Meta-Analysis to Establish Population Level Estimates of NNAL in Smokers and Non-smokers

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Meta-Analysis Project

❖ Purpose

- ➢ **Establish pooled average-observed levels for biomarkers of cigarette smoke exposure to serve as baseline for comparisons against changes in exposure for reduced risk products**

❖ Research question

- ➢ **What is the average-observed level estimate for urinary total NNAL? Previously levels for nicotine equivalents and blood carboxyhemoglobin were established (Published CORESTA Report: BMK-161)**

❖ Approach

- ➢ **Conduct a meta-analysis**
  - A method for systematically combining pertinent observations from several selected studies to develop a single conclusion that has greater statistical power

Participating Companies

Company	Delegate(s)	Country
JUUL Labs Inc.	Felix Ayala-Fierro (Lead)	U.S.A.
Imperial Brands PLC	Thomas Verron	U.K./France
Altria Client Services	Mohamadi Sarkar (Co-Lead) Pavel (Paul) Lizhnyak	U.S.A.
RAI Services Company USA	Kimberly Frost-Pineda G.L. Prasad Robert (Rob) Freeland	U.S.A.
Philip Morris International	Ashraf Elamin	Switzerland

Biomarkers: NNK, NNAL

NNK

- NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) is a tobacco-specific nitrosamine; NNK is found in the particulate phase of cigarette smoke
- NNK is identified as a IARC Group 1 carcinogen and is listed as a HPHC in cigarette smoke by FDA

NNAL

- Total urinary NNAL represents metabolites of NNK and is a well-established biomarker to assess NNK exposure
- NNAL has a long half-life (>10d) and is not prone to the variability in measurement from transient changes in smoking behavior
- NNAL levels correlate with cigarette consumption (Rostron *et al* 2020) and provide a reasonable proxy for overall smoke exposure
- NNAL has been shown to differentiate between smokers, nonsmokers and combustible products (Goniewicz *et al* 2018)

Literature Searches

Electronic Databases

Data reported in publications from 2008 - 2020

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

Evidence Table

Method for Inclusion

- A method was developed for systematic assessment of literature
- Data was extracted from publications to create a master evidence table with elements for evaluation
- Elements determine eligibility for inclusion

Data Template

Labels

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- ID
- Year of Publication
- Type of Publication
- Publication Name (i.e. Journal)
- Country
- Authors
- Company/Institution
- Title
- Relevance for Study
- Available

Design

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- Type of Study
- Method Randomization
- Method Assessment Outcome
- Handling Protocol Deviations
- COHORT
- Time (D0, D1, D2, etc.)
- Time Normalized
- Type of Subject
- Sample Size (# Individuals)

Results

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- Biomarker
- Type of Biomaker
- Specimen Material, Type Specimen, Material Volume Volume Unit
- Analyte Value
- Units, Standard Deviation
- Range
- Lower limit
- Upper limit
- Statistical Method
- Analytical Method
- LLOQ (Lower Limit of Quantification)
- Range Inf, Range Sup

Demographics

25+

- Gender (Male, Female)
- Race (White, Black, Other, NA- missing)
- Age *(Mean, Med, SD, Min, Max)*
- BMI *(Mean, Med, SD, Min, Max)*
- Cigarettes per day (CPD) *(Mean, Med, SD, Min, Max)*
- Years Smoked *(Mean, Med, Min, Max)*
- Smoker Status verification

Number corresponds to data elements in each category

Rule: 1 study group per row, 1 data set per cell

Data Collection

- **A total of 76 studies were identified since 2008 and were reviewed for inclusion in meta-analysis**
  - Relevant data captured in evidence table according to “Data Template”
- **42 studies were found to contain clinical and/or observational findings with reportable <original> values**
  - Both Free NNAL and Total NNAL, Values reported in different units
- **34 studies were identified with data in units relevant for the meta-analysis**
  - Ex. geometric and arithmetic mean values, median values
- **19 studies were selected with relevant units for the statistical evaluation (geometric mean values)**
  - Reason for Exclusion was documented (”Exclusion Tab”)

Data Processing: Conversion

NNAL Data Availability in Literature

- Common units: pmol/mg creatinine; ng/mg creatinine; ng NNAL/24 h; pg/mg creatinine
- Less common units: pg/mL; pmol/mL; ng/mL; fmol/mL; nmol/24 h

Data Conversion

- **Data converted to pmol/mg creatinine using a "conversion template"**
  - Assumptions:
    - Median urine volume = 1.8 L/day (0.8 – 2 L/day)
    - Median urine creatinine = 2,208 mg/24 h (601 – 2,936 mg/24 h)
    - NNAL MW = 209.24 g/mol (C10-H15-N3-O)
  - Example (pg/mL to pmol/mg creatinine):
    - **Step 1: Convert pg/mL to pmol/L**
      - NNAL = $1*10^{-12}$ g/mL = $1*10^{-9}$ g/L = $1*10^{-9}$ / 209.24 mol/L = $4.779*10^{-12}$ mol/L = 4.779 pmol/L
    - **Step 2: Normalize for urine and creatinine levels**
      - 4.779 pmol/L x 1.8 L/d urine = 8.6 pmol / 2,208 mg creatinine/day = 0.0039 pmol/mg creatinine

Data Processing: Selection

Geometric Means & Total NNAL Selected for the Meta-Analysis

- Free NNAL was captured but not considered
- Arithmetic mean and median values captured but not considered. Log-transformed data could be considered equivalent to geometric means (further analysis required).

Sequence of Calculations

- Volume-based: Convert to molar-based and adjust for urine/creatinine
  - **1 pg/mL** NNAL x $1*10^{-12}$ g/pg x 1000 mL/L / (209.24 g/mol x $1*10^{-12}$ pmol/L) = 4.779 pmol/L x 1.8L urine = 8.6 pmol / 2,208 mg creatinine/d = 0.0039 pmol NNAL/mg creatinine
- Molar based: Adjust for creatinine levels
  - **1 nmol/24 h** NNAL x 1000 pmol/nmol = 1000 pmol / 2,208 mg creatinine/d = 0.45 pmol NNAL/mg creatinine
- Mass-based creatinine-adjusted: Adjust for creatine and convert to moles
  - **100 ng/g creatinine** NNAL x 2.208 g creatinine/d= 220.8 ng/d / 209.24 mol/L = 1.05 nmol (1055 pmol) = 1055 pmol / 2,208 mg creatinine/day = 0.47 pmol NNAL/mg creatinine

Data Analysis: Statistical Summary

Year	Included / Relevant
2008	0 / 2
2009	1 / 7
2010	4 / 5
2011	2 / 7
2012	2 / 4
2013	3 / 4
2014	0 / 1
2015	2 / 3
2016	3 / 5
2017	0 / 1
2018	1 / 2
2019	- / -
2020	1 / 1
Total	19 / 42

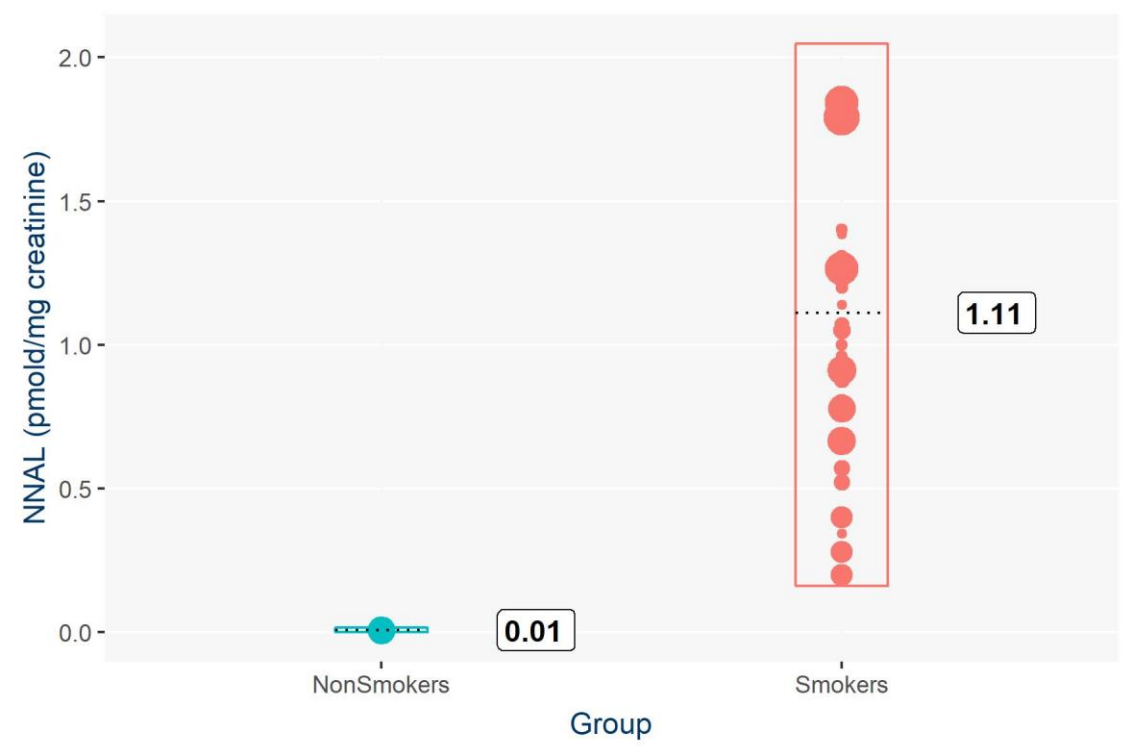
Group	Non-Smokers	Smokers
# Groups	3	36
Total Individuals	1,160	12,218
Average	0.008	1.112
Min	0.002	0.182
Max	0.014	1.842
SD	0.0038	0.5505
95% CI (LL)	0.001	0.161
95% CI (UL)	0.017	2.047

NNAL results are expressed in pmol/mg creatinine.

Comparison	Difference (pmol/mg creatinine)	P-value	Observations
Smokers vs Nonsmokers	1.095	0.0002	Statistically different

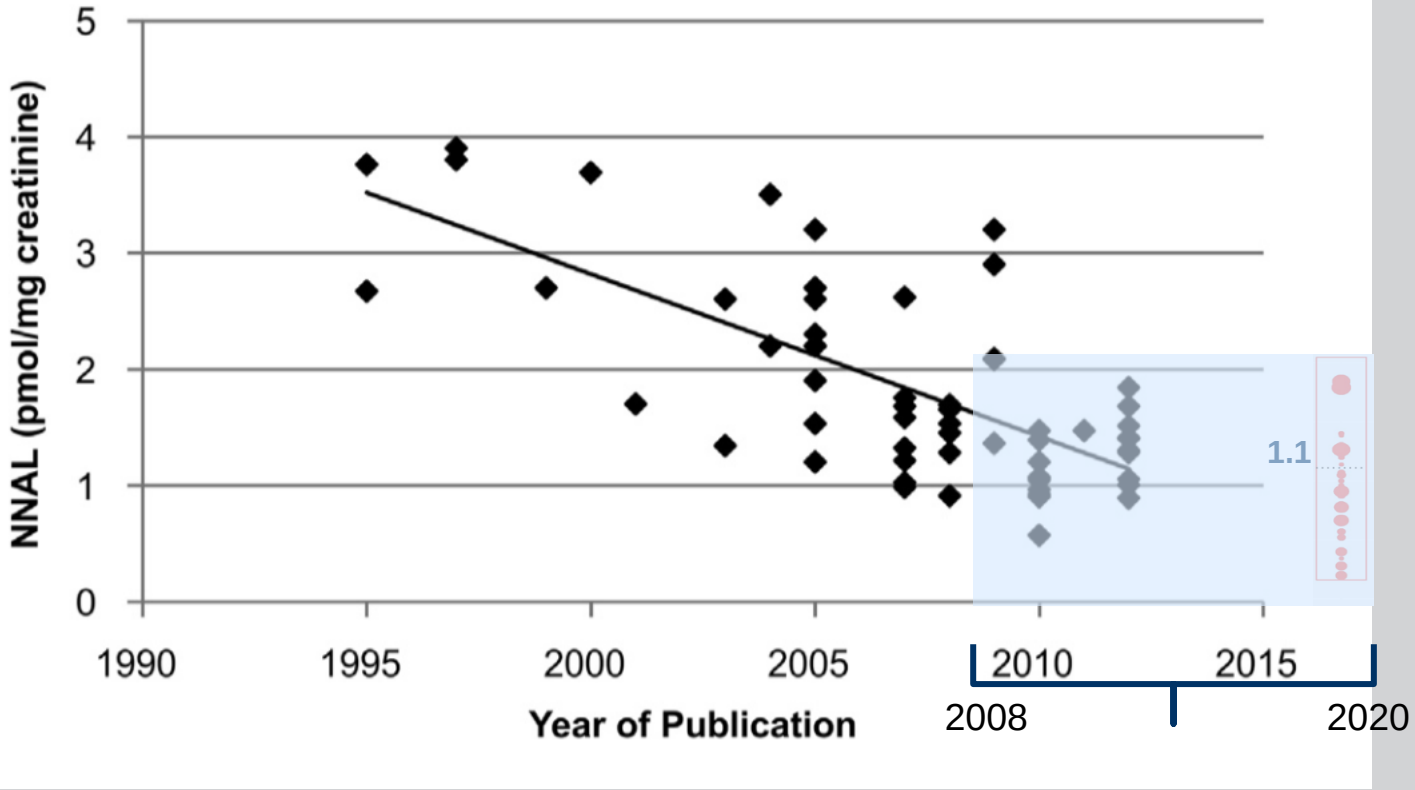
Averages and standard deviations weighted according to the size of the groups, using epanechnikov smothing kernel density

Data Analysis: Group Comparison



NNAL is Smokers - Publication Year Correlation

Appleton *et al* 2014



Summary & Next Steps

Summary

- We have established average-observed levels for NNAL in cigarette smokers
- Similar analysis for COHb and NEQ have been previously completed
- A manuscript describing the results for all three biomarkers is currently in progress (Target Journal - Biomarkers)