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An Approach for Predicting Mainstream Cigarette Smoke Harmful and Potentially Harmful Constituent (HPHC) Yields

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Abstract

To ensure quality, consistency, and supply of cigarette products, a manufacturer may change materials, which can affect its product portfolio. Rather than testing each product individually to determine the effect of a change, designed experiments can be conducted using a subset of products, and statistical modeling can be performed to determine the harmful and potentially harmful constituent (HPHC) yields for the remaining products. To demonstrate this, we selected 30 representative cigarette products covering a wide range of tobacco blends, ingredients, and design parameters from a manufacturer's portfolio. Sets of cigarette products used papers produced with one type of manufacturing technology (control products) and two additional cigarette papers (changed products). The physical characteristics of the changed products' papers were similar to the control products but were manufactured using alternative methods, which could lead to differences in their chemical composition. The experiment was controlled to minimize variations among products, manufacturing, and testing. Linear regression was used to model the relationship between HPHC yields of the tested products. Twelve randomly selected products were used for validation by comparing predicted to measured yields. Model predictions were robust; differences between measured and predicted values were within standard repeatability limits, demonstrating the feasibility of this approach.

Keywords: HPHC, cigarette manufacturing, statistical modeling, regulatory reporting, manufacturing change

Abbreviations: GM, genetically modified; PCR, polymerase chain reaction; NGS, Next generation sequencing; WGS, whole genome sequencing; ISC, insertion site characterization; KOGs, euKaryotic clusters of Orthologous Groups; PMI, phosphomannose isomerase; CEGMA, Core Eukaryotic Genes Mapping Approach; T-DNA, transferred DNA; B[a]P, benzo[a]pyrene; CORESTA, Cooperation Centre for Scientific Research Relative to Tobacco; DNPH, 2,4-dinitrophenylhydrazine; FDA, US Food and Drug Administration; FSPTC Act, Family Smoking Prevention and Tobacco Control Act; GC-FID, gas chromatography with flame ionization detection; GC/MS, gas chromatography-mass spectrometry; HCI, Health Canada Intense; HPHC, harmful and potentially harmful constituent; ISO, International Organization for Standardization; NFDPM, nicotine-free dry particulate matter; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine; RMSE, root-mean-square error; RTD, resistance to draw; SPE, solid-phase extraction; TPM, total particulate matter; U.S., United States; WHO, World Health Organization

1. Introduction

Regulatory reporting of cigarette constituent emissions has been or is currently required in a number of jurisdictions, e.g., Brazil, Canada, Nepal, Taiwan, United States (U.S.), and Venezuela [8, 22]. The World Health Organization (WHO) has proposed a standardized approach to tobacco regulation [48] and developed recommended approaches for measuring constituents in tobacco smoke [7, 49]. In 2009, the U.S. Congress passed the Family Smoking Prevention and Tobacco Control Act (FSPTCA) [18], which provided the U.S. Food and Drug Administration (FDA) with authority to regulate tobacco products. In 2012, a list of 93 harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke was published by the FDA [46]. That same year, the FDA also published a draft guidance document on the reporting of an abbreviated list of 18 HPHCs in cigarette smoke (Table 1) [45].

The FSPTCA also introduced a pre-market approval process called "substantial equivalence" [44], wherein FDA evaluates constituent yields in cigarettes (among other information) before granting permission to market new tobacco products. As indicated in the FDA's Guidance for Industry and FDA Staff, "Demonstrating Substantial Equivalence for Tobacco Products" [43], it is the responsibility of the finished product manufacturer to ensure it has accurate information regarding the com-

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Ammonia	Crotonaldehyde	NNK [a]
1-Naphthylamine	Formaldehyde	1,3-Butadiene
2-Naphthylamine	Benzo[a]pyrene	Acrylonitrile
4-Aminobiphenyl	Carbon monoxide	Benzene
Acetaldehyde	Nicotine (total)	Isoprene
Acrolein	NNN [b]	Toluene

Table 1: Abbreviated list of harmful and potential harmful constituents (HPHC) in cigarette smoke.

[a]NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
[b]NNN, N-nitrosonornicotine

ponents included in its product. For example, if the filter supplier changes the composition of ingredients in its filters, it is the responsibility of the finished cigarette manufacturer to evaluate potential changes in exposure resulting from this change. Such changes in materials, or using an alternate material from a different supplier, may be made by a company to ensure quality, consistency, and supply of its product portfolio over time. In certain cases, such a change may affect all or much of the company's portfolio of products, which results in the need to conduct a significant number of tests in order to demonstrate that the change for each individual product meets the criteria for substantial equivalence necessary for a FDA market order.

Alternatively, the FSPTCA permits a manufacturer to seek an exemption from the substantial equivalence pre-market approval process for "minor modifications" to its tobacco products that involve adding, deleting, increasing or decreasing a tobacco additive [47]. The exemption process allows the manufacturer to file a single application encompassing all products in its portfolio that are impacted by the minor modification. In this way, the exemption process is intended to be more efficient and expedient than the substantial equivalence pre-market approval process, which requires a separate application for each product affected by the change and separate review of each product application by FDA. However, one requirement for obtaining an exemption from the substantial equivalence process is that FDA must determine that submission of a substantial equivalence application, "*is not necessary to ensure that permitting the tobacco product to be marketed would be appropriate for protection of the public health*". To support such a finding, FDA may request, or a manufacturer may wish to submit, data demonstrating that HPHC yields are not impacted by the minor modification. The approach discussed below could be particularly useful for that purpose as it would avoid having to conduct analytical testing of multiple products where the change is minor to begin with, and therefore, unlikely to significantly affect HPHC yields.

The two objectives of this study were to establish statistical relationships for predicting mainstream cigarette smoke yields of the products affected by the change and to demonstrate that those smoke yields are comparable before and after the change. This will be illustrated using the example of a change in cigarette paper, in which testing is conducted on a representative subset of a company's portfolio of products to develop predictive models and to demonstrate equivalence between the products before and after the change.

The general methodology presented in this study is similar to that used in other related tobacco studies; however, the application in other studies has been for a different purpose than that of the current study. Statistical methods have been applied in benchmarking and/or market studies[1] of available cigarettes, in an effort to evaluate short-, medium- and long-term changes in smoke constituent yields [6, 15, 14, 22, 35]. Studies carried out in the U.S. [1, 6, 9, 41], Australia [2], the United Kingdom [20] and other countries [15, 16, 22] have demonstrated strong correlation between the individual smoke constituents and nicotine-free dry particulate matter (NFDPM, also referred to as tar), nicotine, or carbon monoxide.

In the proposed approach, rather than measuring HPHCs for each individual product on the entire portfolio impacted by a change in materials or ingredients, experiments are conducted on a specific subset of products covering the range of the portfolio to demonstrate that the changed products are comparable to the control products by using equivalence testing. The data set is further used to create predictive linear regression models that model the relationship between the HPHC yields of the control products and the changed products. The HPHCs of a smaller subset of the portfolio, not included in the initial model development experiment set, are then measured and compared to the results from the predictive models to demonstrate the accuracy of the model. Following verification, the regression models can then be used to predict smoke HPHC yields of the remaining products within the portfolio. Predicting HPHC yields for the majority of a portfolio based on validated models from a representative subset of the portfolio can ensure timely reporting

[1]Benchmarking survey and market mapping studies involve cigarette brand styles with design properties representing the commercial cigarette market. Published studies include typical cigarette design features on the market, and are beneficial by providing a snapshot of the relative ranges of machine smoke yields [1]. Benchmarking studies such as Borgerding et al. [6] and Counts et al. [15] focused on estimating equations for predicting smoke yields for brands not tested, whereas market mapping studies such as Counts et al. [14] and Morton and Laffoon [35] focused on the variation around those prediction equations through the calculation of statistical prediction intervals. One use for market mapping is as a statistically-based criterion for determining whether a newly added ingredient or material impact the composition of a cigarette in such a way that its machine-measured smoke yield is outside the calculated market range for a particular smoke constituent.

for regulatory submissions by reducing additional testing. In this study we used a change in cigarette paper as an example to demonstrate the feasibility of this approach. The change in cigarette paper is a relevant example, as it is a change that could impact a significant portion of a manufacturer's portfolio; it would not be expected to result in substantially different HPHC smoke yields, but would still require demonstration of equivalence for the entire portfolio. Testing in this manner is more efficient because not all products must be tested, and also more effective because observing the cumulative effect of the change across many products smooths out testing variation to give a better estimate of the effect of the change.

2. Materials and Methods

To demonstrate the feasibility of the proposed analyses of the mainstream cigarette smoke yields of HPHCs resulting from a change in material that might affect all or much of a company's product portfolio, the above-mentioned approach was applied to the HPHCs resulting from a change in the cigarette paper, as an example. The physical properties of the control and changed papers, such as permeability and band width, are similar. The HPHC yields resulting from the changed products made with two different commercial cigarette papers, referred to as Paper A and Paper B, were estimated from the HPHC yields associated with cigarettes manufactured with the control product. To minimize the effects of other design parameters or materials on the mainstream cigarette smoke yields, only the cigarette paper was changed in this study; all other design and manufacturing parameters for the control product and the two changed products (Paper A and Paper B) remained the same.

The general approach used in this study includes the following steps, with each step described in more detail in the following sections:

- 2.1. Development of a methodology for identifying a representative subset of the products affected by the proposed change used to develop regression models;
- 2.2. Identification of a validation subset of products for testing the accuracy of the models;
- 2.3. Manufacturing of those products selected as the representative and validation subsets using current design and proposed changes;
- 2.4. Determination of the mainstream cigarette smoke HPHC yields for a defined list of constituents for both the control and changed products using validated analytical methods;
- 2.5. Demonstrating that the changed products are comparable to the control products by using equivalence testing;
- 2.6. Development of linear regression models to estimate the HPHC yields of the products with the changed paper from the products with the control paper; and,
- 2.7. Testing the proposed models using a validation set of data consisting of randomly selected products from the remaining products in the portfolio.

The developed and validated regression models can then be used to determine and report the HPHC yields for the remaining products in the portfolio (e.g., those not included in the representative and validation subsets) impacted by the change.

2.1. Development of a methodology for identifying a representative subset of products

At the time of this study, the Philip Morris USA portfolio comprised 147 different cigarette designs. These cigarettes may be differentiated by attributes in the design parameters and/or cut filler type [1]. Here cut filler refers to the blend of tobacco, flavored and cut, used to make cigarettes. Identification of products to be included in the 'modeled' data set (e.g., the representative subset) was made based upon those major design characteristics which may influence mainstream cigarette smoke yields from the proposed change in cigarette paper. The objective was to select a set of products that encompasses the design parameters and cut filler types used in the entire portfolio.

For the current analysis, a total of nine variables that could influence HPHC yields were considered (Table 2). Five of the variables were categorical[2] or treated as categorical, and included cut filler type, cigarette paper band width, cigarette paper permeability, cigarette circumference, and filter plug length. Four of the variables (i.e., cut filler weight, filter plug resistance to draw [RTD], tobacco rod length, and filter ventilation) were continuous. Details on the selection criteria used to identify a representative subset of products can be found in Appendix A. Based upon the selection criteria detailed in Appendix A, 30 products were identified to be considered as the representative subset of the entire portfolio. This subset contained one product from each of the 25 categorical combinations and five additional products selected from the continuous variables in order to approximate the distribution of variables in the overall set of 147 cigarette designs.

2.2. Identification of a validation subset of products

A validation subset of products was identified for testing the accuracy of the proposed model for estimating HPHC yield for cigarettes. The number of products used in the validation subset was 12 (40 percent of the original test set of 30 products used to develop the model). These 12 products were randomly selected from the remaining 117 cigarette designs present in the portfolio. The analytical measurement of constituent mainstream cigarette smoke yields in the validation subset of products was then compared to the predicted yields for those products.

[2] A categorical variable is a variable defined by a fixed list of possible values. An example of a categorical variable is gender, which can be defined as either male (M) or female (F).

Variable Name	**Possible Values**
Band width, mm (categorical)	≤ 6.0; > 6.0
Permeability, CU [a] (categorical)	33; 46; 60
Ventilation, % (continuous)	0, 12-71 (Grouped into 8 levels: 0, 10 ≤ x < 20, 20 ≤ x < 30, 30 ≤ x < 40, 40 ≤ x < 50, 50 ≤ x < 60, 60 ≤ x < 70 , and x ≥ 70)
Cut filler weight, g (continuous)	0.4414-0.9430 (Grouped into 6 levels: x < 0.5, 0.5 ≤ x < 0.6, 0.6 ≤ x < 0.7, 0.7 ≤ x < 0.8, 0.8 ≤ x < 0.9, and x ≥ 0.9)
Filter plug RTD, mmWG (continuous)	0-169 (Grouped into 7 levels: 0 (missing or 0), 60 *leq* x < 80, 80 ≤ x < 100, 100 ≤ x < 120, 120 ≤ x < 140, 140 ≤ x < 160, x ≥ 160)
Filter plug length, mm (categorical)	0; 19; 21; 25; 27; 31.5
Cigarette circumference, mm (categorical)	17; 23; 24.0; 24.8
Tobacco rod length, mm (continuous)	Grouped into 8 levels: 50 ≤ x < 55, 55 ≤ x <60, 60 ≤ x < 65, 65 ≤ x < 70, 70 ≤ x < 75, 75 ≤ x < 80, 80 ≤ x < 85, and x ≥ 85
Cut filler type (categorical)	Type 1; Type 2; Type 3; Type 4; Type 5; Type 6

Table 2: Cigarette variables that could influence HPHC yields.

[a]CU, CORESTA air permeability unit

2.3. Manufacturing of products

As indicated in Eldridge et al. [17], Morton and Laffoon [35], Oldham et al. [36], and Purkis et al. [39], some of the variability associated with smoke yields measured for the same brand over time, could result from temporal variability due to changes in design or materials used from one collection time point to the next or from temporal variation in analytical testing. To address the potential variability due to those sources, samples of each product identified in the representative subset and validation subset were made using current production methods and conditions, at the same time, using the control paper and the changed papers A and B, and the products were tested at the same laboratory. As indicated previously, to demonstrate feasibility of modeling HPHC yields, the change of paper was the only design or manufacturing parameter modified; all other parameters of each product were the same. A sufficient number of cigarettes were collected from each of the production runs such that a minimum of three replicate analyses for HPHC yields could be conducted using both the International Organization for Standardization (ISO) and the Health Canada Intense (HCI) smoking regimens.

2.4. Determination of HPHC yields

Mainstream cigarette smoke was generated for the representative subset and validation subset of cigarettes made with the control paper and changed papers A and B. Constituents considered in the analysis of the smoke were the 18 constituents listed on the FDA's abbreviated list [45] (Table 1) and tar. All testing was conducted by the same ISO 17025 accredited laboratory, with all the test methods used in this analysis being included in their scope of accreditation at the time of testing.

As indicated previously, both the ISO and HCI regimens were used for smoke analysis. Smoking machine yields provide an established platform for comparing tobacco smoke constituent yields from different products or verification against regulated yield limits [42, 8]. Cigarettes were conditioned and mainstream cigarette smoke generated and collected in accordance with appropriate ISO methods ISO 3402 [23] and ISO 3308 [26], with deviations made when required to accommodate differences in the HCI method. The ISO puffing parameters are 35±0.25 mL volume, 2±0.05 second duration, and one minute (60±0.5 second) interval between puffs. The ISO method was developed to provide a common basis for testing and comparing different commercial cigarettes and not to determine exposure by any particular human smoker [1, 16]. The HCI smoking machine protocol was developed to better characterize more intense smoking behavior by increasing the puff volume to 55 mL, shortening the puff interval to 30 seconds, and blocking ventilation holes with a strip of Mylar adhesive tape [28, 16, 37]. Like the ISO, the HCI smoking regime also utilizes a two second puff duration.

Data on smoke constituents can differ substantially between laboratories, especially for low-level smoke constituents, because of differences in approaches to the measurements employed by these laboratories [38]. Additionally, over-time results within laboratories can vary notably even when there is consistency of methodology. In an effort to minimize the effects of over-time within lab variability for each analyte, every triple set of products (each sample product with the control and two changed papers) sampled during the study were conditioned, smoked, and analyzed at the same period of time in a lab that is ISO 17025-accredited.

HPHC yields were measured as follows:
Ammonia yields were measured by collecting mainstream smoke from conditioned cigarettes on a Cambridge filter pad followed by two impingers, each containing 25 mL of 0.1N aqueous sulfuric acid. After smoking, the filter pad was added to the impinger solution and mixed. The solution was filtered and analyzed by ion chromatography (IC) with suppressed conductivity detection, using methanesulfonic acid as the eluent. The ion chromatograph was equipped with a cation-exchange column (Thermo scientific IonPac CS12A 4 mm x 250 mm). Aromatic amine (4-aminobiphenyl, 1-naphthylamine and 2-naphthylamine) yields were measured by collecting the total particulate matter (TPM) from conditioned cigarettes on a 44-mm Cambridge filter pad. After smoking, the filter pad was placed in an amber vial with 15 mL of a 0.24 N hydrochloric acid solution and the following internal standards: d7-1-naphthylamine, d7-2- naphthylamine, and d9-4- aminobiphenyl. The sample was shaken for 30 minutes and then a 6-mL aliquot of the extract was filtered (Sera-Separa® filter) and subjected to solid-phase extraction using a strong cation exchange sorbent (Strata-X-C 33μ, 60-mg sorbent). The solid phase extraction (SPE) eluate (1.5 mL dichloromethane) was dried using anhydrous sodium sulfate and the aromatic amines were derivatized using pentafluoropropionic acid anhydride and trimethylamine. The derivatized aromatic amines were analyzed by gas chromatography-mass spectrometry (GC-MS) equipped with a DB-WAX capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness) and with the mass spectrometer operating in single ion monitoring (SIM) mode.

Benzo[a]pyrene (B[a]P) yields were measured by collecting the TPM from conditioned cigarettes on a 44-mm Cambridge filter pad. Deuterated internal standard (B[a]P)-d_{12}) was added and the pad was subsequently extracted with 15 mL of hexane. A portion of the extract was passed through a NH2 SPE cartridge (500 mg). The hexane eluent was concentrated and then reconstituted in 50:50 toluene:iso-octane prior to analysis. The samples were analyzed by GC/MS equipped with a 17MS capillary column (30 m x 0.25 mm I.D., 0.25 μm film thickness) and with the mass spectrometer operating in single ion monitoring (SIM) mode. This method is equivalent to the published procedure in ISO 22634-2 with minor modifications.

Carbonyl (acetaldehyde, acrolein, crotonaldehyde and formaldehyde) yields were measured by passing unfiltered smoke from conditioned cigarettes through two impingers, each containing 30 mL of an acidified solution of 2,4-dinitrophenylhydrazine (DNPH, 17.5 mM) in acetonitrile. An aliquot of the DNPH-smoke extract was then stabilized with pyridine. The samples were analyzed by reversed phase ultra-performance liquid chromatography with ultraviolet detection using a Water Acquity BEH Shield RP18 column (2.1 x 100mm, 1.7μm particle size). This method is equivalent to the published procedure in ISO 21160:2018, with minor modifications.

Tobacco specific nitrosamine [N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] yields were measured by collecting TPM from conditioned cigarettes on a Cambridge filter pad. Deuterated internal standards (NNN-d4 and NNK-d4) were added and the filter pad was subsequently extracted with ammonium acetate. The samples were analyzed by reversed phase ultra-performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) using a Waters XBridge BEH C18 column (2.1 mm x 50 mm, 2.5 μm particle size). The mass spectrometer was operated in the Multiple Reaction Monitoring (MRM) mode using electrospray ionization. This method is equivalent to the published procedure in ISO 19290:2016, with minor modifications.

Tar and nicotine yields were measured by collecting TPM from conditioned cigarettes on a Cambridge filter pad that was subsequently extracted with 2-propanol containing carvone and ethanol as internal standards. Nicotine in the extract was determined using gas chromatography with flame ionization detection (GC-FID). Water in the extract was determined using gas chromatography with thermal conductivity detection (GC-TCD). Tar was calculated from the mass of TPM less the mass of water and nicotine. This method is equivalent to the published procedures in ISO 10315 and 20779:2018, with minor modifications.

Carbon monoxide yields were determined from smoke that had been collected in a gas sampling bag after passing through a Cambridge filter pad. The concentration of carbon monoxide was determined by non-dispersive infrared spectroscopy. This method is equivalent to the published procedure in ISO 21160:2018, with minor modifications.

Volatile organic compound (acrylonitrile, benzene, 1,3-butadiene, isoprene, and toluene) yields were measured by passing the mainstream smoke from conditioned cigarettes through a 44-mm Cambridge filter pad followed by one fritted impinger containing 25 mL of methanol that was cooled in a dry ice/ isopropyl alcohol bath to < -70 °C. After collection, the pad was discarded and the trapping solution was fortified with labelled internal standards (benzene-d6, and toluene-d8). The samples were analyzed by GC/MS equipped with a DB-624 capillary column (30 m x 0.25 mm I.D. x 1.4 μm film thickness) and with the mass spectrometer operating in single ion monitoring (SIM) mode. This method is equivalent to the published procedure in ISO 21330:2018, with minor modifications.

2.5. Demonstrate that changed products are comparable to the control products

When contemplating introducing a new material as a substitute for the original material, it is key to demonstrate that the product with the new material (changed product) performs equivalently to the product with the current material (control product). In this publication we evaluate equivalence between the control and changed products by assessing the agreement between the HPHC smoke yields measured from the control products and changed products, similar to the approach described in Bland and Altman [4], Schuirmann [40] and Feng et al. [19]. In this approach, we demonstrate equivalence by placing confidence intervals around the proportional differences in HPHC yields between the control and changed products. The products were considered equivalent if the confidence intervals were contained within ±10 percent. Equivalence testing was

carried out for each constituent measured under two smoking regimens; on both the combined representative and validation subsets, based on the relative smoke yield differences between the control and changed papers.

2.6. Development of regression models to correlate HPHC yields

All statistical analyses and linear regression modeling was performed using MATLAB® 8.6 by MathWorks, Inc. MATLAB® is a high-performance language for technical computing. The strength of the functional relationship between the mainstream smoke constituents of the cigarettes with the control paper compared to those cigarettes using cigarette Paper A or Paper B was assessed using the coefficient of determination, R^2 (defined as the ratio of the variance of the dependent variable explained by the independent variable and the total variance of the dependent variable), and on the spread of results around the regression line. Linear regression relating the constituent yield in the control product to those yields resulting following a change in paper was performed.

2.7. Testing the proposed models using the validation subset

The linear regression equations were used to estimate the constituent yields for the products in the validation set. These functional relationships were gauged for their capacity to predict the smoke constituent yields of the validation product set by comparison of the root-mean-square error (RMSE) to the expected method variability [21, 34, 12, 13, 25, 27] based on collaborative studies with this or a similar analytical method for the analyte.

3. Results

3.1. Measurements of HPHC yields

As indicated previously, three replicates were performed for each constituent in each product (i.e., the 30 products in the representative subset and the 12 products in the validation set), and smoking regimen combination. The average and standard deviation calculated for each product, for each regimen and for each analyte are available in Appendix B (Tables S2.1 - S2.12). The resulting values were generally in line with cigarette smoke yields reported in other studies [1, 5, 17, 36].

3.2. Equivalence testing

Equivalence testing was carried out on the relative smoke yield differences *(d)* between the control *(c)* and test *(t)* papers using the formula:

$$d_i = \frac{t_i - c_i}{(c_i + t_i)/2} \quad (1)$$

For a given analyte, smoking regimen, and test paper, the calculation was carried out for the combined 30 training and 12 validation products (for i = 1 ,2, . . ., 42). The equivalence intervals are shown in Table 3. Note that all of the constituent yields are equivalent within ±10%. A t-test on the average relative differences between papers A and B and the control paper (t-value = -6.18 and -4.13, respectively) indicates that overall the changed papers give slightly lower HPHC yields.

3.3. Relationship between HPHC yield for control products and products with changes in paper

The coefficient of determination, R^2, was used to assess the strength of the linear relationship between the HPHC yield for products using the control paper and the yield from those products following a change in paper, Paper A or B. The calculated R^2 values for each of the 18 HPHCs and tar are presented in Table 4. The yield for each of the HPHCs for the control products was strongly correlated ($R^2 \geq 0.85$) with the yield for products with the change in paper for the analytical data generated using the ISO smoking regimen. Likewise, using the HCI smoking regimen, the yield for all of the HPHCs for the control products were strongly correlated with the HPHC yield for products changing to Paper A or Paper B, with the exception of acetaldehyde, acrolein, and crotonaldehyde. The R^2 values for acetaldehyde (0.769 for Paper A and 0.771 for Paper B), acrolein (0.764 for Paper A and 0.723 for Paper B), and crotonaldehyde (0.788 for Paper A and 0.745 for Paper B) indicated a slightly weaker correlation ($R^2 > 0.7$) between yield from current products and those with a change in paper. These lower R^2 values for the HCI smoking regimen may be due, in part, to the method variability, which is known to be large for the set of measured constituents. Another explanation may be in the number of cigarettes that are smoked per collection for these constituents. In this study only one cigarette is smoked per replicate versus five or three for all other constituents under ISO and HCI smoking conditions, respectively.

3.4. Regression analysis of HPHC yields from control products and products with changes in paper

Linear regression analysis was performed to determine the relationship between the yields produced from products with changed paper (the scalar dependent variable, y), Paper A or Paper B, to the yields produced from products with the control paper (the independent variable, x). The linear regression was performed for each constituent and smoking regimen combination over the products contained within the representative subset, using the equation, $y = ax + b$, where y is the HPHC yield of products with the changed paper, x is the HPHC yield for products with the current paper, a is the slope of the regression line, and b is the intercept. In our analysis we did not force the intercept through zero since the true relationship between the control and changed papers is unknown, and exclusion of the intercept term has potential to bias the predictions. Examples of plots of the individual HPHC yields for control products (X axis) to the HPHC yields from products with the paper change (Y-axis for either Paper A or Paper B), are presented in Figures 1 and 2 for NNN and crotonaldehyde (as examples), respectively, for both the ISO and HCI smoking regimens. The fitted linear regression line is also provided in these figures. Figures for all constituents and tar are presented in the Appendix C (Figs. S-3.1 through S-3.19). The slope and intercept of the regression models along

HPHC [a]	**Regimen**	**Relative Difference between Control and Paper A**			**Relative Difference between Control and Paper B**		
		Average	Lower Limit	Upper Limit	Average	Lower Limit	Upper Limit
Ammonia	HCI [b]	-4.76%	-6.26%	-3.27%	-4.35%	-5.90%	-2.80%
1-Naphthylamine	HCI	-2.60%	-3.94%	-1.27%	-7.48%	-8.75%	-6.21%
2-Naphthylamine	HCI	-3.10%	-4.36%	-1.84%	-7.36%	-8.88%	-5.85%
4-Aminobiphenyl	HCI	-2.98%	-4.52%	-1.44%	-6.47%	-7.97%	-4.96%
Acetaldehyde	HCI	-0.01%	-1.53%	1.51%	0.40%	-1.05%	1.86%
Acrolein	HCI	-3.89%	-5.68%	-2.11%	-1.48%	-3.39%	0.43%
Crotonaldehyde	HCI	-1.87%	-3.38%	-0.36%	0.09%	-1.59%	1.76%
Formaldehyde	HCI	0.73%	-1.97%	3.44%	1.33%	-1.25%	3.91%
B[a]P	HCI	-6.65%	-8.21%	-5.10%	-5.65%	-7.07%	-4.23%
Carbon Monoxide	HCI	0.48%	-1.25%	2.20%	1.32%	-0.37%	3.00%
Nicotine	HCI	-2.23%	-3.21%	-1.26%	-3.26%	-4.40%	-2.13%
Tar	HCI	-1.92%	-3.00%	-0.84%	-1.07%	-2.09%	-0.06%
NNN [c]	HCI	-4.46%	-5.70%	-3.22%	-5.50%	-6.82%	-4.17%
NNK [d]	HCI	-4.26%	-6.50%	-2.03%	-5.10%	-6.99%	-3.22%
1,3 Butadiene	HCI	-6.97%	-8.16%	-5.77%	-3.14%	-4.45%	-1.84%
Acrylonitrile	HCI	-2.57%	-3.67%	-1.47%	-1.41%	-2.66%	-0.16%
Benzene	HCI	-4.54%	-5.59%	-3.49%	-3.17%	-4.32%	-2.02%
Isoprene	HCI	-2.73%	-4.03%	-1.43%	-0.70%	-1.62%	0.21%
Toluene	HCI	-3.88%	-4.89%	-2.87%	-2.77%	-3.82%	-1.72%
Ammonia	ISO [e]	1.45%	-0.84%	3.75%	0.72%	-1.44%	2.89%
1-Naphthylamine	ISO	-2.97%	-4.77%	-1.17%	-6.99%	-8.67%	-5.30%
2-Naphthylamine	ISO	-2.15%	-3.38%	-0.92%	-6.35%	-8.11%	-4.58%
4-Aminobiphenyl	ISO	-1.15%	-2.65%	0.35%	-5.57%	-6.99%	-4.14%
Acetaldehyde	ISO	1.57%	-0.83%	3.98%	1.12%	-1.22%	3.46%
Acrolein	ISO	-2.49%	-5.79%	0.80%	-0.10%	-3.26%	3.06%
Crotonaldehyde	ISO	-1.34%	-4.99%	2.32%	1.58%	-2.39%	5.55%
Formaldehyde	ISO	2.16%	-1.79%	6.11%	5.37%	1.17%	9.56%
B[a]P	ISO	-3.35%	-5.00%	-1.69%	-5.12%	-6.62%	-3.63%
Carbon Monoxide	ISO	-0.69%	-3.30%	1.91%	0.02%	-4.03%	4.08%
Nicotine	ISO	-1.74%	-3.00%	-0.49%	-2.44%	-3.78%	-1.09%
Tar	ISO	-0.17%	-1.54%	1.21%	0.45%	-1.12%	2.03%

Table 3: Average relative difference and upper and lower equivalence limits showing equivalence of smoke yields between the control and test papers.

[a]HPHC, harmful and potentially harmful constituent
[b]HCI, Health Canada Intense
[c]NNN, N-nitrosonornicotine
[d]NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
[e]ISO, International Organization for Standardization

HPHC [a]	**Regimen**	**Relative Difference between Control and Paper A**			**Relative Difference between Control and Paper B**		
		Average	Lower Limit	Upper Limit	Average	Lower Limit	Upper Limit
NNN	ISO	-0.36%	-1.89%	1.16%	-0.59%	-1.99%	0.81%
NNK	ISO	0.28%	-1.90%	2.46%	-2.08%	-4.07%	-0.09%
1,3 Butadiene	ISO	-3.78%	-5.42%	-2.15%	-1.00%	-2.62%	0.63%
Acrylonitrile	ISO	-1.21%	-3.09%	0.67%	1.12%	-0.85%	3.09%
Benzene	ISO	-3.62%	-5.16%	-2.07%	-1.69%	-3.24%	-0.14%
Isoprene	ISO	-0.60%	-2.18%	0.99%	0.79%	-0.97%	2.54%
Toluene	ISO	-2.58%	-4.14%	-1.02%	-1.19%	-2.79%	0.40%

Table 3: Continued.

[a]HPHC, harmful and potentially harmful constituent

HPHC [a]	**Coefficient of determination (R^2)**			
	ISO [b]		**HCI** [c]	
	Paper A	Paper B	Paper A	Paper B
Ammonia	0.977	0.982	0.960	0.935
1-Naphthylamine	0.970	0.953	0.970	0.977
2-Naphthylamine HCI	0.976	0.955	0.974	0.971
4-Aminobiphenyl HCI	0.974	0.962	0.961	0.964
Acetaldehyde	0.932	0.951	0.769	0.771
Acrolein	0.910	0.922	0.764	0.723
Crotonaldehyde	0.939	0.957	0.788	0.745
Formaldehyde	0.954	0.960	0.920	0.918
Benzo[a]pyrene	0.966	0.977	0.931	0.946
Carbon Monoxide	0.937	0.879	0.874	0.879
Nicotine	0.984	0.981	0.969	0.964
Tar	0.982	0.983	0.951	0.966
NNN [d]	0.991	0.989	0.981	0.981
NNK [e]	0.974	0.977	0.954	0.965
1,3 Butadiene	0.978	0.966	0.963	0.938
Acrylonitrile	0.976	0.967	0.931	0.925
Benzene	0.971	0.966	0.929	0.896
Isoprene	0.974	0.962	0.953	0.979
Toluene	0.972	0.972	0.943	0.962

Table 4: Coefficients of determination for control paper to Paper A and Paper B.

[a]HPHC, harmful and potentially harmful constituent
[b]ISO, International Organization for Standardization
[c]HCI, Health Canada Intense
[d]NNN, N-nitrosonornicotine
[e]NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

HPHC [a]	**Coefficients of Linear Model and 95% Confidence Intervals**							
	ISO [b]				**HCI** [c]			
	Paper A		Paper B		Paper A		Paper B	
	a	b	a	b	a	b	a	b
Ammonia	1.01 ±0.06	-0.13 ±0.86	0.99 ±0.05	0.12 ±0.73	1.02 ±0.08	-2.42 ±3.30	0.98 ±0.10	-0.80 ±4.08
1-Naphthylamine	0.95 ±0.07	0.29 ±0.95	0.96 ±0.08	-0.47 ±1.20	0.91 ±0.06	1.29 ±1.49	0.96 ±0.06	-0.76 ±1.35
2-Naphthylamine	1.00 ±0.06	-0.08 ±0.45	1.00 ±0.08	-0.41 ±0.63	0.94 ±0.06	0.33 ±0.77	0.96 ±0.06	-0.51 ±0.84
4-Aminobiphenyl	1.00 ±0.06	-0.02 ±0.09	0.97 ±0.07	-0.02 ±0.10	0.92 ±0.07	0.14 ±0.20	0.95 ±0.07	-0.03 ±0.20
Acetaldehyde	0.92 ±0.10	55.45 ±60.97	1.02 ±0.09	-3.12 ±56.84	0.90 ±0.19	163.10 ±306.5	0.90 ±0.19	164.84 ±304.4
Acrolein	0.88 ±0.11	5.03 ±6.67	0.96 ±0.11	2.37 ±6.73	0.73 ±0.16	38.66 ±26.45	0.77 ±0.18	35.70 ±30.88
Crotonaldehyde	0.94 ±0.09	0.56 ±1.70	0.99 ±0.08	0.68 ±1.48	0.84 ±0.17	8.00 ±10.01	0.89 ±0.20	6.15 ±11.89
Formaldehyde	0.93 ±0.08	1.71 ±2.87	0.95 ±0.08	2.30 ±2.73	0.90 ±0.10	10.33 ±11.10	0.92 ±0.11	8.75 ±11.43
Benzo[a]pyrene	0.96 ±0.07	0.06 ±0.52	0.93 ±0.06	0.13 ±0.41	0.95 ±0.10	-0.19 ±1.65	0.96 ±0.09	-0.19 ±1.47
Carbon Monoxide	0.95 ±0.10	0.59 ±1.12	1.00 ±0.14	0.09 ±1.69	1.01 ±0.15	-0.38 ±4.41	0.98 ±0.14	1.25 ±4.18
Nicotine	0.97 ±0.05	0.01 ±0.04	0.96 ±0.05	0.01 ±0.04	0.99 ±0.07	-0.02 ±0.15	0.98 ±0.07	-0.02 ±0.16
Tar	0.98 ±0.05	0.18 ±0.59	0.98 ±0.05	0.17 ±0.57	1.01 ±0.09	-0.85 ±2.85	1.01 ±0.07	-0.63 ±2.34
NNN [d]	0.98 ±0.04	1.49 ±3.76	0.99 ±0.04	0.64 ±4.17	0.95 ±0.05	2.58 ±12.02	0.99 ±0.05	-5.54 ±12.40
NNK [e]	0.96 ±0.06	3.76 ±5.17	0.93 ±0.06	3.45 ±4.79	1.04 ±0.09	-10.68 ±17.09	1.04 ±0.08	-13.06 ±14.87
1,3 Butadiene	0.91 ±0.05	1.86 ±2.71	1.01 ±0.07	-0.76 ±3.71	0.92 ±0.07	1.62 ±8.46	0.91 ±0.09	7.42 ±11.05
Acrylonitrile	0.99 ±0.06	0.06 ±0.67	1.02 ±0.07	-0.05 ±0.81	1.01 ±0.11	-1.14 ±3.58	0.90 ±0.10	2.89 ±3.35
Benzene	0.96 ±0.06	0.12 ±2.79	0.99 ±0.07	0.06 ±3.11	0.99 ±0.11	-3.14 ±10.68	0.84 ±0.11	±12.90 ±11.12
Isoprene	0.96 ±0.06	11.23 ±24.15	1.02 ±0.08	-0.73 ±31.50	1.00 ±0.09	-24.49 ±87.37	1.00 ±0.06	-2.96 ±57.32
Toluene	0.97 ±0.06	0.62 ±3.96	0.98 ±0.06	0.67 ±4.02	0.93 ±0.09	5.29 ±14.58	0.86 ±0.07	17.37 ±10.96

Table 5: Linear regression parameters (a, slope and b, intercept) to predict ISO and HCI smoke constituent yields.

[a]HPHC, harmful and potentially harmful constituent
[b]ISO, International Organization for Standardization
[c]HCI, Health Canada Intense
[d]NNN, N-nitrosonornicotine
[e]NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

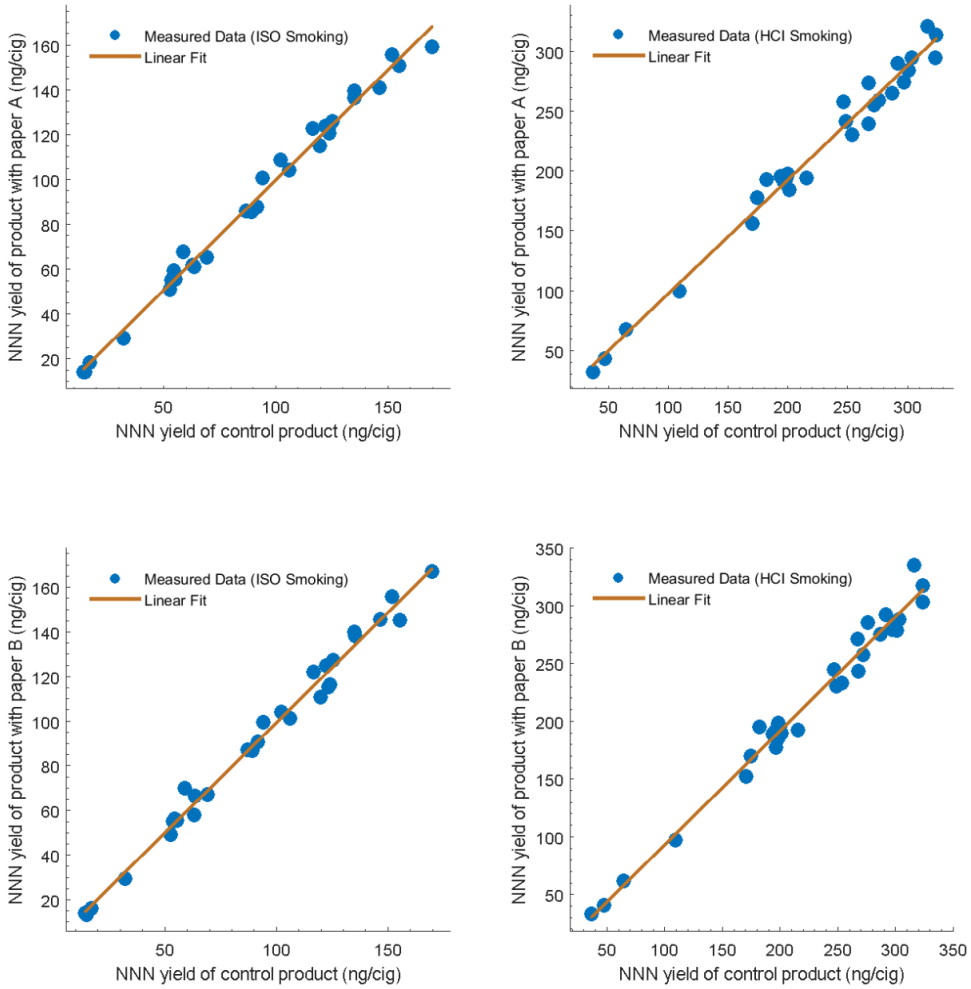

Figure 1: Correlation of product yields for NNN for control product versus product yields of NNN for Papers A and B for each smoking regimen (HCI and ISO) with regression lines.

with the 95% confidence intervals for each constituent and tar for both ISO and HCI smoking regimens are presented in Table 5.

A comparison of the X-Y plots and the regression line in the figures demonstrates that for those constituents with an R^2 approaching one, the data points represented by the yield for the control product and paper change tend to match the regression line (an example is Figure 1 for NNN with R^2 values between 0.991 and 0.981). For those with a lower R^2, the data points are more distant from the linear regression line (e.g., Figure 2 for crotonaldehyde yields for the HCI regimen with R^2 values of 0.788 and 0.745 for Papers A and B, respectively).

3.5. Estimation and validation of HPHC Yields for additional products

To test the consistency of the regression equations and how well these would predict HPHC yields for the remaining products, the measured HPHC yields for the 12 validation products were compared with their predicted yield estimated using the developed regression equation for each constituent. As an example, X-Y plots of the predicted constituent yields to the measured yields from a change in the paper, either Paper A or Paper B, are presented in Figures 3 and 4 for NNN and crotonaldehyde for the ISO and HCI smoking regimens. Upper and lower limits based on the method repeatability, using interlaboratory study results regarding the variability associated with testing for the analytes considered here [21, 34, 12, 13, 25, 27], are provided in each plot of Figures 3 and 4. As illustrated in Figures 3 and 4, the error associated with the predicted yields is in the same range as the expected method error. Similar figures for all constituents are available in Appendix D (Figs. S-4.1 through S-4.19).

The repeatability of each analytical method was estimated based on interlaboratory studies. These are approximate, since the analytical methods in the interlaboratory studies were not always the same as the methods employed in this study, and the published information for variability of the methods for some analytes is limited. The repeatability results from the interlaboratory studies were adjusted in a similar fashion to the adjustments in ISO 5725-6 section 4.2.1. That is, the repeatability limits illustrated in Figures 3 and 4 and Appendix D were adjusted based on the total number of cigarettes comprising a re-

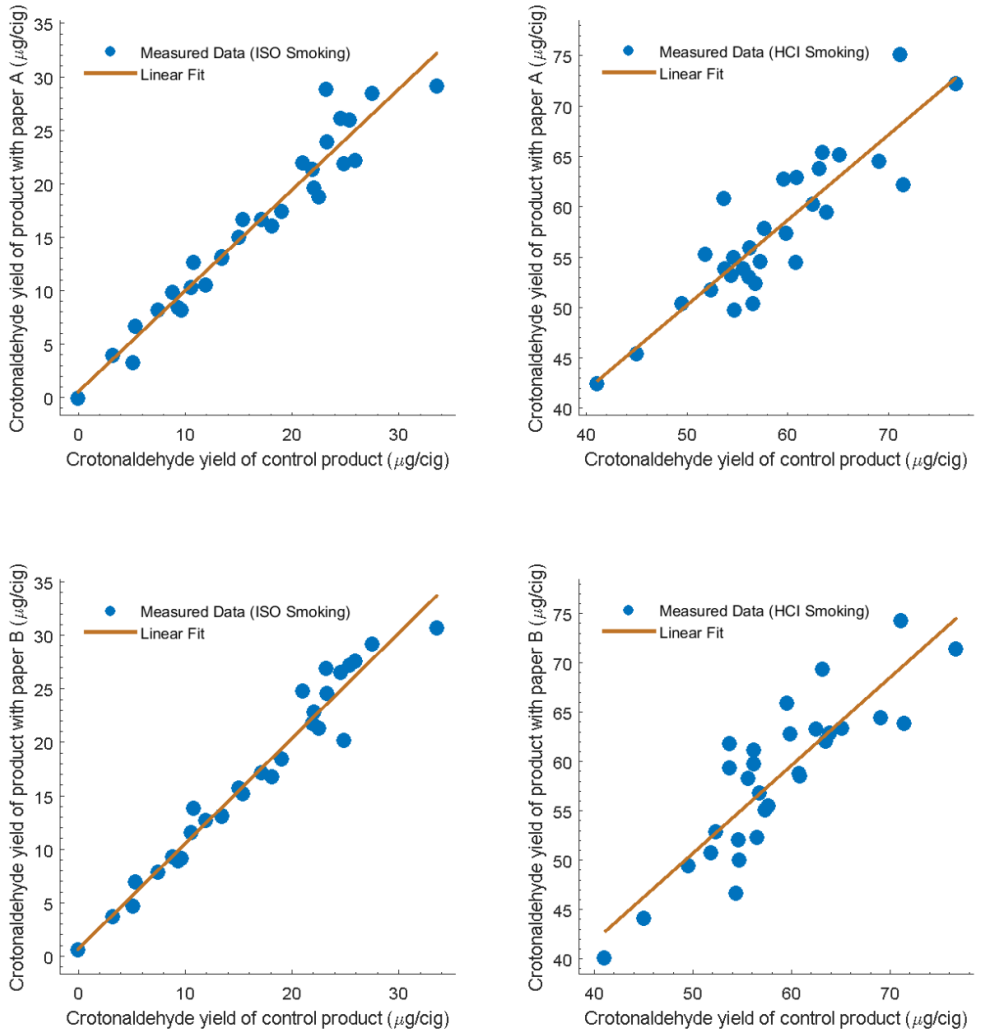

Figure 2: Correlation of product yields for crotonaldehyde for control product versus product yields of crotonaldehyde for Papers A and B for each smoking regimen (HCI and ISO) with regression lines.

sult in this study, compared to the number making up a result in the interlaboratory study. For example, in this study, a result for Nicotine under ISO conditions is the average yield from smoking 15 cigarettes (3 pads with 5 cigarettes/pad) in a single smoking machine run, while a result used to calculate the repeatability coefficient provided in ISO/TR 19478-1 is based on defining a test result as 20 cigarettes or four ports on a linear smoking machine. For this example, the repeatability coefficient was adjusted using the repeatability standard deviation, according to the following equations:

$$sr_{20} = \frac{r_{20}}{2.8} \tag{2}$$

$$sr_{15} = \frac{r_{15}}{2.8} \tag{3}$$

$$sr_{15} = \sqrt{\frac{20}{15}} * sr_{20} = \sqrt{\frac{4}{3}} * sr_{20}\ ; \tag{4}$$

where sr_{20} represents the standard deviation from smoking 20 cigarettes and sr_{15} represents the standard deviation from smoking 15 cigarettes. Details on the calculation of the repeatability limits as well as the averaged reported and adjusted repeatability coefficients can be found in Appendix E.

The root-mean-square error (RMSE) was calculated to assess the predictive capability of the linear regressions. The RMSE is a measure of the accuracy of the predictions made using the regression equation. The RMSE for each constituent, smoking regimen, and paper was estimated as:

$$RMSE = \frac{\Sigma(Y - Y')^2}{n}\ , \tag{5}$$

where Y is the measured concentration, Y′ is the predicted concentration, and n is the number of test samples. RMSEs for each constituent, paper and smoking regime are presented in Table 6. In order to assess the quality of the predictions, the RMSE was compared to the average adjusted repeatability predicted from the 30 test samples for each constituent, paper and

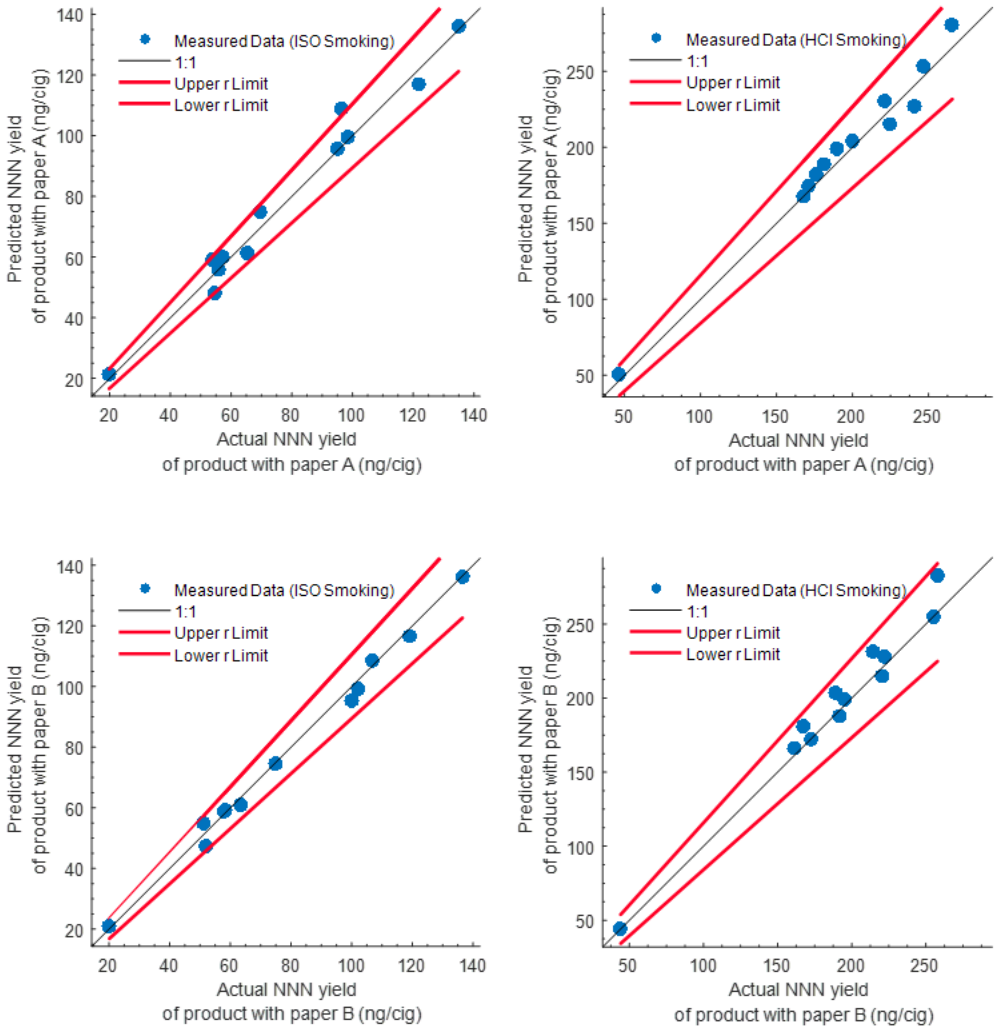

Figure 3: Predicted and measured yields for NNN for the validation data set.

smoking condition. The adjusted repeatability defines an interval within which approximately 95 percent of the measurements are expected to reside. Although the magnitude of the repeatability coefficients varies over the concentration range, the averaged adjusted repeatability is expected to be within a similar range of approximately twice the RMSE value, since it approximates the range where 95 percent of the data should reside. The averaged adjusted repeatability coefficients and the 95% RMSE intervals are also provided in Table 6.

4. Discussion

The evolution of cigarette manufacturing technology over the past several decades has resulted in modifications to cigarette design, incorporating variations in physical design parameters, variations in tobacco blends, and variations in non-tobacco materials utilized [9, 41]. A cigarette manufacturing company may choose to manufacture various products representing variations of the design parameters and tobacco blend. At the time of this study, Philip Morris USA had a portfolio comprising 147 cigarette designs based upon variations in physical design parameters, tobacco blends, and non-tobacco materials. Changes in any of these, either alone or in combination, may result in a change in the yield of mainstream cigarette smoke constituents. Prior to introducing a changed product into the marketplace, it is required that HPHC yields be submitted to the FDA. This publication has proposed an approach to allow a subset of the changed products to be tested, a regression equation developed, and the remainder to be estimated instead of separately tested.

The example presented in this publication discusses a change in the paper (Paper A and B) used in manufacturing of the cigarette products that would potentially affect the entire portfolio. To evaluate the HPHCs in the mainstream cigarette smoke constituents, each of the 147 cigarette designs would have to be manufactured with the changed papers (Paper A and B) resulting in the manufacture and testing of the entire abbreviated HPHC list for 294 products.

As an alternative to evaluating the entire portfolio of products, the proposed assessment approach provides a methodology for the selection of a subset of the portfolio to be manufac-

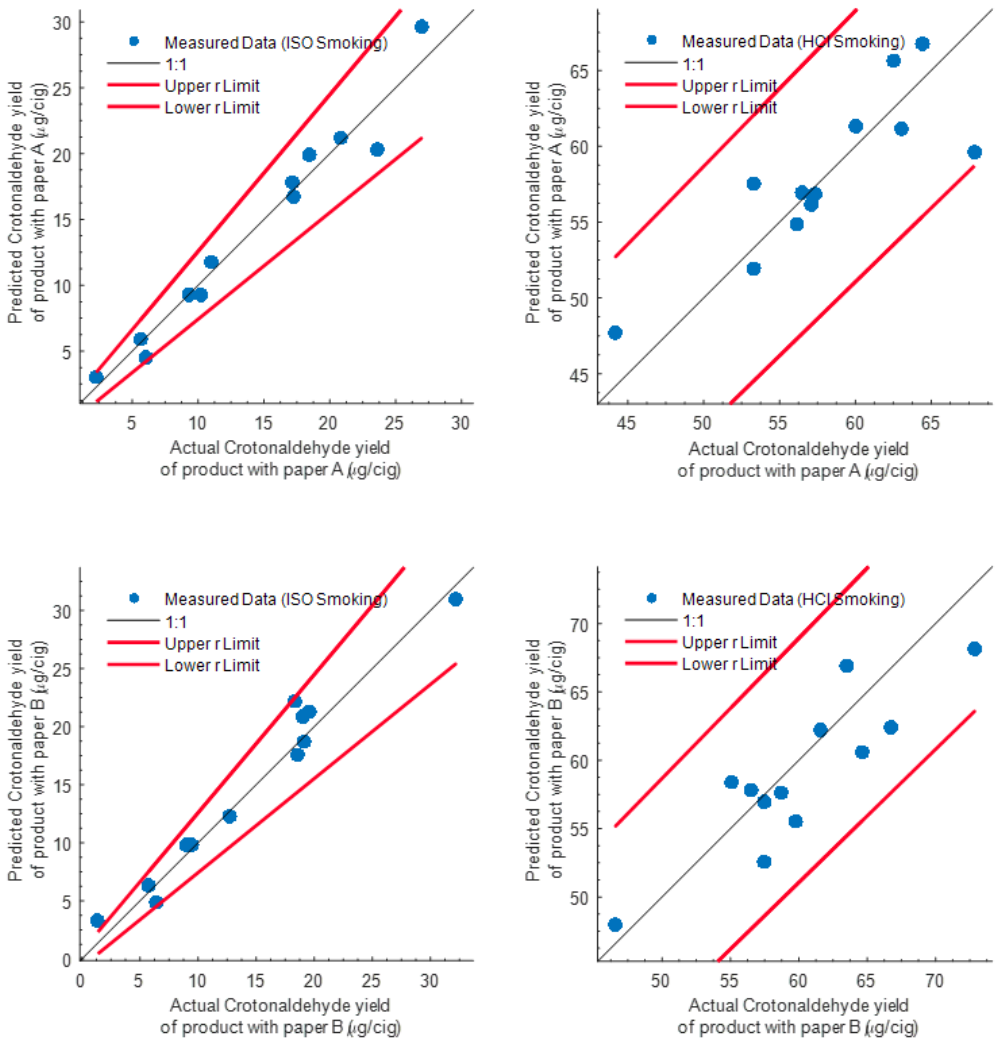

Figure 4: Predicted and measured yields for NNN for the validation data set.

tured and analytically evaluated with statistical analysis for use as the estimation tool for the remaining products in the portfolio. The selection of a representative subset of the portfolio was focused on nine variables (Table 2) that are likely to influence HPHC yield and resulted in a selection of a subset of 30 products that represent the entire portfolio for testing purposes. Following a review of the 147 cigarette designs, it was determined that there were only 25 unique combinations of cut filler type, cigarette paper band width, and cigarette paper permeability. The selection of products for evaluation included at least one product from each of these 25 combinations. For the selection of the remaining products included in the analytical subset, the distributions within each of the remaining six variables were considered. Ensuring that a minimum of one product was selected from the 25 combinations of cut filler type, cigarette paper band width, and cigarette paper permeability variables, and that the distribution of the remaining variables was retained, resulted in the selection of 30 products for evaluation. An additional 12 products were randomly selected from the 117 cigarette designs not included in the evaluation subset to be used to validate the regression equations generated from the statistical analysis.

Concurrent production runs with the control paper and the changed papers were performed for each of the 30 products (and 12 validation products), using the current production methods and conditions to minimize the effects of manufacturing variation (i.e., the only change in production was the paper, and since production occurred around the same time and with the same batch of cut filler, no, or little, temporal variability based upon production should exist). Analytical testing of the samples was performed for 18 smoke constituents (e.g., the HPHCs on FDA's abbreviated list for constituents in cigarette smoke [Table 1]) and tar by an ISO 17025 accredited laboratory. Performing all analytical procedures in the same laboratory following the same methodology at approximately the same time removes the possibility of inter-laboratory variability and reduces the potential for intra-laboratory variability.

In order to determine if the HPHC yields measured from the changed products were comparable to HPHC yields measured from the control product, equivalence testing was carried out

HPHC [a]	RMSE [b]				Average predicted adjusted r				95% RMSE Intervals			
	ISO [c]		HCI [d]		ISO		HCI		ISO		HCI	
	Paper A	Paper B	Paper A	Paper B	Paper A	Paper B	Paper A	Paper B	Paper A	Paper B	Paper A	Paper B
Ammonia (μg/cig)	0.90	0.89	2.0	1.4	2.08	2.06	5.46	5.50	1.76	1.74	3.90	2.76
1-Naphthylamine (ng/cig)	0.89	0.58	0.9	1.1	0.70	0.67			1.75	1.14	1.73	2.24
2-Naphthylamine (ng/cig)	0.30	0.38	0.4	0.7	0.34	0.33			0.59	0.75	0.82	1.37
4-Aminobiphenyl (ng/cig)	0.08	0.06	0.1	0.1	0.15	0.15			0.16	0.11	0.21	0.27
Acetaldehyde (μg/cig)	32.15	34.69	97.7	82.3	81.50	81.74	185.67	185.54	63.01	68.00	191.58	161.39
Acrolein (μg/cig)	4.30	4.41	12.0	9.9	10.21	10.51	18.30	18.31	8.43	8.64	23.50	19.47
Crotonaldehyde (μg/cig)	1.45	1.59	3.2	3.3	3.71	3.86	8.87	8.87	2.85	3.12	6.26	6.38
Formaldehyde (μg/cig)	3.83	3.35	6.6	6.4	6.41	6.68	19.29	19.16	7.51	6.57	12.97	12.54
Benzo[a]pyrene (ng/cig)	0.39	0.33	0.7	0.5	0.49	0.47	1.49	1.50	0.77	0.65	1.47	1.07
Carbon monoxide (mg/cig)	0.78	0.54	2.3	2.7	1.12	1.12	2.91	2.97	1.53	1.06	4.59	5.36
Nicotine (mg/cig)	0.02	0.02	0.1	0.1	0.07	0.07	0.22	0.22	0.05	0.04	0.12	0.17
Tar (mg/cig)	0.35	0.27	1.2	1.3	1.07	1.07			0.68	0.52	2.31	2.49
NNN [e] (ng/cig)	5.16	2.68	8.5	10.8	9.77	9.73	28.19	28.27	10.10	5.26	16.74	21.26
NNK [f] (ng/cig)	3.49	3.23	11.0	11.4	9.94	9.69	24.09	23.77	6.83	6.34	21.61	22.39
1,3-Butadiene (μg/cig)	1.98	2.23	6.1	6.0	5.76	6.01	15.75	16.43	3.89	4.37	11.86	11.84
Acrylonitrile (μg/cig)	0.45	0.67	1.7	2.0	1.30	1.33	3.90	3.93	0.88	1.31	3.26	3.82
Benzene (μg/cig)	1.36	2.25	4.0	3.3	3.80	3.87	8.25	8.28	2.66	4.41	7.84	6.55
Isoprene (μg/cig)	16.16	19.47	44.5	35.5	37.87	38.82	104.79	105.25	31.68	38.16	87.16	69.66
Toluene (μg/cig)	2.95	3.91	5.4	6.9	7.50	7.60	14.22	14.33	5.79	7.66	10.56	13.57

Table 6: Validation comparison of RMSE and adjusted r.

[a]HPHC, harmful and potentially harmful constituent
[b]RMSE, root-mean-square error
[c]ISO, International Organization for Standardization
[d]HCI, Health Canada Intensive
[e]NNN, N-nitrosonornicotine
[f]NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

on the relative smoke yield differences between the control and changed papers for each analyte measured under both smoking regimes. The relative differences for all constituent yields were found to be equivalent (within ±10%) indicating that the changed products were comparable to the control product.

Linear regression modeling was performed to estimate the functional relationship between the mainstream smoke constituents of the control cigarettes with those following a change in paper, Paper A or B. Using the R^2 to assess the strength of the linear relationship indicated that the yield for each of the HPHCs for control products was strongly correlated ($R^2 \geq 0.85$) with the yield for products with the change in paper (Paper A or Paper B) for all constituents when using the ISO smoking regimen, and for the majority of constituents when using the HCI smoking regimen. Generally, the correlations under the HCI smoking regimen were lower than with the ISO regimen, since filter ventilation, a major source of product-to-product smoke yield differences, is eliminated with HCI, because the ventilation holes are blocked. Those constituents in the HCI smoking regimen with lower R^2 values were acetaldehyde (R^2 values of 0.769 for Paper A and 0.771 for Paper B), acrolein (R^2 value of 0.764 for Paper A and 0.723 for Paper B), and crotonaldehyde (R^2 values of 0.788 for Paper A and 0.745 for Paper B). It is likely that the high method variability contributed to the lower correlation coefficient for acetaldehyde. The method variability for crotonaldehyde and acrolein is moderately high compared to the other constituents, and inspection of the x-y plots illustrates that many of the results for these two constituents are clustered in a small range, which would contribute to the lower observed R^2 values.

To test the consistency of the regression equations and how well these would predict HPHC yield for the remaining products, the measured HPHC yields for 12 validation products were compared with their predicted yields estimated using the developed regression equation for each constituent. Inspection of the validation plots shows that less than 6 percent of the predicted results fall outside the adjusted method repeatability limits. This is as expected, since the repeatability limits represent the range at which we expect 95 percent of the measured values to fall within. Comparison of the 95% RMSE intervals with the adjusted repeatability coefficients indicates that the yields predicted with the regression equations are within – or very close to – the method repeatability. This analysis of the validation samples confirms that the regression equations can reliably predict the yields for the non-analyzed products.

The results presented in this analysis demonstrate that reliable predictions of the HPHC yields in non-analyzed products of a company's portfolio of products can be made using linear regression equations developed from the yields observed in the analyzed products when the yields of the changed products are comparable to the unchanged products. While this report focuses on an example of a change in the cigarette paper used, which may affect all or much of the entire portfolio, the technique would also be useful for prediction with other changes, such as material, ingredient or tobacco additive changes that affect a subset of the products within a company's cigarette portfolio. For each new change, an appropriate methodology for the identification of the products used to define the regression equations must be developed. Using the statistical analysis approach presented would allow a company to define a specific subset of the products affected by a proposed change for evaluation. Therefore, only those specific products would need to be produced and analyzed. In the example presented, only 42 (30 in the evaluation subset and 12 in the validation subset) of the 147 cigarette designs, approximately 29 percent, affected by the change, were analyzed. An evaluation conducted in this manner would significantly reduce the amount of time and expense necessary to generate and analyze the product samples.

This testing was conducted in a way intended to minimize variability to the extent practical. To minimize the production variability, all three sample products (current control paper and paper A and paper B) were made at the same time using the same production method and the same materials other than papers, including the same batch of cut filler. Variability associated with use of the smoking machine and analytical testing was minimized by generating the samples for each of the three sample products (e.g., those manufactured using the control paper, Paper A, and Paper B) by using the same smoking machine, smoking the three sample products as closely together in time as practicably possible and by using the same ISO 17025 accredited analytical lab for testing.

5. Conclusions

A tobacco product manufacturer may need to make changes that affect all or much of its portfolio to ensure quality, consistency, and supply security of its product portfolio over time. The results presented in this analysis demonstrate that reliable predictions of the HPHC yields in non-analyzed products of a company's portfolio of products can be made using linear regression equations developed from the yields observed in the analyzed products when the yields of the changed products are comparable to the unchanged products. Rather than testing each product individually, designed experiments can be conducted using a subset of products that encompasses the major design characteristics of the portfolio and statistical modeling performed to determine the HPHC yield for the rest of the portfolio.

This investigation describes a statistical approach for demonstrating equivalence and estimating the HPHC yields in non analyzed products based upon linear regressions determined for the products in a portfolio for which analytical measurements of HPHC concentrations have been determined. The example change considered in this investigation, a change in paper used in the manufacturing of the cigarette products, may affect all or most of the entire portfolio, but the methodology would be applicable for any other material, ingredient or tobacco additive change that affected a subset of the portfolio. The critical component of the methodology is the identification of an adequate subset of products that are being affected by the change. The usefulness of this approach will be seen through the timely reporting of regulatory submissions and reduction in cost and materials required to obtain tested/predicted smoke yields for the portfolio.

6. Declaration of Conflicting Interest

The authors declare no conflicts of interest.

7. Acknowledgement

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8. Article Information

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10. Supplemental Materials

Supplemental figures and tables are located at http://www.feedhaccp.org/distance/elearning/JRS/2019/jrs-v07hannel_appendix.pdf on the following pages:

- Appendix A: Figure S-1.1 (page 1)
- Appendix B: Figures S-2.1 through S2.12 (pages 2-13)
- Appendix C: Figures S-3.1 through S-3.19 (pages 14-23)
- Appendix D: Figures S-4.1 through S-4.19 (pages 23-32)
- Appendix E: Figure S-5.1, Tables S-5.1 and S-5.2 (pages 33-34)

Appendix A: Cigarette Selection Criteria

In an effort to select a representative subset of the entire portfolio, it was necessary to understand the distribution of the products within three of the categorical variables (i.e., cut filler type, cigarette paper band width, and cigarette paper permeability) and the distribution within the other variables. Cigarette paper band width and permeability are important variables since they directly affect (1) air supply needed for static burning and (2) air flow through burning coal during a puff; both of which affect smoke yield and potentially its composition. Based on the three categorical variables considered, there are two paper band width categories which can be combined with three different permeability values possible for each cut filler type. Figure S-1.1 illustrates the six possible cigarette paper design configurations for each cut filler type. A total of 36 product configurations are possible (six band width-permeability design configurations * six cut filler types). Review of the 147 cigarette designs indicated there were only 25 actual combinations of these three categorical variables (i.e., no products exist for some cut filler, band width and permeability combinations). Our selection of the products for evaluation required inclusion of at least one product from each of the 25 possible categorical combinations. Of the identified 25 categorical combinations, seven combinations had only one associated product; therefore, each of these products was included in the representative subset of products for evaluation.

For the selection of the remaining products to be included in the representative subset, the distributions of the other variables were taken into consideration. Each continuous variable was reviewed and values were grouped to represent their distributions. For example, as indicated in Table 2, the values presented for filter ventilation were 0 (indicating a non-ventilated cigarette) or a value between 12% and 71%. Based upon the distribution of the values, eight groups were defined to represent the possible ranges of the ventilation variable (e.g., 0, $10 \leq x < 20$, $20 \leq x < 30$, $30 \leq x < 40$, $40 \leq x < 50$, $50 \leq x < 60$,

$60 \leq x < 70$, and $x \geq 70$). Products for inclusion were selected in an effort to approximate the proportion of total products in each of the eight groups. In those cases where the selection decision was between two products with similar results for the distributional variables, manufacturing volume of the products was used to determine the product with the higher production volume and that product was selected. Based upon these selection criteria, 30 products were identified to be considered as the representative subset of the entire portfolio. As indicated previously, this subset contained one product from each of the 25 categorical combinations with multiple products selected from the other variables in order to approximate the distribution of variables in the overall set of 147 cigarette designs.

Appendix B: Product Average and Standard Deviations

The sample average and standard deviation calculated for the 30 test products and 12 validation products for each smoking regimen and paper combination is reported in Figures S-2.1 through S-2.12.

Appendix C: Calibration Figures

Plots of the individual HPHC yields for control products (X axis) to the HPHC yields from products with the paper change (Yaxis for either Paper A or Paper B), are presented in Figures S-3.1 through S-3.19.

Appendix D: Validation Figures

The actual measured smoke yields versus the predicted yields for Paper A and B are shown in Figures S-4.1 through S-4.19 for each HPHC constituent and tar under ISO and HCI smoking regimens. The upper and lower r limits in the figures below are based on CORESTA and ISO reported method repeatability for each constituent for ISO and HCI smoking regimens. The r values were adjusted to account for the number of cigarettes tested to generate a single test result.

Appendix E: Adjusted Repeatability

Adjusted Repeatability

Adjusted repeatability (r) coefficients were calculated to adjust for differences between the total number of cigarettes comprising a result in the referenced CORESTA and ISO [21, 34, 12, 13, 25, 27] reports and in this study. Table S-5.1 indicates the total number of cigarettes comprising a result in this study versus the total determined from each referenced report. The reported repeatability coefficients were adjusted using repeatability standard deviations according to the following equations:

$$sr_{Adj} = \frac{r_{Adj}}{2.8} \tag{S-5.1}$$

$$sr = \frac{r}{2.8} \tag{S-5.2}$$

$$sr_{Adj} = \sqrt{\frac{N}{N_{Adj}}} * sr\,; \tag{S-5.3}$$

Where r and sr are the reference reported repeatability and repeatability standard deviation, respectively, r_{Adj} and sr_{Adj} are the adjusted repeatability and standard deviation and N is the total number of cigarettes which comprise a single result in the reference and used in this study, N and N_{Adj}, respectively. Table S-5.2 provides the average reference reported yield and r coefficients and the calculated r_{Adj}. This is analogous to the equations given in ISO 5725-6.

Adjusted Repeatability Limits

Linear regression analysis was performed to determine the relationship between the reported mean yields and the calculated adjusted repeatability coefficients for each constituent and smoking regime. Table S-5.3 contains the calculated R^2 values and regression coefficients for each constituent and smoking regime. The yields were strongly correlated to the adjusted repeatability coefficients for the ISO smoking regime with all R^2 values greater than 0.66. Only two data points were available for 1-napthylamine, 2-napthylamine and 4-aminobiphenyl for the ISO smoking regime and no data was found under HCI smoking conditions for these three constituents and tar. Correlations were less significant for most of the constituents under HCI smoking conditions. Several transformations were applied to the data to determine if a better fit could be achieved. The fit of ammonia was improved slightly when a log transformation was applied; however, since the improvement was not significantly better than the linear fit this transformation was not used.

Upper and lower adjusted repeatability limits were plotted on actual versus predicted plots shown in Figures 4 and 5 and Figures S-4.1 through Figure S-4.19 by first calculating the predicted adjusted repeatability for each actual yield measurement using the regression coefficients (slope and intercept) reported in Table S-5.3. The upper and lower limits were then plotted as the actual measured yield versus the actual measured yield plus or minus the predicted adjusted repeatability. As an example, Figure S-5.1 shows the actual versus predicted plot for nicotine yield of the product with Paper A with the fitted upper and lower adjusted r limits and the actual adjusted repeatability limits for nicotine calculated from the repeatability coefficients from [27]. As illustrated in Figure S-5.1 the fitted limits approximate the actual data reasonably well and serve as a gage for assessing the accuracy of the model predicted yields.