Supporting Information:

Free-Base Nicotine is Nearly Absent in Aerosol From IQOS Heat-Not-Burn Devices, as Determined by ^{1}H NMR Spectroscopy

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Materials: DMSO-d_6 (D 99.9%) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA); *t*-butylamine (98%) was obtained from Sigma-Aldrich (St. Louis, MO), and glacial acetic acid was from Mallinckrodt Chemicals (Staines-upon-Thames, England). 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) was from Wilmad Glass Co., Inc (Buenea, NJ).

Sample generation for ^{1}H NMR analysis: IQOS heatsticks (purchased on ebay.com) were vaporized per usage recommendations, and the holder was cleaned after every 20 heatsicks per the user guide recommendation. The filter was connected to a capped 2-mL autosampler septum vial via 1-inch of 3/8 inch outer diameter ACF0017-F Tygon S3 E-3603 tubing connected to a 18G x 1 ½ BD PrecisionGlide needle penetrating the septum of the vial with its tip positioned at the very bottom of the vial. A second, outlet needle just penetrated the septum. The outlet needle was connected to a Single Cigarette Smoking Machine (SCSM; CH Technologies; Westwood, NJ) with 2 inches of the same tubing, which ran puff programs according CORESTA or mHCI protocols. Sufficient heatsticks (10 – 15) were required to produce at least 60 µL of condensed aerosol. The IQOS holder was cleaned per the instruction manual. Each collected sample (in a 2 mL autosampler vial) was spiked with < 0.01 mg (one flake of solid) DSS, and sonicated as necessary. The sample was subsequently transferred to a precision coaxial NMR tube insert (Wilmad-LabGlass, WGS-5BL), which was inserted into a 5 mm NMR tube (Wilmad-LabGlass, 535-PP7) containing 500 µL DMSO-d_6 + 0.05% v/v tetramethylsilane.

Acid and base standard samples: Free-base and monoprotonated nicotine standards were prepared by adding an excess of either acid (acetic acid, 0.5 µL to a 40

μL HNB sample) or base (tert-butylamine, 4.3 μL to a 35 μL HNB sample) – relative to nicotine - to aliquots of a vaporized HNB sample based on approximate nicotine content until nicotine methyl chemical shifts were no longer altered by further addition of acid or base. The presence of acetic acid was confirmed by spiking with pure acetic acid and testing again by ^{1}H NMR.[1]

Reference chemical shifts for Nic and NicH$^+$ were found to be comparable to those obtained using an Eclipse cigarette as tested by Pankow *et al.* (2003).[2] Vaporized samples were found to be similar between brands so acid and base standards from a single brand were used to calculate α_{fb} for all HNB samples. Approximate nicotine and acetic acid content were determined in triplicate via quantitative ^{1}H NMR using samples containing 10 μL of condensed HNB aerosol (from Parliament brand), and 10 μL of a 177 mM solution of 2,3,4,5-tetrachloronitrobenzene (TCI America, Inc.) in DMSO-d_6. The Global Spectral Deconvolution function from MestreNova NMR processing software was used for all quantitative experiments to deconvolute overlapping peaks. Figure S1 displays a sample NMR spectrum used to quantitate nicotine and acetic acid in the HNB aerosol.

NMR spectroscopy: ^{1}H NMR experimental parameters were identical to those used by Duell *et al.* (2018),[3] including the use of a 600 MHz NMR spectrometer, TXI probe at 40 °C, 30° observation pulse, and a 3 second relaxation delay. TopSpin (Bruker Biospin) was used to phase correct and baseline straighten the spectra. Referencing was accomplished by calibrating relative to the DSS resonance at 0 ppm. Nicotine methyl identification in the ^{1}H spectra was conducted using peak integration

relative to the aromatic nicotine resonances as well as *via* HSQC spectra obtained by using the Bruker library experiments, HSQCEDETGP or HSQCETGP.

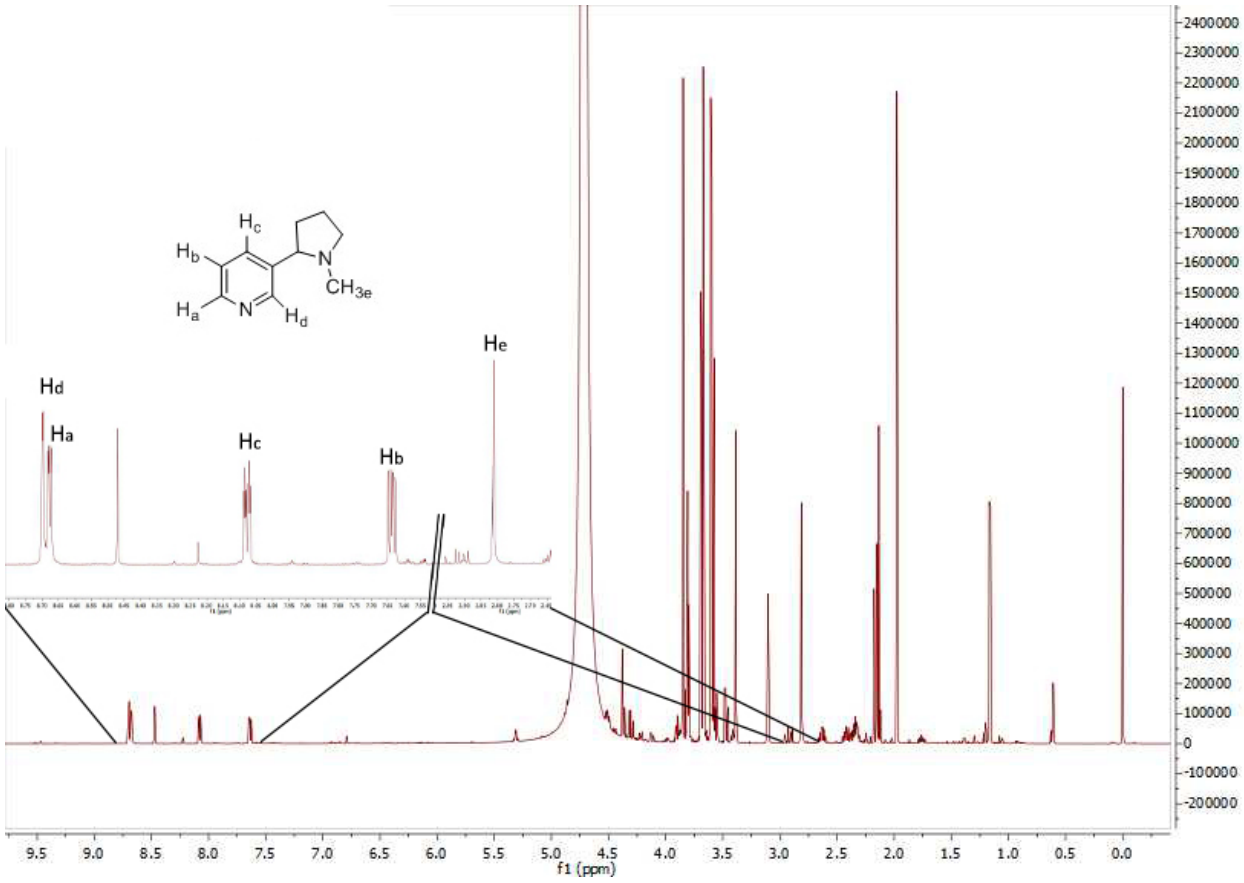

Figure S1. Sample NMR spectrum that was used for α_{fb} determination by NMR. The highlighted portion shows nicotine aromatic and methyl peaks labelled.

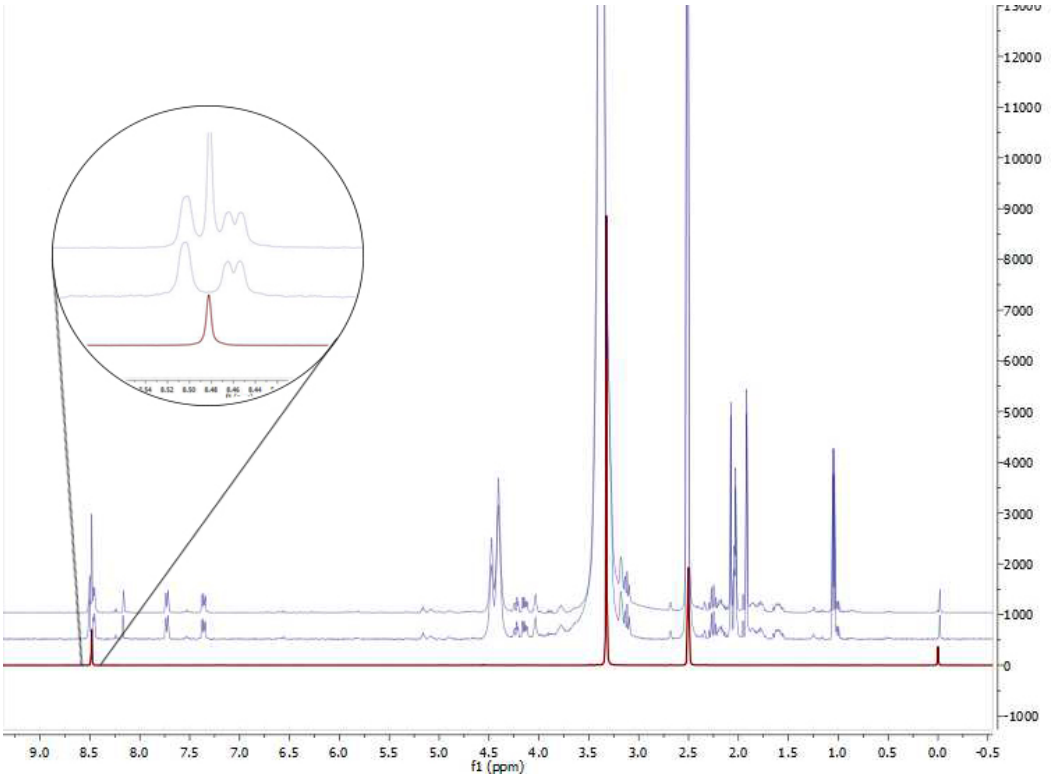

Figure S2. A sample spectrum used to quantitate acetic acid and nicotine in the bulk aerosol. The overlaid traces are (listed from bottom to top): 2,3,4,5-tetrachloronitrobenzene, bulk aerosol, and bulk aerosol spiked with 2,3,4,5-tetrachloronitrobenzene. All samples are in DMSO-d_6.

α_{fb} by HS-SPME-GCMS: This novel method is a hybrid of that reported in Watson *et al.*[4] and Pankow *et al*. (2003).[5] A single Parliament IQOS heatstick was vaporized per Teflon membrane filter (TMF, 47mm diameter, 10 µm pore size, which was cut from 8x10 inch Zefluor filter sheet (Pall Corporation, Port Washington, New York), supported by a Cambridge Filter Pad holder, in triplicate, under CORESTA smoking conditions with an SCSM.[6] After vaporizing, each filter was immediately inserted into a 40 mL septum-capped amber vial and left to equilibrate for one hour. The relative gas phase

content of nicotine in the headspace of each vial was measured in triplicate, and compared to headspace nicotine content after the addition of ammonia (NH_3; 3 mL) to vials to convert all $NicH^+$ to Nic. The quotient of pre-NH_3 to post-NH_3 nicotine in the headspace provides a direct value of α_{fb} for the HNB aerosol trapped on the filter. Each vial was analyzed in triplicate pre- and post-NH_3 addition using a 65 µm PDME/DVB fiber (Sigma-Aldrich) exposed for 20 min., and subsequently desorbed with a 230 °C inlet temperature with a 10:1 split ratio and a constant flow of He of 1 mL/min. The GC temperature program was 100 °C for 2 min., and then programmed to 280 °C at 10 °C/min., and cooled to 45 °C for 1-minute post run. The MSD transfer line was set at 230 °C. The mass spectrometer, in positive ion mode, had a scan range of 34 -300 amu, with an ionization energy of 70 eV, an electron multiplier voltage of 1682 V, a source temperature of 226 °C, and a quadrupole temperature of 150 °C.

Nicotine quantification by HPLC-UV: Total nicotine delivery was determined by HPLC-UV by collecting particulate and gas-phase nicotine. IQOS heatsticks were heated in their holder with the filter connected to a Cambridge Filter Pad (CFP, GE Healthcare) in a CFP-holder to capture particulate nicotine, followed by two consecutive impingers for gas-phase nicotine collection with 20 mL water and 20 µL of 200 mM solution of quinoline analytical standard (Sigma Aldrich) in isopropanol. The standard solution was stored at -20 °C but brought to room temperature before use. Each impinger was submerged in an ice-water bath as in Crouse *et al*.[7] After smoking, the CFP was extracted in 20 mL isopropanol along with 20 µL of the internal standard, sonicated for 30 min. and shaken on a rotary shaker for 5 min., similar to ISO 4387.[8]

Chromatographic conditions were similar to those in Saunders *et al.*[9] Before testing, samples were filtered through 13 mm 0.22 µm PVDF syringe filters (Fisher Scientific) and tested on a Waters 1525 binary HPLC pump with a Waters 2996 photodiode array detector. Separation was achieved using a 250 x 4.6 mm Luna 5 µm CN 100 Å (Phenomenex) column heated to 40 °C with a CH-30 heater and a TC-50 controller (Eppendorf) using isocratic elution at 1 mL/min with 40% methanol in water with 0.2% phosphoric acid (EMD) adjusted to pH 7.25 with triethylamine (Fisher Scientific) using an Orion 410A pH meter and vacuum filtered through a 47 mm, 0.45 µm nylon membrane filter (Whatman).

Statistics and error analysis: α_{fb} and nicotine aerosol concentration values were found to be consistent across all heatsticks tested. α_{fb} values consistently suggested that the majority of nicotine in the aerosol from these products is $NicH^+$, with very little Nic. α_{fb} values determined by NMR were cross-validated using the HS-SPME-GCMS method, which found no significant difference (p=0.068) between them for the brand tested (Parliament). Additionally, puff topography did not significantly influence α_{fb} of the brands/flavors chosen (Parliaments, HEETS *Yellow*, and Marlboro *Smooth Regular*). A two-tailed paired *t*-test on individual measurements of α_{fb} by NMR of the three brands comparing the two puffing parameters found no significant difference (p=0.13), likewise nicotine delivery for each did not differ significantly (Parliaments: p=0.63, HEETS *Yellow*: p=0.95, Marlboro *Smooth Regular*: p=0.37). In all these significance tests, the null hypothesis was taken to be that there is no difference in α_{fb} or nicotine delivery between puffing parameters mHCI and CORESTA, therefore, p values greater than 0.05 indicate that no significant difference exists. In all these statistical tests for significance, the null hypothesis was taken to be that no difference exists between analysis methods or puffing topographies for α_{fb} and nicotine delivery. In all cases, the

null hypothesis was accepted (given that all *p*-values are greater than 0.05), meaning that no significant difference exists between measurements.

Variability analysis for the ^{1}H NMR method suggests that the majority of the experimental uncertainty stems from variability in aerosol generation by the product rather than NMR detection and data processing. Variation (95% CI) from re-processing a single spectrum in triplicate was 0.001, whereas the variation stemming from re-testing a single sample after re-shimming the instrument in triplicate was 0.003. Variation in α_{fb} from three different bulk aerosol samples obtained from the same brand (Parliament) was 0.03, an order of magnitude higher than the variation attributed to shimming and processing. Variation in heat delivery from the heating flange to the tobacco may greatly affect the volatilization of the aerosol components that influence pH. This was evidenced by the fact that some heatsticks were inserted with difficulty into the holder, whereas some offered no resistance at all, which may be a consequence of small deviations in the rolling/folding pattern of the reconstituted tobacco. Given the small deviations among the results for the products tested, it was assumed that the magnitude of the variation in α_{fb} between brands/flavors would be representative for all α_{fb} measurements by this method. Thus α_{fb} values in Tables 1 and 2 were calculated using a single measurement each, except for Parliament brand, for which triplicate measurements were made. Viewed relative to the scale of all possible α_{fb} values (0 to 1), the variability in these NMR α_{fb} calculations is small, and gives great confidence that the nicotine in the aerosol generated from these devices is nearly fully monoprotonated. Acetic acid and nicotine were both quantified by NMR (see Methods) ([nicotine] = 0.14 ± 0.01 M; [acetic acid] = 0.14 ± 0.02 M), which allowed for a mathematical treatment of the equilibrium, giving a calculated α_{fb} value of 7.3×10^{-4}, assuming all activity coefficients to be unity and that nicotine was protonated only by

acetic acid. Any other acids present would drive this very small α_{fb} value to an even smaller number (although such a change would be difficult to measure).

Experimental challenges: The methods presented herein does have several challenges. The high amount of heatsticks required needed to generate sufficient sample for ^{1}H NMR analysis (>50 µL) requires a substantial input of time collecting aerosol. Aerosol particle deposition by diffusion and impaction are the two mechanisms by which the aerosol deposits onto the walls of the vials used, but as evidenced by the generation of aerosol droplets on the outlet tube of the vial, not all of the vaporized sample was captured. Capture efficiency by diffusion may be increased by lowering the temperature of the vial, which warrants investigation. To avoid loss of free-base nicotine by evaporation, a noteworthy concern given its high volatility, transfers and analyses were performed shortly after aerosol collection. However, some loss of loss of Nic may have led to underestimation of α_{fb} values.

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