



Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Free-base nicotine in tobacco products. Part I. Determination of free-base nicotine in the particulate phase of mainstream cigarette smoke and the relevance of these findings to product design parameters [☆]

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ARTICLE INFO

Article history:

Received 1 May 2009

Available online xxxx

Keywords:

Free-base nicotine

Cigarettes

Mainstream cigarette smoke

Gas-particle partitioning theory

Nicotine

Smoke pH

Tar/nicotine ratio

Solid-phase microextraction

ABSTRACT

The free-base nicotine (FBN) content of mainstream cigarette smoke (MSS) has been discussed in the peer-reviewed literature and popular press. It has been alleged that manufacturers adjust product design features to increase the percentage of total nicotine (TN) in the MSS gas-vapor phase that is unprotonated [$P_{g,nic}(\%)$] and/or the fraction of nicotine in the MSS total particulate matter (TPM) that is unprotonated (FBN/TN). Our research showed the Health Canada Intensive smoking conditions negated the effects of blend and cigarette design features reported to raise the pH of TPM collected under ISO or US FTC conditions. Our research also showed that when additive-free Canadian cigarettes were smoked under ISO conditions, the FBN/TN ratio increased as the tar/nicotine ratio decreased. Our findings are in line with other studies that have questioned allegations of a relationship between use of ammonia and its compounds as tobacco additives and amounts of unprotonated nicotine in MSS. In addition, the experimental work demonstrated how use of solid-phase microextraction to estimate FBN can yield erroneously high results due to improper conditioning and/or smoking of the cigarettes. Our research showed that there is no longer any scientific support for regulators to require smoke pH and FBN determinations on cigarette products.

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1. Introduction

Numerous articles in the peer-reviewed literature, technical presentations, and internal tobacco industry reports have discussed the methodology and significance of pH values (i.e., smoke pH) determined on the mainstream cigarette smoke aerosol (MSS) or fractions of the same collected by various means. The topic has been the subject of two reviews (Rodgman, 2000; Dong et al., 2000), both of which discussed methodology, but also claimed that there was no physiological significance to values obtained with any of the methods. Other methods reported since the dates of those reviews include those by Cochran et al. (2003), who defined smoke pH as the pH of a solution of the soluble fraction of MSS particulate matter. [MSS particulate matter is also known as MSS total particulate matter (TPM); in addition, we will follow terminology recommended by Seeman (2007) who stated, “The terms “total particulate matter” and “particulate matter” refer to material

captured during a machine-smoking method on, for example, a Cambridge filter pad or in an impaction trap. In contrast, MS smoke aerosol is composed of heterogeneous *particles* suspended in a gas phase. Trapped particulate matter is thus not the same in terms of physical or chemical properties as the particles in the dynamic aerosol.] Pankow et al. (2003) reported pH-values (so-called pH effective) based on a volatilization of nicotine from MSS TPM before and after treatment with ammonia. In a later report by the same research group, experimental difficulties were reported (effects of particulate-phase water) when an attempt was made to repeat the earlier work (Chen and Pankow, 2009).

There was little regulatory or public interest in smoke pH until the discovery of statements in industry documents that claimed that cigarettes providing smoke with increased unprotonated nicotine had greater consumer acceptability and greater addictiveness (Freedman, 1995). Furthermore, there were incorrect interpretations made by some industry scientists and marketers about the effects of ammonia-containing additives on smoke pH and unprotonated nicotine (e.g., Aulbach et al., 1991). Such statements served as the basis for articles in the popular press (Freedman, 1995). As a result, there were attempts to show how tobacco ingredients and cigarette design features might alter smoke pH and levels of

[☆] This study was funded by Health Canada under contract number H4133-070511/001/SS.

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nicotine (assumed to be unprotonated nicotine) in the gas–vapor phase of the MSS aerosol (Pankow et al., 1997, 1999) and the fraction of nicotine in the TPM that was not protonated (α_{fb} , Pankow et al., 2003). The resulting publicity produced calls by regulatory authorities for manufacturers to report smoke pH and/or concentrations of unprotonated nicotine in the MSS aerosol. Examples of such regulations included those promulgated by the Commonwealth of Massachusetts (Connolly, 2002) and Health Canada (1999). However, there were surprising results when smoke pH values were obtained using smoking regimens more intense than the regimen specified in the ISO standards (ISO Standard 4387, 2000) or in the US Federal Trade Commission regulations (Federal Trade Commission, 2000; Federal Register, 1967). It was found that use of the more vigorous smoking regimens resulted in smoke pH values that were almost the same for differing cigarette products (Seeman, 2007; Lee et al., 2006; Counts et al., 2005).

Several research teams have examined other approaches to determining the potential of ammonia and its compounds to alter the levels of unprotonated nicotine in MSS. Some of the other approaches have been summarized in reviews by Seeman and Carchman (2008) and Seeman (2007). Although cited in the reviews, other very relevant papers deserve special mention. They include those by Seeman et al., 2004 (kinetic studies); Armitage et al., 2004a,b (nicotine uptake by smokers); Fournier et al., 2001, and Seeman et al., 1999 (thermal transfer of nicotine and its salts). Other researchers (Ingebrethsen, 2006; Ingebrethsen et al., 2001) have focused on numerical simulations and inclusion of carbon dioxide and other MSS acids in the calculations. The research by Armitage and co-workers (2004b) showed that there was very little difference in nicotine uptake among members of a trained smoke panel when they smoked cigarettes made without ammonia-containing ingredients and when they smoked cigarettes made with two different commercial methods of incorporating ammonia-containing compounds into tobacco blend. All of the above studies taken together show that the uses of ammonia and its compounds at levels typically used in commercial products have little, if any, effect on nicotine uptake during smoking.

On the other hand, some researchers feel that the evidence just cited is inconclusive or supports their position that the use of ammonia and/or its compounds in the processing of cigarette tobaccos results in an increase of unprotonated nicotine in the gas–vapor phase and TPM of the cigarette smoke aerosol. For example, in their recent publication on unprotonated nicotine in MSS, Ashley et al. (2009), stated that all the experimental work reported by Pankow et al. (1997, 2003), and Watson et al. (2004), was correct (including the claim that up to 40% of nicotine in MSS could be available as the volatile free-base form) even though Pankow had revised the 40–20% (Pankow et al., 1999). Ashley also reported that the 2001 Ingebrethsen (Ingebrethsen et al., 2001) results support his position; however, Ingebrethsen's results for unprotonated nicotine were for diluted mainstream, while his total nicotine results were for undiluted mainstream smoke. Ashley did not provide additional support for his conclusions except for statements found in certain litigation documents. Other researchers have written similar papers based on industry documents (Wayne et al., 2006; Henningfield et al., 2004a,b).

Therefore, the purpose of this report is to clarify the issues regarding the determination of FBN in MSS TPM; and, in particular, limitations in the interpretation of the results. Since various authors have used different terminology for the chemical and physical state of nicotine in MSS, we are carefully defining the terms we use in this report. In particular, we want to emphasize that the term FBN refers to the concentration of unprotonated nicotine in MSS TPM under a defined smoking regimen and analytical conditions for the determination of the FBN. We will use the term α_{fb} for the fraction of nicotine in MSS TPM that is not protonated.

We will use the term $P_{\text{g, nic}}(\%)$ to refer to the percentage of total MSS nicotine that was not trapped on the Cambridge filter pad as determined by the technique reported by Callicutt and colleagues (2006a,b, 2002). In discussing the results of our work and their significance to the tobacco regulatory community, we will make reference to gas–particle partitioning theory (Pankow, 1994a,b). Pankow's theory requires that there is equilibrium between the gas phase and particulate phase of the aerosol. We have found no evidence in the scientific literature that the mainstream smoke aerosol is at equilibrium. In fact, quite to the contrary, there is much data in the literature that demonstrates the mainstream smoke aerosol is not at equilibrium. Thus, from the purely physical chemistry perspective application of a Pankow's theory to MSS is wrong. However, some in the tobacco control and regulatory communities do not agree with the pure physical chemistry approach. In addition, there is empirical evidence that trends predicted by Pankow's theory can be found for gas–particle partitioning of semivolatile organic compounds in MSS and other combustion aerosols.

Our main audience for this report is the regulatory community, and we recognize that our approach may not satisfy those who believe that the health and regulatory communities (unless they say they are acting purely on the grounds of the precautionary principle) should demand both theoretical and experimental rigor for the test methods they require for products they regulate. We have used data (TPM, nicotine, water, CO, pH, and FBN) we obtained on MSS generated [both ISO and HCI (Health Canada Intensive) conditions] from cigarettes and other smoking products sold in Canada in the year 2007. The brand-styles were chosen and products were provided by Health Canada. Health Canada also specified the test methodologies to be used. We also tested cigarettes made and sold in Canada dating back to 1970 that had been kept in frozen storage (-4°C) at Labstat International ULC. The FBN data were generated using an improved solid-phase microextraction (SPME) method for the determination of FBN in cigarette mainstream particulate matter (TPM) (Bao et al., 2010). The results of our studies and their relevance to regulation of tobacco products are described in the remainder of this report.

We wish to alert the reader that the Editor has requested that we use nonstandard terminology to describe the fraction of the mainstream smoke aerosol that has not been retained by the Cambridge filter pad. ISO Standard 10815:2004 Tobacco and tobacco products – Vocabulary defines the vapor phase of mainstream smoke as “the portion of smoke which passes the particulate phase trap during smoking in accordance with ISO 4387 using a machine conforming to ISO 3308”. Our studies [this work and Bao et al. (2010)] as well as those of others have involved the use of a particulate phase trap (a Cambridge filter pad in our work).

2. Relevance of this work to tobacco regulations

The research we are reporting was commissioned by Health Canada, which specified the analyte to be determined [unprotonated nicotine in mainstream total particulate matter (TPM) collected under ISO and HCI conditions], the method to be used (SPME), and the cigarettes to be tested. In principle, our research is along the same lines as that reported by Counts et al. (2005). Our findings, as were those of Counts and co-workers were empirical, as opposed to being based on theoretical relationships.

The results of bioassays and chemical test methods required or considered by regulatory agencies sometimes are not well correlated with underlying human physiology or toxicity they are purported to represent. Moreover, the methods required by regulatory agencies sometimes defy chemical theory, but the results are

essential for public health (for example, pH-values determined on aqueous slurries of food products).

As noted above, we are presenting the results of research funded by Health Canada. Health Canada funded the development and validation of a technique for determining the amount of FBN in mainstream TPM, and the use of the technique to determine the FBN content of a series of cigarettes it selected. As most in the regulatory community know, governmental agencies sometimes have requested test data when there is no solid link between the data sought and any biological effect.

Tobacco regulators are often not concerned with the toxicological relevance of the regulations they have promulgated or are planning to promulgate. The new Center for Tobacco Products of the US Food and Drug Administration recently issued its final *Guidance for Industry on Listing of Ingredients in Tobacco Products* (Center for Tobacco Products, 2009). The Guidance document asks for reporting of ingredients whose use level and toxicities are such that they have little, if any, toxicological relevance to human use of the products. Smoke pH is an example of a parameter that has little, if any, relevance to the toxicological properties of the smoke. The same is true for the FBN content of the TPM (also known as α_{fb}). There are data that demonstrates that the relative concentrations of nicotine, ammonia, and solanesol in MS smoke aerosol varies with time (Seeman and Carchman, 2008; Seeman, 2007; Seeman et al., 2004). That time dependency makes the use of any single parameter of questionable value in predicting bioavailability, pharmacodynamics, and pharmacokinetics of any given smoke constituent. Thus, parameters such as α_{fb} , pH, and % FBN have not yet been demonstrated to be of value for the estimation of the bioavailability of nicotine in MSS.

We ask that the reader keep the above discussion in mind when considering our research and the values of using smoke pH, FBN, and α_{fb} as part of any regulatory scheme. As noted by Rodgman (2000), “‘Smoke pH’ value for MSS, like the ‘tar’ value for MSS, provides little, if any, useful information about the chemical composition of the source of the MSS, or about its chemical or biological properties.” The research we present in this report supports Rodgman’s conclusion. Our research contradicts the scientific support for regulators to require smoke pH and FBN determinations on cigarette products that has been claimed by Ashley et al., 2009, and the WHO Study Group on Tobacco Product Regulation (WHO, 2007).

3. Materials and methods

3.1. Cigarettes

Two sets of cigarettes were used for this study. These are detailed in Tables 1a and 1b. The first set of cigarettes (cross-sectional set, Table 1a) was provided by Health Canada and consisted of ten commercial brand-styles of machine-made cigarettes, a brand of nontobacco cigarettes, cigarettes assembled from fine-cut tobacco and filter tubes, cigarettes sold in bags (illicit product) and two brands of mini-cigarillos. This set represented contemporary product available on the Canadian market. The second set of cigarettes (Historical set, Table 1b) was taken from Labstat’s cigarette inventory. It contained seven subsets of brand-styles. Each subset consisted of product produced at several time points over the past three decades. In addition, the 3R4F reference cigarette (Reference Cigarette Program, 2008) was used as an embedded control in each

Table 1a

Average mainstream cigarette smoke data for cross-sectional brands (data from this work).

Sample ID	Smoking regimen	Sample description	Puff count	TPM (mg/cig)	Water (mg/cig)	Water/TPM ratio (%)	Tar (mg/cig)	Nicotine (mg/cig)	CO (mg/cig)	FBN (μ g/cig)	α_{fb}	Smoke pH	Tar/nic ratio
62403	ISO	Brown Tips In Bags –ILLICIT 1	9.1	19.8	1.32	6.65	17.2	1.29	14.9	29.2	0.023	5.83	13.3
72954	ISO	Cafe Crème regular (a mini-cigarillo)	9.2	41.2	6.73	16.3	32.4	2.07	35.7	1032	0.498	7.75	15.7
72955	ISO	Prime Time FILTRE Vanille (a mini-cigarillo)	8.7	23.6	1.86	7.86	20.7	1.08	31.5	6.25	0.006	5.53	19.2
72956	ISO	Player’s Special Blend Regular Size	7.3	19	2.64	13.9	15.2	1.11	12.3	15.6	0.014	5.93	13.7
72957	ISO	Camel Filters 20 King Size	8.4	16.2	0.86	5.33	14.3	1.08	11.7	45.3	0.043	6.07	13.2
72958	ISO	Player’s King Size	8.1	18.2	1.7	9.36	15.3	1.12	13.3	15.7	0.014	5.83	13.7
72959	ISO	Craven A Gold King Size	8.9	12.4	0.64	5.2	10.8	0.94	10.3	32.8	0.035	6.07	11.5
72960	ISO	Player’s Fine Cut Tobacco + Filter Tubes	8.9	23.7	2.88	12.2	19.4	1.39	15.7	9.46	0.007	5.65	14.0
72961	ISO	Gauloises Blondes (Red)	7.1	10.4	0.36	3.42	9.27	0.79	9.38	43.3	0.055	6.1	11.7
72962	ISO	Player’s Plain Regular Size	7.2	18.1	0.91	5.04	16	1.19	9.26	24	0.02	5.64	13.4
80229	ISO	More Filters 120’s	14.7	14.5	0.37	2.53	13.1	1.03	18.9	83.6	0.082	6.23	12.7
80230	ISO	Number 7 Silver King Size	8.2	5.34	0.116	2.16	4.8	0.43	4.32	31.5	0.074	6.18	11.2
80231	ISO	Honeyrose Menthol (nontobacco cigarette)	6.5	6.52	0.11	1.68	6.41	BDL	7.64	BDL	N/A	5.89	N/A
80232	ISO	Podium Gold King Size	8.8	17	1.4	8.23	14.4	1.13	13.7	19.4	0.017	5.88	12.7
80233	ISO	du Maurier Extra Light Regular Size	7.8	8.1	0.17	2.09	7.25	0.68	6.06	49.1	0.073	6.09	10.7
62403	HCI	Brown Tips In Bags –ILLICIT 1	12.9	52	12	23	37.4	2.68	28.9	24.7	0.009	5.76	14.0
72954	HCI	Cafe Crème regular (a mini-cigarillo)	10.2	86.7	18.5	21.3	65.6	3.5	49	1500	0.428	7.94	18.7
72955	HCI	Prime Time FILTRE Vanille (a mini-cigarillo)	10.5	66.4	18.5	27.8	45.8	2.22	51	17.7	0.008	5.67	20.6
72956	HCI	Player’s Special Blend Regular Size	9.2	48.3	13	27	33	2.3	23.5	15.7	0.007	5.78	14.3
72957	HCI	Camel Filters 20 King Size	9.3	55.1	14.1	25.6	38.6	2.41	30.5	34.1	0.014	5.78	16.0
72958	HCI	Player’s King Size	11.3	53.1	13.6	25.6	36.9	2.67	29	18.4	0.007	5.68	13.8
72959	HCI	Craven A Gold King Size	10.3	51.4	14.4	28	34.5	2.48	31	21.1	0.009	5.76	13.9
72960	HCI	Player’s Fine Cut Tobacco + Filter Tubes	11.6	57.7	15.2	26.4	39.6	2.81	32.8	19.1	0.007	5.55	14.1
72961	HCI	Gauloises Blondes (Red)	8.7	45.9	12	26.2	31.8	2.05	30.1	24.7	0.012	5.88	15.5
72962	HCI	Player’s Plain Regular Size	9.9	45.1	6.74	14.9	34.9	2.6	20.6	18.7	0.007	5.57	13.4
80229	HCI	More Filters 120’s	14.3	51.7	12.2	23.6	37.1	2.41	50.1	29.5	0.012	5.8	15.4
80230	HCI	Number 7 Silver King Size	8.6	40	10.2	25.6	27.9	1.88	27.1	16.2	0.009	5.79	14.8
80231	HCI	Honeyrose Menthol (nontobacco cigarette)	9.5	25.1	6.11	24.3	19	0.02	20.5	0.932	0.059	5.69	N/A
80232	HCI	Podium Gold King Size	14.8	43.3	9.35	21.6	31.7	2.23	27.2	20.9	0.009	5.72	14.2
80233	HCI	du Maurier Extra Light Regular Size	10.3	39.5	8.93	22.6	28.5	2.05	22.2	19.2	0.009	5.77	13.9

Table 1b

Average mainstream cigarette smoke data for historical brands (data from this work).

Sample ID	Smoking regimen	Product year	Sample description	Puff count	TPM (mg/cig)	Water (mg/cig)	Water/TPM ratio (%)	Tar (mg/cig)	Nicotine (mg/cig)	CO (mg/cig)	FBN (μ g/cig)	α_{nb}	Smoke pH	Tar/nic ratio
80344	ISO	1970	du Maurier KSFT	10.3	26.2	1.7	6.48	23.1	1.41	23.9	13.2	0.009	5.66	16.4
80345	ISO	1980	du Maurier KSFT	9.3	26.8	1.63	7.3	23.2	1.29	22.6	10.1	0.008	5.6	18.0
80376	ISO	1993	du Maurier KSFT	8.1	15.6	1	6.39	13.7	0.94	14.4	11	0.012	5.63	14.6
80346	ISO	1999	du Maurier KSFT	8.8	17.7	0.86	4.85	15.6	1.25	15.2	26.7	0.021	5.88	12.5
80347	ISO	2007	du Maurier KSFT	8.5	21.6	1.9	8.8	18.4	1.24	16.4	12.8	0.01	5.65	14.8
80348	ISO	1970	Export A FF RSFT	8.7	24.7	1.86	7.53	21.5	1.26	17.7	9.29	0.007	5.54	17.1
80349	ISO	1980	Export A FF RSFT	8.6	32	4.63	14.5	26	1.32	21.9	6.2	0.005	5.43	19.7
80350	ISO	1990	Export A FF RSFT	7.6	20.4	1.98	9.71	17.6	1.06	14.8	9.36	0.009	5.63	16.6
80351	ISO	1999	Export A FF RSFT	7.4	18.6	1.79	9.59	15.6	1.23	13.9	20	0.016	5.77	12.7
80352	ISO	2007	Export A FF RSFT	6.8	18.6	2.03	10.9	15.6	1.02	13.5	11.1	0.011	5.76	15.3
80353	ISO	1969	Mark Ten KSFT	11.2	31.3	2.66	8.52	26.9	1.73	25.8	11	0.006	5.62	15.5
80354	ISO	1978	Mark Ten KSFT	8.9	21.7	2.54	11.7	18.3	0.86	20.6	5.27	0.006	5.55	21.3
80355	ISO	1990	Mark Ten KSFT	8.5	18.3	0.91	4.96	16.4	1.02	13.9	10.7	0.01	5.64	16.1
80356	ISO	1998	Mark Ten KSFT	8.3	17.6	1.01	5.74	15.5	1.09	14.7	20	0.018	5.72	14.2
80357	ISO	2007	Mark Ten KSFT	8.9	18.5	0.92	4.98	16.2	1.41	16.2	32	0.023	5.87	11.5
80358	ISO	1970	Peter Jackson KSFT	10.5	26.5	1.81	6.83	23.3	1.45	25.1	15.2	0.01	5.71	16.1
80377	ISO	1979	Peter Jackson KSFT	9.4	25	1.88	7.53	21.8	1.3	23.3	11.5	0.009	5.62	16.8
80378	ISO	1995	Peter Jackson KSFT	8.3	19.2	1.14	5.95	16.8	1.25	17.3	20.4	0.016	5.75	13.4
80359	ISO	1999	Peter Jackson KSFT	8.8	18.4	1.13	6.14	16	1.28	16.2	30.4	0.024	5.85	12.5
80360	ISO	2007	Peter Jackson KSFT	8.7	16.6	0.74	4.48	14.8	1.04	14.2	25.7	0.025	5.85	14.2
80361	ISO	1970	Player's RSFT	8.8	32.1	3.44	10.7	27.1	1.59	21.5	7.68	0.005	5.56	17.0
80362	ISO	1978	Player's RSFT	8.3	28.6	3.03	10.6	24.1	1.37	21.9	7.32	0.005	5.64	17.6
80363	ISO	1995	Player's RSFT	7.6	20	1.75	8.78	17	1.26	14.6	13.1	0.01	5.69	13.5
80364	ISO	1999	Player's RSFT	7.5	20	1.88	9.42	16.8	1.3	16.4	15.5	0.012	5.68	12.9
80365	ISO	2007	Player's RSFT	7.5	17.5	1.24	7.08	15.1	1.16	12.3	26.4	0.023	5.78	13.0
80366	ISO	1971	Rothmans KSFT	10.8	25.5	1.67	6.53	22.5	1.4	21.8	14	0.01	5.46	16.1
80367	ISO	1979	Rothmans KSFT	9.8	20.5	1.33	6.47	18.1	1.14	18.6	12.2	0.011	5.61	15.9
80368	ISO	1996	Rothmans KSFT	8.4	18.2	1.02	5.59	16	1.25	14.3	26	0.021	5.64	12.8
80369	ISO	1999	Rothmans KSFT	8.2	16.4	0.9	5.51	14.4	1.07	14.3	20	0.019	5.77	13.5
80370	ISO	2007	Rothmans KSFT	8.4	18.1	0.65	3.6	16.2	1.27	16.2	32	0.025	5.81	12.8
80371	ISO	1972	Player's Plain RSPT	8.9	30.2	1.38	4.57	27.4	1.37	23.2	10	0.007	5.41	20.0
80372	ISO	1980	Player's Plain RSPT	7.9	26	1.17	4.49	23.6	1.19	18.5	7.78	0.007	5.36	19.8
80373	ISO	1989	Player's Plain RSPT	7.5	21.3	0.97	4.56	19.1	1.29	12.5	18.1	0.014	5.45	14.8
80374	ISO	1999	Player's Plain RSPT	7.5	19.2	0.6	3.1	17.3	1.33	10.9	27.3	0.021	5.75	13.0
80375	ISO	2007	Player's Plain RSPT	7.6	18.5	0.53	2.89	16.8	1.13	10.8	28.6	0.025	5.66	14.9

smoking machine run. Cigarettes were stored frozen prior to conditioning and preparation for analysis.

3.2. Cigarette testing – routine methods

Except as noted, official Health Canada methods were used. These have been posted by Health Canada on the Internet at <http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/index-eng.php>. The test methods used included (1) method T-113, determination of mainstream tobacco smoke pH; (2) method T-115, determination of “Tar”, nicotine and carbon monoxide in mainstream tobacco smoke [note: method T-115 includes both the ISO puffing regimen (ISO Standard 4387, 2000) (35 mL puff of 2-s duration taken once every 60 s; no blocking of filter ventilation) and the Health Canada Intensive (HCI) puffing regimen (55 mL puff of 2-s duration taken once every 30 s; complete blocking of filter ventilation)]; (3) ISO 9512:2002(E) – cigarettes – determination of ventilation – definitions and measurement principles; (4) ISO 6565:2002(E) – tobacco and tobacco products – draw resistance of cigarettes and pressure drop of filter rods – standard conditions and measurement; and (5) ISO 2965:1997(E) – materials used as cigarette papers, filter plug wrap and filter joining paper, including materials having an oriented permeable zone – determination of air permeability. The ISO standards (Technical Committee 126 on tobacco and tobacco products) are available from the International Organization for Standardization (ISO), 1 ch. de la Voie-Creuse, Case postale 56, CH-1211 Geneva 20, Switzerland. The ISO web site for the purchase of the ISO tobacco standards is http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=52158.

3.3. Cigarette testing – determination of free-base nicotine in mainstream smoke total particulate matter (TPM)

Free-base nicotine (FBN) in mainstream smoke TPM was determined using an improved solid-phase microextraction (SPME) method (Bao et al., 2010). Briefly, five (three in the case of HCI smoking) preconditioned cigarettes were smoked using an automated 20-port constant volume smoking machine using the ISO or Health Canada Intensive (HCI) smoking protocol. TPM was collected by passing the mainstream smoke through a 44 mm glass fiber filter disc (Cambridge filter pad). After smoking, the pad was cut into halves; and one-half was transferred into a 20 mL amber glass vial. Internal standard solution (2 μ L of a 5 mg/mL solution of toluene- d_8 in hexane) was added to the pad. The vial was closed with a PTFE/silicone-lined septum screw cap and left for at least 2 h at room temperature followed by 15 min at 30 °C to allow for the equilibration of analytes between the pad and the headspace. The FBN was then extracted from the headspace over the pad by SPME after allowing time for the equilibration of analytes between the pad, the headspace, and the SPME fiber. The SPME was done with an automated SPME sampler (CTC CombiPAL) using a 65 μ m polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber and analyzed by gas chromatograph/mass spectrometer (GC/MS). The response of free-base nicotine from the pad in headspace SPME analysis was dependent on the water content of the TPM. The FBN data obtained by using headspace SPME/GC/MS analysis were then corrected by a relative response factor, which was calculated from the sample pad water content and a pre-determined relationship between the relative free-base nicotine response and the pad water content. The relationships between the relative free-base

nicotine response and pad water content by using headspace SPME are given by the data and regression equation shown in Table S1 of the Supplementary material.

It is important to note that care must be used to avoid overloading the pad with condensate. Thus, five cigarettes per observation are used for mainstream ISO conditions whereas only three cigarettes per observation should be smoked for HCI conditions. For other smoking products, it is necessary to select a number of units smoked per pad such that breakthrough does not occur. Please note that the remaining half of the pad is used to determine smoke nicotine and smoke water using Health Canada test method T-115.

4. Results

4.1. Overall results and product composition

As noted earlier, cigarette descriptions are given in Tables 1a (cross-sectional brands) and 1b (historical brands) along with routine smoke data, estimated FBN deliveries, and smoke pH (Health Canada method). The amount of particulate-phase water as a percentage of TPM weight is also given for each of the brand-styles. The importance of this parameter will be discussed in Sections 5.2 and 5.3. Table 1c contains tip ventilation, open cigarette pressure drop, and paper porosity data for the cross-sectional sample set. All data appeared reasonable given available knowledge of the cigarette blends and designs used. As expected with ISO smoking, the typical all flue-cured Canadian products (with the exception of highly ventilated products such as du Maurier Extra Light Regular Size and Number 7 Silver King Size) had lower values for the α_{fb} that did the three blended products (Camel Filters 20 KS, the Gauloises Blondes, and the More 120's). The More 120's gave the highest α_{fb} (0.082) on account of the very high puff count and very low particulate-phase water. Both factors contribute to higher than normal α_{fb} values. With HCI smoking, all the cigarette products had α_{fb} values of less than 0.012.

Based on information available on the Internet, Cafe Crème regular brand of mini-cigarillos contained a fermented, dark air-cured tobacco filler that is typical of cigars, not cigarettes. The wrapper on Cafe Crème was leaf tobacco (Thompson Cigar, 2009). As noted

in Table 1c, the porosity of the wrapper was too low to be measured. This could explain why there was little decrease in the values α_{fb} between the ISO and HCI smoking protocols. The blend for the Prime Time FILTRE Vanille brand of mini-cigarillos could not be determined with certainty from the available information, and the product did not give the high smoke pH associated with fermented dark air-cured tobaccos. The Honeyrose Menthol cigarettes reportedly contain a tobacco-free, nicotine-free filler made with rose petals, marshmallow leaves, red clover flowers, honey and apple juice (Honeyrose, 2008). Under HCI smoking conditions, enough nicotine was apparently pulled-in from the sidestream smoke of cigarettes being smoked on adjacent ports to give about one $\mu\text{g}/\text{cig}$ FBN. According to the Imperial Tobacco Canada web site, the Player's Fine-Cut Tobacco was manufactured using humectants such as glycerin (2.74%), invert sugar, and propylene glycol (Imperial Tobacco Canada, 2009).

Based on data that Health Canada collected in 2004 and made public in 2006 (<http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/constitu-eng.php>), the Camel Filters 20 KS, the Gauloises Blondes, and the More 120's were blended product. The tobacco in the Gauloises Blondes product was reported to contain 20 mg/g glycerol and 7 mg/g propylene glycol. The other two products were reportedly free of glycerol and propylene glycol. The Gauloises Blondes product also had a reported tobacco ammonia level of more than 1700 $\mu\text{g}/\text{g}$, which was about 50% more than reported for the Camel and More products. The tobacco ammonia levels reported for the latter two products were much above those for products known to be free of burley tobacco. There were tobacco and smoke analytical data (nitrate, TSNAs, NO_x) that distinguished the three products just discussed from the remainder of the cigarettes studied. Our conclusions on these brands have also been supported by the analyses reported by Hammond and O'Connor (2008). The tobacco blends used in all the other cigarette products are believed to be Canadian-style all flue-cured blends.

4.2. The cross-sectional sample set

Visual inspection of only the cigarette data in Table 1a indicated that differences in the values of α_{fb} obtained with ISO smoking

Table 1c

Average physical properties for cross-sectional brands (data from this work).

Sample ID	Sample description	Tip ventilation (%)	Open cigarette pressure drop (mm)	Cigarette paper porosity (CU)
062403	Brown Tips In Bags – ILLICIT 1	<5	152	29.2
072954	Cafe Crème regular (a mini-cigarillo)	NF	131	NM
072955	Prime Time FILTRE Vanille (a mini-cigarillo)	<5	110	46.3
072956	Player's Special Blend Regular Size	6	81	66.1
072957	Camel Filters 20 King Size	28	110	24.0
072958	Player's King Size	<5	107	47.1
072959	Craven A Gold King Size	34	79	51.2
072960	Player's Fine Cut Tobacco + Filter Tubes	<5	97	33.4
072961	Gauloises Blondes (Red)	31	104	60.1
072962	Player's Plain Regular Size	NF	153	66.7
080229	More Filters 120's	NM	95	18.6
080230	Number 7 Silver King Size	60	163	44.4
080231	Honeyrose Menthol (nontobacco cigarette)	5	140	65.8
080232	Podium Gold King Size	15	106	43.8
080233	du Maurier Extra Light Regular Size	42	152	72.9

All cigarettes had standard circumference and were filter tipped unless otherwise specified; More Filters 120's were about 20 mm circumference and about 120 mm long; smoke pH values in Table 1b were obtained with ISO smoking.

All cigarettes except for Honeyrose Menthol contained tobacco; all tobacco-containing cigarettes were fabricated with Canadian-style all flue-cured blend except for Camel Filters 20 King Size, More Filters 120's, and Gauloises Blondes (Red).

BDL, below detection limit.

KS, king size (80–85 mm length).

RS, regular size (70–72 mm length).

PT, no filter (e.g., plain tip).

FT, filter tip.

NF, no filter.

NM, not measured.

might be influenced by relative amount of water in the TPM [Water/TPM(%)] and the tar/nicotine (Tar/nic ratio, T/N) ratio and that those effects were not likely found in the data obtained with HCl smoking. The data from both ISO and HCl smoking were plotted as shown in Figs. 1–4. In these figures, the data points are identified by the last two digits of the Sample ID numbers as shown in the leftmost column of Table 1a. The horizontal numbers are for ISO smoking and the vertical numbers are for HCl smoking. The best regression lines (MS Excel 2003) were determined for the following relationships as shown in Figs. 1–3, respectively: α_{fb} versus tar/nicotine ratio, α_{fb} versus the concentration of nicotine in the TPM [c_p (ng nicotine/ μ g TPM)], and α_{fb} versus the percentage of water in the TPM. Fig. 4 shows a plot of α_{fb} versus smoke pH. The pH-values were determined with Health Canada method T-113, which was derived from the original *Sensabaugh and Cundiff method* (1967) as modified by *Brunnemann and Hoffmann* (1974). Regression analysis with a quadratic function had an $R^2 = 0.88$ for the relationship between α_{fb} and smoke pH (ISO smoking).

Without knowledge of the nature and concentration of the acidic components of the MSS in the cross-sectional set, interpretation of the relationships in Figs. 1–4 requires some speculation. In Fig. 1, the data from both the More Filter 120's (29) and Camel Filters 20 KS (57) showed higher α_{fb} values than would have been expected from corresponding tar/nicotine ratios. Those data are consistent with our hypothesis that these two cigarette products contain an additive-free US-style blend. The data for Gauloises Blondes (61), whose filler is like a cased US-style blend, showed properties similar to those of all flue-cured cigarettes. The smoke from cigarettes made from cased fine-cut tobacco (60) gave a very low value for α_{fb} . This value was likely the result of a combination of high TPM water content and the smoke acids formed from the invert sugar added to the tobacco.

Decreases in the tar/nicotine ratio are likely caused by use of blends with a higher nicotine content and/or less potential of

blends to form acidic compounds on smoking (use of lower sugar flue-cured tobaccos and/or reduced sugars in burley casings used in blended products). Decreases in the tar/nicotine ratio will cause increases in the α_{fb} ratio as shown by the solid line (ISO smoking) in Fig. 1. However, the effects of changes in tar/nicotine ratio can be modulated by other factors such as the relative amount of water in TPM and filter ventilation as occurs with HCl smoking (since filter ventilation is blocked, the 55 mL puff goes through the burning coal). The results of the changes in smoking protocol are shown by points along the dotted line (HCl smoking) in Fig. 1. Those changes resulted in a slight upward trend in α_{fb} with increasing tar/nicotine ratio.

Fig. 2 shows similar effects when α_{fb} was plotted against $c_{p,nicotine}$, the concentration of nicotine in the TPM. For ISO smoking, the upwards trend in α_{fb} with $c_{p,nicotine}$, was similar to that observed for the data reported previously by *Pankow and co-workers* (2003) and was not unexpected given the relationship found between α_{fb} and the T/N ratio. However, the slight downward trend in α_{fb} with increased $c_{p,nicotine}$, under HCl smoking conditions was unexpected. The smoke data for the cigarette most like many US-styles, Gauloises Blondes (61), fell very closely to the regression line. This implies that cased US-style blends, even if they contain diammonium phosphate (DAP), do not yield α_{fb} values that are different from all flue-cured cigarettes.

Fig. 3 shows the importance of particulate-phase water to α_{fb} . The data for MSS (ISO) from all flue-cured additive-free cigarettes (56, 58, 59, 62, 30, 32, and 33) do not appear to be different from the blended products (29, 57, and 61) in the sample set. The one product whose data was off the regression line was a nonfilter cigarette (62). The data in Fig. 4, as noted earlier, showed that α_{fb} for ISO smoking was highly correlated with smoke pH as determined by the Health Canada method. The MSS data farthest from regression was for du Maurier Extra Light Regular Size, a cigarette fabricated from a no-additive Canadian flue-cured blend. Likely additive-free US-

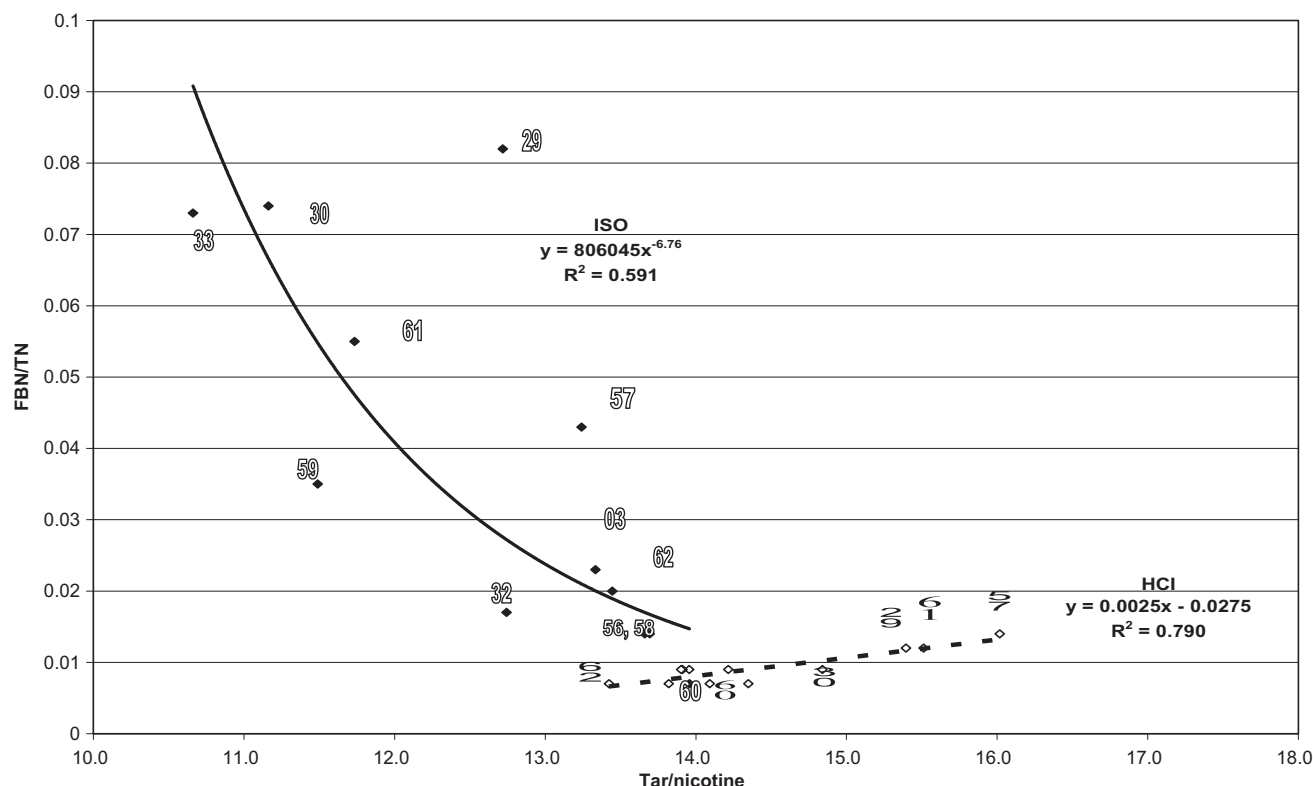


Fig. 1. Plot of FBN/TN ratio (α_{fb}) versus tar/nicotine ratio for cigarettes in the cross-sectional set with ISO and HCl smoking.

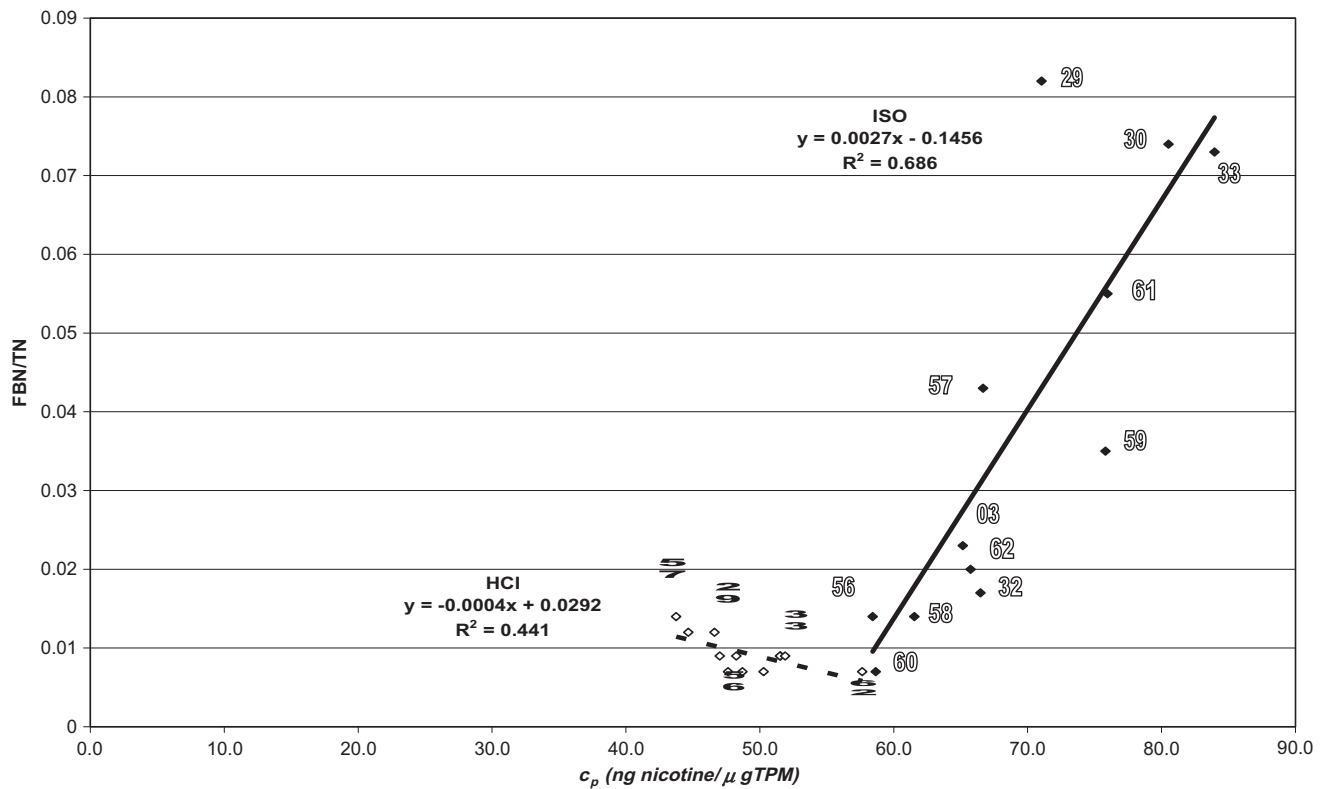


Fig. 2. Plot of FBN/TN ratio (α_{fb}) versus $c_{p,nicotine}$ for cross-sectional set with ISO and HCI smoking.

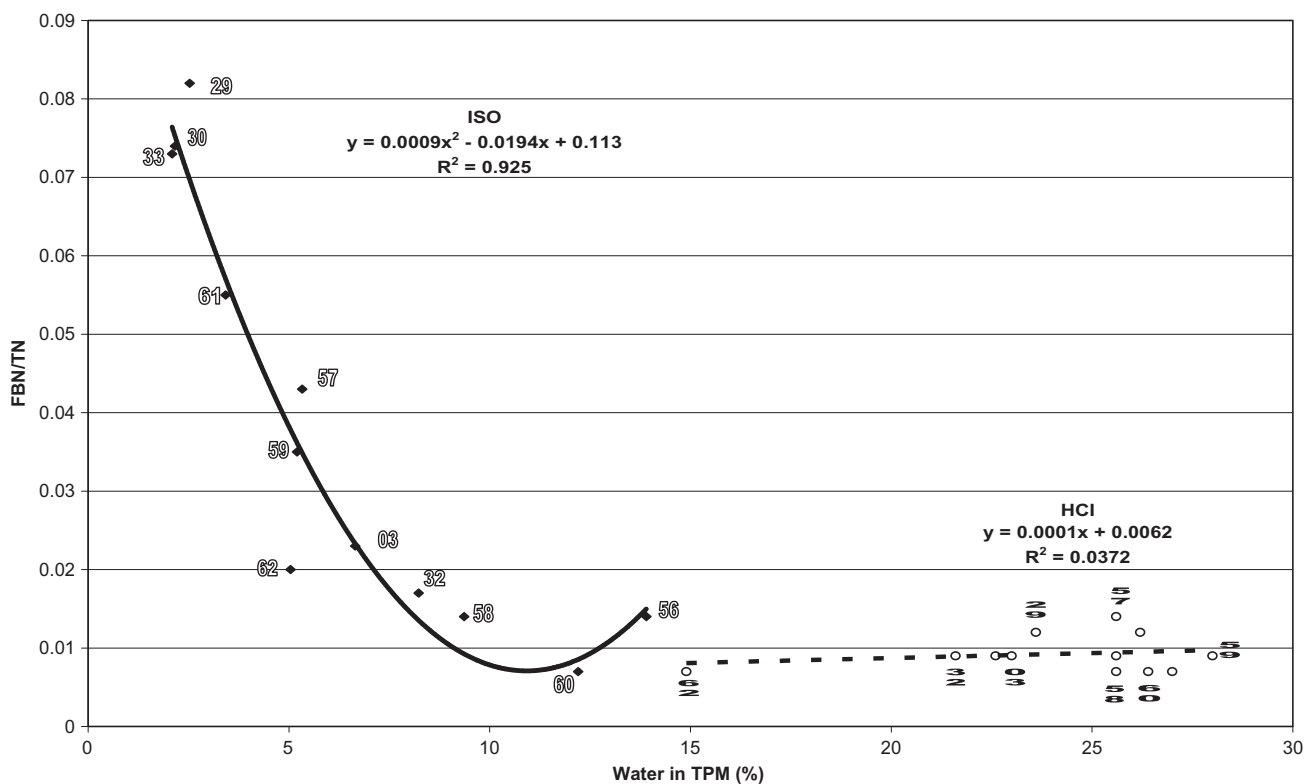


Fig. 3. Plot of FBN/TN ratio (α_{fb}) versus percentage of water in TPM for cross-sectional set with ISO and HCI smoking.

style products and a US-style product likely containing DAP as a tobacco additive did not perform differently from additive-free, all flue-cured cigarettes. More importantly, we have shown that smoke

pH measurements can provide data relevant to cigarette blend and design parameters. The regression lines shown in Fig. 4 were the ones available in MS EXCEL 2003 that gave the best fit to the data.

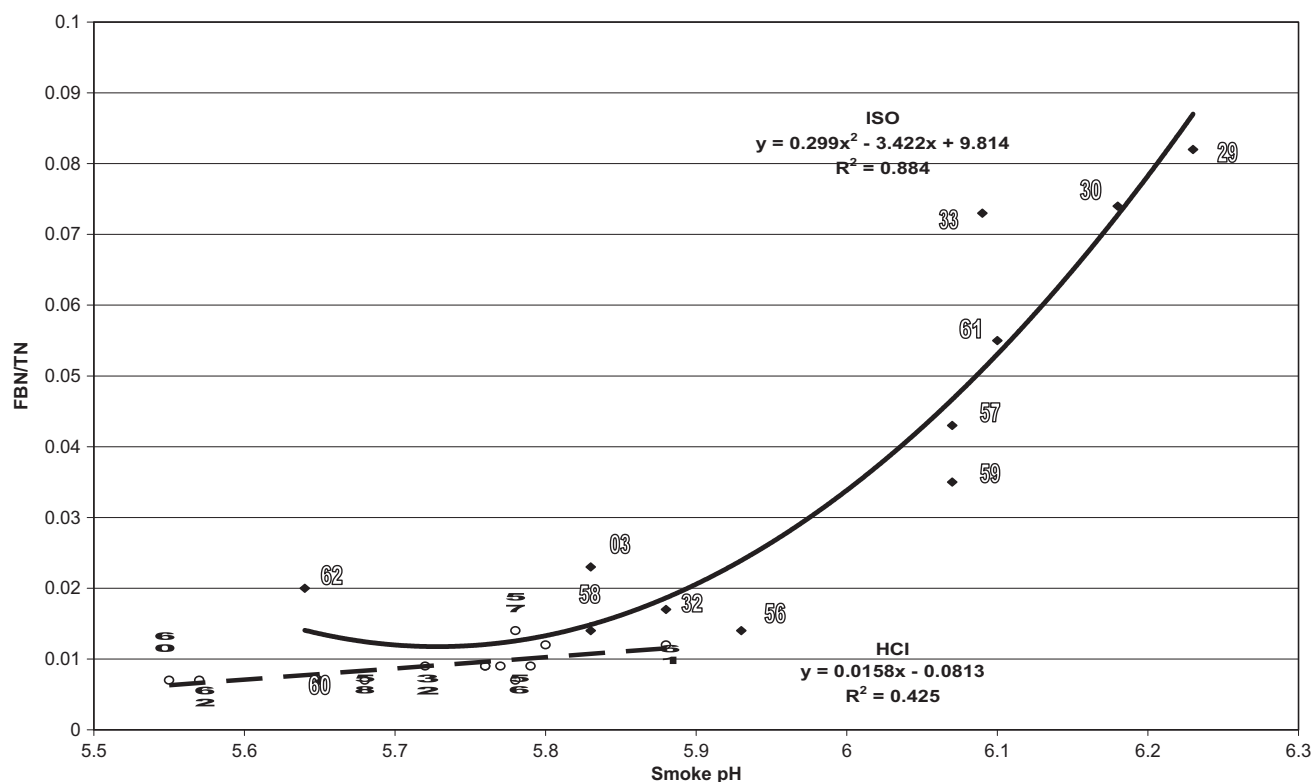


Fig. 4. Plot of FBN/TN ratio (α_{fb}) versus smoke pH for cross-sectional set with ISO and HCl smoking.

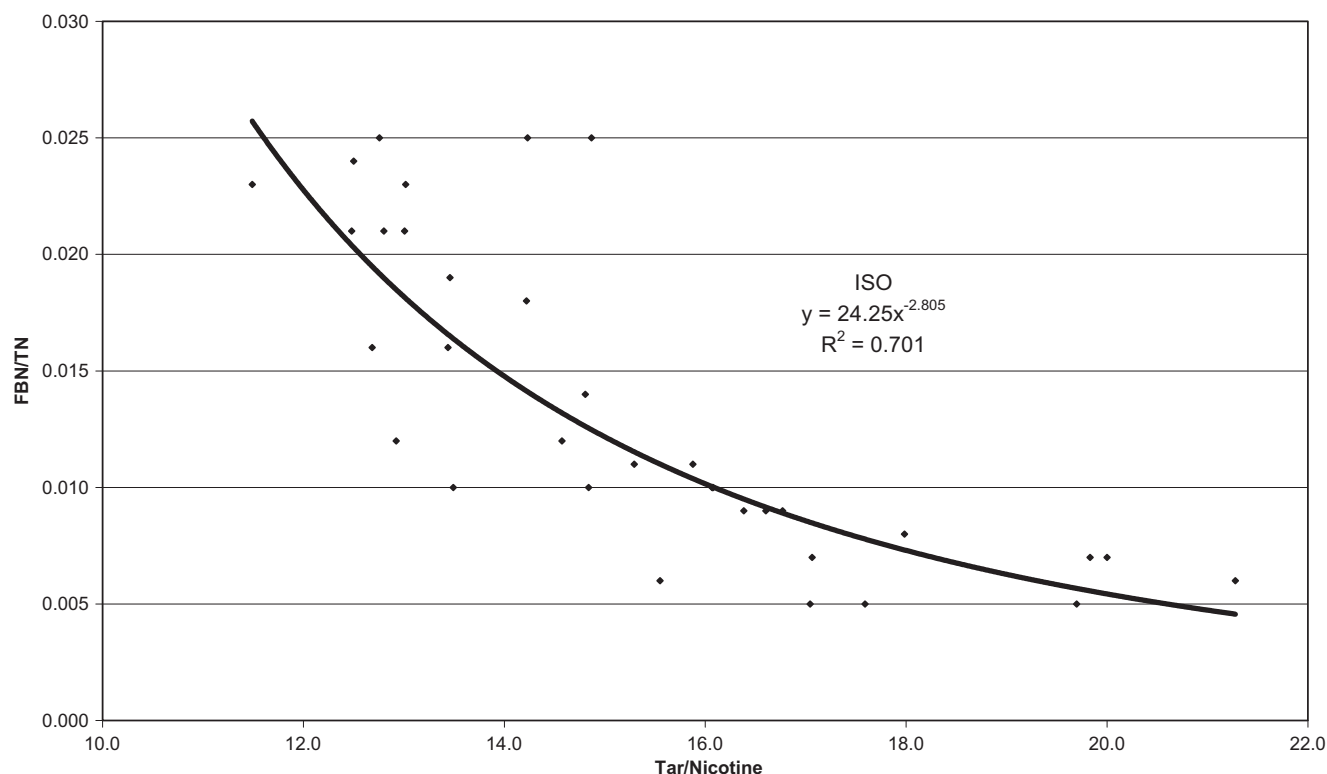


Fig. 5. Plot of FBN/TN ratio (α_{fb}) versus tar/nicotine ratio for cigarettes in the historical set with ISO smoking.

4.3. The historical set

The data on the historical products (Table 1b, ISO smoking only) also showed some interesting trends. As deliveries over the past

three decades were reduced, smoke pH-values as determined by the Health Canada method increased. This could follow from the decrease in concentration of particulate matter in the smoke aerosol, but there may have been other factors. Nicotine deliveries did

not drop as fast as tar deliveries did, so the tar/nicotine ratio (T/N) decreased. We do not know the specific product changes employed to reduce deliveries for the cigarettes used in this study. O'Connor and his co-workers have described cigarette design features, principally filter ventilation, which the tobacco industry has used to reduce deliveries (O'Connor et al., 2006, 2008). However, that would not explain the delivery and T/N decreases for the nonfiltered product (Player's Plain RSPT). Rickert and Kaiserman (1996) reported that the nicotine of the lamina in Canadian cigarettes was about 9 mg/g from 1968 to 1979 and then it rose to 13–14 mg/g in 1993–1994. Other changes in the design parameters of Canadian cigarettes over the time period covered by the historical set included an increase in tobacco nicotine content, and increase in cigarette nicotine content but a decrease in the percentage of nicotine in a cigarette that was transferred to MSS (Malaisson et al., 2007). Use of cigarette papers with higher levels of citrate and porosity could further aid in reducing tar more than nicotine (Biesse et al., 2004). How could a decrease in T/N cause an increase in smoke pH and FBN? Smoke acids, except for the very volatile ones, would be part of tar; and reductions in tar at constant nicotine could decrease the acid to nicotine ratio. Carbon dioxide levels might also be expected to decrease with decreasing tar, as would carbon monoxide levels. Regression analysis was used to investigate the possibility of relationships among the variables in the data set. Both smoke pH and the fraction of smoke nicotine that is not protonated α_{fb} were negatively correlated ($R^2 > 0.6$) with the T/N ratio. Fig. 5 shows a plot of α_{fb} versus T/N ratio. Fig. 6 shows a plot of FBN/TN versus the percentage of water in the TPM. Many of the samples in the historical set gave relatively “dry” TPM (<7% water). Fig. 7 shows a plot of α_{fb} versus smoke pH. The R^2 -value was not as high as it was for the cross-sectional set, but it indicated that smoke pH was reflecting the values determined for α_{fb} . Taken together, these findings suggest that use of FBN data to indicate the use of ammonia-containing tobacco additives is likely to be inappropriate.

Since the cigarettes in the historical set did not contain tobacco additives, the observed effects were due in part to changes in tobacco composition over time, which probably resulted in a decrease in the equivalent of smoke acids to the equivalents of nicotine. In the 1970s, the US Department of Agriculture conducted extensive studies on several types of flue-cured (bright) tobaccos and the MSS produced from unfiltered cigarettes made from those tobaccos (Tso and Chaplin, 1977). Smoke pH determinations were made with the Sensabaugh and Cundiff procedure (1967). The major tobacco variables positively correlated with smoke pH were total alkaloids, total volatile bases, and total nitrogen. The major tobacco variables negatively correlated with smoke pH were tobacco pH, tobacco cellulose, potassium, cellulose, and sucrose. Additional studies would be needed to confirm the previous findings and to identify the acidic components in the TPM that is used or the FBN determinations.

4.4. Comparison of our FBN SPME results with those of others

There are three sets of data that are comparable to our data (shown in Table 1a): (1) Watson et al. (2004), ISO smoking; (2) Lee et al. (2006), ISO smoking; and (3) Lee et al. (2006), HCl smoking. Watson et al. reported $\alpha_{fb} \sim 0.012$ for the TPM from the KY1R4F reference cigarette. We found (Bao et al., 2010) $\alpha_{fb} \sim 0.048$ for the TPM from the very similar (Chen and Moldoveanu, 2003) KY2R4F reference cigarette. Watson et al. reported $\alpha_{fb} \sim 0.072$ for the TPM of their Brand T (32% FV, 9 mg tar, 0.7 mg nicotine). We found (Table 1a) $\alpha_{fb} \sim 0.055$ for Gauloises Blondes KS (31% FV, 9 mg tar, 0.8 mg nicotine). Lee et al. (ISO) reported $\alpha_{fb} \sim 0.13$ for the TPM from the KY2R4F, while we found $\alpha_{fb} \sim 0.048$ for the TPM from the KY2R4F. Lee et al. (ISO) also reported a $\alpha_{fb} \sim 0.051$ for their Test Cig 3 (30% FV, 9 mg tar, 0.7 mg nic), we found $\alpha_{fb} \sim 0.055$ for Gauloises Blondes KS (31% FV, 9 mg tar, 0.8 mg nic). We do not know why our ISO results for the KY2R4F differed from Lee's results.

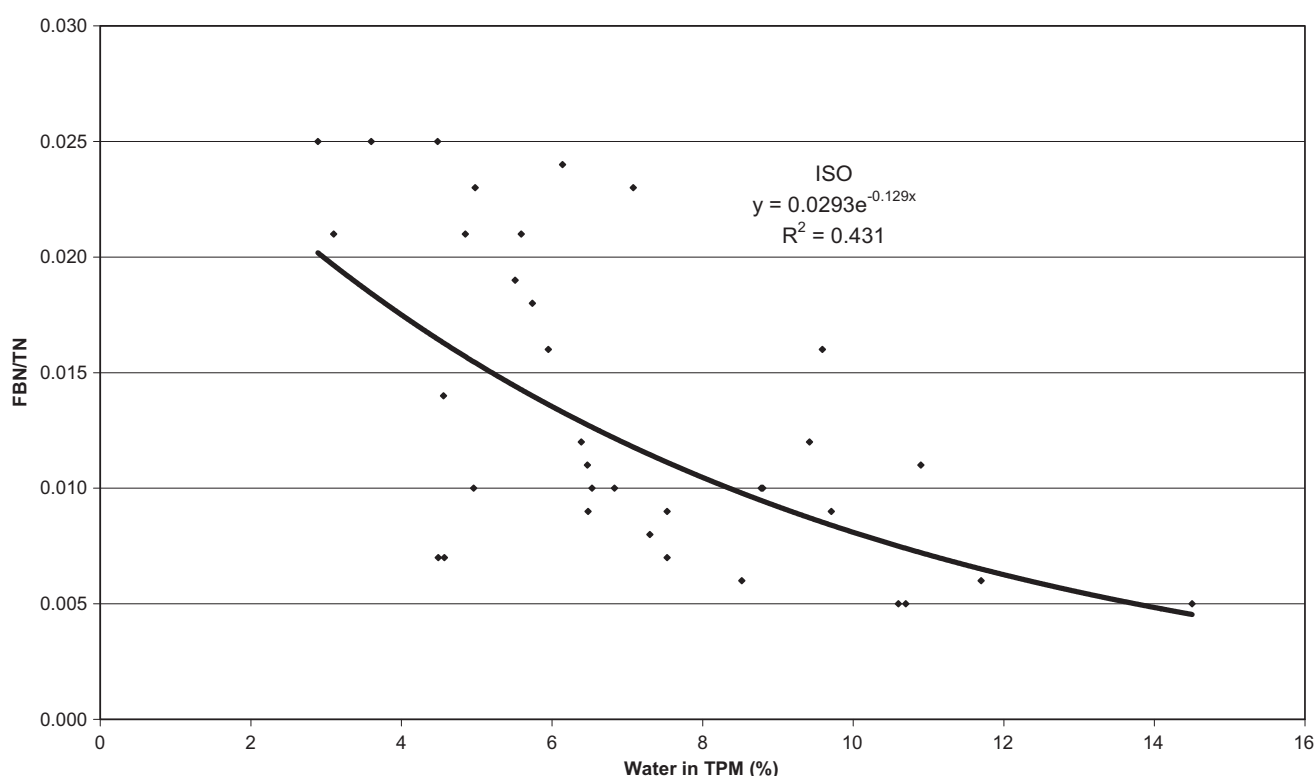


Fig. 6. Plot of FBN/TN ratio (α_{fb}) versus percentage of water in TPM for historical set with ISO smoking.

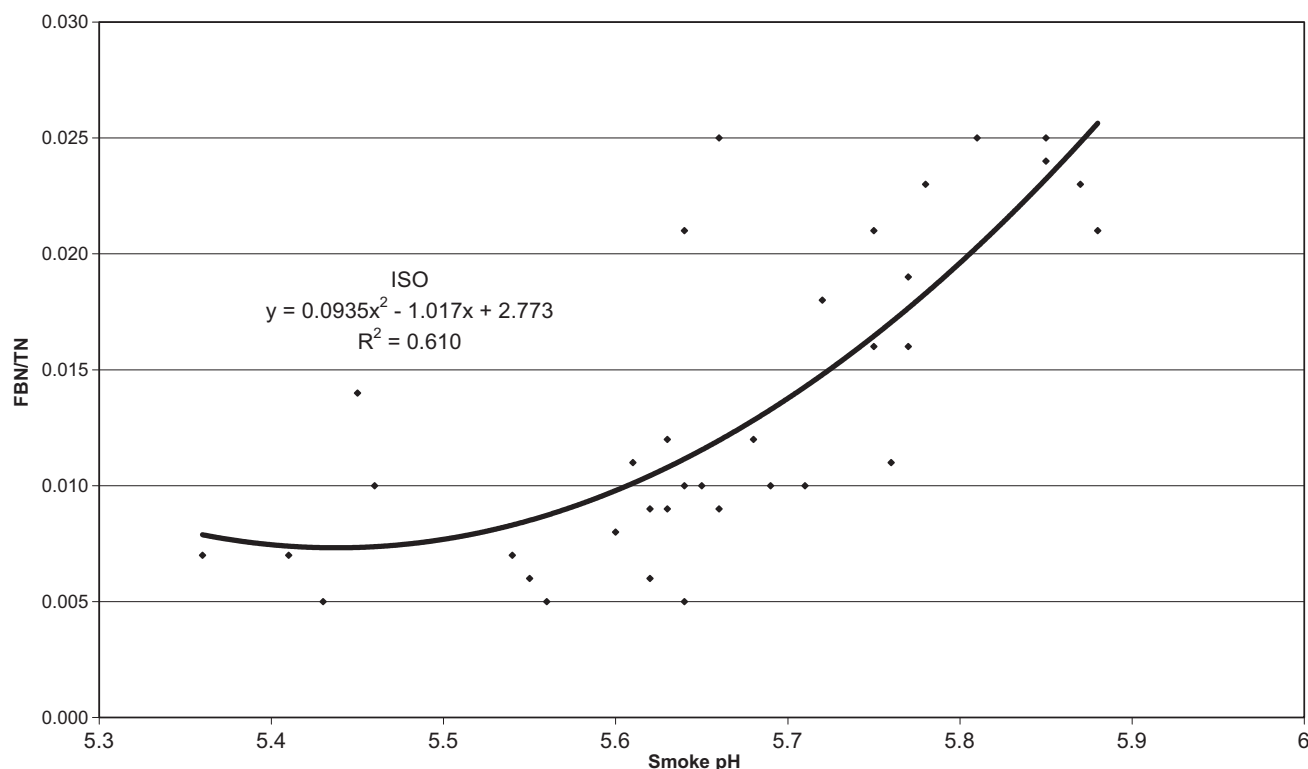


Fig. 7. Plot of FBN/TN ratio (α_{fb}) versus smoke pH for historical set with ISO smoking.

The low results Watson et al. reported for the KY1R4F may have been due to degradation over two decades of storage of the cellulose acetate and glycerol triacetate in the filter section adding acetic acid to the smoke (Baker et al., 1998). For TPM collected under HCl conditions, Lee et al. reported an α_{fb} of ~ 0.03 for KY2R4F, and we found $\alpha_{fb} \sim 0.02$ for KY2R4F. Lee et al. reported an α_{fb} of ~ 0.01 for Test Cig 3 (100% VB, 32 mg tar, 1.9 mg nic), while we found $\alpha_{fb} \sim 0.01$ for Gauloises Blondes KS (100% VB, 32 mg tar, 2.1 mg nic). Overall, there is good agreement with our results and the results reported by Lee et al.

Very recently, Chen and Pankow reported FBN data on a series of cigarettes and other smoking tobacco products purchased in the USA in 2007 (Chen and Pankow, 2009). Products were reportedly smoked using the Massachusetts Department of Public Health (MDPH) puffing and vent-blocking conditions (Massachusetts General Laws, 1997). However, an automated smoking machine and Cambridge filter pads as specified in the Massachusetts regulations were not used. Methodology used was reportedly similar to that which Pankow and his colleagues used in their 2003 report (Pankow et al., 2003). In this very recent work, Chen and Pankow reported that $P_{g,nicotine}(\%)$ ranged from 0.0028% for Newport KS (HP, hard pack) to 0.086% for American Spirit Red HP. The corresponding α_{fb} values [α_{fb}^{nic} in the Chen and Pankow paper and estimated with a value of $\log K_{p,nic}^{nic}$ of -5.03 (Pankow et al., 2003)] ranged from 0.011 for Craven A KS HP to 0.174 for American Spirit Red HP. For the blended cigarettes we studied, we found for ISO smoking conditions that α_{fb} was 0.043 for Camel Filters 20 KS, 0.082 for the More 120's, and 0.055 for the Gauloises Blondes KS. Under Health Canada Intensive smoking conditions, those values had dropped to 0.014, 0.012, and 0.012, respectively. Use of the regression equation in Fig. 2 (α_{fb} versus $c_{p,nicotine}$), and Pankow's value of $c_{p,nicotine}$ of 117.6 ng/ μ g for American Spirit Red HP, the estimated value of α_{fb} is 0.172, which compares to the value of 0.174 that was estimated from the values reported by Chen and Pankow for $\log K_{p,nicotine}$ and $\log K_{p,nic}^{nic}$. The American Spirit brands are well

known for having additive-free US-style tobacco blends so the high value for α_{fb} is related to the high concentration of nicotine in the TPM, not to the use of ammonia-containing additives (Tobacco Products, 2009). The cigarette brand-style Basic KS HP was also tested by Chen and Pankow. Basic KS HP reportedly used both diammonium phosphate and ammonium hydroxide as tobacco additives. Chen and Pankow reported values of 81.6 ng/ μ g for $c_{p,nicotine}$, -3.43 for $\log K_{p,nicotine}$ and 0.0063% for $P_{g,nicotine}(\%)$ to give an estimated value of 0.0251 for α_{fb} . Use of the regression equation from Fig. 2 and the value of 81.6 ng/ μ g for $c_{p,nicotine}$, provides an estimated value for α_{fb} of 0.075. The TPM of 18.8 mg/cig reported by Chen and Pankow is closer to value expected for ISO smoking (about 19 mg/cig) than it is to the much higher value expected for MDPH smoking conditions. The estimated value for $c_{p,nicotine}$ would be about 60 ng/ μ g, which would give an estimated value for α_{fb} of 0.02.

4.5. Summary of results

In summary, the results shown in Tables 1a and 1b appear to be very consistent with the designs of the cigarettes tested and the smoking conditions used. In addition, they support the hypothesis that high values observed for α_{fb} when cigarettes are smoked using the ISO or FTC protocols are not caused by the use of ammonia or its compounds as tobacco additives, but are caused by use of tobaccos and other cigarette design features that increase the concentration of nicotine in the TPM (Lauterbach and St. Charles, 2004; Lauterbach, 2003). Moreover, as shown by the data graphed in Fig. 3, insufficient conditioning of the cigarettes and/or cigarette design features (e.g., insufficient use of humectants to achieve a particulate-phase water level of at least 10% of TPM weight) results in drier than normal TPM. This, in turn, drives α_{fb} up. Furthermore, when cigarettes are smoked under HCl conditions, α_{fb} values are under 0.02, and $P_{g,nicotine}(\%)$ are under 1% of total smoke nicotine. Thus, under the normal range of human use of commercial ciga-

rette products, the percentage of FBN in MSS TPM is toxicologically insignificant.

5. Discussion

5.1. The concept of free-base nicotine in tobacco smoke

The concept of free-base nicotine in tobacco smoke has been one of the more controversial ones in the tobacco industry over the last two decades. It has made headlines in the Wall Street Journal (Freedman, 1995). A constant theme throughout the discussion of free-base nicotine and contemporary tobacco products is that the higher the ratio of free-base nicotine to total nicotine, the higher the addictiveness of the product, and the greater its market share. Yet if that theme were true, most smokers would be smoking dark air-cured cigarettes and/or cigars. Thus, factors other than FBN are important in consumers' choices. These other factors could include marketing efforts (brand image, price, availability, etc.) and smokers' preference for a different mixture of hedonic attributes (taste, body, irritation, etc.).

The science behind free-base nicotine has been misunderstood by scientists both in the industry and outside of the industry. First, free-base nicotine is a misnomer. Organic bases such as nicotine are either protonated or they exist as the neutral molecule. Second, analogies between free-base nicotine and the unprotonated forms of a common illicit drug have been shown to be incorrect. Both of these aspects of nicotine chemistry will be discussed below.

Some of the older literature is often quoted as examples of deliberate attempts of tobacco industry chemists to increase smoke pH and increase free-base nicotine. One report often cited was written by Claude Teague of R.J. Reynolds Tobacco Company (Teague, 1973). Teague stated, "In addition to enhancing nicotine 'kick', increasing the pH (increasing alkalinity) of smoke above about 6.0 causes other changes, particularly when the increase in smoke pH is achieved by adding ammonia to the blend. As smoke pH increases, in general stemmy taste, mouth irritation, flue-cured flavor, and Turkish flavor are diminished, and burley flavor and character are enhanced." We do not know how much information Teague had when he made his statement. What we know now is that the sugars in the tobacco and casings reacted with ammonia and certain of its compounds such as DAP (diammonium phosphate) forming Maillard polymers. Maillard polymers pyrolyze to a mixture of nitrogenous compounds similar to those formed from the pyrolysis of burley tobacco in a cigarette (Lauterbach, 2000, 2007; Agyei-Aye et al., 2002; Moldoveanu et al., 1989; Hayase and Kato, 1981; Tsuchida et al., 1976). Thus, treatment of flue-cured tobacco or blends of flue-cured, sugar-cased burley, and Turkish tobaccos such as found in American-blend cigarettes with ammonia and/or certain of its compounds results in smoke with increased burley character (Cox et al., 1987). As described by Agyei-Aye and co-workers, diammonium phosphate (DAP) reacts much faster than other ammonium salts with common reducing sugars found in flue-cured and sugar-cased burley tobaccos (Agyei-Aye et al., 2002). Furthermore, as reported by Hayase and Kato (1981) and by Tsuchida et al. (1976), pyrolysis of Maillard polymers and Amadori compounds yields large amounts of carbon dioxide, acetic acid and other acids. While Moldoveanu and colleagues did not specifically mention the acids in their 1989 report, a review of the chromatograms and mass spectral identifications they reported showed that acetic acid was a major pyrolysis product of the Maillard polymer they had produced. Taken together all these reports suggest that when ammonia and/or certain of its compounds are added to cigarette tobacco, the ammonia reacts with the reducing carbohydrates to form Maillard polymers (which remain in the tobacco); and when such polymers are pyrolyzed

during the smoking process, acidic compounds are formed. Such acidic compounds would neutralize any unprotonated nicotine (FBN) that may be in the MSS aerosol.

Robert R. Johnson, a well-known tobacco industry chemist also made a similar mistake when he explained what happened when highly ventilated cigarettes were smoked with the FTC protocol. In the November 1983 monthly report for part of Brown & Williamson's Product Development department, Johnson wrote, "Tar/Nic ratios can best be rationalized by assuming that smoulder-released nicotine greatly enriches the first part of a puff, which is all that gets delivered from a highly ventilated cigarette. This also explains higher smoke pH of these cigarettes (more free-base nicotine), and higher Tar/Nic ratios from smokings at two puffs/min (less smoulder time)" (Johnson, 1983). What Johnson failed to consider was that the smoke pH method used at that time only considered the mainstream particulate matter trapped on the Cambridge pad (Lauterbach, 1997a). The three main smoke acids are carbonic acid, acetic acid, and formic acid (Ingebrethsen et al., 2001; Lagoutte et al., 1994; Sakuma et al., 1983). These acids are far more volatile than nicotine. Thus, when highly ventilated cigarettes are smoked under the FTC or ISO conditions, these acids are predominately in the smoke vapor phase while the nicotine is predominately in the particulate phase. Consequently, the effect reported by Johnson was *not* caused by the increase in free-base nicotine, but by the decrease in organic acids retained on the Cambridge pad. Furthermore, we now know that smokers do not smoke highly ventilated cigarettes in the same manner as they are smoked with the FTC protocol that was implied by Johnson's comments (the only smoking machines at B&W in 1983 were FTC machines). Most smokers of highly ventilated cigarettes partially occlude filter ventilation and take larger and more frequent puffs than called for in the FTC regulations (St. Charles et al., 2006; Thun and Burns, 2001). As will be shown in Section 4.2, such smoking conditions inhibit the formation of vapor-phase nicotine (also known free-base nicotine).

One of the more prominent examples of a misrepresentation of data related to nicotine in tobacco and tobacco smoke can be found in a 1997 publication by Pankow and co-workers on the gas-particle partitioning of nicotine in mainstream cigarette smoke (Pankow et al., 1997). In that article, the authors alleged that ammonia and its compounds are added to tobacco to volatilize nicotine in an analogous manner to the addition of base to cocaine hydrochloride to make cocaine volatile. However, a review of the literature (Lauterbach, 2000) showed that while nicotine is easily volatilized from its salts without decomposition during thermolysis (Seeman et al., 1999), cocaine salts decompose (Nakahara and Ishigami, 1991; Martin et al., 1989).

5.2. Gas-particle partitioning of nicotine in mainstream cigarette smoke (MSS)

Irrespective of type of cigarette and/or puffing conditions, the percentage of unprotonated nicotine (or for that matter, the percentage of any semivolatile compound that is in the vapor phase) in mainstream cigarette smoke (MSS) can be estimated by using Pankow's theory of absorptive partitioning (Pankow et al., 1997, 2003, 2004; Pankow, 1994a,b, 2001; Lauterbach, 2000) in conjunction with the formula shown in Eq. (1) (see Glossary for definition of terms). Pankow's theory and formulae assume that volatile and semivolatile compounds (SVC) in the aerosol are at thermodynamic equilibria between the gas-vapor phase and the particulate phase of an aerosol. That assumption is *not* true for mainstream cigarette smoke. However, sufficient research has been done on MSS and other combustion aerosols to show the equations that Pankow derived from his theory can be used to *estimate* the extent to which nicotine and other semivolatile compounds partition be-

tween the gas–vapor phase and particulate phase of the MSS aerosol and how the presence of acidic or basic compounds may partition differently than do neutral compounds.

We will refer to the work of several different research groups to support our contention that reasonable estimates of gas–particle partitioning (GPP) can be made using the equations that were derived by Pankow. First, we will cite the work of Susan Vekris of BAT. A summary of Vekris's work was presented at the 1966 Tobacco Chemists' Research Foundation (Vekris and Hook, 1966; Johnson, 1967). However, the full experimental details and development of a theoretical framework similar to Pankow's were not made public until copies of the reports were available at tobacco document libraries such as the Legacy Tobacco Documents Library at University of California at San Francisco (<http://legacy.library.ucsf.edu>). A newer summary of the Vekris's work, which was based on the original reports, was presented at the 2006 Tobacco Science Research Conference (Lauterbach, 2006). As best as we can tell from Vekris's reports, her research was initiated to support studies aimed at developing better selective filtration systems. To be selectively filtered, compounds needed to be at least partly in the gas–vapor phase, the transfer from particulate phase to the gas–vapor phase was assumed to be via distillation mechanism, and equilibrium between gas phase and particulate phase was assumed to be established very rapidly. In a series of six reports (Vekris, 1965, 1966a,b, 1967, 1968a,b), Vekris provided the experimental proof of applicability of GPP theory to MSS by measuring the concentrations of SVC in both phases of MSS. In addition, her measurements made on absorption of pyridine vapor from whole smoke and from smoke passed through normal or heat-treated (to remove organic binder) Cambridge filter pads showed that heat-treated Cambridge filter pads were shown to have minimal effect of pyridine vapor concentrations. Vekris recognized that the MSS aerosol was not at equilibrium. She cited the following examples of evidence for a dynamic equilibrium of SVC between the two phases of MSS: particle-size growth in the MSS aerosol by condensation of existing particles, selective filtration by the distillation mechanism is dependent on evaporation rate from particulate phase to the gas–vapor phase, displacement of low-volatility flavors from tobacco rod or filter tip is more efficient with MSS than air, selective filtration of phenol by plasticized CA filters occurs even though less than 5% of phenol in the gas phase, and distribution of an SVC in MSS phases through the Cambridge filter is constant and approximates calculated equilibrium distribution. Vekris showed that dynamic equilibrium was attained on a time-frame that was short (probably between 10 ms and 0.1 ms). According to the abstract of Vekris and Hook as shown in the 20th Tobacco Chemists' Research Conference Program Booklet and Abstracts (Vekris and Hook, 1966). "For a given compound in a given type of smoke, the ratio of the concentrations in the two phases is independent of the total amount present. In other words, there is a linear partition between the phases. For compounds of the same chemical type in the same smoke, the ratio increases as the vapor pressure of the pure compound increases. In general, this trend does not extend to compounds of different types or to smokes from different tobaccos. The results are consistent with the hypothesis that a dynamic equilibrium exists between the two phases. The deviations from a simple theory based on Raoult's law are of the order of magnitude which would be expected for equilibrium in a multicomponent system."

In addition to the research of Vekris, we also have the research of Professor Richard M. Kamens and his group at the University of North Carolina. They studied wood smoke and diesel engine exhaust. For some of their work, they used small (two cubic meter) environmental chambers to collect the smoke aerosol. The wood smoke aerosol contained organic acids. They found that they had to use Pankow's equation with activity coefficients other than

unity (Jang et al., 1997; Jang and Kamens, 1998; Kamens et al., 1999). The concept of activity coefficients is very important to the understanding of the GPP of nicotine in MSS as we will show in Section 5.6. Another demonstration of the relevance of GPP and the relevance of estimates of GPP using Pankow's equations can be found in the work of Kalaitzoglou and Samara on MSS *n*-alkanes and polynuclear aromatic hydrocarbons (2006, 2005). The work of Kalaitzoglou and Samara is important because they used cigarettes with a wide variety of designs (nonfilter, filter 100's, slim 100's) and deliveries (17 mg/cig to 1 mg/cig ISO TPM) and their results compared favorable with estimates from GPP theory.

5.3. Equations for estimating gas–particle partitioning of nicotine and other SVC in MSS

We must always remember that when we discuss GPP of SVC in MSS we are dealing with nonequilibrium conditions and that use of equations derived for equilibrium conditions are not truly applicable and provide only estimates of the extent of partitioning. It must also be remembered that MSS water is a SVC and that water partitions extensively between the two MSS phases (Lauterbach, 2005, 2001). Please refer to the Glossary for the definitions of terms used in the following paragraphs.

For any given SVC, *i*, the percentage of *i* in the vapor phase is as follows.

$$P_{g,i}(\%) = 100\% \{1/(1 + K_{p,i}TSP)\} \quad (1)$$

$$P_{g,i}(\%) = 100\% \{c_{g,i}/(c_{g,i} + c_{p,i}TSP)\} \quad (2)$$

Examination of Eq. (1) shows that as TSP decreases [e.g., dilution of the smoke aerosol by a high level of filter ventilation for a cigarette smoked under ISO (or FTC conditions)], $P_{g,i}(\%)$ will increase provided the gas–particle partitioning coefficient, for compound *i*, $K_{p,i}$, remains constant. Another definition of $K_{p,i}$ is given in Eq. (3).

$$K_{p,i} = F_i/TSP/A_i = c_{p,i}/c_{g,i} \quad (3)$$

Eq. (3) was developed by Pankow for environmental aerosols (Pankow, 1994a). He applied it to mainstream cigarette smoke in his later articles (Pankow et al., 1997, 2003; Pankow, 2001). In the case where compound *i* is nicotine, $K_{p,nicotine}$ can be calculated from experimental data such as those reported by Callicutt et al. (2006a,b). Those data are given in Table 2 along with other parameters that can be calculated from them.

However, other factors affecting $K_{p,i}$ need to be considered as shown in Eq. (4), which comes from Pankow's theory of absorptive partitioning (Pankow, 1994b).

$$K_{p,i} = (f_{om}760RT)/(MW_{om}\gamma_i p_{L,i}10^6) \quad (4)$$

It is clear from Pankow's development of his theory of absorptive partitioning that he was considering atmospheric aerosols where the organic matter surrounded a dust particle or other material that did not participate in the partitioning of the organic matter. Indeed, Pankow estimated f_{om} to be in the range of 0.1–0.3 for urban particulate matter and at about 0.2 for secondary organic aerosols in urban environments (Pankow, 1994a,b). However, in the particulate phase of MSS, virtually all of the inorganic matter is water, and the water behaves in a manner similar to a semivolatile organic compound. This, it appears that assuming a value of unity for f_{om} is reasonable (Lauterbach, 2001). This assumption is also supported by the pioneering work of Vekris and Hook (1966) on the application of gas–particle partitioning theory to mainstream cigarette smoke as detailed in the 40th anniversary reprise of their work at the 2006 Tobacco Science Research Conference (TSRC) (Lauterbach, 2006).

To use Eq. (4) to estimate $K_{p,nicotine}$ for mainstream cigarette smoke, we need estimates of MW_{om} , $\gamma_{nicotine}$, and $p_L^{\circ}nicotine$. Based on the work of Oja and Hajaligol (1999, 1998), values of 202 g/mol and 262 g/mol would be good estimates of the number-average and weight-average molecular weights for dry tar from presumed additive-free cigarettes. However, water in the TPM as well as glycerin and propylene glycol (if those glycols are used as tobacco humectants in the amounts typically used in US-style cigarettes) in the TPM, can reduce the values of MW_{om} to well under 100 g/mol (Lauterbach, 2005, 2001). The best available value for the supercooled vapor pressure of nicotine, $p_L^{\circ}nicotine$, was determined experimentally to be 0.021 torr at 25 °C (Ray, 1990). Based on estimates of $P_{g,nicotine}(\%)$ reported in the literature and in conference proceedings along with other factors needed (e.g., estimates of TSP and MW_{om}), estimates of $\gamma_{nicotine}$ have been made (Lauterbach, 2000). These data showed that for full flavor filter cigarettes, $\gamma_{nicotine}$ is less than 0.5. Pankow and colleagues reported a value of 0.8 for $\gamma_{nicotine}$ for Camel KS and Marlboro KS smoked under conditions that approximated those prescribed by the MDPH, and $P_{g,nicotine}(\%)$ was estimated at 0.4% (Pankow et al., 2004; Massachusetts General Laws, 1997). However, when the cigarettes were smoked under the mandated MDPH conditions, and a value of unity was assumed for $\gamma_{nicotine}$, $P_{g,nicotine}(\%)$ was estimated to be 0.04% (Lauterbach, 2005). The difference was caused by two factors: (1) Pankow used a higher vapor pressure for nicotine [0.0245 torr at 20 °C instead of the 0.012 torr at 20 °C reported by Ray (1990)]; and (2) the much higher water content of the TSP when the cigarettes were smoked according to the MDPH protocol. Indeed, Pankow has repeated his measurements; and has now reported $P_{g,nicotine}(\%)$ for Camel KS and Marlboro KS of $0.0062 \pm 0.0009\%$ and $0.0037 \pm 0.0014\%$, respectively (reportedly Massachusetts DPH smoking conditions; Chen and Pankow, 2009).

When the relative water content of the TSP increases, MW_{om} decreases. For example, using the data from Counts et al. (2005) for Marlboro KS soft pack made for the US market, and considering only water, nicotine, and tar, the estimated values of MW_{om} resulting from use of FTC, MDPH, and HCl smoking conditions were 98 g/mol, 52 g/mol, and 46 g/mol, respectively. Particulate-phase water as a percentage of TSP for the same cigarettes and smoking conditions were 12%, 29%, and 34% respectively. Estimated values for $K_{p,nicotine}$ (assuming a value of unity for $\gamma_{nicotine}$) were $9.02 \times 10^{-6} \text{ m}^3/\mu\text{g}$, $1.69 \times 10^{-5} \text{ m}^3/\mu\text{g}$, and $1.92 \times 10^{-5} \text{ m}^3/\mu\text{g}$; and TSP values were $7.67 \times 10^7 \mu\text{g}/\text{m}^3$, $1.64 \times 10^8 \mu\text{g}/\text{m}^3$, and $3.25 \times 10^8 \mu\text{g}/\text{m}^3$, respectively. These data gave estimated values of $P_{g,nicotine}(\%)$ of 0.14%, 0.04%, and 0.02%. The calculations behind the values just mentioned are shown in Table S2 of the Supplementary data.

It is important to note that these values are on the same order of magnitude as those reported almost a decade ago at the 53rd TSRC by two different research groups using two completely different methods (Joseph and Cochran, 1999; Kinser et al., 1999). This is a key reason why all the smoke pH-values in Tables 1a and 1b are below pH 7 with the exception of one of the two cigarillo samples. It also explains why all our smoke pH-values with the exception of one of the two cigarillo samples were under pH 6 when the HCl smoking protocol was used. Why is one of the cigarillo samples different? As noted earlier, it may be a combination of blend, wrapper, and use of cigarette smoking conditions as reported by Schmeltz et al. (1976) such that mainstream ammonia was so high as to overwhelm the normal smoke acids.

5.4. Differences between $P_{g,nicotine}(\%)$ and α_{fb}

When Pankow's original experimental approach (Pankow et al., 1997) for the determination of FBN in whole mainstream cigarette smoke was challenged by several research groups operating inde-

pendently (Joseph and Cochran, 1999; Kinser et al., 1999; Lauterbach, 2000; Ingebrethsen et al., 2001), he then focused his efforts on just the mainstream particulate matter (TPM). In his new experimental approach (Pankow et al., 2003), the cigarettes were smoked using MDPH puffing and vent-blocking conditions, and the smoke drawn into an evacuated Teflon bag. The aerosol in the bag was sampled before and after addition of excess ammonia gas. One bag was used for the first three puffs and another for the remaining puffs. $K_{p,nicotine}$ was determined using Eq. (3). A second equilibrium constant, $K_{p,fb}^{nic}$, was determined after the ammonia addition and the ratio of $K_{p,fb}^{nic}/K_{p,nicotine}$ was equivalent to the fraction of nicotine in the TPM that was not protonated (α_{fb}). From that ratio and the second pK_a value for nicotine, a quantity called pH_{eff} could be calculated. Pankow claimed that pH_{eff} was the only true measure of smoke pH since all the experimental methods were flawed. This approach was based on an earlier publication (Pankow, 2001) where the assertion was made that carbon dioxide and other acidic compounds in the vapor phase of mainstream cigarette smoke were not relevant to the volatility of nicotine in the TPM and that whole-smoke pH techniques were not valid. As noted in Section 3 of this report, our data (as presented in Figs. 4 and 7) does not support Pankow's contention that carbon dioxide and other acidic compounds in the vapor phase of mainstream cigarette smoke were not relevant to the volatility of nicotine in the TPM.

After there were additional challenges to the approach just described, several research groups have attempted to use solid-phase microextraction (SPME) techniques applied to TPM collected on a Cambridge filter pad to determine the concentration of FBN in TPM. One key assumption in the use of these SPME techniques is that the composition of the TPM collected on the Cambridge filter pad represents equilibrium conditions with the gas-vapor phase of the smoke aerosol. Another key assumption is that the chemical composition of the TPM is not altered by any aspect of the SPME technique. As detailed below, care must be taken with all aspects of the smoke collection and SPME experiment to prevent artifact formation. Moreover, it appears that attempts to determine FBN in mainstream cigarette smoke using solid-phase microextraction (SPME) may also be affected by the relative amount of water in the TPM.

For example, in their report of their use of SPME to estimate FBN trapped in TPM, Watson and co-workers reported that the chromatographic responses of nicotine and toluene- d_8 decreased as the amount of TPM on the Cambridge pad increased as more puffs were collected from the smoke of a denicotinized cigarette (Watson et al., 2004). Those authors claimed that the relative response factors were unchanged. However, their findings point out the weakness of using SPME for determining the fraction of smoke particulate-phase nicotine that is unprotonated. The amount of nicotine in the headspace above the TPM-loaded Cambridge pad is controlled by the partial pressure of the nicotine in the headspace. The partial pressure of the nicotine in the headspace is proportional to MW_{om} . This should not be a surprising fact as it comes about from the derivations of $K_{p,i}$ developed by Vekris and Hook (1966) and by Pankow (1994a,b). Another caveat with the SPME technique as applied by Watson is that the temperature used to collect the nicotine on the SPME fiber cannot be different from the temperature at which the TPM is collected. Any difference in temperature would change vapor pressures and activity coefficients for the compounds that affect the volatility of nicotine; and hence, it would be difficult to maintain the integrity of the determinations especially when the internal standard (toluene- d_8) was not chemically similar to the analyte.

For full-flavor cigarettes with an ISO/FTC delivery of about 1 mg/cig particulate-phase nicotine, Watson reported finding between 15 and 80 $\mu\text{g}/\text{cig}$ FBN (estimated from Fig. 1 of Watson

et al., 2004). This corresponds to α_{fb} values of around 0.015–0.080. For light cigarettes (~ 0.8 mg/cig nicotine), the α_{fb} values ranged from about 0.013 to 0.09. For all the ultralight cigarettes (~ 0.45 mg/cig nicotine) except for Watson's cigarette "U", α_{fb} values varied from about 0.09 to 0.14. Cigarette "U", which was designed to yield less than 1 mg tar and 0.1 mg nicotine, had a α_{fb} value of about 0.3. Does this mean that 30% of the nicotine is in the gas-vapor phase of the MSS aerosol? Probably not as $\alpha_{fb} = -K_{p,fb}^{nic}/K_{p,nicotine}$ and $K_{p,fb}^{nic} = 9.33 \times 10^{-6} \text{ m}^3/\mu\text{g}$. Thus, $K_{p,nicotine} = 3.11 \times 10^{-5} \text{ m}^3/\mu\text{g}$. If we assume a TPM of 1.2 mg/cig and 8 puffs/cig, $TSP = 4.29 \times 10^6 \mu\text{g}/\text{m}^3$. By Eq. (1), $P_{g,nicotine}(\%) = 0.74\%$. A similar cigarette (TPM = 2 mg/cig), was reported to have a total nicotine delivery of 0.18 mg/cig and a vapor-phase nicotine delivery of 0.00137 mg/cig (Callicutt et al., 2006b). Those experimental values give $P_{g,nicotine}(\%) = 0.76\%$.

Lee and colleagues repeated Watson's work and also extended it to the MDPH and HCl protocols (Lee et al., 2006). They used both linear and rotary smoking machines for the ISO smoking and linear smoking machines for the MDPH and HCl smoking. They also determined whole-smoke pH by collecting the smoke from one cigarette per brand-style into two impingers containing 20 mL deionized water and determining the pH of the aqueous smoke suspensions with a pH meter. The cigarettes in their sample set included the 2R4F reference cigarette, several commercial products with filter ventilation levels ranging from 34% to 82% and several test products that were identical except for the levels of filter ventilation, which were 0, 10, 30, 50, and 70%. The results from Lee and his co-workers are shown in Table 1d. The values for FBN and FBN/TN had to be estimated from the bar charts that were in the presentation materials.

The results presented by Lee and his co-workers (2006) are quite helpful in explaining the results presented in Tables 1a and 1b. First, the whole-smoke pH-values were very much as expected with all samples having pH-values less than 5.26 and with most results in the range between 4.97 and 5.10. These findings reinforce the conclusion that for most flue-cured and blended cigarettes, the pH of the whole mainstream smoke (what the smoker inhales) is acidic. It also implies that the particulate-phase water entrapped in the TPM is acidic and is likely saturated with carbon dioxide based on the data reported by Ingebrethsen and colleagues (2001). Indeed, the pH-values of aqueous extracts of the TPM (Cambridge filter pads) from mainstream cigarette smoke are generally less than 7 and often less than 6, but residual alkalinity on the pads can raise those values (Lauterbach, 1997a). Therefore, one would expect the SPME determinations would show very little FBN. Then how did Lee and his colleagues get much higher levels of FBN than would be expected from classical measurements (e.g., pH of aqueous extracts of TPM)? Lee and his co-workers may have provided the answer to that question. When they used a rotary smoking machine (Borgwaldt RM20/CS) instead of a linear machine (Cerulean ASM 500), the FBN levels were higher, especially for highly ventilated cigarettes. One explanation for this is that the sidestream smoke from one cigarette was dragged over the adjacent cigarette as the carousel of the RM20/CS turned. Another explanation is that the airflows in an ISO-harmonized rotary smoking machine such as the Borgwaldt RM20/CS go from the lit end of the cigarette back towards the butt end before entering the exhaust duct. Thus, sidestream smoke can be drawn through the porous cigarette paper and filter ventilation holes of the cigarette being puffed. Sidestream smoke contains a much higher concentration of ammonia than does mainstream smoke (Sakuma et al., 1984). Thus, the effect found by Lee and his colleagues may have been due to added alkalinity contributed by the ammonia in sidestream smoke (Lee et al., 2006). Furthermore, there is mention in the literature of ammonia results of ultralow delivery cigarettes having more smoke ammonia than would be expected and labora-

Table 1d
Data reported by Lee et al. (2006).

Cigarette	Ventilation %	ISO Tar mg/cig	Nicotine mg/cig	Tar/Nic	Smoke pH	^a FBN mg/cig	^a α_{fb}	MDPH Tar mg/cig	Nicotine mg/cig	Tar/Nic	Smoke pH	^a FBN mg/cig	^a α_{fb}	HCl Tar mg/cig	Nicotine mg/cig	Tar/nic	Smoke pH	^a FBN mg/cig ^a	^a α_{fb}
KY2R4F	28.3	8.9	0.72	12.4	5.05	0.09	0.13	23.2	1.76	13.2	5.06	0.18	0.10	35.4	1.91	18.5	5.02	0.06	0.03
A	34.1	7.5	0.6	12.5	5.07	0.07	0.12	22.8	1.45	15.7	5.1	0.095	0.07	36.8	1.78	20.7	5.07	0.03	0.02
B	34.4	6.4	0.57	11.3	5.10	0.08	0.14	18.6	1.39	13.4	5.13	0.07	0.05	31.1	1.54	20.2	5.02	0.03	0.02
C	41.2	6.1	0.56	10.9	5.19	0.12	0.21	17.7	1.43	12.4	5.17	0.08	0.06	34.9	1.76	19.8	5.14	0.05	0.03
D	49	6.2	0.54	11.6	5.18	0.065	0.12	18.4	1.38	13.3	5.06	0.07	0.05	29.8	1.7	17.5	5.06	0.03	0.02
E	53.7	5.7	0.53	10.7	5.11	0.08	0.15	17.2	1.61	10.7	5.06	0.06	0.04	38.3	2	19.2	5.07	0.03	0.02
F	59.3	4.9	0.49	10.0	5.18	0.125	0.26	17.8	1.49	12.0	5.11	0.14	0.09	32.7	1.97	16.6	5.04	0.03	0.02
G	60.1	2.9	0.25	11.4	5.13	0.09	0.36	11.3	0.88	12.8	5.16	0.13	0.15	26.5	1.25	21.2	5.02	0.05	0.04
H	63.1	3.2	0.3	10.6	5.14	0.08	0.27	12.7	1.09	11.6	4.99	0.07	0.06	29.9	1.67	17.9	5.02	0.03	0.02
I	73.8	0.8	0.09	9.1	5.18	0.05	0.56	6.1	0.52	11.8	5.03	0.04	0.08	19.8	0.88	22.5	5.01	0.03	0.03
J	76.9	1.0	0.09	11.6	5.24	0.09	1.00	7.7	0.67	11.4	5.09	0.13	0.19	28.3	1.27	22.3	5.07	0.05	0.04
K	80.1	0.8	0.07	11.0	5.24	0.04	0.57	6.9	0.72	12.2	5.02	0.05	0.09	24.3	1.27	19.1	4.97	0.03	0.02
L	81.8	1.3	0.11	11.4	5.26	0.03	0.27	7.9	0.72	11.0	5.02	0.05	0.07	29.5	1.29	22.9	4.98	0.03	0.02
Test Cig 1	0	11.7	0.88	13.3	5.03	0.025	0.03	25.1	1.82	13.8	5.06	0.038	0.021	32.8	1.92	17.1	4.98	0.028	0.01
Test Cig 2	10	10.4	0.82	12.7	5.08	0.027	0.03	23.3	1.75	13.3	5.04	0.04	0.02	31.2	1.89	16.5	NR	0.025	0.01
Test Cig 3	30	8.5	0.71	12.0	5.12	0.036	0.05	21.7	1.7	12.8	5.02	0.06	0.04	31.6	1.9	16.6	NR	0.028	0.01
Test Cig 4	50	5.8	0.52	11.1	5.12	0.047	0.09	18.4	1.49	12.3	5.06	0.06	0.04	30.7	1.84	16.7	NR	0.028	0.02
Test Cig 5	70	3.5	0.31	11.4	5.19	0.059	0.19	14.2	1.26	11.3	4.98	0.06	0.05	30.3	1.82	16.7	NR	0.028	0.02

^a Values for FBN and α_{fb} were estimated from charts included in the text.

Table 2
Calculation of gas-particle partitioning parameters from Callicutt's data (Callicutt et al., 2002).

Cigarette	TPM μg/cig	Nicotine in TPM μg/cig	GVP nicotine μg/cig	Total nicotine μg/cig	$P_{g,nic}(\%)$	Puff count/cig	Total puff volume (m ³)	TSP μg/m ³	$(100 - P_{g,nic})/P_{g,nic}$	$K_{p,nic}$ m ³ /μg	F_{nic} ng/m ³	A_{nic} ng/m ³	F_{nic}/TSP ng/μg	$K_{p,nic}$ m ³ /μg
Cambridge Lowest 100s	1800	180	1.37	181	0.76	7.9	2.77E-04	6.51E+06	1.31E+02	2.01E-05	6.51E+05	4.95E+03	1.00E-01	2.02E-05
Merit Ultra Lights KS	5700	480	1.17	481	0.24	7.5	2.63E-04	2.17E+07	4.10E+02	1.89E-05	1.83E+06	4.46E+03	8.42E-02	1.89E-05
KY1R4F (KS)	11200	860	0.54	861	0.06	8.9	3.12E-04	3.60E+07	1.59E+03	4.43E-05	2.76E+06	1.73E+06	7.68E-02	4.43E-05
Industry Monitor 16 (KS)	20600	1070	0.25	1070	0.02	7.9	2.77E-04	7.45E+07	4.28E+03	5.74E-05	3.87E+06	9.04E+02	5.19E-02	5.74E-05
Marlboro Lights KS	12000	830	0.49	830	0.06	8.4	2.94E-04	4.08E+07	1.76E+03	4.15E-05	2.82E+06	1.67E+03	6.92E-02	4.15E-05
Marlboro Lights KS RL1	11500	790	0.58	791	0.07	8.4	2.94E-04	3.91E+07	1.45E+03	3.48E-05	2.69E+06	1.97E+03	6.87E-02	3.48E-05
Marlboro Lights KS RL2	10800	790	0.49	790	0.06	8.4	2.94E-04	3.67E+07	1.71E+03	4.39E-05	2.69E+06	1.67E+03	7.31E-02	4.39E-05
Marlboro Lights KS RL3	11300	770	0.47	770	0.06	8.2	2.87E-04	3.94E+07	1.64E+03	4.16E-05	2.68E+06	1.64E+03	6.81E-02	4.16E-05
Marlboro Lights KS RL4	10500	860	0.91	861	0.11	8.1	2.84E-04	3.70E+07	9.45E+02	2.55E-05	3.03E+06	3.21E+03	8.19E-02	2.55E-05

TPM = total particulate matter (μg/cig).

Nicotine in TPM = nicotine retained in TPM on Cambridge filter pad (μg/cig) = PP nicotine.

GVP nicotine = nicotine in XAD-4 tube (μg/cig).

Total nicotine = PP nicotine + GVP nicotine (μg/cig).

 $P_{g,nic}(\%)$ = percentage of total nicotine that was trapped in the XAD-4 tube.

Puff count = number of 35 mL puffs it takes to burn down the cigarette to specified butt length (puff counts estimated from other data).

Total puff volume = puff count times puff volume (35 mL) with results expressed in cubic meters (m³) (calculated).

TSP = total suspended particulate matter in the aerosol = TPM divided by total puff volume (calculated).

 $K_{p,nic}$ = gas-particle partitioning coefficient for nicotine in the aerosol (m³/μg) (calculated). F_{nic} = particle-associated concentration of nicotine in the aerosol (ng/m³) (calculated). A_{nic} = gaseous concentration of nicotine in the aerosol (ng/m³) (calculated).

Marlboro Lights KS RL1 = Marlboro Lights KS with ammonia-containing additives.

Marlboro Lights KS RL2 = Marlboro Lights KS without ammonia-containing additives.

Marlboro Lights KS RL3 = Marlboro Lights KS without ammonia-containing additives and without additives in reconstituted tobacco.

Marlboro Lights KS RL4 = Marlboro Lights KS without additives.

tory air having sufficient background ammonia to contribute up to 10 μg/cigarette to the ammonia contents found on the Cambridge filter pads (Lauterbach, 1997b; Adams et al., 1987). We do not have enough information to know if the inadvertent admission of ammonia to the analytical system caused the high FBN values reported by Lee and co-workers. However, it points to the need for further study.

5.5. Determination of α_{fb} with solid-phase microextraction (SPME)

The use of SPME for the determination of FBN in cigarette mainstream TPM offers the benefit of not having to deal with aqueous extracts of TPM, but unlike other applications of SPME, there are some added complications that other workers appear to have neglected. The first of these is that the conditioning of the cigarettes must be thorough and the product checked for maximum conditioned moisture at the end of the specified conditioning time. The smoking of the products must be done strictly according to the protocols, and heat-treating the Cambridge pads as recommended by Vekris and Hook (1966) should be considered. In particular, this means making sure that the expected values are obtained for TPM, puff count, CO, particulate-phase water and particulate-phase nicotine. It also means that the smoking system must keep sidestream and atmospheric ammonia from entering the mainstream smoke and from contaminating the Cambridge filter pads. Once the smoking process has been completed, it is essential that the Cambridge pad and the TPM are not subjected to conditions that would alter the TPM composition. Care should be taken to make sure that volatile acids (e.g., carbonic, formic, acetic, etc.) are not lost. Furthermore, factors affecting the reliability of headspace SPME process, such as the SPME fiber type, pre-equilibration time, SPME extraction time and temperature, the water content in TPM, and the solvent used for the preparation of calibration standards should be examined. These factors were controlled in the work described here and in the development of the underlying method (Bao et al., 2010).

The data in Tables 1a and 1b show that for the cigarette products the ratio of free-base nicotine to total nicotine (α_{fb}) in the particulate matter is less than 0.05 (5%) for many of the brand-styles under ISO smoking conditions. For the conventional cigarette brand-styles smoked under HCl smoking conditions, all values of α_{fb} were under 0.02 (2%). Thus, we did not find the high values of FBN that have been reported by others for commercial machine-made cigarette products. Indeed, for three [Gauloises Blondes (Red), Number 7 Silver King Size, and du Maurier Extra Light Regular Size] of the four brand-styles where α_{fb} did exceed 0.05 under ISO smoking conditions, the relative amount of particulate-phase water in the TPM was well under 5%. Thus, it appears that the samples were not conditioned properly or there was a problem with the samples that was not obvious to the analysts. The fourth sample was the More Filter 120's. More Filter 120's is an ultraslim product so the smoking dynamics are different from cigarettes with a normal circumference. However, one would expect that the TPM would contain at least 7% water instead of the 2.5% found. Low particulate-phase water in combination with low TPM per puff will give conditions that favor increased α_{fb} . This follows from use of Eqs. (4) and (1). If the values of $\gamma_{nicotine}$, the activity coefficient for nicotine in TPM, were to differ greatly from unity, we may have a different situation. Fortunately, we have sufficient data to make some estimates of $\gamma_{nicotine}$.

5.6. Estimation of $\gamma_{nicotine}$ – the activity coefficient for nicotine in TPM

Since we have α_{fb} and the other necessary parameters to estimate $\gamma_{nicotine}$, we should not miss the opportunity to compare experimentally derived estimates for $\gamma_{nicotine}$ with the estimate of

0.8 made by Pankow and co-workers (2004). If we use the data from two products of nominally the same cigarette design and delivery but different blend: (1) Camel Filters 20 King Size; and (2) Player's King Size, we can see how the numbers work (detailed calculations for estimation of γ_{nicotine} are included in the [Tables S3 and S4 of the Supplementary information](#)). The Camel Filters 20 King Size gave a very dry TPM (5% water) and had an estimated MW_{om} of 149 g/mol (very high). Combined with a TSP of $6.75\text{E}+07 \mu\text{g}/\text{m}^3$ and an estimated $K_{p,\text{nicotine}}$ of $5.94\text{E}-06 \text{m}^3/\mu\text{g}$, the estimated $P_{g,\text{nicotine}}(\%)$ was 0.25%. However, the value for α_{fb} with this improved SPME method was 0.043. This, in turn, gave an estimated $K_{p,\text{nicotine}}$ of $2.17\text{E}-04 \text{m}^3/\mu\text{g}$. The estimated value of the activity coefficient for nicotine in TPM, γ_{nicotine} , is 0.02. Similar calculations for Player's King Size showed a more moist TPM (10% water), gave an estimated MW_{om} of 110 g/mol. Combined with a TSP of $7.58\text{E}+07 \mu\text{g}/\text{m}^3$ and an estimated $K_{p,\text{nicotine}}$ of $8.02\text{E}-06 \text{m}^3/\mu\text{g}$ and an estimated $P_{g,\text{nicotine}}(\%)$ of 0.16%. Since the experimentally determined α_{fb} value was 0.014, the estimated $K_{p,\text{nicotine}}$ of $6.66\text{E}-04 \text{m}^3/\mu\text{g}$ gave an estimated γ_{nicotine} of 0.012. US-style blended products, which typically have glycerin, propylene glycol, and sugars added to the blend, tend to give mainstream smoke that has more particulate-phase water, typically ranging from 10% to 20% of the TPM for KS full-flavored product (Lauterbach, 2001). Such products have been shown to have very low values of $P_{g,\text{nicotine}}(\%)$ and γ_{nicotine} values less than unity. When products are smoked with the Health Canada Intensive protocol, there is so much water in the TPM that the vapor pressure of nicotine is reduced to such an extent that it has little volatility. This conclusion is supported by the data obtained with Health Canada Intensive smoking conditions. Furthermore, the activity coefficients reported for nicotine in water at 25 °C were all less than unity for mole fraction nicotine between 0.001 and 0.100 (Norton et al., 1940). Based on the ISO smoke data for the 1R4F and 2R4F reference cigarettes (Chen and Moldoveanu, 2003), the mole fraction of nicotine is about 0.05 if one considers only nicotine and water, and γ_{nicotine} would be around 0.13 (Norton et al., 1940). If one considers a simplified system of water, nicotine, and a molecule with the same structural groups as neophytadiene as a substitute for tar and uses the same relative proportions as reported by Chen and Moldoveanu for the 2R4F, the estimated γ_{nicotine} was 0.005. If an alternate dataset is used to simulate "dry" TPM, the estimated γ_{nicotine} was 0.12. If a second alternate data set is used to simulate HCl smoking of the same "dry" cigarettes, the estimated γ_{nicotine} was 0.002. These estimates were obtained with the UNIFAC Activity Coefficient Calculator Version 3.0. These crude estimates point out that small changes in the relative amount of water in mainstream TPM can have a major effect on the amount of FBN found in the TPM and on α_{fb} .

The low values we have estimated for γ_{nicotine} can be compared to values of the activity coefficients for naphthalene, acenaphthene, and fluorene that were estimated from the experimental data of Kalaitzoglou and Samara (2006). For a wide range of commercial products, estimated values for the activity coefficients for these three compounds ranged from a low of around 6 to a high of around 50 (Lauterbach et al., 2006). The vapor pressures of those three compounds are similar or less than the vapor pressure of nicotine, but the fraction of the compounds in the gas–vapor phase is much greater than that for nicotine. Based on vapor pressure alone, one would have expected a lower fraction of such compounds in the gas phase of MSS. This is why naphthalene, acenaphthene, and fluorene have activity coefficients higher than unity for MSS TPM.

In most of the discussions about unprotonated nicotine and other basic substances in MSS (ammonia, for example), the identities and concentrations of the acids in smoke are not considered. Smoke acids are more difficult to measure than nicotine and

ammonia, and do not seem to attract the attention of those who set the smoke chemistry priorities. We cannot understand what is happening unless we have the complete picture. While some may argue the usefulness of smoke pH measurements, we need to know the major acids that are causing the differences in smoke pH. Ingebrethsen and co-workers found when they analyzed the MSS from flue-cured, burley, oriental, and blended cigarettes. The acids present and their amounts in the TPM will help determine the activity coefficient for nicotine in TPM. If we had good estimates for those values, we could determine which analytical approaches are better for determining unprotonated nicotine in MSS. Very recently, Adamczyk and colleagues reported that aqueous solutions of carbonic acid are likely much more acidic than previously thought (2009). They estimated that the pKa for carbonic acid was about 3.45 not 6.35 as previously reported. Since carbonic acid has a higher concentration in MSS than do other acids, this finding gives another reason why there is so little FBN in MSS.

6. Conclusions

In this report, we have documented the experimental results we obtained with use of an improved SPME method for determining the free-base nicotine (FBN) content of mainstream cigarette smoke (MSS) particulate matter (TPM). We have shown that the main drivers of FBN in TPM under ISO smoking conditions are cigarette design features that increase the concentration of nicotine in the TPM and the water content of the TPM. Therefore, the use of FBN alone or as the ratio to total smoke nicotine (TN), ($\text{FBN}/\text{TN} = \alpha_{\text{fb}}$) to determine if ammonia and/or its compounds have been used to treat the tobacco is inappropriate. We have pointed out some of the pitfalls of the SPME-based methodology in our hands and in the hands of others. The main problem with the SPME methodology is that the uptake of unprotonated nicotine by the SPME fiber is very dependent on the moisture content of the TPM collected on the Cambridge filter pad. While the gas–vapor phase and the particulate phase of MSS are not in thermodynamic equilibrium, we have also shown that our results can be explained by Pankow's theory of absorptive partitioning (which requires thermodynamic equilibrium). When products are smoked under conditions that mimic human smoking, the amounts of FBN in the TPM and the amounts of unprotonated nicotine in the MSS gas–vapor phase [$P_{g,\text{nic}}(\%)$] are so much less than when the cigarettes are smoked under ISO conditions that these concentrations of nicotine are physiologically insignificant. Thus, there is no longer any scientific basis for regulators to require reporting of FBN/TN and [$P_{g,\text{nic}}(\%)$]. We have also reviewed examples in the literature where incorrect statements and experimental findings about FBN were made, and we have shown the likely causes of the misinformation. Our research and the research of others has shown that statements relating smoke pH and FBN to product acceptability made in the distant past by various tobacco industry researchers and executives are incorrect and should not be used as the basis for making regulations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.yrtph.2010.05.007](https://doi.org/10.1016/j.yrtph.2010.05.007).

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Glossary

α_{nb} : Fraction of nicotine in the TPM that is not protonated

γ_{nicotine} : Activity coefficient for nicotine in the TPM

$c_{\text{g,nicotine}}$: Concentration of nicotine (unprotonated) in the gas–vapor phase (ng/m^3)

$c_{\text{p,nicotine}}$: Total nicotine concentration in the TPM ($\text{ng}/\mu\text{g}$)

f_{om} : Fraction of organic matter in the TPM

$P_{\text{L}}^{\text{nicotine}}$: Vapor pressure of nicotine as a liquid at temperature of interest (torr)

A_{nicotine} : Concentration of nicotine in gas–vapor phase of MSS (ng/m^3)

F_{nicotine} : Concentration of nicotine in TPM (ng/m^3)

FBN/TN : Fraction of nicotine in the TPM that is not protonated = α_{nb}

GVP : Gas–vapor phase of MSS is the material that passes through the Cambridge filter pad during machine smoking of a cigarette. According to ISO 10185:2004, the vapor phase is the “portion of smoke which passes the particulate phase trap during smoking in accordance with ISO 4387 using a machine conforming to ISO 3308.”

$K_{\text{p,nicotine}}$: Gas/particle partitioning coefficient for nicotine in MSS aerosol ($\text{m}^3/\mu\text{g}$)

MSS : Mainstream cigarette smoke

MW_{om} : Number average molecular weight of the TPM (g/mol)

$P_{\text{g,nic}}(\%)$: The percentage of total MSS nicotine that is in the gas–vapor phase of undiluted MSS as determined by the technique reported by Callicutt and colleagues (2002,2006a,b)

R : Gas constant ($8.2 \times 10^{-5} \text{ m}^3 \text{ atm mol}^{-1} \text{ T}^{-1}$)

T : Temperature ($^{\circ}\text{K}$)

TPM (1) : Total particulate matter (mg/cig). Definition 1. According to ISO 10185:2004, “TPM is that portion of the mainstream smoke which is trapped in the smoke trap (in this work the smoke trap is the Cambridge filter pad)”

TPM (2) : Total particulate matter (mg/cig). Definition 2. According to Seeman (2007), “The terms ‘total particulate matter’ and ‘particulate matter’, refer to material captured during a machine-smoking method on, for example, a Cambridge filter pad or in an impaction trap. In contrast, MS smoke aerosol is composed of heterogeneous particles suspended in a gas phase. Trapped particulate matter is thus not the same in terms of physical or chemical properties as the particles in the dynamic aerosol”

TSP : Concentration of total suspended particulate matter in MSS aerosol. In the case of MSS, we can approximate the TSP by taking the net weight of total particulate matter (TPM) on the Cambridge pad, dividing it by the total volume of the puffs used to smoke the cigarette, and expressing the results in $\mu\text{g}/\text{m}^3$