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Significance of ammonium compounds on nicotine exposure to cigarette smokers

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Abstract

The tobacco industry publicly contends that ammonia compounds are solely used as tobacco additive for purposes of tobacco flavoring, process conditioning and reduction of its subjective harshness and irritation. However, neither objective scientific reports, nor the contents of a large number of internal tobacco company documents support this contention.

The present review focuses on the hypothesis that addition of ammonium compounds to tobacco enhances global tobacco use due to smoke alkalization and enhanced free-nicotine nicotine exposure. Obviously, ammonia enhances the alkalinity of tobacco smoke. Consequently, the equilibrium shifts from non-volatile nicotine salts to the volatile free base that is more readily absorbed from the airways. The observed change in the kinetics of nicotine (i.e., shorter $t_{1/2}$ and higher c_{max}) after ammoniation is, however, predominantly due to the higher concentration of nicotine in the smoke, rather than to an increase in the absorption rate of free-base nicotine in the respiratory tract.

Although several findings support the hypothesis, additional studies are required and suggested to provide a proper, objective and independent scientific judgment about the effect of tobacco ammoniation on nicotine bioavailability. Scientific and public awareness of the effects of tobacco-specific ammonia compounds may stimulate global control, legislation and restriction of their use in cigarette manufacture.

Abbreviations: BAT, British American Tobacco company; B&W, Brown & Williamson; RJR, RJ Reynolds Tobacco Company; WHO, World Health Organization; FDA, US Food and Drug Administration; FTC, Federal Trade Commission; USDHHS, US Department of Health and Human Services; CDC, Centers for Disease Control and Prevention; TFI, Tobacco Free Initiative; NTE, Nicotine Transfer Efficiency; MS, Mainstream; SS, Sidestream; ID, Internal document from tobacco industry; MAC, Occupational Maximal Acceptable Concentration; STEL, Short Term Exposure Level; PEL, Permissible Exposure Limit; ETS, Environmental Tobacco Smoke

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1. Introduction

Tobacco smoking, the main cause of lung cancer (Boyle et al., 2004, Hurt and Robertson, 1998, Kessler, 1994b and Muggli et al., 2004), takes an enormous toll on public health and is the world's leading cause of preventable deaths (Schroeder, 2004 and WHO-TFI, 2004). Yearly, five million people die world-wide due to tobacco use (CDC, 1988, Ezzati and Lopez, 2003 and WHO-TFI, 2004), a number that has been estimated by the World Health Organization (WHO) to be doubled in about 15 years.

The scientific literature and the recent availability of previously confidential internal documents from various tobacco companies have demonstrated that the incidence of tobacco use is manipulated by adding several compounds (Bates et al., 1999, Hurt and Robertson, 1998, Muggli et al., 2004 and Schroeder, 2004). The world-wide use of tobacco additives has initiated discussions and legislation measures on tobacco-related additives and their putative adverse health effects (Cummings et al., 2002).

The present review focuses on the hypothesis that addition of ammonium compounds to tobacco enhances global tobacco use. Though the tobacco companies shun to properly inform the public on this subject on their web-sites, ammonia improves nicotine's bioavailability by increasing the fraction of free-base nicotine in whole tobacco. The underlying mechanisms of these effects are outlined and discussed to understand the rationale of their use in tobacco product manufacture. In addition, the exposure to and the adverse health effects of ammonia as tobacco additive are briefly reviewed.

Public peer-reviewed data have provided the basis of the review on this topic. Information retrieved from internet and, particularly, the internal tobacco industry documents has been contemplated with caution and skepticism (Cummings et al., 2002, Diethelm et al., 2004, Drope and Chapman, 2001, Hurt and Robertson, 1998, Kessler, 1994b, Kessler et al., 1996, Kessler and Myers, 2001, Muggli et al., 2004 and Wilner and Feingold, 2000). Internal documents have therefore been specified and referred to as ID's, when cited.

2. Exposure of tobacco smokers to ammonia

Highly water soluble ammonia (NH_3) is a weak base with a pK_a-value of 9.4 (Hunt et al., 2002). Ammonia has a very pungent odor and is used as household cleaning agent and fertilizer.

Ammonia is also a natural component of tobacco. Its content varies from 0.1% in flue-cured tobacco to 0.5% in dark tobacco, which results in ammonia amounts in smoke of 51 and 153 μg, respectively (Sloan and Morie, 1974). In addition, ammonia and ammonium salts are added as ingredient, giving a final ammonia content of 0.1–6 mg per gram tobacco (cf. Table 1). Other processes that increase the level of ammonia in tobacco (smoke) are: (i) pyrolysis of proteins and other organic nitrogen compounds in the tobacco blend; (ii) reduction of nitrate during the smoking process; (iii) residual ammonia in the blend; and (iv) decomposition of ammonia-containing additives (e.g., ammonia compounds) (Lauterbach, 2000).

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Table 1.

Background information on ammonia

Item	Level (ppm)[a]	Remark
Exposure routes:		
Air	0.01–0.005	Exposure levels are sensitive to location and activity; major fluctuations observed
Soil	1–5	
Water	<6	
Ingestion from food/drinks	–	18 mg per day
Formed from nitrogenous matter in food/drinks	–	4.2 g of ammonia is endogenously formed in the digestive tract per day
Normal human blood level	400–700	400–700 mg/L (based on 7 L of blood)
Tobacco-derived intake (20 cigarettes per day)	–	35 mg, assuming max. ammonia-level of 700 ppm; 10 puffs of 35 ml per cigarette
Averaged urban level	0.03	25 μg/m^3; per day about 0.5 mg of NH_3 is inhaled (20 m^3 air breathed per day)
Exhaled air level	0.13–2.86	0.1–2.2 mg/m^3 in healthy volunteers; ≤80% of NH_3 in food/drinks is exhaled
Alveolar breath level	0.18–0.55	0.14–0.42 mg/m^3; during fasting
Lower odor detection	45	35 mg/m^3
MAC-value (occupational)	23–52	18–40 mg/m^3; in The Netherlands: 18 mg/m^3 (26 ppm)
STEL[b]	35	27 mg/m^3
PEL[b]	25	18 mg/m^3
Indigenous amount in tobacco	–	0.01–0.6% (w/w) of tobacco
Amount used in cigarette manufacture		0.02–1.0% (w/w); total ammonia content of one cigarette is 0.2–10 mg/g
Mainstream smoke level	19–742	7–200 μg per gram of tobacco smoked
Sidestream smoke level	–	320–450 μg per gram of tobacco smoked; 40–170 fold higher level than in MS smoke

Data from references: (Smith et al., 2001, Steele et al., 1994, BAT, 1982, Johnson, 1989, WHO, 1986, WHO, 2004, Hoffmann and Hoffmann, 1998, Huang et al., 2003, Sloan and Morie, 1974, Sloan and Morie, 1976, Djordjevic et al., 2000, Carmines, 2002, Counts et al., 2004, Djordjevic, 2004, Spanel et al., 1998, Baker, 1999, Neurath, 1969, Anonymous, 1979, Covington and Burling, 1993, Covington and Burling, 1994, Sudholt, 1996, Hoffmann and Hoffmann, 1997, Hoffmann and Hoffmann, 2004, Bases, 1984, Baker et al., 2004b, Davis and Nielsen, 1999, Stabbert et al., 2003 and USDHHS, 1990).
[a] 1 ppm NH_3 corresponds to 700 μg NH_3 per m^3.
[b] STEL: Short-Term Exposure Limit; PEL: Permissible Exposure Limit.

Upon smoking tobacco, about 90% of the ammonia in inhaled smoke is retained in the nasal mucosa and the lining fluid of the upper airways. Studies in volunteers have shown that prolonged exposure to 500 ppm ammonia results in a much lower (about 30%) retention of the ammonia; the rest being excreted by expiration (USDHHS, 1990). This limited capacity to absorb ammonia has been confirmed by others (Anonymous, 1979, Silverman et al., 1949 and WHO, 1986).

As depicted in Table 1, some 18 mg of ammonia is ingested daily via food and drinks, and about 4 g of ammonia is daily produced in the body from from nitrogenous matter in the nutrition, including urea (WHO, 1986 and USDHHS, 1990) [ID (Heck, 1993)].

Ammonia levels in cigarette mainstream smoke (MS smoke) vary from 19 to 742 ppm (cf. Table 1), implicating that the upper level in MS smoke greatly exceeds the average urban air level (0.03 ppm) and the occupational maximal

acceptable concentration (MAC-value) of 26 ppm. Assuming continuous inhalation of the maximal amount of ammonia in MS smoke (700 ppm), the daily dose of a pack-a-day smoker is 35 mg (10 puffs of 35 ml per cigarette); a considerable lower dose of 0.8 mg per day was calculated by Sloan and Morie (Sloan and Morie, 1974). Ammonia levels in second-hand smoke (ETS) are some 40–170 fold higher than in MS smoke, and breathing 20 m^3 of this atmosphere (10 μg of ammonia per m^3) gives an intake of 0.2 mg of ammonia per day.

In non-smoking healthy subjects, blood levels of ammonia ranged from 400 to 700 mg/L (Anonymous, 1979, USDHHS, 1990 and WHO, 1986). Ammonia is excreted via exhaled air, sweat or urine [ID (Steele et al., 1994)]. Urinary ammonia excretion rate varies from 0.3 to 1.0 mg/day [ID (Covington and Burling, 1994)].

3. Human toxicity of ammonia

Ammonia levels, at the upper levels during smoking, cause throat irritation (400 ppm), increase the breathing rate (500 ppm), and induce cough (1700 ppm) (Silverman et al., 1949). Human inhalation studies with ammonia exposure periods varying from 6 h/day during 6 weeks to 3 h/day for 2–3 years showed that ammonia at a level of 29 ppm produces mild irritations in the eyes, nose and throat (Anonymous, 1979, Brautbar et al., 2003, Issley et al., 2004, USDHHS, 1990 and WHO, 1986). Epidemiological studies have shown that exposure to 50–200 ppm ammonia (with durations varying from acute to 90 days) can elicit chronic obstructive lung disease, chronic latent lung function damage, pulmonary fibrosis, bronchitis/bronchiolitis (Brautbar et al., 2003) and decrease the minute volume associated with a higher mean respiratory frequency (Anonymous, 1979 and CDC, 1974). The MAC-value and the 15-min STEL (short term exposure level) for ammonia are 26 and 35 ppm, respectively.

3.1. Impact of nicotine

Tobacco is a complex chemical mixture of several thousand potentially toxic constituents and more than 5000 in its smoke (Baker et al., 2004a, Counts et al., 2004, Hoffmann and Hoffmann, 2004 and Leffingwell, 1999). The nicotine in tobacco is mainly present as the pharmacologically more active [*S*]-enantiomer that elicits tobacco dependence (Henningfield et al., 2004, Henningfield et al., 1993, Hurt and Robertson, 1998, Soc. Neurosc., 2004 and USDHHS, 1988). Tobacco smoking is further characterized by a transient constriction of the upper airways, a brief increase in blood pressure, respiration rate and heart rate, and various other physiological effects (e.g., relaxation in stress situations (Karan and Benowitz, 2000 and Soc. Neurosc., 2004); see ID (Creighton, 1987)). These effects (referred to as "impact" or "kick") are attained via neuronal cholinergic activation and the release of neurotransmitters, like norepinephrine and dopamine (Henningfield and Benowitz, 2004, McGehee et al., 1995, Karan and Benowitz, 2000 and Pich et al., 1997).

3.2. Absorption and elimination of nicotine

Cigarettes represent very efficient nicotine 'delivery devices' that enable much faster and completer nicotine absorption [ID (Backhurst, 1966)], as compared to tobacco gum and nicotine spays. Inhalation of the tobacco smoke of one cigarette (0.9–1.1 g of whole tobacco containing 6–11 mg of nicotine) rapidly delivers 1–3 mg of nicotine to the airways of the smoker (Henningfield, 1995, Djordjevic et al., 2000, Benowitz and Jacob, 1994b, Benowitz and Henningfield, 1994a, Hoffmann and Hoffmann, 1998, Hoffmann and Hoffmann, 2004 and Van Andel et

al., 2003) [see ID (Backhurst, 1966)]. Within a few seconds about 90% of the nicotine is absorbed in the upper and lower airways (Armitage and Turner, 1970, Henningfield et al., 2004, Henningfield and Benowitz, 2004, Henningfield et al., 1993, Henningfield, 1995 and Johnson, 1977), the rest is swallowed and time-delayed absorbed via the intestinal route [ID (Reininghaus, 1994)]. At 4 and 6 min following the smoking of one cigarette peak arterial (some 45 ng/mL) and venous (some 25 ng/mL) blood nicotine levels are attained (Domino et al., 2004, Guthrie et al., 2004, Henningfield, 1995, Lee et al., 2004, Lunell et al., 2000, Malson et al., 2003 and USDHHS, 1988).

The typical pack-per-day smoker absorbs about 20–40 mg nicotine per day (Benowitz et al., 1983, Benowitz and Henningfield, 1994a, Djordjevic et al., 2000 and Djordjevic, 2004). Usually, early on the day the nicotine blood level of these heavy smokers reaches a plateau (steady-state) level of about 30 ng/mL, which levels-off overnight to a baseline value of some 5 ng/mL (USDHHS, 1988). A similar kinetic profile is seen upon smoking cigarettes that contain low (0.4 mg) or high nicotine (2.5 mg), although the respective plateau levels are different (about 10 and 40 mg/mL, respectively) (USDHHS, 1988).

MS tobacco smoke contains both volatile free-base nicotine and particle bound nicotine. Fresh MS smoke of US blended, non-filter cigarettes contains about 5 billion spherical droplets with a particle size of 0.1–1.0 μm (Hoffmann and Hoffmann, 1998). Deposition measurements during cigarette smoking indicate that 50–95% of these particles is deposited mainly in the upper airways: 11–23% is deposited in the nasal, oral, pharyngeal, and laryngeal regions; 45–81% in the tracheo-broncheal region, and around 26–35% in the pulmonary region. Iwase et al. (1991) showed that deeply inhaled nicotine-containing particles are also deposited in the lower respiratory tract (Iwase et al., 1991); [ID (Reininghaus, 1994)].

Pankow (2001) proposed four different mechanisms by which compounds in tobacco smoke aerosols attain the respiratory tissues (cf. Fig. 3): (i) direct gas deposition; (ii) evaporative gas deposition; (iii) particle deposition with evaporation; and (iv) particle deposition with diffusion. Nicotine, being present in both the particulate fraction and the gas phase, may be deposited in the respiratory tract via each of these four ways (Pankow, 2001). The way of nicotine deposition is of major importance for the absorption rate of nicotine. If deposited via direct gas deposition, free-base nicotine directly enters the lining fluid of the respiratory tract, where it is rapidly absorbed. Quite in contrast is the slow absorption via the particle deposition way, because nicotine has to diffuse from the particulate matter to the lining fluid of the respiratory tract before it is absorbed.

The current literature suggests that, depending on pH, free-base nicotine levels in the particulate fraction of commercial cigarette smoke can be as much as 40%, which is deposited in and absorbed via the airways (Pankow, 2001). The uptake of nicotine via the buccal epithelium of the mouth is complex and the amounts of nicotine absorbed here remain to be elucidated [ID (Reininghaus, 1994)].

Nicotine is mainly and rapidly ($t_{1/2}$ of 9 min) metabolized to cotinine, but the metabolic pathways of nicotine will not be discussed here since these do not seem to be affected by ammonia compounds. Only 5–10% of nicotine is cleared renally with a $t_{1/2}$ elimination value of 2–3 h, depending on urinary pH and flow rate (Henningfield, 1995, Karan and Benowitz, 2000, Soc. Neurosc., 2004 and USDHHS, 1988). Only minor quantities of nicotine are excreted via saliva (USDHHS, 1988).

3.3. Publicly claimed effects of ammonia by tobacco industry

Annually, about five million kilos of ammonia are used in the manufacture of US cigarettes, an amount that corresponds to 10 mg of ammonia per cigarette produced [ID (Johnson, 1989)]. In addition to ammonia, various ammonium salts like NH_4HCO_3 and $(NH_4)_2HPO_4$, and urea, are commonly used as tobacco additives in the cigarette manufacture (usually max. 5% w/w) serving as a source of ammonia.

Table 2 summarizes the results of previously confidential documents of the tobacco industry. The tobacco industry publicly contends that ammonia compounds are only used as tobacco additive to flavor tobacco, to reduce its subjective harshness and irritation, and to 'condition' the tobacco process (CDC, 1974, Dixon and Lambing, 2000, Hurt and Robertson, 1998, Leffingwell, 1999 and Muggli et al., 2004); ID [(BAT, 1982)]. Indeed, part of the internal documents have described rather vague applications like (i) combustion modifier, (ii) paper improvement, (iii) tobacco/smoke ammonia content enhancer, (iv) flavor/body/taste enhancer, (v) irritation reducer; and (vi) reducer of toxic constituents in smoke [ID (B&W, 1991)]. Other internal documents, however, have clearly described (cf. Table 2) that ammonia and ammonium compounds "modifies the tobacco/smoke nicotine content", i.e., ammonia is added to tobacco to decrease the acidity of tobacco smoke with the aim to increase the concentration of nicotine in smoke [ID (Creighton, 1987)].

Table 2.

Use of ammonium compounds in cigarette manufacture

Particular property/effect	**Description**
Combustion modifier	Expansion of tobacco, ash conditioner, burn retardants, sustainers/accelerators (smolder retardant)
Sheet physical quality improver	Releasing natural binders, pectins and softening fibers
Tobacco nicotine content modifier	Reducers (e.g., tobacco expansion)
	Enhancers (tobacco to smoke transfer)
Smoke nicotine content modifier	Reducers (filter acidification)
	Enhancers (distribution to free fraction in smoke; impact booster)
Tobacco/smoke NH_3 enhancer	
Flavor/body/taste enhancer	Smoothing, cooling effect to the taste; several patents have been described
Irritation reducer	Scavenging of irritant components or by tobacco expansion
pH regulator[a]	Whole tobacco and smoke

[a] Other ammonium compounds used as tobacco additive that affect (decrease or increase) pH: NH_4-associated organic acids, NH_4Ac, NH_4Cl, NH_4OH, NH_3, $(NH_4)_2HPO_3$ (DAP), urea, NH_4HCO_3, NH_4OH, $(NH_4)_2SO_4$, NH_4Cl.

The research to ammonia as enhancer of the nicotine dose has been extensive and goes back to 1966, when an internal document from the tobacco company BAT [ID (Backhurst, 1966)] described that the reaction of a smoker to the strength of tobacco was more related to the amount of free-base nicotine in the smoke, than to the total nicotine content. This study further showed that following extraction of several tobaccos with chloroform, the amount of nicotine in

the chloroform phase, being the nicotine free-base fraction, depended on the pH of the aqueous phase (ranging from 5 to 8) [ID (Backhurst, 1966)].

Much later in 1991, the American tobacco company's handbook of leaf blending and product development [ID (B&W, 1991)], as well as the FDA (Kessler, 1994b) coined the use of ammonia-compounds by the tobacco industry as "impact booster", i.e., increasing the impact and 'satisfaction' reported by smokers; see also Bates et al., 1999, Hebert, 2004, Henningfield and Benowitz, 2004, Hurt and Robertson, 1998, Kessler, 1994a and Kessler et al., 1996. In the same period, the US Department of Health and Human Services (USDHHS) and the FDA acknowledged that the psychoactive and reinforcing effects of nicotine increase with the speed of its absorption (Henningfield and Benowitz, 2004). Although the alkalinity of tobacco smoke was accepted as a major factor in nicotine absorption, other internal documents [cited in (EPA-FTC, 1978)] argued that smoke contact time with mucus membranes, pH of the membrane and body fluids, depth and degree of inhalation, degree of habitat of the smoker, nicotine and moisture content and puff frequency are highly relevant for nicotine absorption, as well. Other objective scientific reports (Peedin, 1999, Boyle et al., 2004 and Davis and Nielsen, 1999) have confirmed the pH-regulatory function of ammonium compounds (in whole tobacco and its smoke).

Finally, some internal documents of tobacco companies have claimed that treatment of whole tobacco with ammonium-compounds (e.g., di-ammonium phosphate; DAP) modified the nicotine content of tobacco [US Patent No. 4,215,706; ID's (B&W, 1990a, B&W, 1991, BAT, 1988, Larson, 1980, Lorillard, 1984 and B&W, 1990b)]. This modification has been described as "nicotine scavenging" or "root technology". The mechanisms by which ammonia increases the bioavailability of nicotine are outlined below.

3.4. Ammonia and nicotine in mainstream smoke

The p$K_{1,2}$-values of nicotine, being 8.02 (pyrrolidine) and 3.04 (pyridine nitrogen) at 25 °C, respectively (Weast, 1970) implicate that nicotine is protonated (i.e., is a non-volatile salt) at pH values below five (Seeman et al., 2004, Henningfield et al., 2004 and Domino, 1999). At higher pH values (>5) nicotine is gradually deprotonated to its volatile free-base form (cf. Fig. 1). MS smoke consists of a vapor phase and a particulate ('tar') fraction. Whereas the vapor phase contains only nicotine in its volatile non-protonated (i.e., free-base) form (Dixon and Lambing, 2000, Hurt and Robertson, 1998, Pankow et al., 1997, Pankow, 2001, Rodgman, 2000 and Seeman et al., 1999), the particulate fraction contains both protonated and non-protonated nicotine in a ratio that depends on the pH-value of this fraction (the diprotonated nicotine is of no importance in smoking).

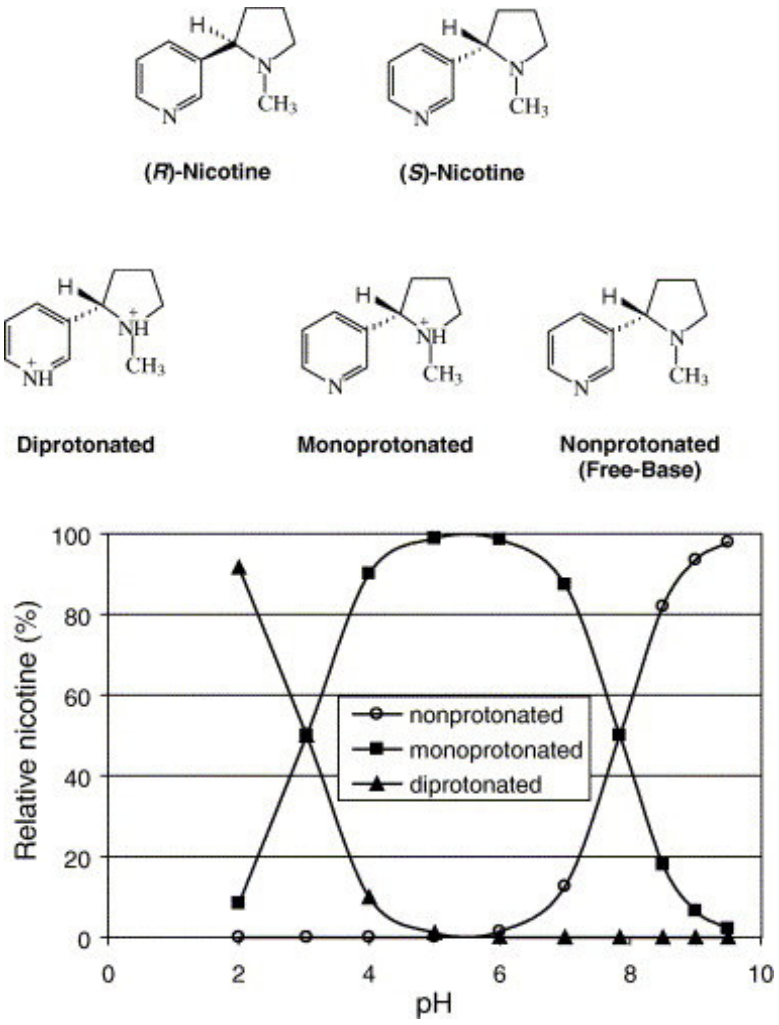

Fig. 1. Chemical structures of nicotine's forms and their percentages as function of pH, ranging from 2 to 9.5. Modified from Hoffmann and Hoffmann (1997).

As outlined in the previous paragraph on absorption and elimination of nicotine, the rate of nicotine absorption depends on the way of nicotine deposition (Pankow, 2001). Studies to the effect of ammonia or ammonium compounds on the deposition of tobacco smoke derived nicotine-containing particles have, however, not been described.

By definition, only aqueous solutions (and not gasses) have a pH-value. To properly compare pH values of smoke, Pankow (2001) introduced the parameter 'effective pH of the tobacco smoke particulate matter phase for nicotine (pH_{eff})', which also considers the free-base nicotine fraction in MS smoke (i.e., drawn and inhaled by smoker). The MS smoke pH_{eff} values (hereinafter referred to as pH) of eleven different cigarette brands ranged from 5.8 to 7.8 (Pankow, 2001). As can be referred from Fig. 1, tobacco smoke at a pH of 5.8 contains mainly non-volatile mono-protonated nicotine, while the free-base nicotine fraction gradually increases at higher tobacco smoke pH: at pH 7.8 about 30% of total nicotine in smoke is present as free-base (Bates et al., 1999, Burch et al., 1993, Hoffmann and Hoffmann, 1997, Lauterbach, 2000, Pankow, 2001, Seeman et al., 1999 and Seeman et al., 2004). An internal BAT document [ID (BAT, 1970)] described several ammoniation studies (e.g., NH_3, NH_4OH; max. 4% w/w) and reported

increased values for nicotine delivery (30–45%), "extractable nicotine" (27–63%), smoke pH (30–31%), and ammonia levels in smoke (59–105 times) as compared to control cigarettes. The effect of ammonia on smoke pH is illustrated by Fig. 2, showing that among different tobacco products the level of total and free ammonia in smoke proved to be positively associated with the pH of the smoke (from 5.2 to 7.4) (Sloan and Morie, 1976). Though the uptake of nicotine in the mouth and lungs is complex and not well understood [ID (Reininghaus, 1994)], it seems evident that alkalization of the smoke by ammonia promotes nicotine absorption.

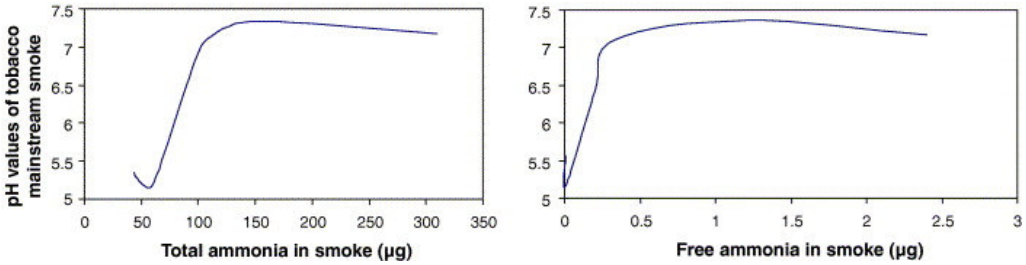

Fig. 2. Effect of total ammonia smoke levels on measured pH value of tobacco mainstream smoke. Modified from Sloan and Morie (1976).

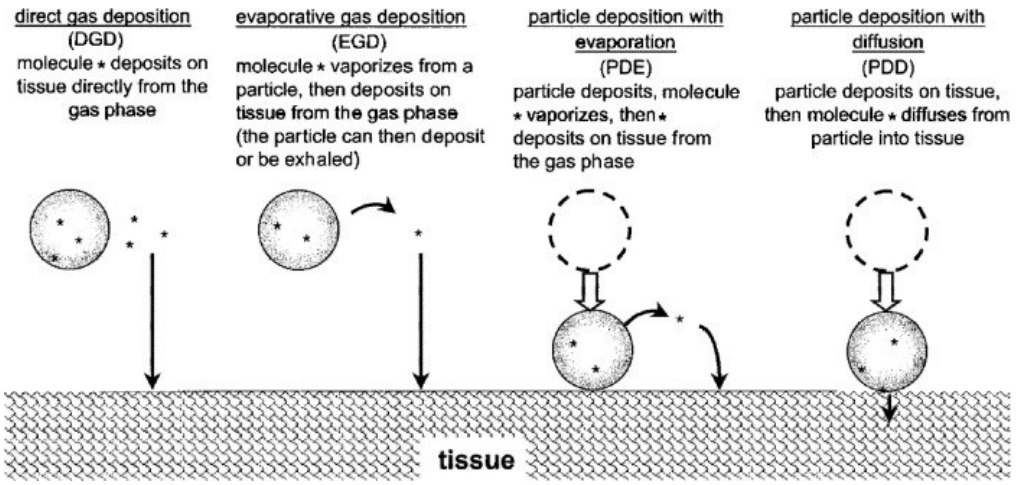

Fig. 3. Four mechanisms by which molecules (each of which is represented as an asterisk (*)) in a tobacco smoke aerosol can come into molecular contact with tissues in the airways; adapted from Pankow (2001) with permission.

Sidestream smoke (SS smoke; secondary smoke during smolder) of a cigarette contains some 3-fold higher total nicotine levels than MS smoke i.e., 0.8–2.3 mg per cigarette (Bates et al., 1999, Burch et al., 1993, Hoffmann and Hoffmann, 1997, Lauterbach, 2000, Pankow, 2001, Seeman et al., 1999 and Seeman et al., 2004), presumably because free-base nicotine easily evaporates near the hot tip of the cigarette. Apparently, much of the free ammonia evaporates from the tobacco, due to the generation of heat during smoking, and explains why SS smoke contains some 40–170 fold higher concentrations of ammonia than MS smoke (cf. Table 1) (Bates et al., 1999, Burch et al., 1993, Hoffmann and Hoffmann, 1997, Lauterbach, 2000, Pankow, 2001, Seeman et al., 1999 and Seeman et al., 2004). The pH-value of SS smoke averages that of MS smoke (pH of 6.7–7.5), so that up to 30% of total nicotine in SS smoke is present in its free-

base form. Obviously, the addition of ammonia to tobacco also increases the (free-base) nicotine level in SS smoke (cf. Fig. 1).

4. Effect on nicotine transfer and distribution

"Nicotine transfer efficiency" (NTE) reflects the ratio between the amount of nicotine in the smoke near the heat source (tip of a lit cigarette) and the amount present in the respective unburned tobacco (Seeman et al., 1999). Burley tobaccos (pH 7.6) have higher NTE values (20.6%) than flue-cured tobaccos (pH 5.6; 18.7%) and Oriental tobaccos (pH 5.1; 18.7%) [ID (BAT, 1995)]. Another study showed that addition of ammonia from 0.1% to 0.5% increased smoke ammonia levels and enhanced NTE from 13% to 18% [ID (Johnson, 1989)]. Finally, ammonia treatment (0.54% w/w) increased the pH value of tobacco (+16%), the amount of nicotine in filter (+45%), and nicotine in tobacco smoke (+26%) [ID (Johnson, 1977)].

5. Effect on oral absorption of nicotine

Transport of ammonia across membranes is pH-dependent: free ammonia (NH_3) freely diffuses across membranes, whereas ammonium salts (NH_4^+) are confined to cellular compartments [ID (Steele et al., 1994)]. The absorption rate of nicotine depends therefore on the form (free-base, protonated) in which nicotine is presented to buccal-pulmonary epithelial tissue. Similarly, the absorption rate of nicotine and the amount of nicotine absorbed is smoke pH-dependent.

Several studies suggest that nicotine absorption in the oral cavity is increased by alkali, including ammonia (cf. Table 2). The promoting effect of alkali on the oral nicotine absorption has been demonstrated in cats exposed to alkalinized nicotine aerosols and solutions, and in humans with alkalinized mouths treated with nicotine sublingual tablets (Molander and Lunell, 2001, Armitage and Turner, 1970 and Burch et al., 1993) and [ID (Reininghaus, 1994)]. An early tobacco industry document describes that about four times more nicotine is absorbed by the smoker from basic smoke as compared to acid smoke [cited in ID (EPA-FTC, 1978)]. The study with sublingual nicotine tablets revealed a slightly higher absorption rate of nicotine, i.e., a significant shorter t_{max} value ($P < 0.05$) in alkalinized versus acidified mouths (Molander and Lunell, 2001). The difference between the total amount of nicotine absorbed (area under the curve; AUC) from these mouths was, however, not statistically significant ($P > 0.05$). The often cited study of Armitage and Turner (1970) clearly demonstrated in anaesthetized cats the pH-dependency of nicotine's oral absorption and bioavailability (shorter t_{max} and higher c_{max} at higher pH). These data have been confirmed by another human study (Burch et al., 1993), showing increased plasma nicotine levels (8, 12 and 18 ng/mL) after inhalation of nicotine aerosols with pH values of 5.6, 7.5 and 11, respectively.

Volunteer studies showed that nicotine retention within the mouth (smoke was not inhaled) from control cigarettes was 20–26% [ID (Backhurst, 1966)] and 46% (Armitage et al., 2004), respectively. Treatment of cigarettes with 4% w/w DAP and urea significantly increased nicotine retention in the mouth during the mouth-retention to 64% and 53%, respectively (Armitage et al., 2004), but DAP or urea treatment did not increase the venous blood level of nicotine. However, when the mouth-hold condition was followed by inhalation (500 ml) DAP and urea did not increase the amount retained (and blood nicotine level) as compared to control cigarettes. In fact, control cigarettes (no ammonia added) gave already complete retention (99.1%) of nicotine (Armitage et al., 2004). It is remarkable that the treatment with urea or DAP failed to increase the blood nicotine level

(Armitage et al., 2004). Unfortunately, Armitage and co-workers have not reported whether smokers perceived increased mouth retention of nicotine by ammoniation in terms of cigarette strength (i.e., increased "impact", "kick").

6. Effect on pulmonary absorption of nicotine

The airway epithelium has a pH of about 7.6 (CDC, 1988, Hunt et al., 2000 and Kostikas et al., 2002), and a high buffer capacity of 7 mval/pH for which albumin, bicarbonate and ammonia in the lining fluid are responsible (Hunt et al., 2002) and [ID (Reininghaus, 1994)]. This multiple buffering system prevents tobacco smoke, including the ammonia in the smoke, to alter the pH at the luminal side of the airways (Hoffmann and Hoffmann, 1998).

As mentioned before, treatment of cigarettes with ammonium compounds increases the amount of free-base nicotine presented to the airways, i.e., relatively more nicotine is 'deposited' as free-base nicotine in the lining fluid of the respiratory tract. However, considering the pH-value of the lining fluid (pH of 7.6), about half of the free-base nicotine will be protonated again, leaving the remaining non-protonated nicotine available for rapid absorption.

To our surprise, no studies from independent researchers could be retrieved that described the effect of tobacco ammoniation on blood nicotine level. As quoted before, one study, sponsored by the tobacco industry, showed that treatment of tobacco with urea or DAP failed to increase the blood nicotine level (Armitage et al., 2004).

In this respect it is remarkable that similar amounts of nicotine are absorbed from (generally) acidic cigarette smoke as compared to alkaline cigar smoke [ID (Reininghaus, 1994)]; see also (Armitage et al., 1968). Considering the differences in smoking techniques of cigarette and cigar smokers, like the usually superficial (less deep) inhalation by cigar smokers, this similarity in nicotine delivery may also be attributed to a higher absorption of nicotine from cigars by the mucous layer in the mouth.

7. Discussion

The present review shows that the exposure of cigarette smokers to free-base nicotine can be easily increased by adding ammonia to whole tobacco. It is concluded, that ammonia increases the bioavailability of nicotine via multiple mechanisms that are based on the fundamental property of weak bases to be de-protonated at high alkalinity: (1) liberation of nicotine from whole tobacco; (2) increase of the vapor–solid particle ratio of nicotine; (3) ammonia top affects the proportion of the free base in smoke that is more readily absorbed.

Ammonia and certain ammonium salts increase the pH value of tobacco, so that a larger portion of the weak base alkaloid nicotine will acquire the free-base form. In contrast to protonated nicotine that is confined to its cellular structure, free-base nicotine easily passes cellular membranes and is a volatile entity. Consequently ammonia liberates nicotine from the cellular structures of the tobacco leaf, and upon heating free-base nicotine diffuses to the vapor phase. As such, it is clear that ammoniation of tobacco increases the dose of nicotine (i.e., concentration of free-base nicotine in smoke), the smoker will be exposed to.

One may, however, question whether ammonia also facilitates the pulmonary absorption per se (rate of absorption and total amount of nicotine absorbed) (Sloan and Morie, 1976). Considering the high buffer capacity of the lining fluid, it

is unlikely that ammonia affects the pH at the luminal side of the bronchioli (CDC, 1988, Hunt et al., 2000, Hunt et al., 2002 and Kostikas et al., 2002). Therefore, the enhancing effect of ammonia on nicotine absorption will finally be confined to a concentration-driven increase in absorption due to an elevation of the free-base nicotine concentration in MS smoke.

Henningfield et al. (Henningfield et al., 2004) recently proposed that ammonium compounds may increase the reinforcing potency of cigarettes via: (i) enrichment of the MS smoke with nicotine; (ii) a better and faster absorption of nicotine; (iii) a higher impact of nicotine at peripheral and central nicotine receptors; and (iv) improvement of the sensory characteristics (Henningfield et al., 2004). Though their proposal largely conforms to our conclusion, no corroborative data on the higher impact at nicotine receptors, and the improvement of the sensory characteristics have been described by independent investigators. To our surprise and despite the large research efforts (see below), no conclusive data on the enhancement of nicotine plasma levels (bioavailability) by cigarette ammoniation could be retrieved.

Clearly, the tobacco industry is interested in the development of cigarettes that give a rapid and high delivery of nicotine (Bates et al., 1999). Several tobacco companies use variations of "ammonia technology", originally described in the late 1960s by Philip Morris (Marlboro), with the aim to manipulate nicotine delivery to the smoker (Kessler et al., 1996); see also [ID (B&W, 1990a)]. The dramatic increase in PM's sales since, may well be due to this innovative ammonia technology (Bates et al., 1999 and Hurt and Robertson, 1998); see also [ID (Wayne et al., 2003)]. Until the late 1980s (Hurt and Robertson, 1998, Muggli et al., 2004 and USDHHS, 1988), this phenomenon of ammoniation (also known as 'nicotine free-basing'), was publicly not known, implicating that the tobacco consumer was intentionally diverted by the tobacco companies that applied the ammonia technology. The nicotine values depicted on cigarette packages (ranging from 0.9 to about 1.1 mg) reflect the values of "tar"-bound nicotine that is obtained via measurements according to the FTC-guidelines. These nicotine values under-estimate the factual exposure during cigarette smoking, since the volatile nicotine fraction in the smoke of these cigarettes is not considered. As a result, the nicotine value described on the package gives no reliable information with respect to the true dose the consumer will be exposed to. In 2001, Philip Morris decided to stop adding ammonia to its cigarettes sold in the EU, but not to those marketed in the USA.

In addition to the higher exposure of smokers to ammonia (and nicotine), it should be mentioned that the addition of ammonia to tobacco increases indoor (and outdoor) ambient air pollution. Ammonia in SS smoke with 40–170 fold higher levels than in MS smoke is mainly responsible for the ammonia in environmental tobacco smoke (ETS). Still, the indoor air level of 10 μg of ammonia per m^3 that will be reached as a result of smoking one cigarette (releasing some 5000 μg of ammonia) in a small room of 500 m^3 remains far below the PEL/STEL value of 18–27 mg/m^3. On the other hand, certain people with hypersensitive airways may show throat irritation and COPD-like symptoms at such low ammonia concentrations. Secondly, smokers- and less so non-smokers- are acutely exposed to relatively high levels of ammonia (up to 750 ppm), which are known cause throat irritation, or may even elicit chronic obstructive lung disease (Brautbar et al., 2003). Typical urban and non-urban ambient ammonia levels, to be mentioned here for comparison, are around 20 and 5 $\mu g/m^3$ (30 and 7 ppb), respectively. The daily intake of 0.1–0.5 mg of ammonia by a non-smoker via ETS and 35 mg of ammonia via MS by typical package-a-day smoker, is comparable to the 18 mg of ammonia that is normally

ingested per day, so that no systemic adverse health effects are to be anticipated.

The majority of the data referring to a promoting effect of ammonia on nicotine delivery is derived from tobacco internal documents, that have not been reviewed by independent scientists working in the public domain, and should, therefore, be considered with caution. To properly assess the rationale and the adverse effects of ammonia as tobacco additive it is recommended to perform independent public-accessible research to ammoniated tobacco products. Relevant research topics that should be addressed include the relation between the smoke and blood nicotine level, and between bioavailability (higher or prolonged exposure) and brain nicotine level.

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