CORESTA Biomarkers Sub-Group

Technical Report

Meta-Analysis Study to Establish Baseline Levels of COHb and NEQs in Smokers and Non-Smokers

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1. INTRODUCTION

In the context of tobacco products, broadly two types of biomarkers are assessed, biomarkers of exposure (BoE) and biomarkers of potential harm (BoPh). BoE measures exposure to tobacco constituents in the gas/vapor or particulate phase, e.g. carbon monoxide (CO) and nicotine. The purpose of the study (a project of CORESTA Biomarker Sub-Group, BMK-186-NWIP) was to establish population level estimates for biomarkers of cigarette smoke exposure to serve as a baseline against potential changes in exposure upon switching to a potentially modified risk tobacco product.

A meta-analysis of data published 2008-2018 was conducted to estimate population levels for blood Carboxyhemoglobin (COHb), a biomarker for CO, and urinary Nicotine Equivalents (NEQs), a biomarker of nicotine exposure. Meta-analysis systematically combines pertinent observations, from several selected studies, to develop a single conclusion that has a greater statistical power. First a protocol for assessment of literature was developed [see Literature Searches] followed by an evidence-based table to identify and select studies. The database was built following a data template that identified elements in four major categories: study identification, design, results and demographics. Once the database was considered complete, studies that met criteria (e.g. clinical studies, values in critical units reported) were selected. Studies in the final database (Microsoft excel spreadsheet) were processed by reading and filtering data according to published years, by group (e.g. smokers), source of data, tobacco industry or academia, and by statistical estimates (e.g. mean, S.D., etc.). Finally, data analysis was conducted for each biomarker.

In total, 119 scientific studies were identified for the two biomarkers considered in this meta-analysis with 56 studies reporting clinical findings. At the end data from 31 studies were found to meet the criteria for the inclusion in this analysis. The database was organized by categories, filtered, and data weighted according to the size of the groups. Based on the weighted data, Epanechnikov smoothing kernel density was used to estimate the average, the standard deviation and the 95 % confidence interval for each group (smokers, non-smokers and former smokers) and each biomarker (COHb and NEQs). For both biomarkers most of the data was derived from smokers, followed by non-smokers and former smokers (cessation group). The population level estimate for smokers was found to be significantly different from non-smokers and former smokers. The smoker population estimate was statistically and substantially higher than non-smokers and former smokers for both COHb and NEQs.

In summary, the population level estimate for smokers can be used as the baseline against which changes in exposure for smokers switching to a potentially reduced-risk tobacco product and/or following cessation can be compared.

2. ABBREVIATIONS AND TERMINOLOGY REFERENCES

- BoE: Biomarkers of Exposure
- BoPh: Biomarkers of Potential Harm
- CO: Carbon Monoxide
- NEQs: Nicotine Equivalents
- COHb: Carboxyhemoglobin

3. PURPOSE

The purpose of the study was to establish population level estimates for biomarkers of cigarette smoke exposure to serve as a baseline against potential changes in exposure upon switching to a potentially reduced-risk tobacco product.

4. PARTICIPATING COMPANIES

The meta-analysis study was initiated with the participating companies shown in Table 1.

Table 1. Participating companies

Company	Delegate(s)	Country
ITG Brands LLC	Felix Ayala-Fierro (Team Lead)	U.S.A.
Philip Morris International	Ashraf Elamin	Switzerland
RAI Services Company USA	Kimberly Frost-Pineda Tao Jin Eckhardt Schmidt G.L. Prasad	U.S.A.
Altria Client Services	Mohamadi Sarkar	U.S.A.
Imperial Brands PLC	Thomas Verron	U.K.

5. BIOMARKERS

The meta-analysis included two biomarkers, carbon monoxide and nicotine equivalents, selected from two matrixes, blood and urine. The selected biomarkers needed to be robust to establish a baseline level of exposure to combustible cigarettes in the population and serve as a comparator for changes in exposure related to potentially modified risk products.

5.1 Carboxyhemoglobin (COHb)

Carboxyhemoglobin is a biomarker for carbon monoxide (CO) exposure. CO is a product of incomplete combustion, it has a half-life of 4-6 h, and therefore can only be utilized as a short-term biomarker. CO exposure can be measured in the *blood* as carboxyhemoglobin (as percent saturation of hemoglobin) and in exhaled breath (as concentration of CO in parts per million). The close correlation between exhaled CO and carboxyhemoglobin is well-documented and thus they serve as proxies for one another. CO biomarkers are well-established with respect to distinguishing cigarette smokers from non-smokers, responding to cessation and cigarette reduction, and having a dose-response with cigarettes per day.

5.2 Nicotine Equivalents (NEQs)

Nicotine equivalents is a biomarker of exposure for nicotine. Nicotine and cotinine measurements do not completely explain total nicotine intake due to their metabolic transformation to water-soluble N-oxides, glucuronides and trans-3′-hydroxycotinine passed in urine. In contrast, total nicotine equivalents (TNE) is the sum of urinary nicotine, cotinine, and several metabolites in the nicotine metabolic profile; TNE is considered the gold standard for daily nicotine intake. TNE exhibits strong correlation with several tobacco exposure biomarkers including hydroxy-PAHs and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). In this meta-analysis the NEQ subtleties were not specified; however, it is believed to include Nic+5 biomarkers.

6. LITERATURE SEARCHES

The literature searches were conducted using the electronic databases listed below.

- PubMed (www.ncbi.nlm.nih.gov/pubmed)
- Science Direct (www.sciencedirect.com)
- ToxNet (www.toxnet.nlm.nih.gov)
- Google Scholar (https://scholar.google.com/)

Searches were conducted for "clinical studies" mentioning each specific biomarker [carboxyhemoglobin, COHb, nicotine equivalents, NEQs] published between 2008 and 2018. Data was used to create an evidence table that captured a series of elements for evaluation that determined eligibility.

7. EVIDENCE TABLE

The table lists eligibly criteria that includes the following categories:

- Timeframe
  - Studies published from January 2008 to December 2018
- Publication information
  - Publication ID (assigned for each study)
  - Publication year
  - Type of publication (research article)
  - Journal name
  - Country of publication
  - Authors
  - Title of publication
  - Company name/Institution name
  - Relevance for study (Yes, No)
  - Available (Yes, No)
- Study design
  - Type of study (if clinical study meets criteria for inclusion)
  - Method of randomization
  - Method assessment outcome
  - Handling of protocol deviations
  - Cohort
  - Time collection sample (D0 = baseline, time (D1), time (D2))
  - Type of subject (smoker, non-smoker)
  - Sample size (Total # individuals, # individuals per group)

- Biomarker/Analyte data
  - Biomarker
  - Type of biomarker (blood, urine)
  - Specimen information (material type, volume, volume unit)
  - Analyte value
  - Analyte units
  - Standard deviation
  - Range
  - Lower/higher limits
  - Statistical method
  - Analytical method
  - LLOQ, Range information
- Demographics
  - Gender (male, female)
  - Race (White, Black, other, NA)
  - Age (mean, median, standard deviation, minimum, maximum)
  - BMI (mean, median, standard deviation, minimum, maximum)
  - Cigarettes per day (mean, median, standard deviation, minimum, maximum)
  - Years smoked (mean, median, minimum, maximum)
  - Smoker status verified (yes, no, explain)

Clinical studies and observational studies with reportable values were considered relevant studies.

8. RESULTS

8.1 Literature Review

In total, 117 scientific studies were identified for the two biomarkers considered in this meta-analysis with 36 studies reporting values (see References) (two studies reported values for both biomarkers and one was not available). At the end, data from 31 studies were found to meet the criteria for the inclusion in this analysis.

For COHb a total of 50 scientific studies were identified with 28 studies reporting clinical findings. A total of 20 articles met the criteria with reported values: 16 articles were published by tobacco companies and 4 were from academia (Table 3).

For NEQs, a total of 69 scientific articles were identified with 14 studies reporting clinical findings but one was not available for consideration. A total of 11 articles met the criteria with reported values: 9 articles were published by tobacco companies and 2 were from academia (Table 3). The studies with values in mg/24h or with units that could be converted were included in the calculations.

Table 2. Total scientific studies

Publications	Biomarker	Number of Studies
Overall Publications	Blood Carboxyhemoglobin	50
	Urine NEQs	69
Reports with Clinical Findings	Blood Carboxyhemoglobin	28
	Urine NEQs	14
Included in Meta-Analysis	Blood Carboxyhemoglobin	20
	Urine NEQs	11

Table 3. Yearly distribution and sources of data from scientific publications

Publication Year	Carboxyhemoglobin		NEQs	
	Tobacco Industry	Academia	Tobacco Industry	Academia
2008	1	1	1	0
2009	2	0	3	0
2010	3	1	0	0
2011	1	0	1	0
2012	3	1	0	0
2013	0	1	0	0
2014	0	0	1*	0
2015	1	0	0	1
2016	4	0	2	0
2017	1	0	0	0
2018	0	0	1	1
Total	**16**	**4**	**9**	**2**

* NEQs data from third-party contractor laboratory

8.2 Data Analysis

The database was organized by categories, filtered, and data weighted according to the size of the groups using Epanechnikov smoothing kernel density.

For carboxyhemoglobin, most of the data was derived from smokers (19282 individuals) followed by non-smokers (1949 individuals) and former smokers (cessation group) (278 individuals). Not surprisingly, smokers had the highest % COHb (5.22 %) compared to former smokers (1.75 %) and non-smokers (1.05 %).

Table 4a. Percent carboxyhemoglobin (% COHb)

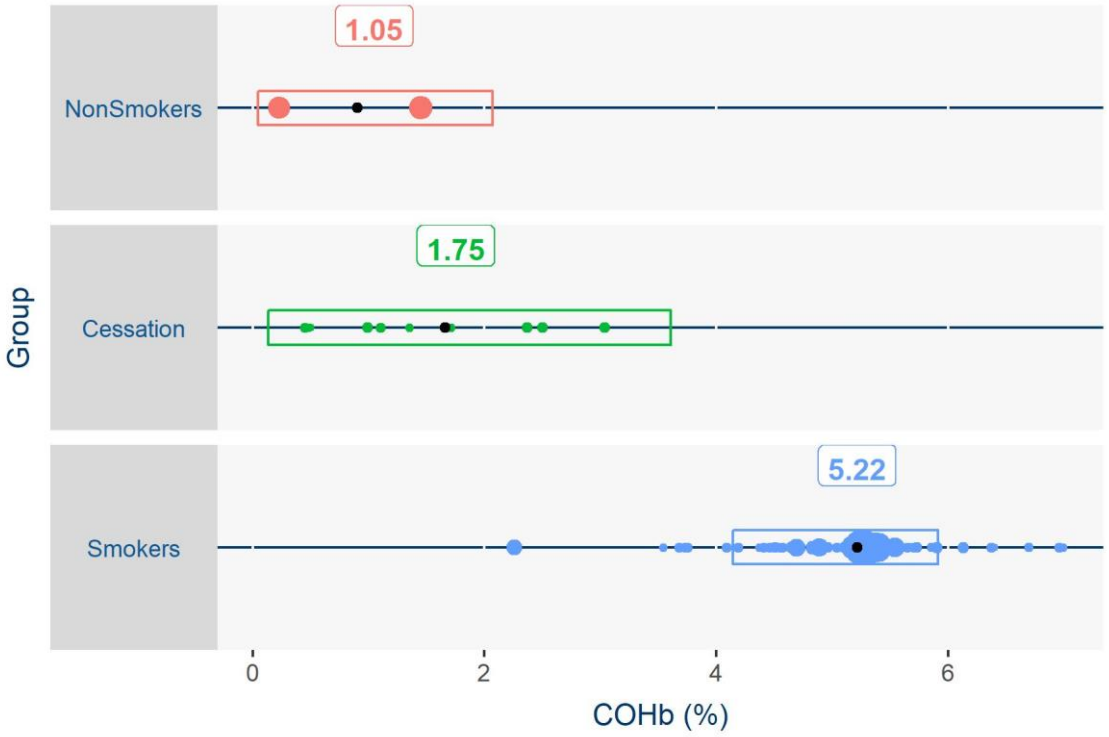
Group	# Groups	Total Individuals	Average	Min	Max	S.D.	95 % CI (LL, UL)
Non-Smokers	2	1949	1.05	0.23	1.45	0.61	(0.04, 2.07)
Former, Cessation	10	278	1.75	0.45	3.04	1.00	(0.14, 3.61)
Smokers	61	19282	5.22	2.26	6.99	0.53	(4.14, 5.91)

Statistical analysis indicates that two groups are significantly different: Smokers vs. non-smokers (p-value < 0.0001) and smokers vs. former smokers (cessation group) (p-value < 0.0001). No statistically significant difference was found for former smokers vs. non-smokers.

Table 4b. Statistical results for %COHb

Group Comparison	Difference	P-value	Statistical Outcome
Former vs. Non-Smokers	0.7559	0.313	Not significantly different
Smokers vs. Non-Smokers	4.3111	< 0.0001	Significantly different
Smokers vs. Former Smokers	3.5552	< 0.0001	Significantly different

Figure 1. Population COHb levels in smokers compared to non-smokers and cessation



For NEQs most of the data was derived from smokers (1768 individuals) followed by non-smokers (129 individuals) and former smokers (cessation group) (55 individuals). Smokers had the highest NEQs levels (mg/24h) (14.84) compared to former smokers (0.62) and non-smokers (0.058).

Statistical analysis indicates that only two groups are significantly different: Smokers vs. non-smokers (p-value < 0.0001) and smokers vs former smokers (cessation group) (p-value < 0.0001).

Table 5a. Nicotine equivalents (mg/24h)

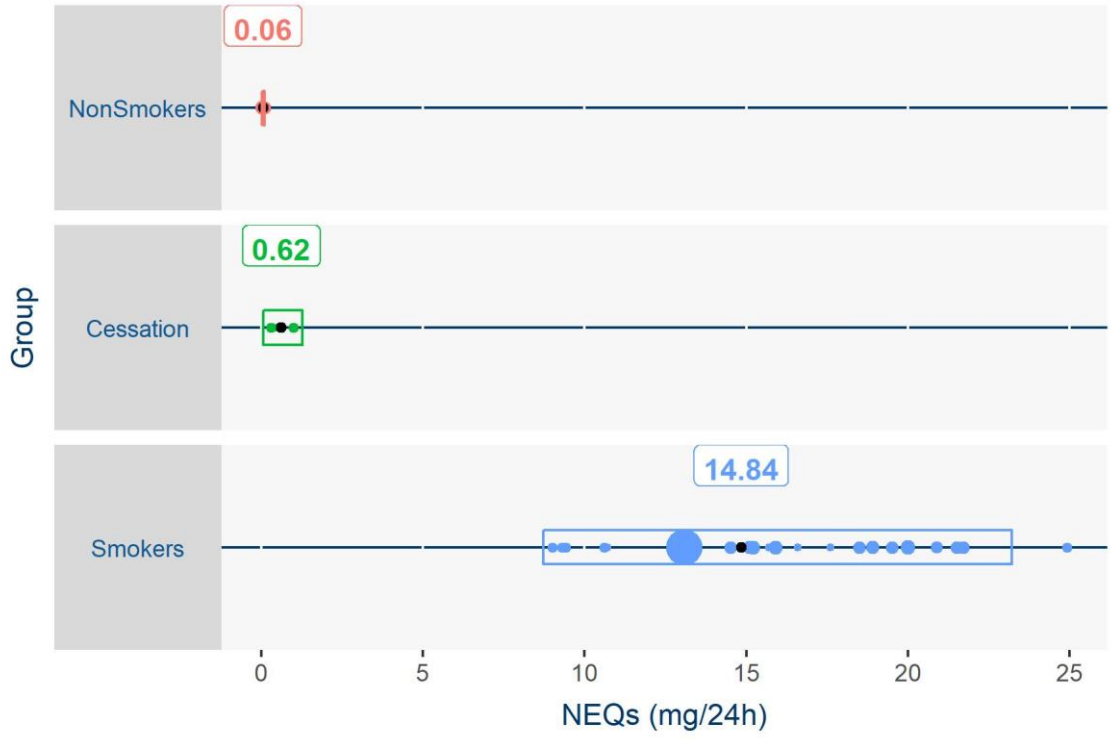
Group	# Groups	Total Individuals	Average	Min	Max	S.D.	95 % CI (LL, UL)
Non-smokers	4	129	0,058	0,03	0,10	0,031	(0,016, 0,112)
Former, Cessation	3	55	0,62	0,30	1,00	0,35	(0,07, 1,28)
Smokers	25	1768	14,84	9,00	24,91	3,76	(8,72, 23,21)

Statistical analysis indicates that only two groups are significantly different: Smokers vs non-smokers (p-value < 0.0001) and smokers vs. former smokers (cessation group) (p-value < 0.0001).

Table 5b. Statistical results for NEQs

Group Comparison	Difference	P-value	Statistical Outcome
Former vs. Non-smokers	0,5512	0,989	Not significantly different
Smokers vs. Non-smokers	14,7837	< 0,0001	Significantly different
Smokers vs. Former	14,2325	< 0,0001	Significantly different

Figure 2. Population NEQs levels in smokers compared to non-smokers and cessation



9. CONCLUSION

This meta-analysis identified clinical studies for two biomarkers of exposure, COHb and NEQs, in relation to smokers, non-smokers, and former smokers (cessation group). The objective was to establish population level estimates for biomarkers of cigarette smoke exposure to serve as a baseline against potential changes in exposure upon switching to a potentially reduced risk tobacco product.

The population level estimate for smokers was established for the two biomarkers of exposure. This estimate was compared to that from two other groups, non-smokers and former smokers, to determine whether a statistical difference existed in comparison to these groups. The population level estimate for smokers was found to be significantly different from non-smokers and former smokers. The smoker population was clearly identified from the non-smokers and former smokers for both COHb and NEQs. Population level estimates for non-smokers were not significantly different from former smokers (cessation group) (p-value = 0.989).

10. RECOMMENDATIONS

The population level estimate for smokers can be used as the baseline against which changes in exposure for smokers switching to potentially reduced risk tobacco products and/or following cessation can be compared.

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