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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: February 18, 2015

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2015.02.18 10:05:38 -05'00'

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Subject: Effects of Increases of Ammonia and Other Basic Compounds on the Transfer of Free-Base Nicotine to Tobacco Smoke

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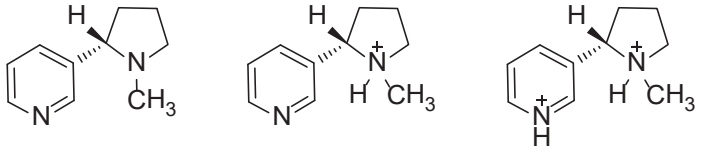
Background

The Federal Food, Drug, and Cosmetic Act (FD&C Act) provides a pathway for tobacco product manufacturers to introduce new tobacco products into interstate commerce by establishing that they are substantially equivalent (SE) to appropriate predicate products under section 905(j) of the FD&C Act. During the scientific review of SE Reports by the CTP's Office of Science, the evaluation of increased ammonia (and other basic compounds) in new tobacco products compared to the predicate tobacco products has become a significant challenge. This memorandum attempts to illustrate the current tobacco science regarding the effects of ammonia and related compounds[1] on free-base nicotine in smoke by examining current literature and finally providing recommendations to address this issue during the scientific review of SE Reports. It should be emphasized that this memorandum does not address smokeless tobacco products.

[1] Ammonia-related compounds used in cigarette manufacture include ammonium hydroxide and ammonia precursors (e.g., ammonium bicarbonate, diammonium phosphate (DAP), and urea) that form ammonia upon heating.

Intrinsic Effects of Basic Compounds on Nicotine

Nicotine can exist as a free-base (**1**) or as protonated salts (forms **2** and **3**). Most cigarettes sold in the US are made with significant amounts of flue-cured tobaccos, which are slightly acidic (pH 5.5-6.0).[2] The pH of tobacco smoke cannot be directly measured, but is often estimated or back calculated from free-base nicotine levels. The pH is used to express the impact of acidic and basic additives on the relative levels of free-base and protonated forms of nicotine. In tobacco smoke under acidic conditions, nicotine is primarily present in its protonated form **2**, which is thought to be non-volatile, pass poorly through membranes, and thus is less easily absorbed into the lungs.[3] Protonated form **3** only exists at very low pH values and is generally assumed to be negligible in tobacco smoke. On the other hand, free-base nicotine is semi-volatile and thought to more easily absorb into the lungs.



1 **2** **3**

Figure 1. Nicotine (1) and its protonated salts (2 and 3)

It has been well documented that ammonia and other basic compounds (e.g., ammonia hydroxide, ammonium bicarbonate, diammonium phosphate (DAP), and urea) can be added to tobacco products to increase the pH of tobacco filler and smoke.[3,4] Increasing the pH would shift the equilibrium of nicotine that is "bound" in nicotine salts (form 2) to

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[2] van Amsterdam et al. Effect of ammonia in cigarette tobacco on nicotine absorption in human smokers. *Food and Chemical Toxicology* 2011, *49*, 3025-3030.

[3] (a) Pankow JF et al. Conversion of nicotine in tobacco smoke to its volatile and available free-base form through the action of gaseous ammonia, *Environ. Sci. Technol*., 1997, 31, 2428-2433; (b) Wayne GF et al. Brand differences of free-base nicotine delivery in cigarette smoke: the view of the tobacco industry documents. *Tobacco Control*, 2006, 15, 189–198; and (c) Stevenson, T., Proctor, R.N. The secret and soul of Marlboro: Phillip Morris and the origins, spread, and denial of nicotine freebasing. *Am. J. Public Health*, 2008, *98*, 1184–1194.

[4] (a) Ashley, DL et al. "Approaches, Challenges, and Experience in Assessing Free Nicotine.", Nicotine Psychopharmacology: Handbook of Experimental Pharmacology vol. 192, 2009. (b) Creighton, DE. "The significance of pH in tobacco and tobacco smoke." BAT Co Ltd. November 2, 1987. (c) Henningfield JE et al. Ammonia and other chemical base tobacco additives and cigarette nicotine delivery: issues and research needs, *Nicotine & Tobacco Research*, 2004, *6*, 199-205; (d) Hurt RD, Robertson CR. Prying open the door to the tobacco industry's secrets about nicotine: the Minnesota Tobacco Trial. *J. Am. Med. Assoc.*, 1998, *280*, 1173–1181; (e) Kessler, DA. The control and manipulation of nicotine in cigarettes, *Tobacco Control,* 1994, *3*, 362-369; (f) Kessler DA et al. The legal and scientific basis for FDA's assertion of jurisdiction over cigarettes and smokeless tobacco, *J. Am. Med. Assoc.,* 1997, *277*, 405–409; (g) World Health Organization, 2007. The scientific basis of tobacco product regulation: report of a WHO Study group. WHO Technical Report Series 945. <http://www.who.int/tobacco/global_interaction/tobreg/who_tsr.pdf> (accessed on 1.6.2013); and (h) Pankow , J.F. A Consideration of the Role of Gas/Particle Partitioning in the Deposition of Nicotine and Other Tobacco Smoke Compounds in the Respiratory Tract. *Chemical Research in Toxicology,* 2001, *14*, 1465-81.

"free" nicotine (1). This effect is also described in Brown & Williamson's 1991 "Handbook for Leaf Blenders and Product Developers," which states that "[a]mmonia, when added to a tobacco blend, reacts with the indigenous nicotine salts and liberates free nicotine." The fraction of particulate matter nicotine in its free-base form is denoted by its activity (α_{fb}^{nic}) and its relationship to the pH of smoke particulate matter is generally defined by the following equation:[4c]

$$pH = pK_a + \log\left(\frac{\alpha_{fb}^{nic}}{1 - \alpha_{fb}^{nic}}\right)$$

Increasing the alkalinity of tobacco and smoke pH has been associated with increasing levels of free-base nicotine in smoke, leading to an increased bioavailability of nicotine. The relationship between pH and the conversion of nicotine from its salts **2** and **3** to its free-base (**1**) is depicted by the following graph, which was referenced in a Lorillard document that states as "the pH increased, the amount of free-base nicotine increases."[5]

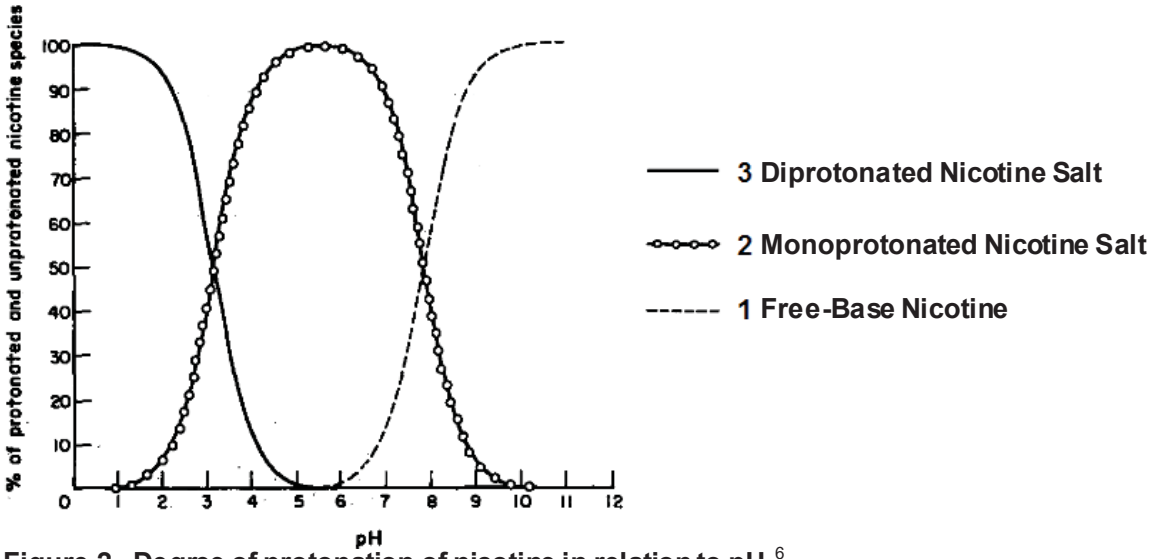

Figure 2. Degree of protonation of nicotine in relation to pH.[6]

Through a survey of the Legacy database searching for keywords such as "ammonia," "pH," and "free-base nicotine," a similar graph and conclusions were identified in RJ Reynolds and Philip Morris documents. Philip Morris also claimed an additional role for the ammonia-forming compound, DAP. They speculate that added DAP liberates pectin from tobacco, allowing pectin to form a stable pectin-nicotine complex that releases free

[5] Chen, Leighton (Lorillard), pH of Smoke, a Review 1-18 (1976), available at http://legacy.library.ucsf.edu/tid/uaq46b00/pdf.
[6] Morie, G.P., Fraction of protonated and unprotonated nicotine in tobacco smoke at various pH values. *Tobacco Science*, 1972, *16*, 167.

base nicotine during smoking. "Thus, more of the nicotine is present in the gas phase as free (or extractable) nicotine and hence a higher smoke impact results than would be expected…"[7] The viewpoint that ammonia and basic compounds are employed in cigarette manufacturing to increase addiction by affecting nicotine delivery is also shared by the FDA.

Measuring smoke pH is complex and challenging because smoke is only ~10% water with the remaining being a mixture of different organic compounds and pH is the typical measure of the acidity or basicity of an aqueous solution. There are three methods to measure smoke pH. In the first method, smoke particulate matter or whole smoke is dissolved in water and then the aqueous solution is measured with an electrode. In the second method, smoke is drawn over a thin film of pH buffer on the electrode. In the third method, the ratio of free nicotine to total nicotine is determined experimentally and the Henderson-Hasselbalch equation is used to back calculate the pH of the smoke. All of these methods are technically challenging and result in limitations to their accuracy and precision particularly between labs. Results from one method cannot be directly compared to the others because the environment of the smoke analysis is different and the smoke is diluted differently, which can influence the acid and base characteristics of the aqueous solution being measured. However, if the same method is used within a study, the values may be compared for relative differences between different smoke particulate matters but there are concerns when drawing conclusions for results which do not differ substantially. Further studies should be carried out to develop a robust validated method to measure smoke pH. Until this method is developed, FDA should not require that applicants to provide smoke pH.

Need for a Policy on Free-Base Nicotine and Basic Compounds

Currently, FDA does not have an established policy on the tolerable level of increased ammonia compounds for new tobacco products when compared to predicate tobacco products. In other words, FDA has not determined what increase in ammonia level would not cause the new product to raise different questions of public health. In a recently submitted SE Report, the applicant needed to provide scientific evidence that a new tobacco product has the same characteristics as a predicate tobacco product in

(b) (4)

(b) (4)

[7] Crellin, RA (Philip Morris), Philip Morris – Reconstituted Tobacco 1-5 (1985), available at http://legacy.library.ucsf.edu/tid/pnm56b00/pdf.

[8] (b) (4)

(b) (4)

(b) (4) The quantitative effect of ammonia on free nicotine in mainstream smoke is unknown in this case. However, FDA needs to be equipped with the appropriate policy in the event that ammonia and ammonia-related compounds increase significantly and the total nicotine levels also increase or remain the same. Developing this policy is particularly important because there is not currently a globally accepted analytical method for the determination of free-base nicotine in tobacco smoke. Recent publications on the effects of ammonia and pH in tobacco products are discussed below in an attempt to formulate a policy for future SE Reports regarding this scientific issue.

Review of Studies Investigating the Effects of pH and Ammonia on Nicotine

In a 2006 study, Callicutt et al. examined the transferability of nicotine from tobacco to mainstream (MS) smoke as a function of ammonia in tobacco, ammonia in smoke, tobacco pH, and smoke pH.[9] Marlboro Lights King Size cigarettes were used as the control, as it is one of the top selling cigarette brands in the US market. Four other types of test cigarettes (T1-T4) with a target tar yield of 10 mg/cig[10] were machine-made with the same blend components, filters, and cigarette wrappers as the Marlboro Lights King Size cigarette, except the reconstituted tobacco materials in T1–T4 were specially made to decrease the levels of ammonia-forming ingredients and other ingredients as shown in Table 1 below. The actual amounts of added ingredients are unknown; however, the ammonia forming ingredients used in this study were ammonium hydroxide and DAP.

[9] Callicutt CH et al. The role of ammonia in the transfer of nicotine from tobacco to mainstream smoke. Regulatory Toxicology and Pharmacology, 2006, 46, 1-17.

[10] A nominal 10 mg FTC tar yield was chosen because it represented an intermediate tar yield found for cigarettes sold in the US and is the level preferred by US smokers according to Daigle, W.P., 2004. Tobacco hdustry Testing Laboratory Market Sample 46 Final Report. Tobacco hdustry Testing Laboratory, Rockville, MD.

Table 1. Callicutt et al. Study Test Sample Blend Additives

Test Sample	Ingredients Added to Reconstituted Tobacco in Tobacco Blend		Ingredients Added to Tobacco Blend[a]
	Ammonia Forming	Non-Ammonia Forming	
Marlboro Lights King (control)	yes	yes	Yes
T1	reduced	yes	Yes
T2	no	yes	Yes
T3	no	no	Yes
T4[b]	no	no	No

[a] None of these ingredients are ammonia-forming.
[b] Did not contain any humectant or proprietary flavorant.

Nicotine and ammonia levels in the cigarette tobacco were determined from 5% acetic acid extracts of ground tobacco. Tobacco pH was determined from a water extract of ground tobacco. All smoke data were determined using the Federal Trade Commission (FTC) smoking regimen. Previous research showed that >99% of the total mainstream smoke nicotine was captured and quantified by the FTC method.[9,11] In this study, FTC nicotine refers to the total amount of nicotine measured in MS smoke (i.e., particulate nicotine). The pH of smoke was determined by measuring the aqueous extracts of mainstream smoke (i.e., pH of aqueous extracts of MS smoke) and mainstream smoke aerosol (i.e., pH of MS smoke/electrode). The pH of MS smoke/electrode were generally ~0.91 pH units higher than the aqueous extracts. The results have been averaged, compiled, and summarized in Table 2 below.

[11] Callicutt, CH et al. The ability of the FTC method to quantify nicotine as a function of ammonia in mainstream smoke. *Beitr. Tabakforsch. Int*., 2006,*22*, 71–78.

Table 2. Callicutt et al. Averaged Study Test Sample Blend Characteristics

Test Sample	Ammonia in Tobacco (mg/cig)	Nicotine in Tobacco (mg/cig)	Tobacco pH	Averaged Tar Yield in Smoke (mg/cig)	Average Ammonia in Smoke (µg/cig)	Average FTC nicotine (mg/cig)	Smoke pH
Marlboro Lights King (control)	2.10	11.2	5.78	10.26	14.91	0.822	5.30[a]
							5.31[a]
							6.27[b]
T1	1.75	11.1	5.73	9.90	12.82	0.795	5.30[a]
							5.35[a]
							6.32[b]
T2	1.11	11.3	5.67	9.56	9.51	0.784	5.30[a]
							5.28[a]
							6.25[b]
T3	1.07	11.1	5.60	9.6	8.84	0.809	5.22[a]
							5.29[a]
							6.26[b]
T4	1.11	12.4	5.57	9.21	8.51	0.832	5.31[a]
							5.31[a]
							6.19[b]

[a] pH of aqueous extracts of MS smoke method
[b] pH of MS smoke/electrode method

Data from this study shows that a larger amount of ammonia compounds in the tobacco blend may lead to more ammonia detected in the smoke as observed with the control having a concentration of 14.91 µg per cigarette. However, the highest amount of FTC nicotine detected was in T4, which did not contain any ammonia forming additives in the tobacco blend. It has been speculated that ammonia can also be formed during the combustion of various endogenous nitrogen containing compounds such as amino acids, proteins, and inorganic nitrates,[12] which could explain why any ammonia was detected in T4 albeit less than the other samples. Interestingly, decreasing ammonia levels in tobacco and smoke over this range did not affect the tobacco pH and smoke pH, respectively. Therefore, increasing ammonia levels by adding ammonia forming compounds to a tobacco product over this range may not raise the pH or free nicotine

[12] Seeman, JI, Carchman, RA. The possible role of ammonia toxicity on the exposure, deposition, retention, and the bioavailability of nicotine during smoking. *Food and Chemical Toxicology*, 2008, *46*, 1863-1881.

levels in mainstream smoke. This discrepancy may be a consequence of the MSS being an aerosol of compounds that are not completely water-soluble that are undergoing various chemical reactions. pH is a measurement of acidity or basicity of an aqueous solution. In other words, pH of smoke cannot be directly measured because it is not an aqueous solution and current smoke pH methods are not sufficiently robust. However, the measured smoke pH can be used to express the relative impact of acidic and basic additives on the relative levels of free and protonated forms of nicotine.

These results agree with a similar study by Ellis.[13] In 1999, Ellis et al. examined the effects of the addition of ammonia compounds to reconstituted tobacco, at commercial application levels, on smoke pH and levels of nicotine and ammonia in mainstream smoke. The ammonia compounds and the amounts added in the Ellis study were not identified, but Marlboro Lights cigarettes were also studied. Ammonia content of the tobacco blend varied from 0.12 % to 0.31 %. The ammonia levels in the mainstream smoke increased slightly, but the pH of the aqueous extracts of smoke and FTC nicotine levels did not change significantly. Therefore, increasing the amounts of ammonia compounds at these levels did not increase the smoke pH and the transferability of nicotine to mainstream smoke particulate.

Although determining the level of total nicotine in mainstream smoke (particulate nicotine) is important in understanding its potential exposure to free-base nicotine, it was still unclear how increased ammonia levels would affect levels of gas-phase nicotine and its eventual uptake into the smoker. Three separate studies report investigating the effect of tobacco additives, including ammonia compounds, in tobacco blends on the concentration of nicotine in the blood. In a 2004 study by Armitage et al., the effects of added ammonia compounds DAP and urea on the delivery of nicotine was measured from a pool of ten regular smokers. The three different cigarettes manufactured for the study contained the same blends of lamina, expanded and stem tobaccos, the same casings, filters, and cigarette papers, and had identical levels of filter ventilation; however, varied in ammonia compounds based on typical levels in US marketed cigarettes (Table 3).[14] The composition of the MS smoke was determined using the ISO smoking regimen (Table 3). In agreement with the previous publications, the level of total nicotine detected in smoke did not change with increasing ammonia concentration.[14]

[13] Ellis, CL et al. The effect of ingredients added to tobacco in a commercial Marlboro Lights cigarette on FTC nicotine yield, "smoke pH" and Cambridge filter trapping efficiency. Proceedings of the CORESTA Smoke and Technology meeting, Innsbruck, Austria, September 5-8, 1999. CORESTA Bull. 3, 108.
[14] Armitage, AK et al. The Effect of Tobacco Blend Additives on the Retention of Nicotine and Solanesol in the Human Respiratory Tract and on Subsequent Plasma Nicotine Concentrations during Cigarette Smoking. *Chem. Res. Toxicol.* 2004, *17,* 537-544.

Table 3. Armitage et al. Study Smoke Characteristics

Test Sample	Total Ammonia Compound in Tobacco Blend (w/w)	Tar Yields (mg/cig)	Ammonia in Smoke (µg/cig)	Nicotine in Smoke (mg/cig)	pH of aqueous extract
control	0	9.6	16	0.67	5.6
DAP treated[a]	1.2% DAP 0.4% NH_4OH 0.2% urea	10.2	26	0.70	5.5
urea treated[b]	2.0% urea	9.3	38	0.65	6.1

[a] Ammonia forming compounds added to reconstituted tobacco.
[b] Urea was added to the tobacco blend. The reconstituted tobacco added was same as control.

For each test sample, the subjects used three different smoking protocols: mouth-hold, 75 mL inhalation, and 500 mL inhalation. The 500 mL inhalation volume was chosen because it is within the range of "normal" post-puff inhalation volumes and 75 mL is the space that allows smoke to penetrate the airways without gas exchange or contact with the alveolar region in the lungs. Venous blood samples were taken at various intervals throughout the time course of the experiment. The data shown in Figure 3 indicate there is no statistically significant difference in any of the cigarette test samples. Based on this data, the uptake of nicotine from the mouth or respiratory tract was not augmented by the addition of DAP or other ammonia compounds over this range.

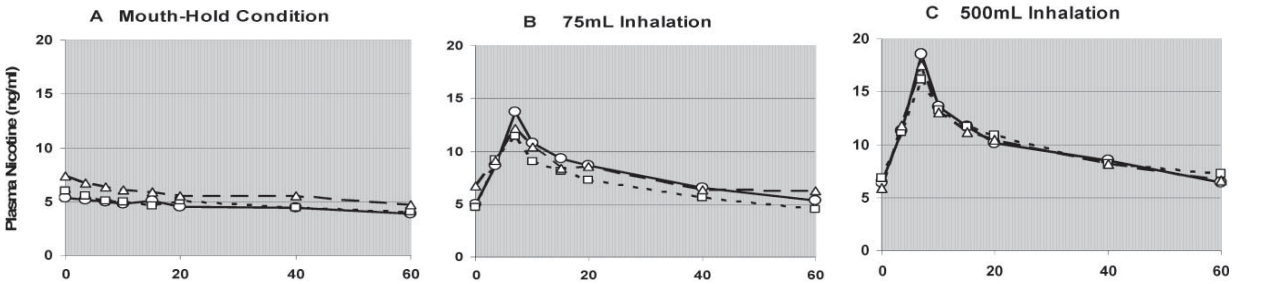

Figure 3. Effect of inhalation condition on the mean plasma nicotine values for the control (□), DAP treated (•), and urea treated (□) cigarettes.

The results from the Armitage et al. study agree with a similar study carried out by van Amsterdam et al. on 51 subjects.[2] In this study, the two cigarette brands tested were Caballero Smooth and Gauloises Brunes. These two brands were selected because they have: 1) the same declared value of nicotine, tar and CO (Table 4); 2) similar filter ventilation; and 3) different ammonium content in the tobacco blend. The

pH and ammonia salt levels in the tobacco extracts are summarized in Table 4 below. The ammonium salts in the tobacco blend were not identified.

Table 4. van Amsterdam et al. Study Tobacco Blend Extract

Test Sample	Declared Tar Yield (mg/cig)	Declared Nicotine (mg/cig)	Declared CO (mg/cig)	Ammonium Salts in Tobacco (mg/g)	pH of aqueous extract
Caballero	8	0.7	8	0.89	5.32
Gauloises	10	0.7	10	3.43	6.14

The level of ammonia in the tobacco blend for the Gauloises Brunes cigarette was 3.8 times higher than that in the Caballero Smooth cigarette. The cigarettes were smoked by the participants on separate days. Venous blood samples were taken at various intervals and analyzed by liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS). Similar to the Armitage et al. study, the plasma nicotine level for the subjects were statistically the same between the two cigarettes (Figure 4).

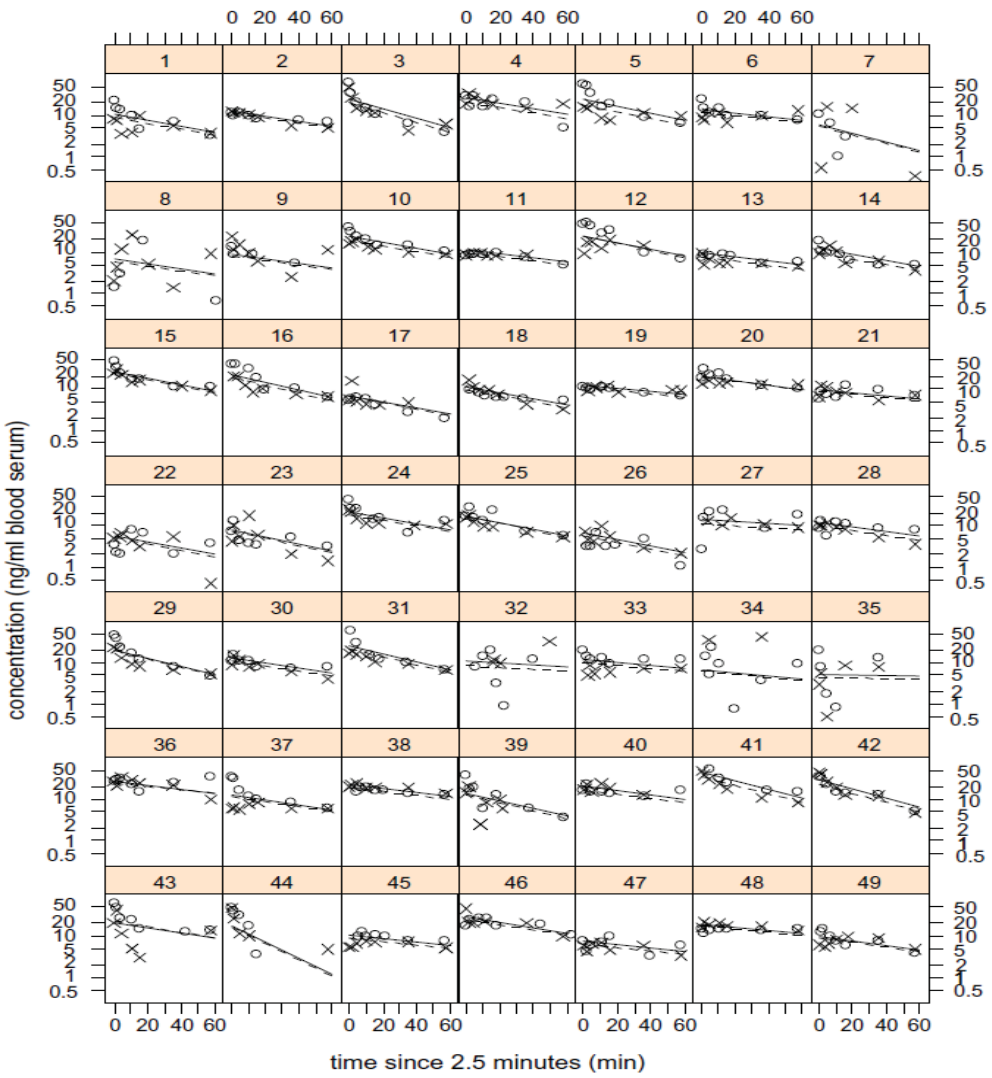

Figure 4. Serum nicotine levels following smoking of a Caballero cigarette (O - brand 1) and a Gauloises cigarette (X- brand 2) in two different smoking sessions.

Therefore, a 3.8 fold difference over the range 0.89 – 3 mg/g in ammonia level in the tobacco blend did not result in a significant difference on nicotine detected in venous blood plasma. However, many researchers indicate that measuring nicotine levels in arterial blood may more accurately measure the effect of ammonia on nicotine pharmacokinetics because inhaled nicotine is absorbed through the pulmonary venous system (arterial blood), which reaches the brain in seconds, rather than the systemic venous system (venous blood).

McKinney et al. conducted a study evaluating the effects of ammonia on nicotine delivery by measuring arterial blood in 34 subjects.[15] All test cigarette design features and characteristics were the same except for the tobacco blend. Test cigarettes were prepared with (1) no reconstituted tobacco (low ammonia) in the tobacco blend, or (2) with 20% reconstituted tobacco (reference), which contains DAP, in the tobacco blend. Although the amount of DAP in the reconstituted tobacco used for the reference cigarette is not provided, the authors claim that it is representative of levels in marketed products. We expect the ammonia compounds amounts to be similar to the DAP-treated cigarettes in the Armitage study above, which are also based the average US marketed cigarette.[16] In the McKinney study, FTC tar, nicotine, and CO levels in the low ammonia and reference cigarettes were similar, but the amount of ammonia in the reference sample is higher as summarized in Table 5 below.

Table 5. McKinny et al. Smoke Characteristics

Test Sample	FTC tar (mg/cig)	FTC Nicotine (mg/cig)	FTC CO (mg/cig)	Ammonia in smoke (µg/g tobacco)
low ammonia (no DAP)	9.2 (0.35)	0.81 (0.04)	9.8 (0.31)	10.1 (0.3)
reference (added DAP)	9.7 (0.30)	0.74 (0.01)	10.2 (0.25)	18.9 (1.7)

Arterial blood samples were taken at various intervals throughout the experiment.[17] Similar to the venous blood analyses, the arterial nicotine levels for the subjects were statistically the same between the two test cigarettes (Figure 5). Therefore, the two test cigarettes equivalently transfer nicotine from tobacco to the smoker and it does not appear that the presence of ammonia compounds in reconstituted tobacco at these levels affects the bioavailability of nicotine.[18] One disadvantage of this study is that the test subjects are "smoking" through a custom-designed cigarette smoke delivery and arterial blood collection system that may not mimic true smoking behavior. However,

[15] This article was provided by the applicants (b) (4) (McKinney, et al. Evaluation of the Effect of Ammonia on Nicotine Aiarmacokinetics Using Rapid Arterial Sampling. Nicotine & Tobacco Research, 2012, 14, 586-595.

[16] Approximately 20% of the tobacco blend is reconstituted tobacco. There are two types of reconstituted tobacco- paper and band cast. According to reference 14, typical U.S. range for DAPin paper reconsrnuted td::>acco is 2.5-3.5%, which also generally contains 1. 75-2.25% urea. The amount of OAP in band cast is 6-8%, which also generally contains 3-4% NH4OH.

[17] Blood samples were collected after each puff at O (start of inhalation), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19,20,22,24,26,28,30,34,38,42,46,50,54,58,and 62 s.

[18] Chen, C., Pankow, JF. Gas/Particle partitioning of two acid-base active compounds in mainstream tobacco smoke: nicotine and ammonia, *J. Agric.* Food Chem. 2009, 57, 2678-2690.

the delivery system analyzes the smoke for ammonia and TNCO as it is being inhaled by the subject. Therefore, McKinney's smoke delivery/blood collection system is expected to adequately measure the amount of cigarette smoke inhaled, the amount of ammonia and TNCO in the MSS, and the amount of nicotine in arterial blood.

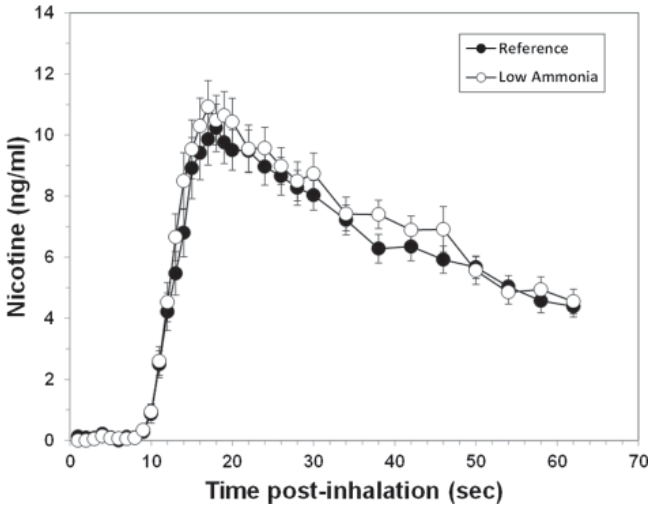

Figure 5. Mean nicotine levels in arterial blood plasma samples.

These studies do not support the hypothesis that higher levels of ammonia in tobacco blend over the range of approximately 1 – 3 mg/g and cigarette smoke over the range of approximately 8 – 20 µg/cigarette would proportionally increase the amount of free-base nicotine in the smoke. Lauterbach et al. suggested that ammonia forming compounds can react with reducing carbohydrates (i.e., sugars) in the tobacco and casings to form compounds called Maillard polymers.[19] During the smoking process, the Maillard polymers could then pyrolyze to produce acidic byproducts, such as acetic acid, which would neutralize the effects of increased ammonia. Instead of pH and ammonia levels, Lauterbach et al. suggest that there are other drivers increasing free-base nicotine in total particulate matter (TPM) under ISO smoking conditions such as cigarette design and water content of the total particulate matter.[19] Chen and Pankow findings concur that there is a close association with water content in the particulate matter and free-base nicotine.[18] However, one major limitation in this study and the other reviewed studies is that positive controls with ammonia at levels that would make a significant change in free nicotine levels were not used. Therefore, additional studies investigating higher levels of ammonia and ammonia precursors in filler and smoke are necessary to understand if there is a correlation between ammonia levels and the bioavailability of nicotine.

[19] Lauterbach, JH et al. Free-base nicotine in tobacco products. Part 1. Determination of free-base nicotine in the particulate phase of mainstream cigarette smoke and the relevance of these findings to product design parameters. *Regulatory Toxicology and Pharmacology,* 2010, *58*, 45-63.

Conclusion

The reviewed publications above indicate that when ammonia levels typically found in US tobacco products (1 – 3 mg/g) were decreased by either 50% or almost 75%, the pH of the tobacco blend decreased modestly, but no difference was detected in the nicotine level in plasma from both venous and arterial blood. However, the relative pH range of products in the studies was narrow (5.3 – 6.3) and not in the pH range that would significantly affect free-base nicotine quantities (as a percentage of total nicotine). RJ Reynolds[20] and Philip Morris[7] originally claimed that ammonia can increase free-base nicotine in smoke, which is evident in gas-sampling denuder studies by Philip Morris in which an increased amount of tobacco smoke nicotine was observed to deposit in acid-coated denuder tubes when the cigarette tobacco blend was treated with ammonia.[21] Therefore, there does not appear to be conclusive evidence, at this time, that the presence of ammonia compounds at typical U.S. levels in reconstituted tobacco products of 2.5-3.5% DAP and 1.75-2.25% urea for paper reconstituted tobacco and 3-4% NH_4OH and 6-8% DAP for band cast reconstituted tobacco substantially increase the bioavailability of nicotine.[14] The products tested in these studies were meant to reflect typical levels in the U.S. market, especially in the studies by Armitage et al. and McKinny et al. However, these studies likely did not cover the full range of tobacco products currently on the U.S. market and future studies will need to be done to capture the full range and diversity of tobacco products and the effects of ammonia compounds on free-base nicotine. Furthermore, many of the cigarettes tested in these studies were highly ventilated. The mainstream smoke of highly ventilated cigarettes is diluted, which may have masked any impact of ammonia. However, it is unclear if there is a threshold concentration for ammonia compounds that could substantially increase in the bioavailability of nicotine that was not been reached in the reviewed studies. Further studies with increasing amounts of ammonia compounds in cigarettes up to the amount that causes an "off-flavor" taste to the smoker are recommended to examine if such a threshold exists.

Current Recommendations for SE Reviews

- It is the applicant's responsibility to demonstrate that differences in ammonia levels do not cause the new tobacco products to raise different questions of public health. At this time, any increases in ammonia and its effect on nicotine protonation should be evaluated on a case-by-case basis for each SE Report, as not every increase will require a deficiency given other tobacco product characteristics that must be considered.
- The Office of Science should collaborate with a tobacco analysis laboratory to conduct research that will assess increases in ammonia and the impact these increases on free nicotine yields in mainstream smoke.

[20] Teague, C.E. (RJ Reynolds), Implications and Activities Arising from Correlation of Smoke pH with Nicotine Impact, Other Smoke Qualities, and Cigarette Sales 1-24 (1973), available at http://legacy.library.ucsf.edu/tid/udq46b00/pdf.

[21] Watson, D.C. (Philip Morris), Gas Phase Nicotine - Status, (Reference: D.C. Watson to R. Fergusson, 'Gas Phase Nicotine', 910903), 1-6 (1991), available at http://legacy.library.ucsf.edu/tid/xrb84e00/pdf.