were asked to place one Camel Snus pouch between either upper or lower lip and gum and to leave in place for 15 to 30 minutes. Occasional movement of the pouch was suggested, but not required. Duration of use was recorded.

Subjects were instructed to stop smoking at least 12 hours before each Test Visit, and to use no Camel Snus the day before each Test Visit. Completion of each Test Visit was dependent on the corresponding expired carbon monoxide (ECO) (≤ 12 ppm) measurement at check-in. If a subject successfully fulfilled the ECO requirement, the first of 10 Mood and Physical Symptoms Scales (MPSS) was completed. An IV catheter was placed, and timed blood draws started with the -2 minute sample collection. Immediately following the 0-minute sample collection, study product was provided and duration of product use was timed. Blood was collected and questionnaires were administered at designated times for three hours following the start of Camel Snus use.

The RJRT R&D Human Research Review Committee (HRRC) approved this study after a review of the experimental protocol. Interested recruits who passed telephone screening were scheduled for a Screening Visit. At the Screening Visit, subjects were given additional information about the study products and study requirements. Subjects provided written informed consent for study participation before any study procedures were performed.

Study Population and Eligibility Criteria: Fifteen generally healthy smokers were enrolled for study procedures.

Key study eligibility criteria are summarized below. For a full listing of eligibility criteria, including definitions and verification procedures, refer to CSD0914 CSR.

Inclusion Criteria:

- generally healthy male or female, age 21 to 55 years (inclusive);
- report smoking 10-30 cigarettes per day of a UB cigarette with Cambridge Filter Method (CFM) tar levels of 8.0-14.0 mg/cigarette;
- afternoon ECO level of ≥ 15 ppm as an indication of smoke inhalation; and

Exclusion Criteria:

- has current oral lesion(s) at Screening Visit or history of major uncontrolled health conditions;
- postponed a decision to quit smoking to participate in this study; or
- current routine use of smokeless tobacco products.

Evaluation Criteria:

Nicotine Uptake: Serum samples taken at intervals from -2 to 180 minutes with respect to the start of product use (cigarette or Camel Snus) were analyzed to measure nicotine and cotinine.

Carboxyhemoglobin: Whole blood samples were drawn at -2, 30 and 60 minutes with respect to the start of product use (cigarette or Camel Snus) for measurement of %COHb.

Questionnaires: The Mood and Physical Symptoms Scale (MPSS) was administered once at the Screening Visit and 10 times during each Test Visit (just prior to product use, and at 5, 15, 30, 45, 60, 90, 120, 150 and 180 minutes after the start of product use).

Expired CO (ECO): Subjects provided breath samples for determination of expired carbon monoxide concentrations once at the Screening Visit and during check-in of each Test Visit to verify smoking abstinence (ECO) (≤ 12 ppm).

Mouth-Level Exposure (Yield-in-Use): Nicotine yield-in-use for each study subject's UB cigarette was determined from cigarette filter tip analysis (collected at Test Visit 1). No other constituents were evaluated.

Mouth-Level Exposure (Snus-after-Use): The Camel Snus pouches used during the Test Visit were analyzed for nicotine and other tobacco alkaloids. Analysis was performed on individual pouches. No other constituents were evaluated.

Safety Evaluation: AEs were recorded by study staff and were assessed for relationship to Camel Snus by a contracted physician who served as the medical advisor for the study.

Statistical Methods: The baseline serum nicotine amount determined for each subject at each Test Visit (an average of the -2 minute and 0 minute time point nicotine concentrations) was subtracted from the observed concentrations at each subsequent time point. The following nicotine uptake parameters were determined from observed values and baseline-adjusted values for individual subjects and statistically analyzed: area under the concentration-versus time curve (AUC), baseline-adjusted AUC, maximum concentration (C_{max}), baseline-adjusted C_{max} and time to maximum concentration (T_{max}). AUC was calculated according to the trapeze formula, commonly known as the trapezoidal rule (*see* CSD0914 CSR, p.10).

Study Results: A summary of study data is provided below. For a full description of all study results, refer to CSD0914 CSR.

Subject Demographic Summary and Disposition:

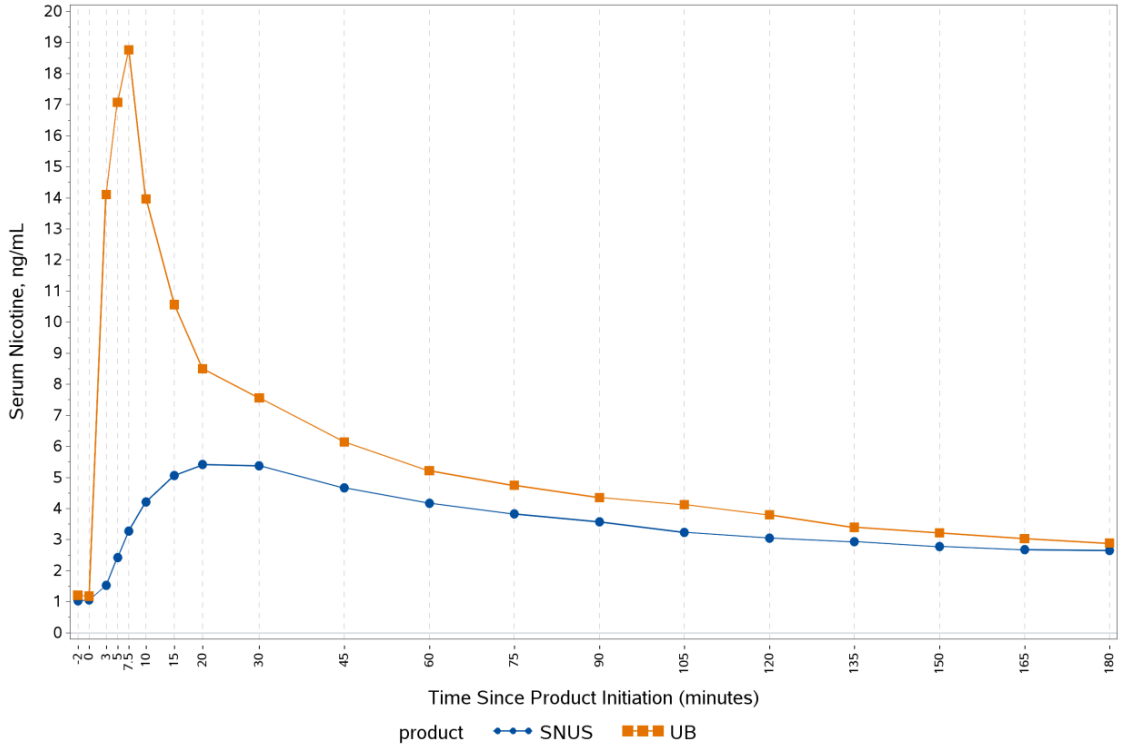
Table 6.1.2-39: Demographic and disposition summary

N (Enrolled / Completed)	15/15
Gender	
Female	7
Male	8
% (female/male)	47/53%
Age (years)	
Mean (SD)	41 (9)
Min, Max	24, 54
Smoking History (years)	
Mean (SD)	21 (9)
Min, Max	3, 35

Tobacco Product Use: During the Test Visit, subjects used Camel Snus for an average of 21.1 minutes, with a minimum use time of 15.1 minutes and a maximum use time of 30.1 minutes. UB cigarettes were used for an average of 5.8 minutes, with minimum and maximum use times of 3.6 and 9.0 minutes, respectively.

Nicotine Uptake: Serum nicotine uptake, measured as the area under the concentration versus-time curve for the 180-minute testing period ($AUC_{0\text{-}180}$), was greater for UB cigarettes (761 ng x min/mL) than for Camel Snus (486 ng x min/mL). Time to maximum concentration (T_{max}) was shorter for UB cigarettes (6.6 minutes) than for Camel Snus (22.7 minutes), consistent with more rapid uptake of nicotine in the lung versus the oral mucosa. Maximum concentration (C_{max}) results for Camel Snus (5.0 ng/mL) were 25% of UB cigarettes (19.9 ng/mL).

Figure 6.1.2-9: Average serum nicotine concentration vs. time curves for Camel Snus and UB cigarettes



Mouth-Level Nicotine Exposure: Mouth-level nicotine exposure from smoking UB cigarettes and Camel Snus use was determined by yield-in-use (YIU) and snus-after-use (SAU) analysis (*see* CSD0914 CSR, Table 7 and Table 8). Average MLE to nicotine from UB cigarette (1.59 mg) was less than that of Camel Snus (2.28 mg), yet as described previously the $AUC_{0\text{-}180}$ from smoking UB was greater than Camel Snus use. The differences between AUC and MLE may reflect differences in rates of absorption in the lung compared to the oral mucosa and the rate of metabolism that occurs once absorbed by the two tissues (*see* Hukkanen *et al.* 2005).

Carbon Monoxide Exposure (%COHb): Statistically significant increases in %COHb were observed following UB smoking, but not after Camel Snus use. These results indicate that no CO uptake occurs during use of Camel Snus.

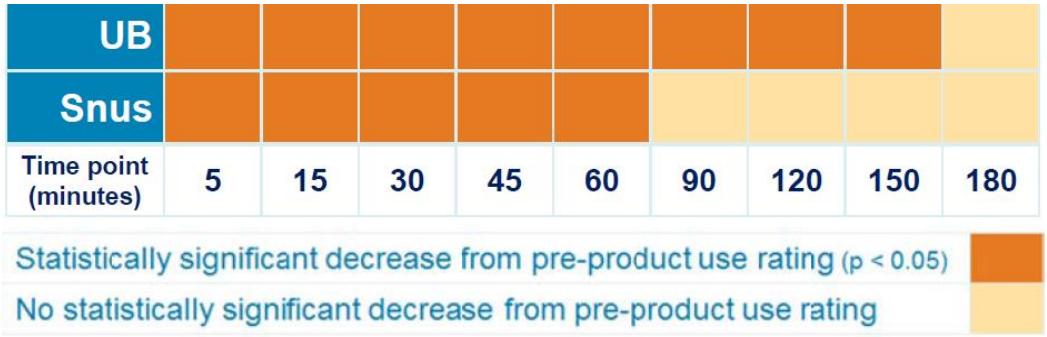
Questionnaire (MPSS Items 1-6): Mean ratings for depression were low and did not significantly change over time for subjects smoking UB cigarettes (1.1 to 1.0) or using Camel Snus (1.1 to 1.0). Mean ratings for poor concentration were low and did not change significantly following Camel Snus use (1.5 to 1.1), but did for UB cigarettes (1.7 to 1.1). Mean changes in poor concentration after smoking UB cigarettes were small and were considered to be caused by a slightly increased mean pre-product use rating, anxiety, irritability and restlessness.

Data for hunger ratings were confounded by subjects snacking during Test Visits, but mean ratings were generally low and slightly increased over time for Camel Snus (1.1 to 1.4) and UB cigarettes (1.4 to 2.1).

Questionnaire (MPSS Urge to Smoke): Mean rating of urge to smoke statistically significantly decreased after use of both UB cigarettes and Camel Snus. The decrease in urge to smoke was greater (maximum mean difference = 3.27) after smoking one UB cigarette than after using one Camel Snus pouch (maximum mean difference = 1.0).

Decreased urge to smoke also lasted longer following cigarette smoking (150 minutes following start of use) than after use of Camel Snus (60 minutes following start of use) (*see* Figure 6.1.2-10).

Figure 6.1.2-10: Duration of decrease in urge to smoke by product



Safety Evaluation: No adverse events (AEs) were associated with UB cigarette use. Eight AEs occurred during the course of the study that were judged by the medical advisor to be possibly, probably, or definitely related to Camel Snus use. These included throat irritation (1 event), nausea (2 events), mouth burn/irritation (2 events), hiccups (1 event), heartburn (1 event) and vomiting (1 event) (*see* CSD0914 CSR, Table 25). AE symptoms were generally mild in intensity and resolved within 45 minutes.

6.1.2.3.2.4 CSD1101: Assessment of Smokers' Nicotine Uptake and Urge to Smoke After Use of Smokeless Tobacco Products

The objectives of this study were to assess (1) nicotine uptake in blood and (2) tobacco abstinence symptoms over a 3-hour period following the smoking of one usual brand (UB) cigarette or use of one Camel Snus pouch. Measurements were made in a clinical setting, following a 12-hour abstinence from tobacco and nicotine.

Study Description and Methodology: CSD1101 was a single-center, randomized, open-label, 10-week crossover study designed to evaluate serum nicotine uptake and tobacco abstinence symptoms following the smoking of one usual brand (UB) cigarette or use of one Camel Snus pouch. Current smokers in generally good health abstained from all tobacco and nicotine use for at least 12 hours prior to using one UB cigarette or one Camel Snus pouch in the clinic during one of five Test Visits. (b) (4)

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Enrolled subjects smoked one UB cigarette at Test Visit 1. Subjects then used Camel Snus Frost (0.6 g) at one subsequent Test Visit (2 through 5). Prior to each Test Visit, expired carbon monoxide (ECO) was measured to confirm smoking abstinence and to determine subject eligibility to undergo visit procedures.

During each Test Visit, serial blood samples were collected for determination of serum nicotine and cotinine concentrations, and subjects completed questionnaires (Mood and Physical Symptoms Scale [MPSS]) to assess tobacco abstinence symptoms. Safety was evaluated based on data collected for adverse events (AEs), physical examinations including oral examinations, clinical laboratory tests and vital sign measurements.

The study was conducted in accordance with the United States (US) Code of Federal Regulations (CFR) governing Protection of Human Subjects (21 CFR 50), Financial Disclosure by Clinical Investigators (21 CFR 54) and Institutional Review Boards (IRB) (21 CFR 56). As such, the study was designed and monitored in accordance with Good Clinical Practice (GCP) as required by the major regulatory authorities and in accordance with the ethical principles of the Declaration of Helsinki.

Bioanalytical laboratory assessments were performed by a Good Laboratory Practice (GLP)-compliant laboratory. Contact information for the bioanalytical laboratory is provided in CSD1101 CSR, Appendix 16.1.4.

Study Population and Eligibility Criteria: Seventeen subjects were enrolled and included in the statistical analyses of nicotine uptake parameters, product ratings and tobacco abstinence symptoms, and included in the safety sample.

Key study eligibility criteria are summarized below. A full description of all eligibility criteria, including definitions and verification procedures, are found in CSD1101 CSR, Section 9.3.

Inclusion Criteria:

- generally healthy male or female, age 21 to 55 years (inclusive);
- self-reported smoking 10 to 30 cigarettes per day for at least six months prior to screening; and

Exclusion Criteria:

- postponed a decision to quit smoking to participate in this study;
- current oral lesion(s) at Screening Visit or history of major uncontrolled health conditions; or
- ECO level was < 15 parts per million (ppm) or > 100 ppm at the Screening Visit, measured between 12 p.m. and 6 p.m.

Evaluation Criteria:

Nicotine Uptake and Pharmacokinetics (PK): Blood samples for determination of serum nicotine and cotinine concentrations were collected with respect to the start time of in-clinic study product administration at: pre-0 (within 10 minutes prior to start of study product administration), 0, 3, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150 and 180 minutes. Cotinine at time 0 was evaluated to monitor subjects' overall tobacco use throughout the study.

(b) (4)

Questionnaires: Two questionnaires were used to assess subjective measures:

- at screening, the Fagerström Test for Nicotine Dependence (FTND) estimated the self-reported level of nicotine dependence; and
- at screening and during each Test Visit (both before and multiple times after study product administration), the Mood and Physical Symptoms Scale (MPSS) assessed tobacco abstinence symptoms.

Expired CO: Expired carbon monoxide was measured at screening to confirm inhalation of cigarette smoke, and at the start of each Test Visit to verify smoking abstinence in order to determine subject eligibility to undergo study procedures.

Safety Evaluation: Safety monitoring included assessment of AE reports, physical and oral examination, clinical laboratory testing (complete blood count, comprehensive metabolic profile, urinalysis, triglycerides, total cholesterol, pregnancy tests) and vital sign measurements (blood pressure, pulse, oral body temperature).

Statistical Methods: (b) (4)

The Statistical Analysis Plan (SAP) is provided in CSD1101 CSR, Appendix 16.1.9.

Study Results: A summary of study results is provided below. For a full description of all study results, refer to CSD1101 CSR, Section 11.

Subject Demographic Summary and Disposition:

Table 6.1.2-40: Demographics summary at screening

Parameter	All Randomized Subjects (N=17)
Gender, n (%)	
Female	11 (64.7%)
Male	6 (35.3%)
Age (years)	
Mean (SD)	36.1 (9.36)
Median	35.0
Min, Max	25, 53
Race, n (%)	
Caucasian	16 (94.1%)
African American	1 (5.9%)

Table 6.1.2-41: Disposition of subjects

	All Randomized Subjects (N=17)
Completed the study	16 (94.1%)
Discontinued from the study	1 (5.9%) – Protocol Noncompliance

Nicotine Uptake and PK: (b) (4)

(b) (4)

(b) (4)
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(b) (4)
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(b) (4)

Questionnaires: Use of UB cigarettes often resulted in greater reductions in tobacco abstinence symptoms (*i.e.,* anxious, irritable, restless, poor concentration and urge to smoke) than use of Camel Snus. The symptom of feeling depressed was not significantly impacted by use of UB cigarettes or Camel Snus. Results for feeling hungry were likely confounded because subjects were permitted to eat during Test Visits after product use.

(b) (4)
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(b) (4)
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(b) (4)
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(b) (4)
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(b) (4)
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Expired CO: (b) (4)

Safety Evaluation: The incidence of subjects with at least one AE, regardless of relationship to study product, was greater with concurrent use of Camel Snus (25.0%) compared with use of only UB cigarettes (0%).

No AE resulted in subject discontinuation from the study, and no AE resulted in cessation or interruption of study product. No subject experienced a serious adverse event (SAE) (including death). The Principal Investigator (PI) considered one of the ten AEs (throat irritation) to be potentially related to Camel Snus product use. All AEs were assessed as mild in intensity.

Safety laboratory parameters with the greatest number of subjects experiencing shifts from normal baseline values were hemoglobin and red blood cells. Five subjects had decreases in hemoglobin to levels at or below the lower limit of the normal range. Four subjects had decreases in red blood cells to levels below the lower limit of the normal range. One subject's shift in hemoglobin (13.3 g/dL to 10.7 g/dL [female normal range: 12.0-15.0 g/dL]), in a 36-year-old female, was assessed as clinically significant by the PI. The lab abnormality was recorded as anemia and was assessed as mild in intensity and unrelated to study product. No other shifts in safety laboratory parameters were considered clinically significant.

Minor fluctuations in blood pressure and heart rate measurements throughout the study were observed. Four subjects had increases or decreases in weight of greater than 5 pounds (range of 5.6 to 10.6 pounds). No changes in vital sign measurements were considered clinically significant or reported as AEs.

Physical and oral examinations showed no clinically relevant changes or abnormalities following use of study products.

6.1.2.3.2.5 HSD0702: Switching from Usual Brand Cigarettes to a Tobacco Heating Cigarette or Snus – A Multi-Center Evaluation of Heath-Related Quality of Life Assessments and Biomarkers of Exposure and Harm

The primary objectives of this study were to: (1) determine the feasibility of the study design and the analysis methodology; (2) assess subject compliance with study products and (3) obtain data on the ability of Camel Snus to modify patient-reported Chronic Obstructive Pulmonary Disease (COPD)-related health status [as measured by the St. George's Respiratory Questionnaire (SGRQ)].

The secondary objectives of this study were to: (1) obtain data on product switching related modification of self-reported health status as measured by the SGRQ, the Leicester Cough Questionnaire (LCQ) and the Smoking Cessation Quality of Life Questionnaire (SCQoL); (2) obtain data in a comparison of health status measures (SGRQ, LCQ, SCQoL) among the three tobacco-using groups; (3) evaluate selected biomarkers (*i.e.*, tobacco exposure and biological effect) among the three tobacco-using groups; (4) compare baseline data from all three tobacco-using groups to baseline data from a never smoking group; (5) measure amount and repeatability of smoke components yielded from tobacco-burning cigarettes (yield-in-use), and determine the relative uptake of selected smoke components and (6) assess issues related to subjects' switching from their usual brand cigarette to either a tobacco heating cigarette or snus.

Study Description and Methodology: This was a randomized, multi-center 4-group study of health status measures (SGRQ, LCQ, SCQoL) and biomarkers in subjects who smoked and were switched to another tobacco product, with comparisons to a non-treatment group of never-smokers. Subjects in one of the study groups were switched from smoking cigarettes to use of Camel Snus Frost (0.4 g), Spice (0.4 g) or Original (0.4 g). Potential subjects were screened to assess their eligibility to enter the study within 28 days prior to study entry (*i.e.*, prior to Week 0). Once randomized/enrolled, subjects were instructed to discontinue their current usual brand (UB) cigarette and use Camel Snus. Subjects used Camel Snus for 24 weeks.

Subjects completed a series of health status assessments at Screening and throughout the study. Exposure biomarkers in urine and blood, expired carbon monoxide (ECO) assessments and spirometry were evaluated, as applicable. For consistency, subjects were generally measured at approximately the same time of day and same day of the week. Subjects were confined to the Clinical Research Unit (CRU) for approximately 24-hours at Weeks 0, 12 and 24 at which time in-house study procedures/assessments were conducted.

The effects of the Camel Snus products were evaluated by comparing (1) changes in SGRQ, LCQ and SCQoL scores, as well as (2) changes in biomarkers of biological effect in urine (24-h and spot) and blood (fasting) from baseline (Week 0) to Weeks 12 and 24 among subjects switched to Camel Snus.

The SGRQ was the primary PRO measure in HSD0702 and is a quality-of-life instrument developed and validated for use in patients with COPD. Each item on the SGRQ can be scored from 0 to 100, with lower scores indicating better outcomes.

The LCQ is a quality-of-life measure of chronic cough that is responsive to change, and measures Physical, Psychological and Social scores. Total scale score ranges from 3 to 21, with higher scores indicating better health status.

The SCQoL assesses health-related quality of life among persons going through the smoking cessation process, and measures Social Interactions, Self-Control, Sleep, Cognitive Functioning and Anxiety scores. Scale score ranges from 0 to 100, with higher scores indicating better health status.

For a full description of the study Investigational Plan, *see* HSD0702 CSR Final, Section 9.

HSD0702 was conducted in accordance with applicable sections of the United States Code of Federal Regulations (21 CFR 50, 21 CFR 54, 21 CFR 56), the International Conference on Harmonization Good Clinical Practice and the Declaration of Helsinki. Study and subject materials, including protocol, protocol amendments, informed consent forms, study product information and recruitment literature were reviewed and approved by Independent Investigational Review Board, Inc. (currently Shulman Associates IRB, Inc., Fort Lauderdale, FL). The Principal Investigator (or designee) at each CRU explained the purpose of the study, described all study procedures to be carried out, and described the study products to the

subjects. After subjects' questions regarding the study were answered, written informed consent was obtained from all subjects prior to any study-specific procedures being performed.

Study Population and Eligibility Criteria*:* Generally healthy male and female subjects were enrolled into one of the following study groups:

Table 6.1.2-42: Enrollment goals

Study Groups	Target Enrollment
Smokers who were switched to Camel Snus	50
Smokers who were switched to a tobacco-heating cigarette	50
Smokers who were switched to a tobacco-burning cigarette	50
Never-smokers as defined by the American Thoracic Society (ATS)	30

Key study eligibility criteria are summarized below. For a full description of eligibility criteria, including definitions and verification procedures, refer to HSD0702 CSR Final, Section 9.3.

Inclusion Criteria:

- generally healthy male or female, age 28 to 55 years (inclusive);
- subjects in tobacco-using groups were cigarette-only smokers who currently smoked at least 15 cigarettes daily and who smoked for at least 10 years prior to Week 0;
- not intending to quit smoking, but were willing to switch to another tobacco product;
- subjects for Group C were self-reported never-smokers as defined by the American Thoracic Society (ATS); and

Exclusion Criteria:

- for tobacco-using groups, regular use of any tobacco or nicotine-containing product (*e.g.*, cigars, pipes, chewing tobacco, snuff, snus, nicotine patch, nicotine gum) other than tobacco burning cigarettes from 6 months prior to the study through Week 24was not allowed.

Evaluation Criteria:

Never-Smokers Group: At Screening, never-smokers completed the ATS questionnaire. At Week 0, never-smokers completed the SGRQ, LCQ and SCQoL (the SF-36 only) assessments. Biomarkers of tobacco exposure and effect were assessed from blood and urine, and a spirometry evaluation was performed.

Camel Snus Group: At Screening, Camel Snus users completed the following assessments: ATS-DLD-78 (ATS); Stages of Change in Quit Smoking; Fagerström Test for Nicotine Dependence (FTND).

Prior to check-in at Week 0, subjects kept smoking diaries for a 2-week lead-in period to document normal smoking behavior with their UB cigarette product. After beginning Camel Snus use at Week 0, subjects documented all tobacco product use daily using an interactive voice response system (IVRS).

At Week 0 (Baseline), Camel Snus users completed the SGRQ, LCQ, SCQoL, and Stages of Change in Quit Smoking assessments. Biomarkers of tobacco exposure and effect were assessed from blood and urine, in addition to evaluations of expired carbon monoxide (ECO) and spirometry.

At Week 4 and Week 8, Camel Snus users completed the SGRQ and LCQ assessments.

At Week 12, Camel Snus users completed the SGRQ, LCQ, SCQoL and Stages of Change in Quit Smoking assessments. Biomarkers of tobacco exposure and effect were assessed from blood and urine, in addition to evaluations of expired carbon monoxide (ECO) and spirometry.

At Week 16 and Week 20, Camel Snus users completed the SGRQ and LCQ assessments.

At Week 24, Camel Snus users completed the SGRQ, LCQ, SCQoL and Stages of Change in Quit Smoking assessments. Biomarkers of tobacco exposure and effect were assessed from blood and urine, in addition to evaluations of expired carbon monoxide (ECO) and spirometry.

For a detailed overview of analytical assay methodologies, refer to HSD0702 CSR, Section 9.5.2.

Safety Evaluation: For all subjects, basic safety was monitored via evaluation of adverse experiences (AEs), clinical laboratory evaluations, 12-lead electrocardiograms, vital signs, physical examinations and “How Do You Feel?” inquiries.

Statistical Methods: Data were evaluated for both the intent-to-treat sample (ITT) and the per-protocol sample. For the Camel Snus group, the ITT sample included all Camel Snus users, regardless of adherence with the compliance criteria, deviation from protocol and/or subsequent withdrawal from the study. The per-protocol sample was a sub-set of subjects from the ITT sample whose cumulative adherence to the compliance criteria was greater than 50% over the 24 study weeks. For complete details on the statistical methodology, refer to HSD0702 CSR, Section 9.7.

Study Results: A summary of study data is provided below. For a full description of all study results, refer to HSD0702 CSR, Section 11.

Subject Demographic Summary and Disposition:

Table 6.1.2-43: Demographic summary of Camel Snus and never-smoker groups

	Intent-to-Treat		Per-Protocol
	Snus Users	Never Smokers	Snus Users
N	43	32	20
Female	22	18	13
Male	21	14	7
Age (mean)	41	42	41
Age (min, max)	30, 55	29, 54	30, 52
White	35	23	17
Black	6	4	2
Other	2	5	1

Table 6.1.2-44: Disposition of subjects

	Snus Users n	Never Smokers n
Intent-to-Treat		
Overall	43	32
Randomized	43	NA[¶]
Completed	29	32
Withdrawal	14	0
Adverse events	0	0
Sponsor discontinued	0	0
Consent withdrawn	11	0
Product related	7	0
Not product related	4	0
Lost to follow-up	1	0
Other[‡]	2	0
Per-Protocol		
Overall	20	32
Randomized	20	NA[¶]
Completed	20	32

[‡]Non-compliance, family emergency

[¶]Not applicable. Never smokers were not randomized.

Patient Reported Outcomes (PRO) Results: Baseline and Screening comparisons of ATS, SGRQ, LCQ and SCQoL confirmed that never-smokers reported significantly better well-being and functioning than did smokers.

There was no clear trend indicating change over time for Camel Snus users. For a full discussion of PRO results, as well as limitations due to ceiling effects, floor effects and population differences, *see* HSD0702 CSR, Section 11.6.

24-hr Urine Biomarkers of Exposure (Smokers Switched to Camel Snus): Most exposure biomarkers measured in 24-hr urine samples were significantly lower after smokers (Week 0) switched to Camel Snus at both Weeks 12 and 24.

- Nicotine equivalents were lower at Week 12 (-9%) and Week 24 (-9%), but did not reach statistical significance.

- NNAL was significantly lower at Week 12 (-39%) and Week 24 (-35%).

- All four aromatic amines were significantly reduced at Week 12 (3-ABP [-58%], 4-ABP [-53%], 2-AN [-66%] and o-T [-46%]) and Week 24 (3-ABP [-51%], 4-ABP [-45%], 2-AN [-55%] and o-T [-42%]).

- Four PAH biomarkers were significantly reduced at Week 12 (2-naphthol [-42%], 2-OH-fluorene [-36%], 1-/9-OH-phenanthrene [-41%] and 2-/3-OH-phenanthrene [-39%]) and Week 24 (2-naphthol [-32%], 2-OH-fluorene [-34%], 1-/9-OH-phenanthrene [-45%] and 2-/3-OH-phenanthrene [-43%]). Two PAH biomarkers (1-naphthol and 1-OH-pyrene) were not significantly different from baseline values at Week 12 or Week 24.

- Acrylamide exposure measured by AAMA and GAMA was significantly reduced at Week 12 (AAMA [-36%], GAMA [-28%]) and Week 24 (AAMA [-39%], GAMA [-21%]).

- DHBMA (1,3-butadiene exposure) was not reduced at either time point. MHBMA, a more specific marker for 1,3-butadiene exposure, was significantly lower at Week 12 (-67%) and Week 24 (-55%).

- HMPMA (crotonaldehyde exposure) was significantly reduced at Week 12 (-55%) and Week 24 (-48%).

- HPMA (acrolein exposure) was significantly reduced at Week 12 (-41%) and Week 24 (-45%).

- SPMA (benzene exposure) was significantly reduced at Week 12 (-57%) and Week 24 (-50%).

- Urine mutagenicity (Ames assay using bacterial strains TA98 and YG1024) showed clear and consistent patterns of significant reduction from baseline at Week 12 (TA98 [-56%], YG1024 [-63%]) and Week 24 (TA98 [-54%], YG1024 [-53%]).

Blood Biomarkers of Exposure (Smokers Switched to Camel Snus): Biomarkers measured in whole blood samples exhibited mixed results after smokers (Week 0) switched to Camel Snus (Weeks 12 and 24).

- Serum cotinine was lower at Week 12 (-12%) but did not reach statistical significance. Serum cotinine was significantly higher than baseline at Week 24 (32%).
- %COHb was significantly reduced at Week 12 (-59%) and Week 24 (-37%).
- 4-ABP-Hb adducts were lower at Week 12 (-23%) and Week 24 (-12%), but did not reach statistical significance.

Biomarkers of Effect (Never-Smokers vs. Smokers):

- Three out of eight measured biomarkers of inflammation/oxidative damage were significantly higher in smokers compared to non-smokers at Week 0.
- None of the five lipid biomarkers were different in smokers vs. non-smokers at Week 0.
- Three of the five biomarkers of hypercoagulable state were significantly higher in smokers compared to non-smokers at Week 0.
- HgBA1c (insulin resistance) and CEP (endothelial function) were not different between smokers and non-smokers at Week 0.
- Sister-chromatid exchange (DNA damage) was significantly higher in smokers compared to non-smokers at Week 0.

Biomarkers of Effect (Smokers Switched to Camel Snus):

- None of the eight measured biomarkers of inflammation/oxidative damage were significantly different from baseline at Week 12 or Week 24.
- None of the five lipid biomarkers were significantly different from baseline at Week 12 or Week 24.
- Four out of the five biomarkers of hypercoagulable state were significantly lower compared to baseline at either Week 12 or Week 24. ICAM1 was lower at both Week 12 and Week 24; WBCs were lower at Week 12 only; isoprostane biomarkers 8,12-iso-$iPF_{2\alpha}$-VI and $iPF_{2\alpha}$-III were lower at Week 24 only.
- HgBA1c (insulin resistance) was significantly lower in Camel Snus users compared with cigarette smokers at Week 24 only. CEP (endothelial function) was not significantly different from baseline at Week 12 or Week 24.

- Sister-chromatid exchange (DNA damage) was significantly higher compared to baseline at Week 12, but not at Week 24.

Safety Evaluation: In the Never-Smokers group, no subject reported AEs. In smokers switched to Camel Snus, a total of 92 AEs (24 subjects) were reported. These included 57 mild (20 subjects) and 35 moderate (16 subjects) AEs, of which 31 (15 subjects) were considered possibly, probably or definitely related to study product by the PI. Complete safety evaluation data are available in HSD0702 CSR, Section 12.2.

6.1.2.3.3 Smoking cessation using Camel Snus vs. NRT

RJRT has sponsored a randomized clinical trial to compare smoking cessation rates for smokers with an intention to quit smoking when they are switched to use of either Camel Snus or NRT. RJRT is not seeking authorization from FDA to market Camel Snus as a nicotine replacement therapy product for smoking cessation, but is providing this clinical study data to aid in FDA's consideration of the risks associated with Camel Snus "as compared to using an FDA-approved tobacco cessation medication" (FDA MRTPA Draft Guidance 2012).

6.1.2.3.3.1 CSD1010: A Randomized, Multicenter Clinical Trial to Compare Smoking Cessation Rates with Camel Snus, with and without Smokeless Tobacco Health-Related Background Information, and a Nicotine Lozenge

The primary objective of CSD1010 was to compare smoking cessation rates after 12 months among 3 study groups. Each study group consisted of smokers who were provided information about the benefits of smoking cessation and were either (1) switched to Camel Snus and provided one-time information about the relative risks of smoking versus smokeless tobacco use, (2) switched to Camel Snus but not provided information about the relative risks of smoking versus smokeless tobacco use or (3) switched to NRT (a Nicorette nicotine lozenge).

Secondary objectives of this study were to examine subjective effects within study groups using questionnaires, measure biomarker levels in subjects who quit smoking and assess product usage over time.

Study Description and Methodology: CSD1010 was a multicenter, randomized, open-label study to compare smoking cessation rates with Camel Snus, with and without health-related smokeless tobacco relative risk information provided on a single occasion, and Nicorette nicotine lozenges.

At Visit 1 (Week 0), Visit 2 (Week 2) and Visit 3 (Week 7), subjects were provided with a supply of their assigned test product in their preferred Camel Snus style, either Frost (0.6 g) or Mellow (0.6 g), or Nicorette lozenge (4 mg nicotine Original or Mint). From Visit 4 (Week 12) on, subjects were no longer provided with their assigned test product. Subjects who wished to continue using study products were informed that they were free to purchase additional study products for their own use.

Smoking cessation was monitored via the "Smoking Status, Cigarette and Test Product Usage Questionnaire" (CSD1010 CSR, Appendix 4.1) administered at Weeks 0, 2, 7, 11, 24/25 and 50/51 and at Months 3, 6 and 12. Subjective effects associated with switching to Camel Snus or Nicorette lozenge (including urge to smoke and nicotine withdrawal symptoms) were assessed at each visit using the B-QSU and MNWS-R. Subjects who reported complete abstention from smoking had their ECO measured to confirm smoking cessation. Blood samples were collected from those subjects with an ECO ≤8 ppm for biomarker determinations (plasma nicotine, cotinine and thiocyanate).

Subjects were monitored for up to 12 months by periodic outpatient clinic visits and telephone contacts to establish test product and/or cigarette use patterns, to answer subjective effects questionnaires, and to verify smoking cessation status with biomarker measures.

The study was conducted in accordance with the United States (US) Code of Federal Regulations (CFR) governing Protection of Human Subjects (21 CFR 50) and IRBs (21 CFR 56). As such, the study was designed, conducted and monitored in accordance with Good Clinical Practice (GCP) and in accordance with the ethical principles of the Declaration of Helsinki.

Assays of biomarker samples were performed by laboratories following Good Laboratory Practice (GLP) procedures.

Study Population and Eligibility Criteria: Enrollment goals were approximately 600 total subjects overall with approximately 200 subjects in each of 3 study groups (Table 6.1.2-45). A total of 649 subjects were enrolled for safety and efficacy: 218 (Camel Snus + relative risk information), 218 (Camel Snus) and 213 (Nicorette Lozenges) (*see* Table 6.1.2-46).

Table 6.1.2-45: Enrollment goals

Study Groups	Target Enrollment
Camel Snus + relative risk information	200
Camel Snus	200
Nicotine Lozenges	200

Key study eligibility criteria are summarized below. A full description of these criteria is available in CSD1010 CSR, Section 9.3.

Current cigarette smokers who were in generally good health and self-reported that they were motivated to quit smoking were eligible for this study. Subjects were required to be free of clinically significant medical conditions; however, those with mild chronic conditions that were not exclusionary could be included at the discretion of the Investigator.

Inclusion Criteria:

- generally healthy male or female, age 21 to 65 years (inclusive);
- self-reported smoking ≥10 cigarettes per day for at least the past year;

- ECO ≥8 parts per million (ppm) at Screening and at Visit 1;
- willing to quit smoking with the aid of Camel Snus or Nicorette nicotine lozenge; and

Exclusion Criteria:

- use of any natural or herbal product with claims to aid in smoking cessation or use of any alternative smoking cessation therapy such as hypnotherapy or acupuncture within 30 days prior to screening or during the study; or
- use of any smokeless tobacco product within 30 days prior to screening or during the first 3 months of study participation (other than the assigned test product).

Evaluation Criteria:

Biomarkers: Blood samples for analysis of nicotine, cotinine and thiocyanate were collected at screening for all subjects and at Visits 4, 5 and 6 for abstinent subjects with an ECO ≤8 ppm.

Exhaled CO: Concentrations of ECO were measured at Screening and at Visit 1 for all subjects (for assessment of inclusion/exclusion criteria). At Visits 2 through 6, ECO was measured only for subjects who abstained from smoking.

Questionnaires: Camel Snus, Nicorette lozenge and cigarette product use were determined by completion of the "Smoking Status, Cigarette, and Test Product Usage" Questionnaires at screening, Visits 1-3 and during each telephone contact.

The FTND was administered for all subjects at screening.

The B-QSU was administered to all subjects at screening and to all subjects participating in Visits 1 through 6.

The MNWS-R was administered to all subjects at screening and all subjects participating in Visits 1 through 6.

Safety Evaluation: Safety assessment and monitoring included assessment of AE reports, physical and oral cavity examination, clinical laboratory testing (chemistry and hematology laboratory tests, urinalysis, hepatitis and HIV screens, alcohol screen, a urine drug screen and urine pregnancy tests), ECG and vital sign measurements (blood pressure, pulse, body temperature and respiration rate). Concomitant medications were assessed at Screening and at each study visit and telephone contact.

Statistical Methods: Data from all subjects with at least one post-baseline visit were included in the statistical analysis. Complete details of the statistical methodology are provided in the Statistical Analysis Plan (SAP) in CSD1010 CSR, Appendix 16.1.9.

Smoking cessation rates were assessed using 6 endpoints:

R1: continuous smoking abstinence (narrow), defined as no smoking after quit date;

R2: continuous smoking abstinence (broad), defined as no smoking in any 2 consecutive weeks after the quit date;

R3: prolonged smoking abstinence, defined as no smoking in any 2 consecutive weeks after a 2-week grace period from the quit date;

R4: repeated point prevalence smoking abstinence, defined as no smoking within the past 7 days after a 2-week grace period from the quit date;

R5A: quitter at Month 12, defined as no smoking since Month 9; and

R5B: quitter at Month 12, defined as no smoking in any 2 weeks since Month 9.

Criteria R5A and R5B were exploratory and not specified in the protocol.

Study Results: A summary of study data is provided below. For a full description of all study results, refer to CSD1010 CSR, Section 11.

Subject Demographic Summary and Disposition:

Table 6.1.2-46: Demographic summary at screening by study group

Parameter	Camel Snus+Info (N=218)	Camel Snus (N=218)	Nicotine Lozenges (N=213)
Gender, n (%)			
Female	109 (50.0)	112 (51.4)	110 (51.6)
Male	109 (50.0)	106 (48.6)	103 (48.4)
Age (years)			
Mean (SD)	41.5 (11.98)	43.4 (11.56)	41.1 (12.09)
Median	41.0	46.0	42.0
Min, Max	21, 65	21, 65	21, 64
Race, n (%)			
White	160 (73.4)	161 (73.9)	155 (72.8)
Black	53 (24.3)	54 (24.8)	51 (23.9)
Other	10 (4.6)	7 (3.2)	8 (3.8)
Average Cigarettes per Day			
Mean (SD)	18.7 (7.18)	19.4 (7.10)	19.2 (7.33)
Median	20.0	20.0	20.0
Min, Max	10, 50	10, 40	10, 40
Duration of Smoking (years)			
Mean (SD)	22.6 (11.65)	23.7 (12.37)	22.1 (11.93)
Median	22.0	24.0	20.0
Min, Max	1, 48	1, 52	1, 50

Table 6.1.2-47: Disposition of subjects

Category, n (%)	Camel Snus+Info (N=218)	Camel Snus (N=218)	Nicotine Lozenges (N=213)
Completed the study	66 (30.3)	72 (33.0)	78 (36.6)
Discontinued the study	152 (69.7)	146 (67.0)	135 (63.4)
Reason for discontinuation:			
Lost to follow up	76 (34.9)	66 (30.3)	55 (25.8)
Withdrew consent	38 (17.4)	48 (22.0)	37 (17.4)
Other	27 (12.4)	24 (11.0)	32 (15.0)
Adverse event	8 (3.7)	5 (2.3)	6 (2.8)
Protocol violation	3 (1.4)	3 (1.4)	5 (2.3)

Smoking Cessation: During Months 6-12, cessation using Camel Snus, with or without smokeless relative risk information, was not statistically different from cessation using Nicorette lozenges by any cessation endpoint (R1-R4). Providing comparative risk information for smokeless tobacco on a single instance did not have any effect on cessation.

Two additional exploratory endpoints were described in the statistical analysis plan (SAP) but not specified in the protocol: R5A, continuous abstinence between Month 9 and Month 12; and R5B, not smoking in any 2 weeks (*i.e.*, not smoking weekly) between Month 9 and Month 12. Both of these endpoints allowed subjects who had previously been classified as treatment failures to be classified as treatment successes if they subsequently (by Month 9) quit smoking later in the study.

For R5A, there were no statistically significant differences in cessation rates between study groups. When the Nicorette group was compared with the combination of both Camel Snus groups, the odds ratio (1.977) was statistically significantly different from 1.

For R5B, cessation for the Nicorette lozenge group was statistically significantly greater than the Camel Snus groups. Note, however, that the study design was not optimized to explore single-point abstinence late in the study. Thus, the R5A and R5B endpoints provide limited insight into late-onset abstinence.

Product Usage: Many subjects who discontinued from the study were treatment failures. No data are available on the subsequent tobacco product use by these subjects.

Subjects in both Camel Snus groups who quit smoking also tended to quit using Camel Snus. Subjects who quit using Camel Snus by Month 6 tended to use less Camel Snus during the study than subjects who continued using Camel Snus after Month 6. All subjects in the Camel Snus and Nicorette study groups reduced daily cigarette consumption.

In the Nicorette lozenge group, all quitters ceased use of Nicorette lozenge by Month 9. There were few dual users of lozenge and cigarettes at the end of the study, and their lozenge consumption was low relative to the amount of Camel Snus used by Camel Snus groups. At

most time points, dual users reduced cigarette consumption relative to subjects who continued to smoke but stopped using lozenges or Camel Snus.

Snus Dual Use: Dual users (Camel Snus and cigarettes) in both Camel Snus groups significantly reduced their per day cigarette consumption by 60% at the end of the study. Among dual users, statistically significant reductions in cigarettes smoked per day were observed during all weeks following screening. The number of Camel Snus pouches consumed by dual users decreased when self-purchased by subjects. Subjects who engaged in dual use of Camel Snus and cigarettes consumed an average of 6 pouches per day at the end of the study.

Questionnaires: Fagerström scores were not associated with cessation rates. Declines in urge to smoke (measured by BQSU) and withdrawal symptoms (measured by MNWS-R) were similar among the Camel Snus and Nicorette lozenge groups.

Plasma Nicotine/Cotinine: Mean plasma nicotine and cotinine concentrations generally declined progressively for the Camel Snus group with health risk information, as well as for the Nicorette lozenge group. However, for the Camel Snus group without health risk information, after an initial decline, mean plasma nicotine increased from Month 3 onward while mean plasma cotinine remained relatively constant. Continued study product use in the Camel Snus groups was more common than in the Nicorette lozenge group, and may have led to increased nicotine/cotinine concentrations in those groups relative to the NRT users.

Plasma Thiocyanate: Mean plasma thiocyanate (SCN) concentrations declined similarly in the Camel Snus and NRT groups, suggesting that increased cotinine results in the Camel Snus groups compared to the NRT group were due to exposure to nicotine from Camel Snus rather than cigarettes.

Exhaled CO: Concentrations of ECO in abstainers dropped sharply at Week 2 and remained below the screening values through Month 12 in each study group.

Exploratory Analysis: Study data were analyzed to evaluate whether Camel Snus or NRT might perform better in race or gender subsets of the subject population. Cessation rates for females and non-whites with NRT by R3 and R4 were greater than for Camel Snus (without risk information). Cessation rates for females in the NRT group by R4 were greater than in the Camel Snus group (with risk information).

Safety Evaluation: Three serious adverse events (SAEs) were reported during the course of the study, but none were related to the use of Camel Snus or NRT. A total of 108 subjects (16.6%) reported at least one adverse event, with a similar number of subjects reporting adverse events in the Camel Snus and NRT groups. A similar proportion of subjects from each group discontinued due to AEs. The most frequently observed AEs were: nausea (18 subjects), upper respiratory tract infection (9 subjects), dyspepsia (8 subjects) and sinusitis (4 subjects). Complete safety evaluation data is available in CSD1010 CSR, Section 12.