The attached document represents CTP's then-current thinking on certain aspects of tobacco regulatory science. The information contained herein is subject to change based on advances in policy, the regulatory framework, and regulatory science, and, is not binding on FDA or the public. Moreover, this document is not a comprehensive manual for the purposes of preparing or reviewing tobacco product applications. FDA's review of tobacco product applications is based on the specific facts presented in each application, and is documented in a comprehensive body of reviews particular to each application.

Given the above, all interested persons should refer to the Federal Food, Drug, and Cosmetic Act, and its implementing regulations, as well as guidance documents and webinars prepared by FDA, for information on FDA's tobacco authorities and regulatory framework. This document does not bind FDA in its review of any tobacco product application and thus, you should not use this document as a tool, guide, or manual for the preparation of applications or submissions to FDA.

MEMORANDUM

DATE: January 2, 2014

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TO: David L. Ashley, Ph.D.
Office Director
Office of Science

SUBJECT: Substantial Equivalence Reports: Recommended requirements for stability testing of smokeless tobacco products

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Purpose

The purpose of this memo is to provide recommendations to the Office of Science (OS) laying out information necessary for smokeless tobacco products (STPs) to demonstrate that a product is chemically and microbiologically substantially equivalent to a predicate product with respect to product stability and does not raise different questions of public health. These recommendations may be incorporated into regulatory actions and/or current internal policies.

Background

Stability is defined as the extent to which a product retains, within specified limits, and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of manufacture. FDA needs to assess the microbial and chemical stability of new STPs because characteristics of these products are known to change over time (i.e., during storage and use), and these changes may cause the new product to raise different questions of

public health. In regards to substantial equivalence (SE) Reports, current practice within OS is to require stability testing data for all STPs. Most SE Reports do not include any stability testing data, and CTP has not issued a regulatory or guidance document specifying what testing should be included in an SE Report.

Why is product stability important?

The chemical stability of STPs needs to be examined to ensure harmful and potentially harmful constituent (HPHC) levels are not changing over time (i.e., during storage and use). Microbial stability is also important in order to assess the microbiological risks of STPs presented by microbial-mediated production of tobacco-specific N-nitrosamines (TSNAs) and mycotoxins (e.g., aflatoxin B1) as well as proliferation of potentially pathogenic microorganisms during product storage. The objective of a stability study is to determine the time during which a product meets appropriate standards when stored under defined conditions. Thus, information obtained through stability testing could be used to ensure the product is stable during the expected storage period and does not result in an increased risk to public health as the product sits in storage.

What kinds of product changes have been observed during storage?

1. TSNA and nitrite formation

TSNAs in smokeless tobacco products are primarily formed during tobacco curing and fermentation of the processed tobacco, as well as during aging/storage of the processed and packaged tobacco product (IARC, 2007; Brunnemann et al., 1996). TSNA and nitrite formation occurs during the smokeless tobacco product storage and is attributed to chemical, enzymatic, and microbial activity. Factors such as nitrate/nitrite concentrations, moisture content, microbial content/activity, storage temperature, and pH can influence TSNA formation in tobacco products.

The nitrate/nitrite content of tobacco strongly influences the levels of carcinogenic TSNAs in the product. Changes in nitrite concentration during aging/storage of STPs have been observed. Andersen et al. (1989) reported very large increases in nitrite concentrations in moist snuff stored at 24^{o}C with maximal accumulation occurring at 24 weeks with subsequent decreases in nitrite during the final storage period (52 weeks). This increase in nitrite concentration is attributed to microbial nitrate reductase activity (NRA). Large numbers of nitrate-reducing bacteria are found in tobacco (Parsons et al., 1986; Di Giacomo et al., 2007) and have been isolated from STPs (Foley, April 2013).

It is generally understood that microorganisms (both bacteria and fungi) can enzymatically reduce ubiquitous nitrate in tobacco to nitrite, which then reacts with secondary or tertiary amine pyridine alkaloids such as nicotine to yield TSNAs (Hamilton et al., 1982; Ramström, 2000; Bush et al., 2001; Phillip Morris, May 1999; Wiernik et al., 1995). However, nitrite itself is not normally capable of catalyzing N-nitrosation reactions, as this requires either further chemical (via nitrous acid) or biological reduction to N-nitrosating species (Smith and Smith, 1992). In addition to reducing nitrate to nitrite, microorganisms can directly enhance nitrosation under

neutral pH (Ayanaba and Alexander, 1973). It has been well established that the nitrosation reaction can occur directly through microbial enzymatic catalysis (Leach et al., 1987; Kaplan et al., 1983; Ralt and Tannenbaum, 1981; Rostkowska et al., 1998; Camels et al., 1988; Smith and Smith, 1992; Mills and Alexander, 1976; Pancholy and Mallik, 1978). Thus, microbial content of tobacco products is of decisive importance for reducing nitrate to nitrite and for the nitrosation of alkaloids.

Increased TSNA levels during storage/aging of STP have been attributed to microbial nitrate reduction (Andersen et al., 1993; Fisher et al., 2012; Djordjevic et al., 1993). Studies conducted using snuff have shown that TSNAs increase when the product was stored for more than 4 weeks at both elevated and ambient temperatures, presumably because nitrate was reduced to nitrite, leading to nitrosation reactions that generate TSNAs (Brunnemann et al., 1996). Rutvist et al. (2011) reports that prior to implementing a controlled heat treatment process in the production of Swedish snus, changes in the quality of the final product were due to microbial formation of nitrite and ammonia, which affected TSNA formation and product stability. In addition, it has been observed that TSNA formation in U.S. moist snuff is attributed to microbial nitrate reduction during fermentation and subsequent product storage (Fisher et al., 2012).

The pH of the product also affects TSNA formation. Chemical-mediated nitrosamine formation through secondary amines and nitrite-derived species is highly pH-dependent with an optimum pH between 3.0-3.4 (Rundlof et al., 2000), while microbial-mediated nitrosation reactions occur around neutral pH (Andersen et al., 1993). A study by Andersen et al. (1993) comparing non-heated and heat-treated (autoclaved) moist snuff supports the concept that increases in nitrite and total nitrosamine levels are mediated by bacterial growth at nearly neutral pH during prolonged storage of the product.

Higher moisture content in STPs had a greater effect for observed increases in TSNA and nitrate levels than elevations in temperature in snuff tobacco products (Djordjevic et al., 1993; Anderson et al., 1989). A different study that looked at temperature and humidity effects on burley tobacco over a 28 day period found that a higher relative humidity was associated with significant increases of nitrite and TSNAs in burley (Burton et al., 1989). Total changes in nitrosated pyridine alkaloid concentration, as well as pH changes, were of greater magnitude in high moisture content (55%) than in low moisture content (21.9%) moist snuff (Andersen et al., 1993). The effects of moisture content on TSNA and nitrite increases are to be expected since moisture content and, more specifically, water activity[1] affects microbial growth.

2. Moisture content

Studies of moist snuff utilizing various packaging materials and environmental conditions have shown that moisture content significantly changes over time (Djordjevic et al., 1993). When stored at ambient temperature (13-27°C) in individually sealed tins, moisture content was reduced by more than 50% within 24 weeks. However, when stored in plastic wrapped tin sleeves in an incubator (37°C, 85% humidity); moisture content was increased by 10% within 8

[1] See definition of "water activity" on page 7 of this memo.

weeks. Thus, packaging materials and environmental conditions can have a significant effect on moisture content.

3. pH and free nicotine

The pH of a smokeless tobacco product is of concern because pH affects the amount of unprotonated nicotine (the bioavailable form of nicotine) in the product and also affects TSNA formation. The Djordjevic studies (Djordjevic et al., 1993) of moist snuff utilizing various packaging materials and environmental conditions also have shown that pH changes over time. Changes in pH of moist snuff during prolonged storage have been reported by Anderson et al. (1993). In this study, the moisture-temperature interaction on pH changes were examined and found that pH and total TSNAs changes were of greater magnitude in high moisture moist snuff than in low moisture moist snuff. At 55% moisture, the pH gradually increased from 6.9 to 7.2 while there was a slight decrease in pH at 21.9% moisture from 6.9 to 6.4 during storage of up to 48 weeks (Andersen et al., 1993). Storage conditions in Swedish snus have influenced the pH and moisture levels which may influence free nicotine content and thus nicotine uptake. Therefore, snus that has aged and been inappropriately stored may deliver less nicotine than freshly manufactured or properly stored (under cooling conditions) snus (Swedish Match, 2009). Additives such as ammonium and potassium salts are also known to influence the pH in snuff and thus increase the bioavailability of nicotine (Djordjevic et al., 1994).

4. Total nicotine

Studies have shown that the total nicotine level in a smokeless product can change over time, and that temperature, humidity, and tobacco blend can affect these observed changes (Djordjevic et al., 1993; Burton et al., 1989). Total nicotine of STPs may also be affected by microbial action. Several bacterial species are capable of using nicotine as the sole carbon and nitrogen source for growth including *Pseudomonas* and *Arthrobacter* (Sguros, 1955; Smith, 1964; Brandsch, 2006; Li et al., 2009; Raman et al., 2013). In addition, several species of fungi isolated from tobacco have been shown to be capable of converting nicotine to norcotine (Uchida et al., 1983).

Why is microbial content of STP a concern?

Microorganisms present in STPs can pose an increased public health risk particularly if the microbial stability of the product is not properly controlled. The tobacco industry has recognized for a long time the important role that microbial processes play in the toxicity and quality of tobacco products (Mitchell, 1972; Hempfling, 1987; Philip Morris, May 1999; Fisher et al., 2012). Industry has attempted to mitigate these risks through research, patents to address microbiological controls, and changes in production practices. However, even with these efforts, microbial contamination of STPs still presents a significant public health risk. In addition to impacting the chemical stability of STPs as previously discussed, microorganisms isolated from STPs can be mycotoxigenic, pathogenic and/or have the ability to catalyze the formation of nitrosamines. Hence, microbiological quality and relevant hazards of STPs will be important to properly assess the public health risks of new tobacco products.

The majority of STPs in the U.S. are produced via a fermentation process driven by microbial action. It is known that the level of bacteria during the tobacco processing steps impacts the amount of bacteria on the final tobacco products (Schulthess, D., 1984; Fisher et al., 2012). For example, thermophilic microorganisms and heat-resistant spores such as those from *Bacillus* and *Clostridium* can survive the fermentation process and can pose a high microbial contamination risk in STPs. Numerous bacterial and fungal species have been isolated from STPs (Tansey, 1975; Rubinstein and Pederson, 2002; Warke et al., 1999; Brotzge, 1983; Varma et al., 1991); many of which are potential human pathogens or are known to produce toxins that can induce dermatitis, neurological disruptions, hepatoxicity, liver damage and/or immune suppression (Bennett and Klich, 2003).

Tobacco is one of the many substrates that can support the growth of mycotoxigenic fungi and aflatoxin production. Varma et al. (1991) reported that *Aspergillus flavus* and *A. niger* are the most common fungal species isolated from chewing tobacco leafs and 18% of the isolates were found to be mycotoxigenic. Isolation of thermophilic fungi from snuff has also been reported and their potential risks to public health have been raised (Tansey, 1975). The most common bacterial contaminant of STPs belongs to the *Bacillus* species (Foley, Jan. 2013; Rubinstein and Pedersen, 2002). These bacteria are able to grow over a wide range of pH (2-10) and temperatures (3-75^{o}C) (Drobniewski, 1993). The presence of *Bacillus* in STPs is of concern because some members of the *Bacillus* spp. can reduce nitrate to nitrite and may contribute to TSNA formation. In addition, some members of the *Bacillus* spp. have been shown to cause human infections including food poisoning, septicemia, endocarditis, and bacteremia (Logan, 1988) or to elaborate a potent exogenous virulence factor(s) that injures the oral mucosa (Rubinstein and Pedersen, 2002). Microorganisms such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus*, *Fusarium*, and *Penicillium* can catalyze the formation of nitrosamines (Rostkowska et al., 1998) and are amongst the microorganisms known to be present in tobacco and STPs (Hamilton et al., 1969; Yang and Lucas, 1970; Lukic et al., 1972; Snow et al., 1972; Foley, Jan. 2013; Rubinstein and Pederson, 2002).

What factors affect microbial stability of STP?

Microbial content and, thus, product stability of STPs is affected by various factors such as the fermentation process, addition of chemical additives to control microbial activity (preservatives/metabolic inhibitors/humectants), water activity of the product, and other manufacturing practices including stabilization process of the fermented tobacco. These factors are discussed below.

1. Fermentation

Typical American moist smokeless tobacco undergoes fermentation, which imparts characteristic flavors but often results in higher concentrations of unwanted bacteria-mediated by-products, such as TSNAs and nitrite (Rodu and Jansson, 2004). Microbial-mediated fermentation produces different changes in the chemical constituents in tobacco depending on the fermentation process and the type of bacterial species involved in the fermentation (Smith, 1964; Cornell et al., 1979). Thus, modifications in the tobacco processing steps have been reported to decrease microbial levels and enzymatic activity in an attempt to reduce TSNA concentrations in

STPs. Brunnemann et al. (1996) reports that reductions in TSNAs have been achieved by changing the processing of tobacco into snuff, most notably by modifying the fermentation step. In addition, the type and amount of the fermentation inoculum can be used to obtain a very specific biochemical response and can change the product as a result of directed fermentation (Geiss, 1989). For example, Fisher et al. (2012) reports the introduction of bacterial 'starter cultures' that do not contain nitrate-reducers in an effort to reduce microbial nitrate reduction activity during the fermentation process of moist snuff. The starter cultures are thought to competitively inhibit the growth of endogenous nitrate reducers and thus reduce TSNA formation during fermentation and subsequent product storage. However, the study by Fisher et al. (2012) did not specify what type of bacterial species were used as the 'starter cultures' nor did it address potential health risks resulting from residual bacteria in the moist snuff product. Therefore, information about the fermentation process (including but not limited to pH, temperature, a_w, duration of the fermentation) and the fermentation inoculum (such as the type and concentration of microorganisms) is very important as these can result in different changes in the chemical constituents of the tobacco and also impact the type and amount of microorganisms in the final product; thereby affecting the stability of the product.

Moist snuff resulting from traditional (natural) fermentation generally contain 10^5-10^8 organisms per gram after processing (Roth et al., 1994) and have a limited storage times due to the presence and growth of residual microorganisms. In addition to TSNA formation, residual microorganisms in moist snuff after processing can destroy the flavor and reduce product acceptability by generating off flavors through further in-package fermentation. Hence, STPs that have undergone fermentation present a high risk of microbial activity in the final product and a shorter storage time particularly if the microbial activity is not properly controlled via a stabilization process.

2. Metabolic inhibitors, preservatives, and other additives

Methods to reduce bacterial and fungal activity remaining after fermentation and TSNA formation in STPs include the use of chemical additives such as bacterial metabolic inhibitors and preservatives. For example, sodium chlorate is added directly to STPs as an inhibitor of nitrate reducing enzymatic activity to prevent formation of TSNAs during product storage. However, this additive is highly toxic to humans and the public health risks of this additive have not been properly evaluated. It is possible that ingesting residual chlorate from STPs could potentially alter the microflora of the human gastrointestinal track. Chlorate can be reduced to cytotoxic chlorite by gastrointestinal bacteria and has been used to alter the gastrointestinal microflora in animals destined for slaughter (McReynolds et al., 2004). Furthermore, a patent has been issued in which tobacco is treated with sodium chlorate, potassium chlorate, and/or calcium chlorate at concentration of 100-400ppm (Cui et al., 2011). Therefore, the levels of sodium chlorate in STPs could potentially be much higher than what is currently reported in SE Reports (maximum of 100 ppm in dry snuff and 50 ppm in moist snuff).

Other additives commonly used in moist snuff as inhibitors of microbial activity include salicylates, propylene glycol, propylparaben, potassium sorbate, sodium benzoate, salt, and ash. Sodium salicylate, a commonly used flavoring in STPs, is another additive of concern as it is known to induce antibiotic resistance in bacteria (Hartog et al., 2010; Price et al, 2000; Riordan

et al., 2007; Schaller et al., 2002; Cohen et al., 1993) and can, therefore, pose an increase human health risk through potential exposure of antibiotic resistant bacteria. Preservatives such as potassium sorbate have been used to inhibit fungal growth, but there exists a risk that potassium sorbate concentration is reduced by the activity of bacteria. The fact that potassium sorbate is metabolized by bacteria shows that these organisms are not at all inhibited in their activity by this preservative. Hence, it has been suggested that quantitative measurement of this preservative should be performed for microbial quality determinations (Schulthess, 1984). It is important to note that preservatives and microbial inhibitors are toxic to humans. Furthermore, the effectiveness of these additives should be properly assessed to assure they do not result in an increased public health risk.

3. Water activity (a_w) and moisture content

Moisture content of STPs affects microbial growth. The growth and activity of microorganisms is generally greatest at high levels of moisture content. It has been reported that bacteria can replicate on tobacco during aging at moisture contents as low as 10-14 percent (Reynolds RJ., 1961). The total moisture content does not, however, give a good indication of the amount of water available to support microbial growth. This is more accurately described by the water activity (a_w) of the substrate (Mutasa et al., 1990). A_w of a product describes the degree to which water is bound to the product; it's availability to participate in chemical and biochemical reactions and to facilitate growth of microorganisms. A_w is defined as the ratio of water vapor pressure of the sample of interest to the vapor pressure of pure water at the same temperature; or as the Equilibrium Relative Humidity (ERH) of the sample of interest divided by 100. A_w can be measured monometrically or by relative humidity sensors. Water activity has been recognized as a key factor for microbial activity in tobacco and tobacco products (Kalin et al.; Hofer, M., 1989; Bevan, 1988; Onno, 2008; Mutasa et al., 1990). Microorganisms generally have optimum and minimum levels of a_w for growth. For example, a_w plays an important role in the growth and development of fungi, and different a_w levels may be critical for germination, linear growth and sporulation. Mutasa et al. (1990) reports that the time before the onset of germination and growth of fungi was increased by reducing substrate a_w, ranging from about one week at 0.85 a_w to about six weeks at 0.75 a_w. In addition, the relationship between moisture content and a_w in cured tobacco was significantly influenced by sugar content. The reduction of a_w is a method used to prevent microbial growth in snuff and is the primary method for cigarettes.

A_w of STPs can be manipulated in a number of ways including the addition of salts, sugars, and humectants. It is well known that high concentrations of sugars in natural products can act as effective preservatives by making moisture unavailable to microorganisms (Frazier, 1958). The effects of chemical additives on a_w in flue-cured and burley tobacco have also been reported (Bevan, June 1988); in this study, two traditional humectants, glycerol and propylene glycol, together with sodium lactate, gave the most consistent reductions on a_w. A feasibility study by St. Charles (1989) demonstrated that reducing the moisture content of snuff from 55% to 50% with the addition of humectants reduced the water activity from 0.90 to about 0.85. In a patent by Onno (2008), a STP is described in which an agglomerated product is thought to have a reduced a_w that helps prevent bacterial growth and, therefore, allows the moist snuff product to be stored without refrigeration and to exhibit improved stability during storage.

4. Manufacturing practices and stabilization process

Manufacturing processes have been reported to reduce microbial contamination of STPs through sanitation programs. For example, Fisher et al. (2012) reports that cleaning and sanitation of product contact surfaces, which involve removal of residual tobacco, is very important to help reduce contamination by nitrate-reducing microorganisms during tobacco processing. It has been recommended that the tobacco industry follow hygiene standards similar to those in food manufacturing due to the high microbial risk of STPs and the fact that they are handled and placed in the mouth, allowing direct contact with the body (B.A.T., 1977). Also, implementation of a heat-treatment (pasteurization or sterilization) has been shown to decrease the microbial content and TSNAs in moist snuff and increase product stability. For example, in the GothiaTek standards, tobacco is processed in a heated closed system that resembles pasteurization of milk, and this reportedly has resulted in a significant decrease in microbial activity, increased product stability, and reduced TSNA levels in Swedish moist snuff (IARC, 2007; Rutqvist et al., 2011). Furthermore, a stabilization process for STPs that undergoes a fermentation process is necessary to ensure the fermentation of the final product is adequately suppressed, microbial content is reduced to safe levels, and to preclude further in-package fermentation that could lead to increases in TSNAs and microbial content during storage. For example, in a patent by Roth et al. (1994) a stabilization process is described in the making of a moist snuff product which involves subjecting the fermented snuff product to refrigerated temperatures to halt the fermentation stage, pasteurization of the fermented snuff product, and addition of preservatives to further retard microflora recovery and oxidation after the pasteurization process is complete. This stabilization process is said to reduce microflora levels in order to preclude further in-package fermentation and enhance product stability during storage of at least 4 months.

Recommendations

Assessment of product stability data is very important because the chemical and microbial characteristics of STPs are known to change during product storage. Product stability data should be required for all new STPs submitted in SE Reports unless *both* of the following conditions have been met:

(1) Differences between the new and predicate products are not likely to affect product stability
(2) Data have been submitted to FDA showing adequate stability testing was conducted on the predicate product to determine acceptable storage time

The purpose of stability testing is to provide evidence on how the quality of STPs varies during storage under the influence of environmental conditions such as temperature and humidity. Stability-indicating tests (stability specifications) should be established based on the specific chemical and microbial properties of the product. It is important to note that there is a wide variety of STPs in the marketplace which undergo different production processes and can vary greatly in their chemical and microbial profile. Also, some new STPs are intended to be consumed and ingested during product use and may have their own associated risks. Therefore, it may be necessary to set requirements that are product-specific based on the production process and intended used of the product.

FDA stability testing requirements should include those attributes that are susceptible to change during storage and are likely to influence the quality and safety of STPs. Stability testing requirements should cover the appropriate physical, chemical and microbiological attributes of STPs. For STPs that are manufactured via fermentation stability requirements should include information and data regarding the fermentation process, fermentation inoculum, and stabilization process for the fermented tobacco as these will impact the chemical and microbial constituents and, therefore, product stability. Although not discussed in detail in this document, packaging attributes (engineering) should be required, as these will impact product moisture and, therefore, product stability of STPs.

Applicable SE Reports:

If the applicant submits an SE Report to FDA for review and reports any of the differences in characteristics listed below, specific stability testing requirements of the new and predicate products should be required:

- Packaging materials (e.g., plastic vs. metal container, adhesive(s) used)
- Packaging design (e.g., changing to/from a pouch or canister, lid design)
- Significant tobacco blend differences
- Flavoring ingredients that can function as preservatives (e.g. salicylates,)
- Preservatives, microbial metabolic inhibitors, and other additives used for the control of microbial growth (e.g., humectants, sodium chlorate, potassium chlorate, calcium chlorate, sodium benzoate, and potassium sorbate, propylparabens, propylene glycol)
- Production process (e.g., non-fermented vs. fermented tobacco)
- Fermentation process (e.g., natural vs. directed or controlled fermentation; batch vs. continuous fermentation)
- Fermentation inoculum (e.g., type and concentration of microorganisms)
- Stabilization process (e.g., low temperature exposure, heat treatment, pasteurization, sterilization)

The following microbial stability test requirements for STPs are recommended:

1. Water activity (a_w):
   Water activity measurements, in addition to moisture content data, should be required in order to make better determinations regarding microbial product stability. Future efforts should include adopting water activity for establishing limits of potential susceptibility to microbial growth, such as those set by FDA for food (21 CFR 113.3 (e) (1) (ii)) and the USP application of a_w determination to non-sterile pharmaceutical products (USP <1112>). This is an important consideration, as it may be feasible to reduce carcinogenic nitrosamine formations in STP by controlling the water activity/moisture content of the product.

2. Microbial content testing:
   Microbial content testing, including total aerobic microbial counts (TAMC) and total yeast and mold counts (TYMC), should be required at a minimum. Minimum microbiological requirements with which the product must comply throughout its storage

period must be specified by the applicant. (b) (5)

(b) (5)

3. Fermentation inoculum and starter culture identification:
   Fermentation inoculum and/or starter culture identification data should be required for new tobacco products that undergo a fermentation process. FDA should evaluate the safety of the starter cultures and the process controls to properly assess the microbial risk of the STP and product stability.

4. Fermentation process:
   Information regarding the fermentation process such as time, pH, water activity, and temperatures of the fermentation should be required, as this will greatly affect the chemical composition (nitrate/nitrite and TSNAs) as well as the microbial loads of STPs.

5. Stabilization process:
   Information regarding the stabilization procedure for the fermented tobacco should be required. This should include data to demonstrate that the process is effective at reducing microbial content of the product to safe levels and to inactivate microbial activity of residual microorganism to preclude further in-package fermentation. In addition, records should be required for new tobacco products claiming to undergo a pasteurization process. The records should include data on temperature and time required to achieve pasteurization. Future efforts should include process control records as part of a quality assurance program and an essential component of manufacturing practice regulations.

6. Preservative effectiveness:
   Preservative effectiveness testing should be required for SE Reports that have differences in preservatives. STPs should be adequately preserved for the duration of the expected storage period in order to prevent microbial proliferation or contamination during its use. The preservative efficacy testing should be performed at the beginning and end of the expected storage period to demonstrate that the antimicrobial activity of the product has not been impaired by storage. If the expected storage period is based on data generated under accelerated conditions, preservative efficacy tests should be performed on samples that have been stored at the higher temperatures.

The following chemical stability test requirements for STPs are recommended:

1. TSNAs:
   TSNA (total, NNN, and NNK) measurements should be required because changes to the curing and/or fermentation processes, and changes to the tobacco blend, affect TSNA levels. TSNAs also form during storage, so TSNA measurements should be taken over time to ensure an appropriate product stability during storage has been established.

2. pH:
   Measurements of the pH of the product should be required because pH affects the amount of unprotonated (free) nicotine, which in this form of nicotine, has numerous biochemical and physiological effects ranging from increased nicotine uptake to acting as a substrate for further chemical transformations. The pH is one factor in the formation of TSNAs.

3. Total and free nicotine:
   Total nicotine and free nicotine values should be required because the levels can change over time and be influenced by temperature, humidity, and microbial action during storage.

4. Flavoring ingredients with preservative effects (e.g. salicylates):
   Some flavoring ingredients, such as sodium salicylate, also function to increase antibiotic resistance in bacteria.

5. Nitrate and nitrite levels:
   Nitrate/nitrite measurements should be required, as changes in the levels could be an indication of the presence or lack of microbial nitrate reducing activity and potential nitrosamine formation. (b) (5) (b) (5) The nitrate content of some types of tobacco such as burley can have up to 4% nitrate (Fisher et al., 1989). Thus, the oral nitrate intake from use of 10g/day of nitrate-rich STP can reach 400mg/day; this presents a high health risk to the user.

6. Preservative levels:
   Quantitative measurements for preservatives, microbial metabolic inhibitors, and other additives used to control microbial growth should be required for new tobacco product applications. The effective level of the preservative must be specified. (b) (5) (b) (5)

What time points should be measured?

Stability testing should cover the expected storage period for the STPs. The frequency of testing will depend on the type of STP and the expected storage period of the product. Ideally, stability testing for microbial content (TAMC and TYMC), pH, a_w, moisture content, and nitrate/nitrite levels should be performed at 0, 1, 3, 6, 12 months. For products with a short expected storage period (6 months or less) such as for moist snuff, the frequency of testing should be at the

beginning (time point zero) and every two weeks until the end of the expected storage period. Alternatively, stability testing can be performed at accelerated storage conditions.

Stability testing data for antimicrobial content (preservative quantitative measurement), TSNAs (total, NNN, and NNK), total nicotine, free nicotine, and flavoring ingredients should be required at the beginning (time point zero), the middle, and the end of the expected storage period. The preservative effectiveness test should be required at the end of the expected storage period (standard incubation or accelerated conditions).

All stability testing should be performed in the same primary packaging in which the product is to be marketed.

Recommended Boilerplate Stability Deficiencies[2]

1. Your SE Reports describe differences in the packaging materials [and/or other relevant characteristics] between the new and predicate products. Differences in product characteristics such as packaging materials can impact the stability and, therefore, composition of smokeless tobacco products. However, your SE Reports lack information about the microbial and chemical stability testing for the predicate and new products. Provide microbial content data to include but not limited to total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) for the expected storage period of the product. In addition to microbial content, provide stability testing data for the physical and chemical attributes which affect microbial activity and/or are susceptible to change during product storage. At a minimum, provide measurements for *all* of the following:
    a. pH
    b. Water activity (a_w)
    c. Moisture content
    d. TSNAs (total, NNN, NNK)
    e. Nicotine (total and free)
    f. Nitrate/nitrite
    g. Preservatives and/or microbial metabolic inhibitors levels (e.g., sodium chlorate, potassium chlorate, calcium chlorate, sodium benzoate, and potassium sorbate)

    Measurements of these should be made at the beginning, middle, and end of the expected storage time. Without this information, we cannot determine whether the predicate and new products are substantially equivalent. Provide full test data (including test protocols, quantitative acceptance criteria, data sets, and a summary of the results) for all testing performed. The accuracy, sensitivity, specificity and reproducibility of the test methods should be determined and documented. Explain how the expected storage time is determined. Additionally, if any of the measurements of stability above differ between the new and predicate products, provide evidence and a scientific rationale demonstrating that these differences do not cause the new products to raise different questions of public health.

[2] The recommended stability deficiencies should be required unless evidence is presented that warrants a deficiency to be a request as opposed to a requirement. In general, these deficiencies should be required by the applicant,

2. Your SE Reports lack information regarding the fermentation process and the fermentation inoculum/starter cultures. Fermentation can impact product composition (i.e., product characteristics). Provide information about the fermentation process including but not limited to pH, temperature, duration of the fermentation, and ingredients added to the fermentation step. In addition, provide microbial characterization data (including species name and inoculum concentration) of the fermentation inoculum/starter cultures. Provide this information for both the new product and the predicate products. If this information is the same, provide it for the new product and a statement that it is the same as for the predicate product.

3. Your SE Reports lack information regarding the stabilization process for the fermented tobacco to preclude further in-package fermentation that could lead to increases in TSNAs and microbial content in the product. In-package fermentation can impact product composition (i.e., product characteristics). Provide detailed information regarding the stabilization process for the fermented tobacco and analytical data to demonstrate that the process is effective at suppressing the fermentation to preclude further in-package fermentation and to reduce the microbial content and activity to safe levels. Provide this information for both the new product and the predicate products. If this information is the same, provide it for the new product and a statement that it is the same as for the predicate product.

4. Your SE Reports lack data regarding the characteristics of the preservatives. Hence, it is unclear if the preservative is effective at the levels indicated. Provide preservative effectiveness testing and quantitative measurement for the preservatives. The preservative efficacy testing and quantitative measurements of the preservative should be performed at the beginning and end of the expected product storage time to demonstrate that the preservative has not been impaired by storage. Provide this information for both the new product and the predicate products. If this information is the same, provide it for the new product and a statement that it is the same as for the predicate product.

Concluding Remarks

FDA guidance on product stability would assist the industry to prepare and submit stability data necessary for proper evaluation of SE Reports. The stability of STPs is essential to evaluate the health risks of these tobacco products; therefore, product stability is necessary to ensure the new products do not raise different questions of public health. It is important to note that commercially available STPs come in a variety of product types (e.g., snuff, snus, chew, plug, dissolvable) with each type having its own associated risks and may be necessary to set different requirements based on product type.

OS should consider recommending (b) (5)

(b) (5)

(b) (5)

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