DEVELOPMENT OF BIOMARKERS OF EFFECT FROM CHRONIC TOBACCO USAGE: Part 1, Study Design and Biomarkers of Exposure

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Study Presentation

Design, methodology, results, and summary and conclusions are presented as four parts:

Platform Presentations

- Part 1: Study Design and Biomarkers of Exposure
- Part 2: Inflammation and Oxidative Stress Biomarkers
- Part 3: Potential Metabolomics Biomarkers of Tobacco Effect

Poster Presentation

- Part 4: Metabolomic Profiles From Cigarette Smokers and Moist Snuff Consumers

Study Guidance

- **US Code of Federal Regulations (21 CFR)**
  - Electronic Records; Electronic Signatures (Part 11)
  - Protection of Human Subjects (Part 50)
  - Financial Disclosure by Clinical Investigators (Part 54)
  - Institutional Review Boards (Part 56)
  - Good Laboratory Practice For Nonclinical Laboratory Studies (Part 58)
- **International Conference on Harmonization Guidelines**
  - Good Clinical Practice: Consolidated Guidance (E6)
  - Guidance on General Considerations for Clinical Trials (E8)
- **US Food and Drug Administration**
  - Guidelines for the Monitoring of Clinical Investigations
  - Guidance for Industry: Statistical Principles for Clinical Trials (E9)
- **Declaration of Helsinki (World Medical Assoc., Revised 2008)**
  - "The international touchstone for the ethical conduct of clinical studies"

Guiding Principles and Beliefs

- The best course of action for tobacco users concerned about their health is to quit. Adults who continue to use tobacco products should consider the reductions of risks for serious diseases associated with moving from cigarettes to the use of smoke-free tobacco or nicotine products.

- Significant reductions in the harm associated with the use of cigarettes can be achieved by providing accurate information regarding the comparative risks of tobacco products to adult tobacco consumers, thereby encouraging smokers to migrate to the use of smoke-free tobacco and nicotine products.

RJRT, Our Guiding Principles and Beliefs (www.rjrt.com)

Study Rationale

Utilizing a parallel-group, cross-sectional study design:

- Explore the risk continuum associated with the consumption of combustible tobacco products relative to non-combustible tobacco products
- Utilize identified biomarkers to differentiate exposure to cigarettes and smokeless tobacco (ST)
- Evaluate select biomarkers of effect to assess differences in inflammation and oxidative stress due to smoking and ST use
- Assess novel biomarkers that could indicate potential harm from chronic tobacco consumption

Study Purpose

Among long-term cigarette smokers or moist snuff consumers and non-consumers of tobacco…

- Assess the effects of ***chronic tobacco exposure*** to combustible and non-combustible tobacco products
  - Exposure biomarkers in blood and urine

- Evaluate potential biomarkers of ***tobacco effect*** that could predict harm due to combustible and non-combustible tobacco products
  - Effect biomarkers in blood and urine
  - Metabolomic profiles in blood and urine

Study Design and Methodology

Overall Design Features

- Single site, three cohort, single-blind, cross-sectional study
  - High Point Clinical Trials Center, High Point NC, USA
- Enrollment goal: 120 normal, healthy males, ages 35-60 years
  - Exclusive cigarette smokers (n=40)
  - Exclusive moist snuff consumers (n=40)
  - Never-smokers/never-tobacco consumers (n=40)
- Study product: None provided during in-clinic phase of study
  - Subject's usual brand (UB) of cigarettes or moist snuff consumed prior to clinic check-in
  - No tobacco or nicotine-containing products for non-tobacco consumers

Study Design and Methodology

Scheduled Study Visits

1. **Screening Visit (within 28-45 days prior to study entry)**
   - Informed consent process
   - Eligibility assessments
     - Basic safety: vital signs, ECG, peak flow, clinical labs, drug/alcohol screens
     - Medical history, concomitant meds, physical/oral exam
     - Cohort-specific inclusion criteria: tobacco usage, expired CO (ECO)
   - Capable of providing a large volume blood draw (~400 mL)
2. **Acceptance Visit**
   - Eligibility reassessment
   - Materials and instructions provided
     - **24-hr "at-home" urine collection** (start Day -2; end Day -1)
     - 7-day diet/ tobacco use/ activity diary (start Day -7 through Day -1)

Study Design and Methodology

Scheduled Study Visits (continued)

3. Test Visit: Day -1 Study Check-in (8:00–8:30 p.m.)

- 24-hr urine collections and 7-day diaries obtained from subjects
- Eligibility reassessment (vital signs, exhaled ECO, cotinine screen)
- Eligibility confirmed; ***subjects enrolled in study***
- 8 to 10 hour fast from food/drink (except water) and abstention from UB tobacco products begins
- Sample collections for select biomarkers of exposure
- Questionnaire administration
  - Nicotine dependence and tobacco product usage questionnaires
  - Self-reported health/ perceived stress questionnaires
  - Dietary questionnaire

Study Design and Methodology

Scheduled Study Visits (continued)

3. Test Visit (continued): Day 1

- Sample collections for biomarkers of exposure and effect
  - **Fasting blood**
  - **First morning urine void**
- Adverse event assessment
- Study discharge (~11 a.m.)

Study Design and Methodology

General Inclusion Criteria

- Males, age 35-60 years (inclusive)
- Free of clinically significant health problems (including oral health) in the opinion of the Investigator
- Met the American Red Cross Blood Donation Eligibility Guidelines for large volume blood donation (~400 mL whole blood)

  Examples:

  - Weighed at least 110 pounds (50 kg)
  - Had a hemoglobin ≥ 12.5 g/dL and a hematocrit ≥ 38%
- No evidence of alcoholism/ drug addiction
- Able to read and answer questionnaires in English
- Able to comprehend and willing to sign an Informed Consent Form

Study Design and Methodology

Cohort-specific Criteria

Exclusive cigarette smokers (SMK, n = 40)

- Any brand of cigarettes measuring ≥ 6 mg “tar”/cigarette by Cambridge Filter Method
- Smoking at least 10 cigarettes/day for at least 3 years prior to screening
- ECO measures 10 to 100 ppm

Exclusive moist snuff consumers (MSC, n = 40)

- Any brand, any cut style, any flavor moist snuff
- Using at least two cans/week for at least 3 years prior to screening
- ECO measures 0 to 5 ppm

Non-tobacco consumers (NTC, n = 40)

- No use of any tobacco or nicotine containing products for at least 5 years prior to screening
- ECO measures 0 to 5 ppm

Study Design and Methodology

Subject Disposition

	SMK	MSC	NTC	Overall
Enrolled	41	41	40	122
Completed	40	40	40	120
Withdrawn from Study	1	1	0	2
Adverse Experience*	1	1	0	2

*AEs = Vasovagal reaction (fainting) due to large volume blood draw

Study Design and Methodology

Demographic and Tobacco Use Summary

	SMK	MSC	NTC
Gender – Male (#)	41	41	40
Race – Caucasian (#) Other (#)	31 10	35 6	28 12
Mean Age (years)	46.9	45.0	47.2
Mean Weight (kg)	88.60	92.68	92.37
Mean Height (cm)	176.5	176.4	177.2
Mean BMI (kg/m^2)	28.42	29.68	29.37
Fagerström Test for Nicotine Dependence Score	5.50	4.91	N/A
	SMK (N=39)*	**MSC** (N=38)*	
Years of Product Use Mean ± SD (Min, Max)	 25.1 ± 9.6 (6, 45)	 20.6 ± 8.5 (5, 37)	
Product Use During Last Month Mean ± SD (Min, Max)	**Cigs/day** 21.5 ± 5.3 (12, 30)	**Cans/wk** 6.3 ± 3.5 (1.75, 14)	

***N < 40 due to missing data**

Biomarkers of Exposure - Blood

Biomarker	Analyte Measured
Carbon monoxide	Carboxyhemoglobin (COHb)
Hydrogen cyanide	Thiocyanate (SCN)
Nicotine	Nicotine Unconjugated (NIC-U) and Cotinine Unconjugated (COT-U)

- **Blood biomarkers were measured after an 8 to 10 hour overnight abstention from food, drink and tobacco products.**

Biomarkers of Exposure - Urine

Biomarker	Analyte Measured
Tobacco-Specific Biomarkers	
Nicotine	Total Nicotine Equivalents (NICEq-T) Nicotine (NIC-U) + Nine Metabolites (CNO, COT-G, COT-U, NCOT, NIC-G, NNIC, NNO, OHCOT-G, OHCOT-U)
Tobacco-Specific Nitrosamines	NNAL-T, NNN-T, NAB-T, NAT-T
Other Biomarkers	
Aromatic Amines	*o*-Toluidine, 2-Aminonaphthalene (2-AN), 3-Aminobiphenyl (3-ABP), 4-Aminobiphenyl (4-ABP)
Polycyclic Aromatic Hydrocarbons	1-OH-naphthalene, 2-OH-napththalene, 2-OH-flourene, 1-OH-pyrene, 1- + 9-OH-phenanthrene, 2- + 3-OH-phenanthrene
Hydrogen Cyanide	Thiocyanate

Biomarkers of Exposure - Urine

Biomarker	Analyte Measured
Mercapturic Acid Metabolites of Select Combustion By-products	
Acrolein	HPMA
Acrylamide	AAMA, GAMA
Benzene	SPMA
1,3-Butadiene	MHBMA
Crotonaldehyde	HMPMA
Metals	
Trace Metals	Cadmium, Chromium, Nickel, Tin, Selenium

- **Urine biomarkers were measured from a 24-hr "at-home" urine collection under normal tobacco product usage and non-fasting conditions.**

All laboratory analyses for blood and urine biomarkers of exposure and effect were performed according to applicable GLP requirements or "fit-for-purpose" principles.

Biomarkers: Exposure & Effect Results

Data Analysis

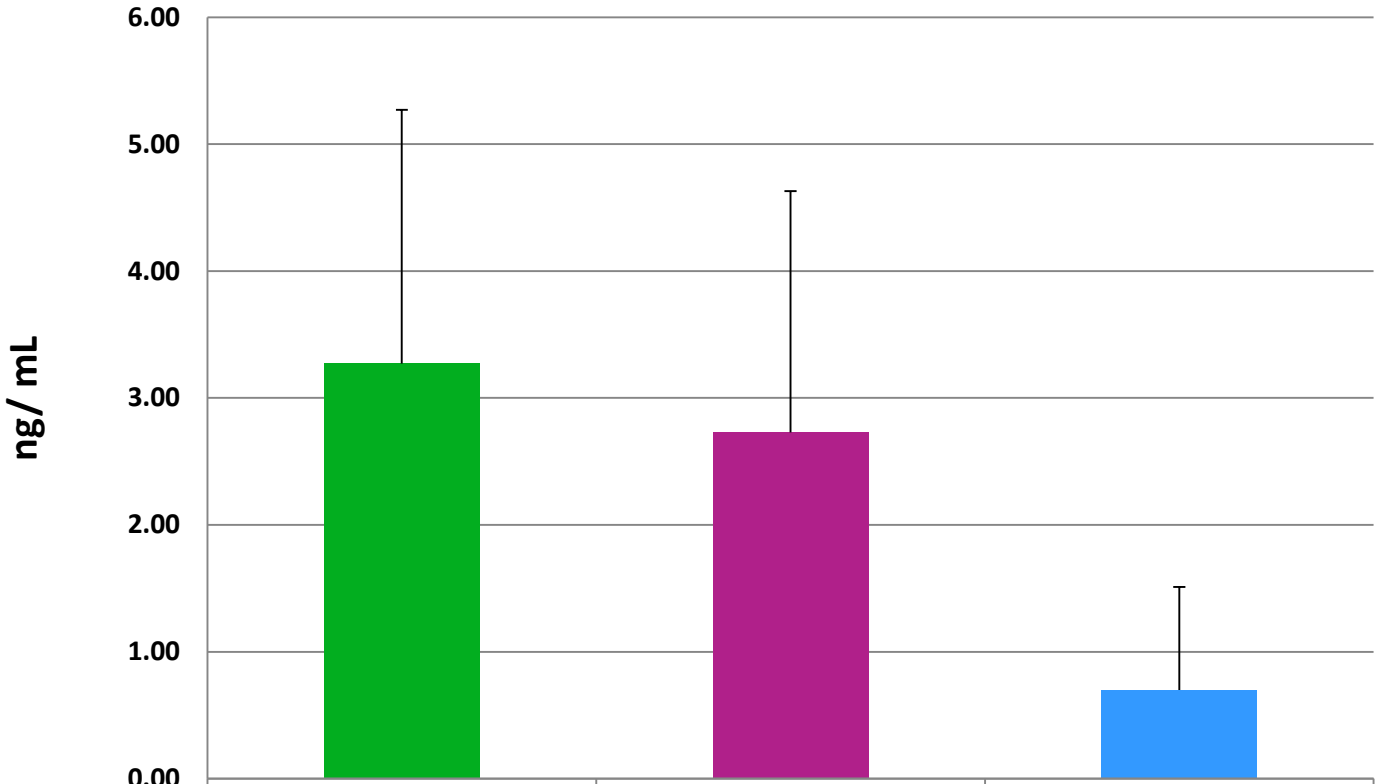
- ANOVA, ANCOVA models used to compare the three cohorts
- A test was performed to identify extreme values that could effect the interpretation of biomarker results
  - Results are presented without the extreme values (3 biomarkers affected)
- Data for select biomarkers of exposure and effect are shown:
  - Results are presented as the mean ± 1SD
  - Statistical significance was defined as $p \leq 0.05$
  - All blood data are based on fasting samples (concentration)
  - All urine data are based on the 24hr urine sample (mass/24h) [exception – metals in first urine void]
- Cohort acronyms:
  - SMK=Smokers; MSC=Moist snuff consumers; NTC=Non-tobacco consumers

In Part 1, only Biomarkers of Exposure results are presented.

Blood Nicotine-Unconjugated (fasting)

SMK & MSC > NTC

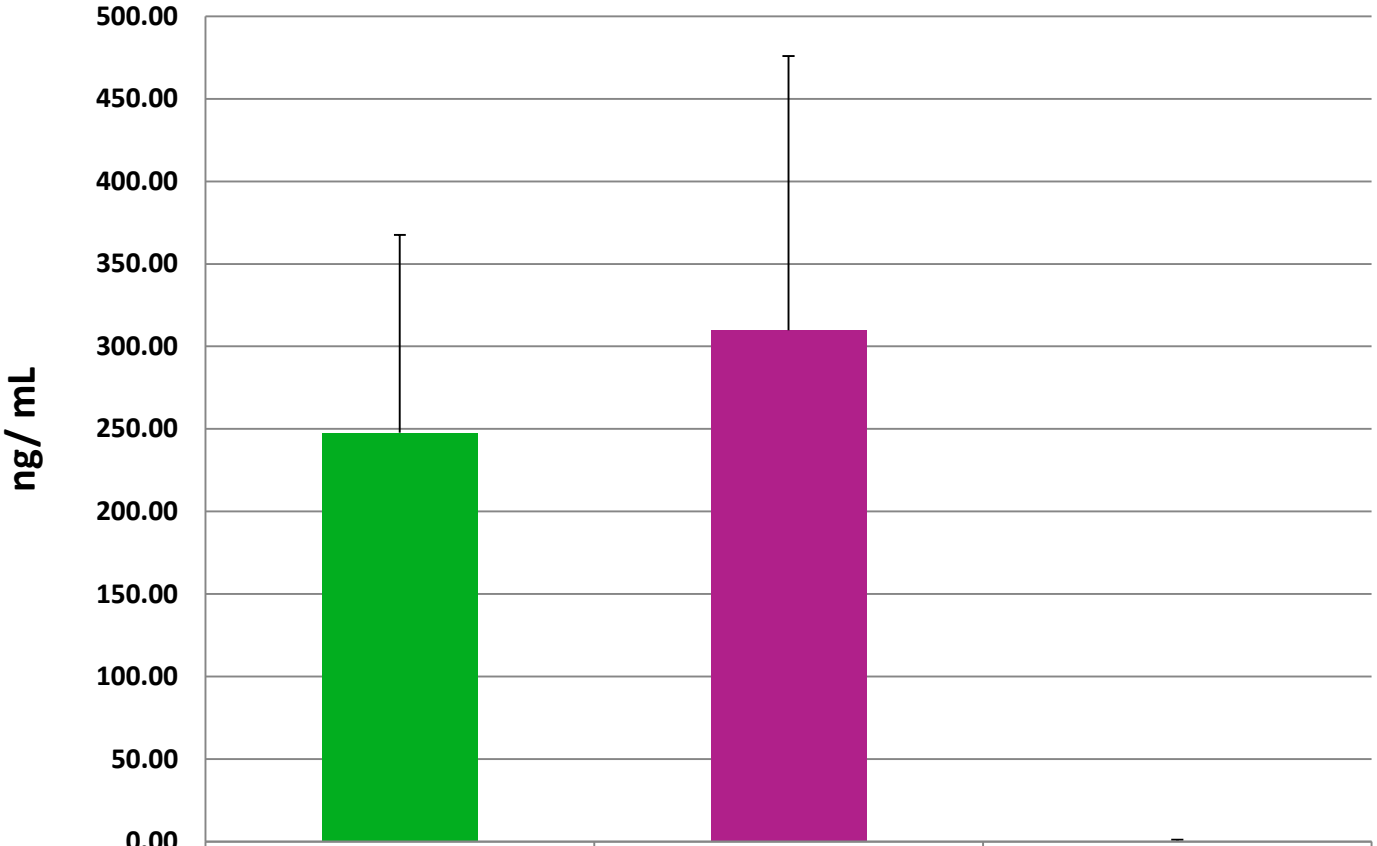
No difference detected between the SMK & MSC



	SMK	MSC	NTC
Cohort mean	3.27	2.73	0.70

Blood Cotinine-Unconjugated (fasting)

MSC > SMK > NTC

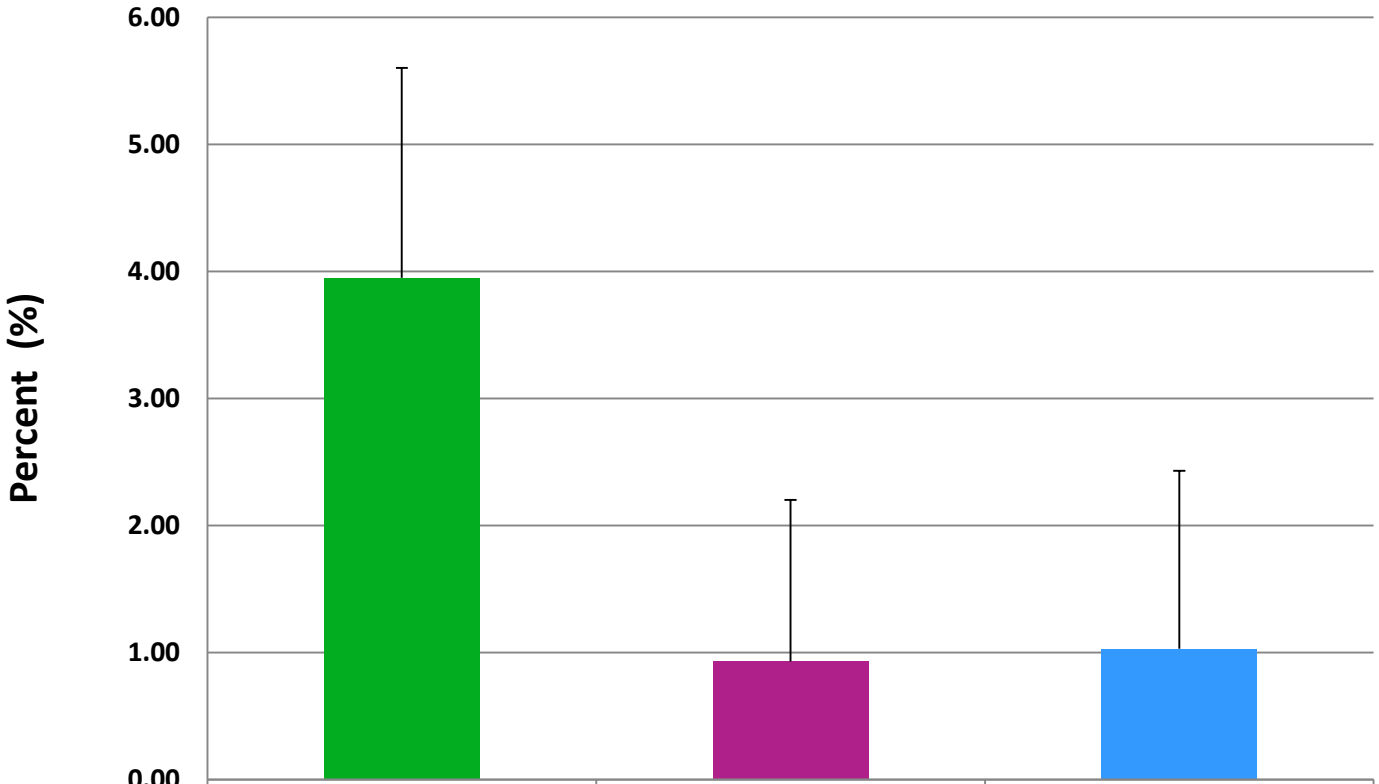


	SMK	MSC	NTC
Cohort mean	247.68	309.72	0.47

Blood Carboxyhemoglobin (fasting)

SMK > MSC & NTC

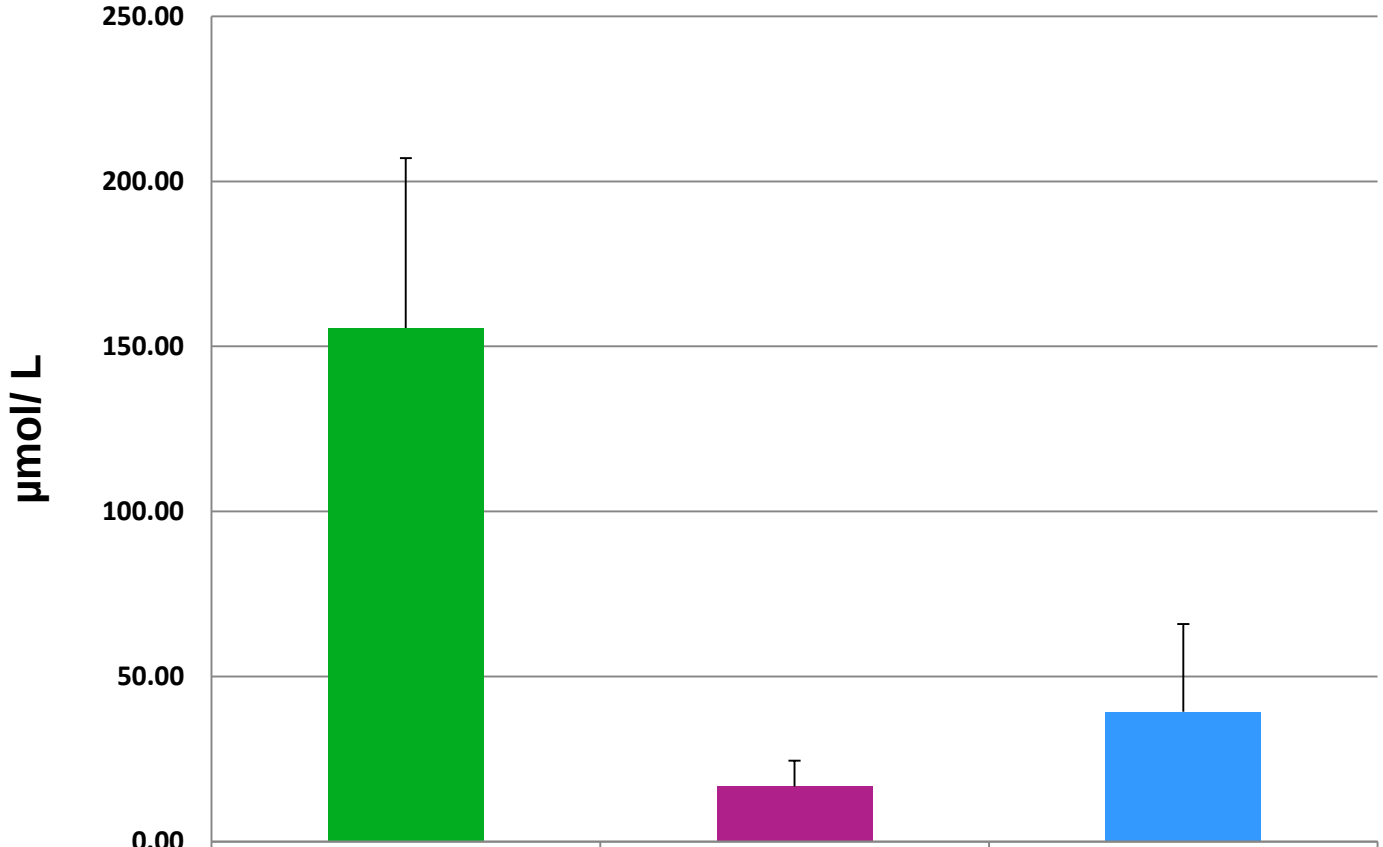
No difference detected between MSC & NTC



	SMK	MSC	NTC
Cohort mean	3.95	0.93	1.03

Blood Thiocyanate (fasting)

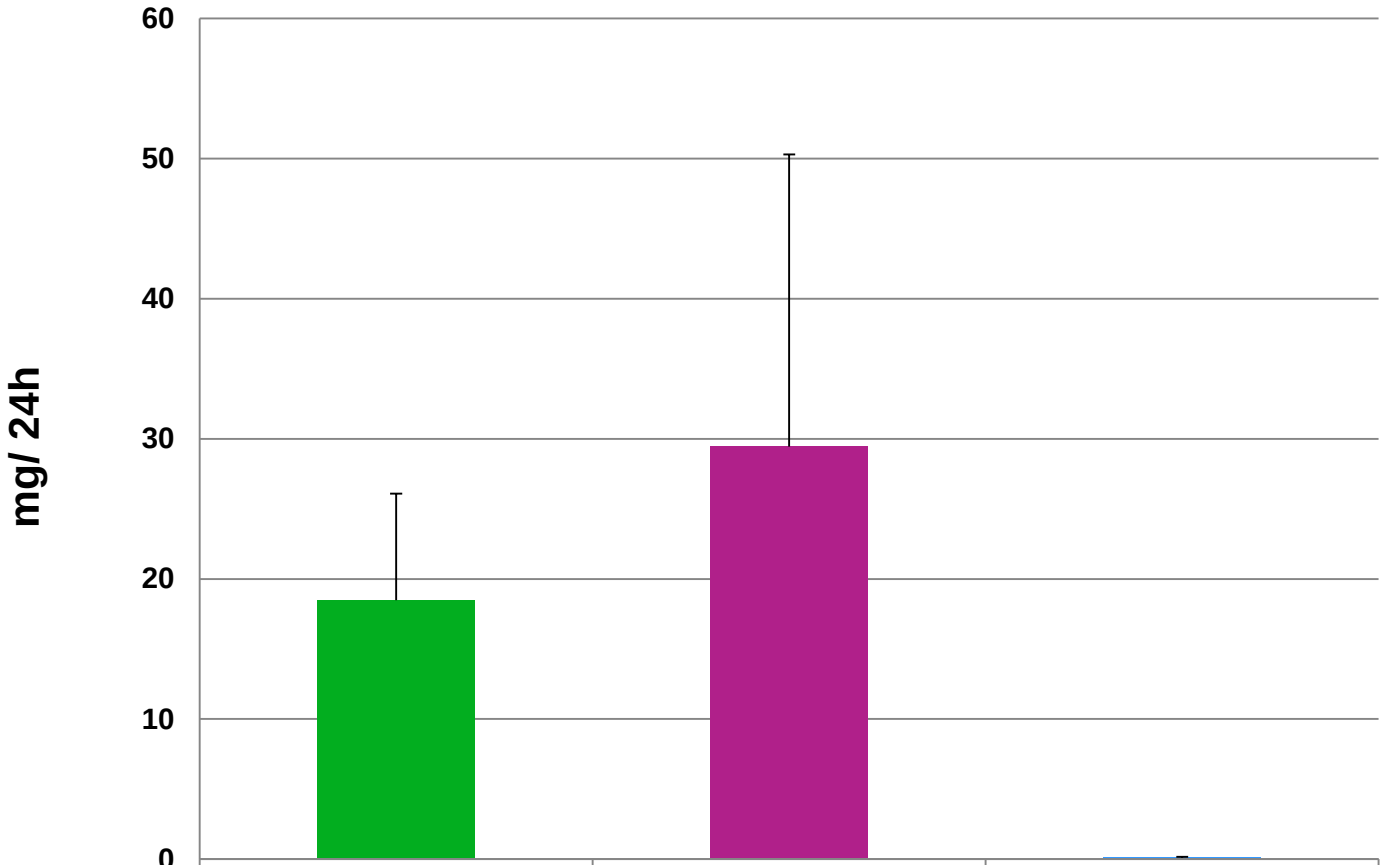
SMK > MSC & NTC; NTC > MSC



	SMK	MSC	NTC
Cohort mean	155.60	16.67	39.40

Urine Total Nicotine Equivalents (NICEq-T)

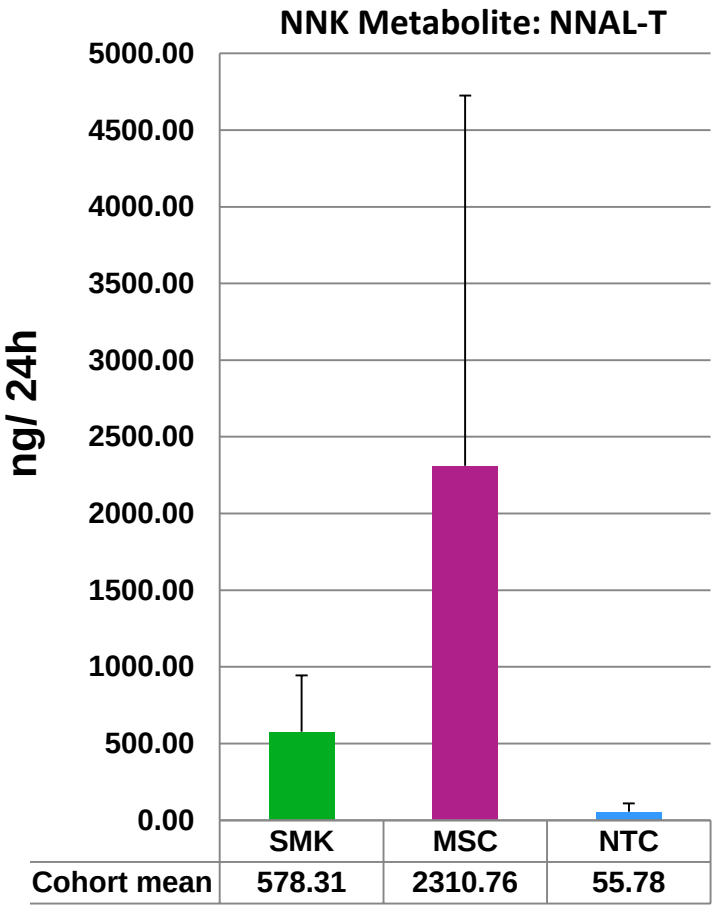
MSC > SMK > NTC

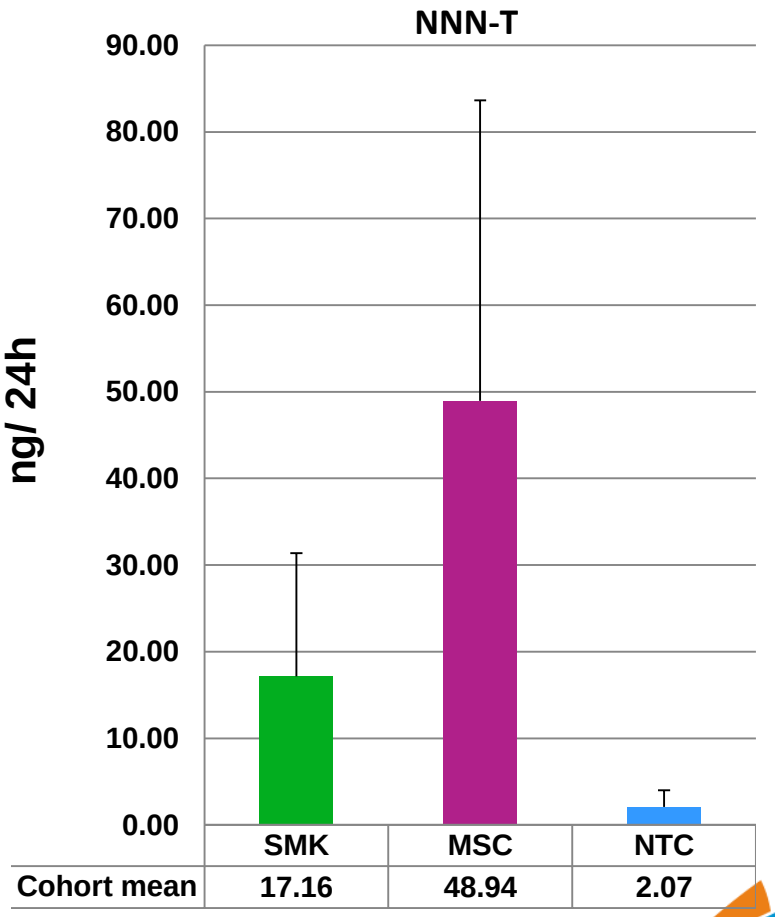


	SMK	MSC	NTC
Cohort mean	18.48	29.44	0.09

Urine TSNAs

MSC > SMK > NTC



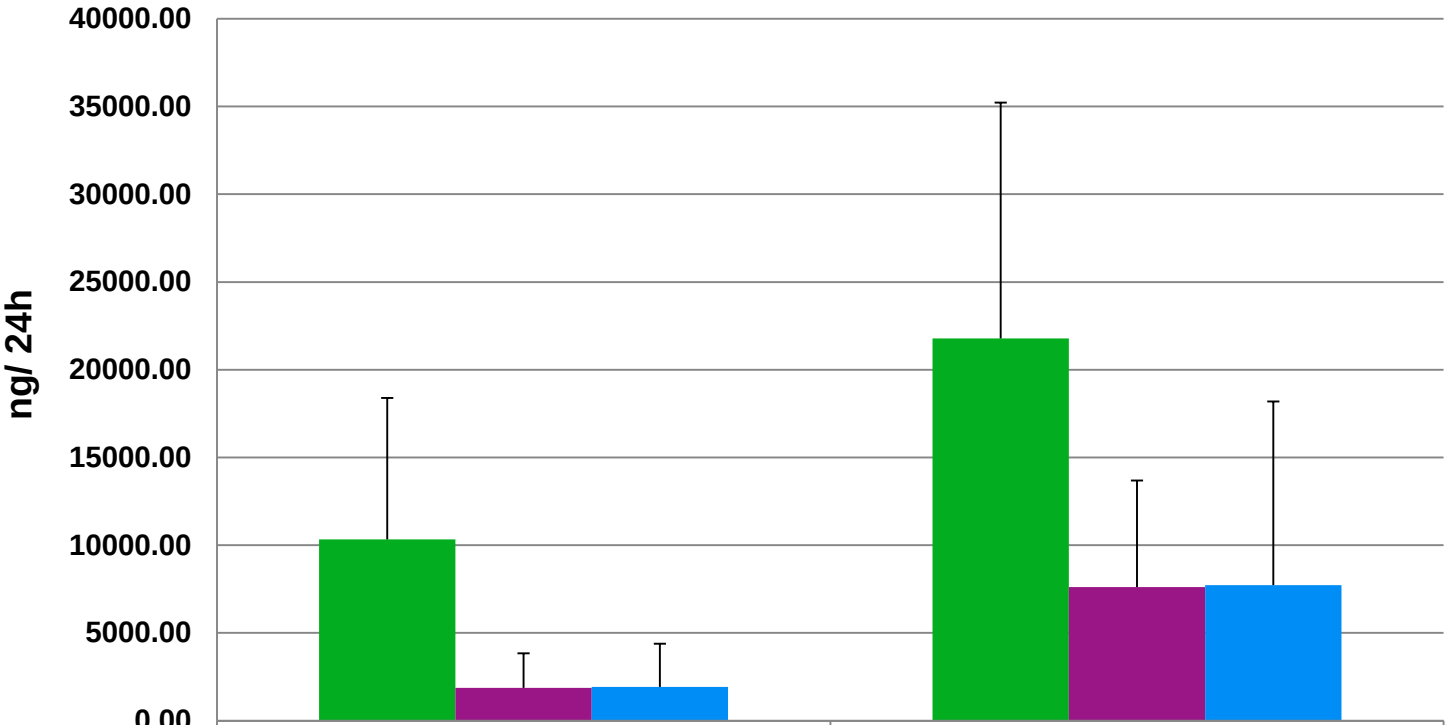


MSC had significantly higher levels of all four TSNAs compared to SMK.
NTC always had the lowest levels.

Urine Polycyclic Aromatic Hydrocarbons

SMK > MSC & NTC

No difference detected between MSC & NTC

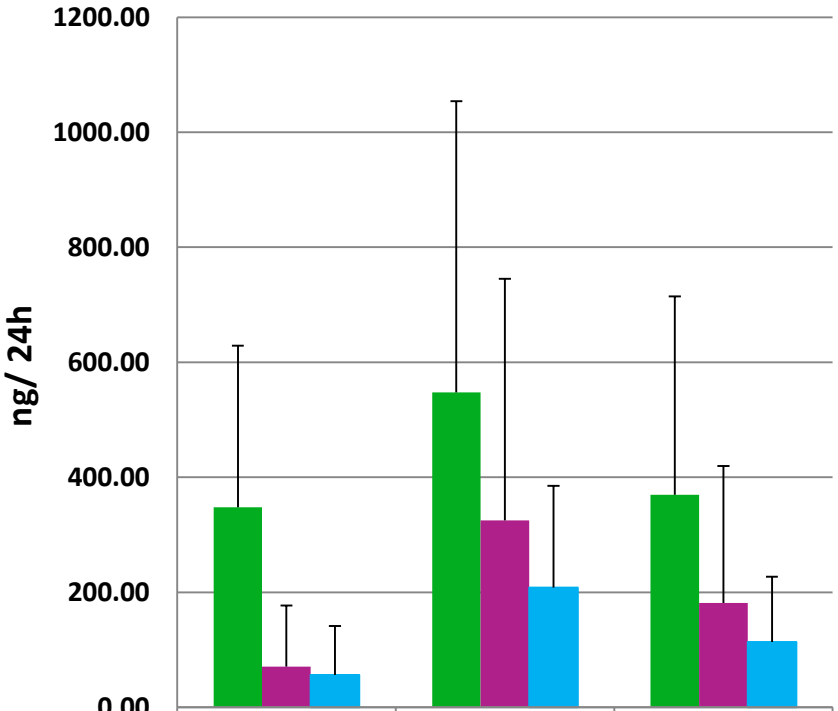


	1-OH-naphthalene	2-OH-naphthalene
SMK	10322.68	21789.21
MSC	1864.16	7620.57
NTC	1924.02	7723.74

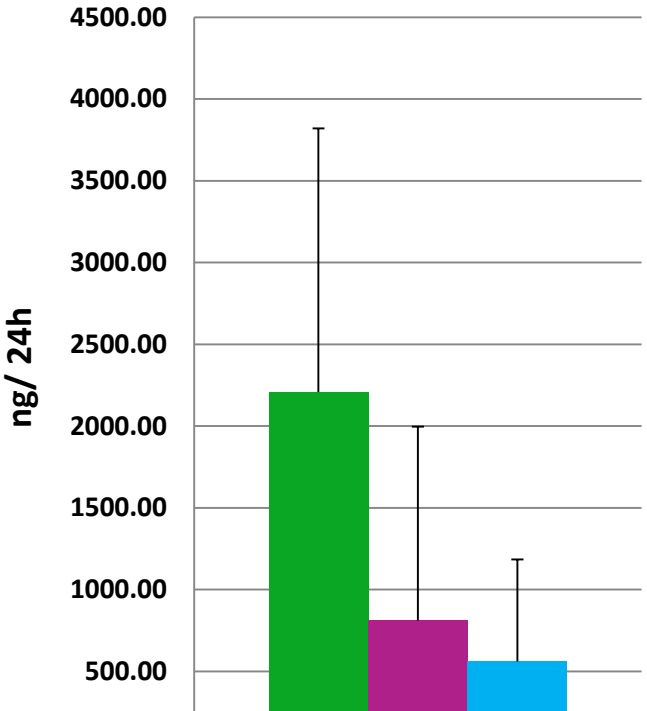
Urine Polycyclic Aromatic Hydrocarbons

SMK > MSC & NTC

No difference detected between MSC & NTC



	1- + 9-OH-phenanthrene	2- + 3-OH-phenanthrene	1-OH-pyrene
SMK	347.81	547.51	369.28
MSC	70.92	325.05	181.38
NTC	56.20	208.01	113.36

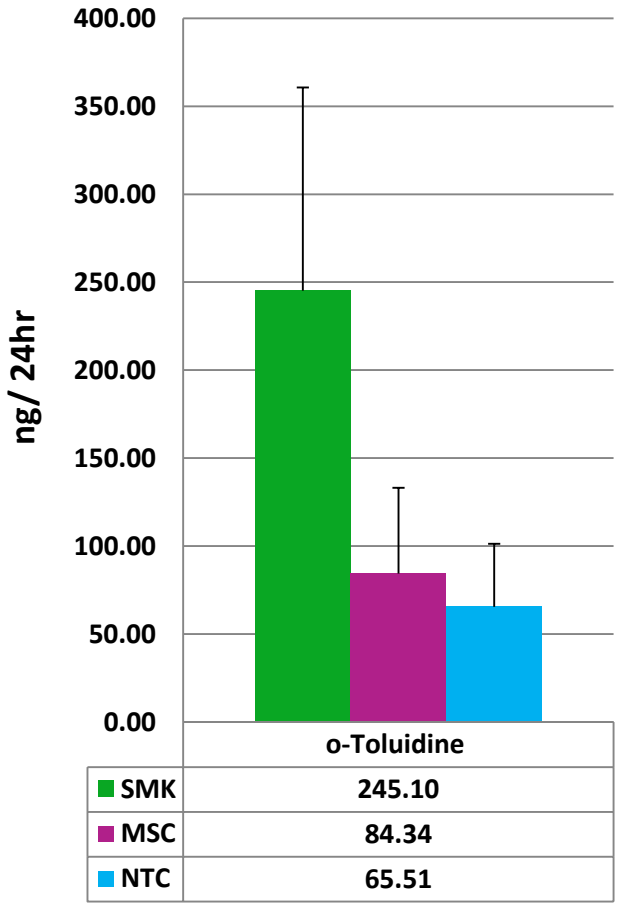


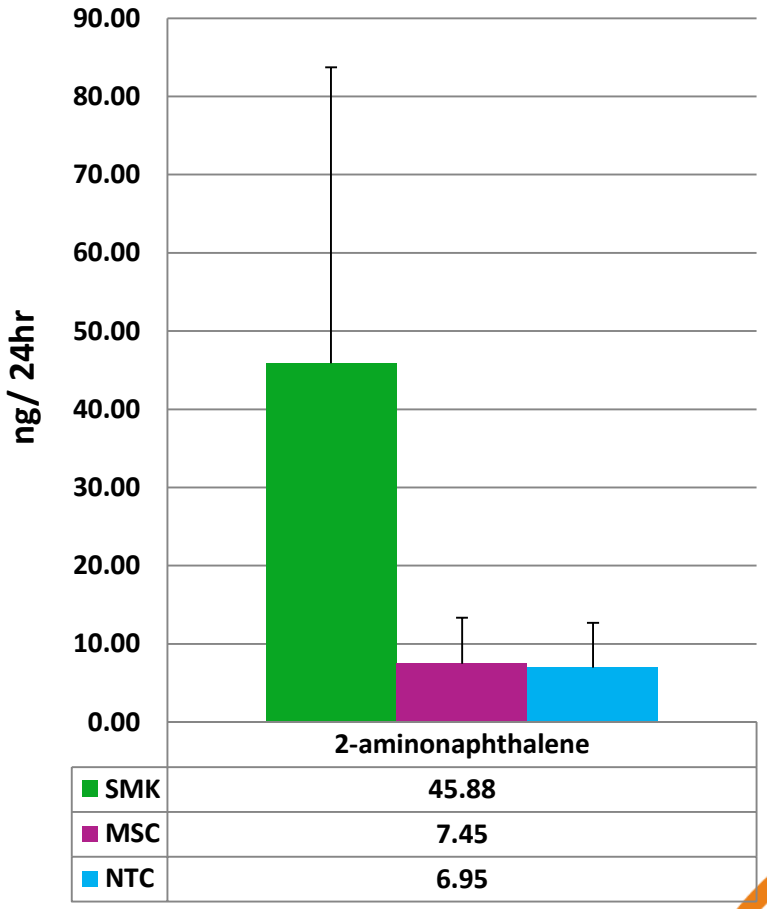
	2-OH-flourene
SMK	2208.32
MSC	810.99
NTC	560.90

Urine Aromatic Amines

SMK > MSC & NTC

No difference detected between MSC & NTC

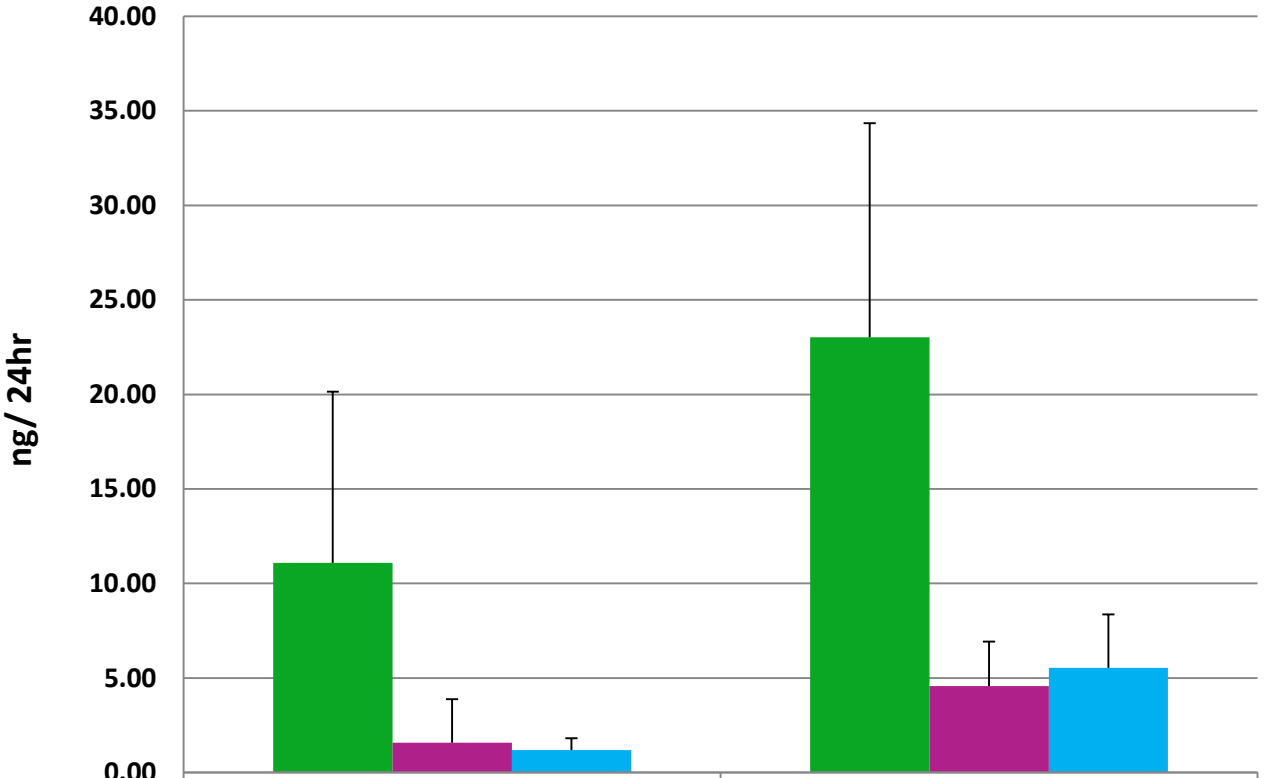




Urine Aromatic Amines

SMK > MSC & NTC

No difference detected between MSC & NTC

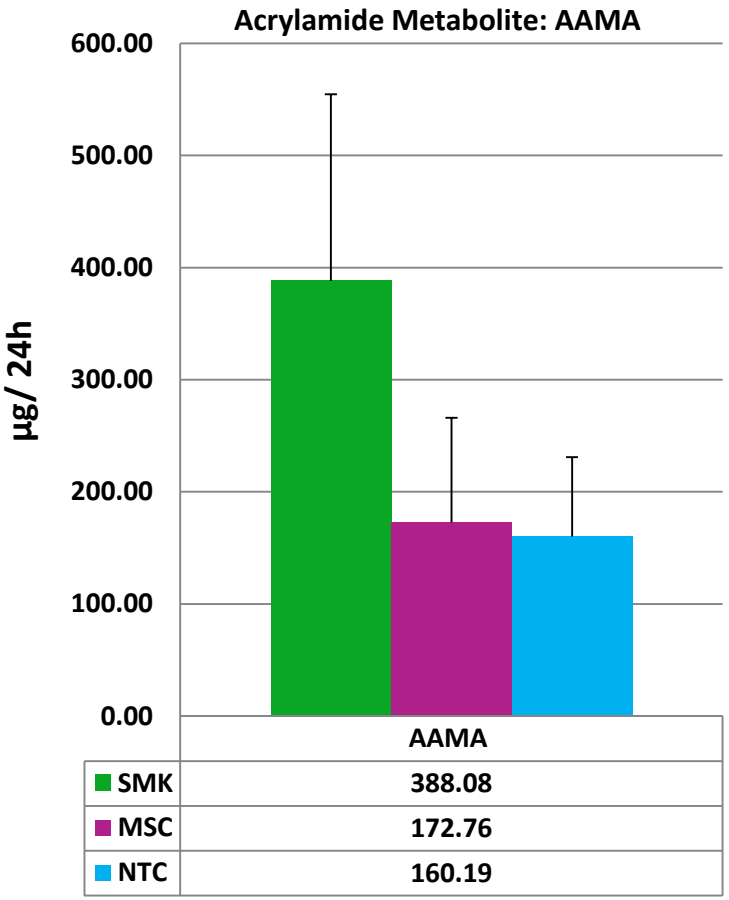


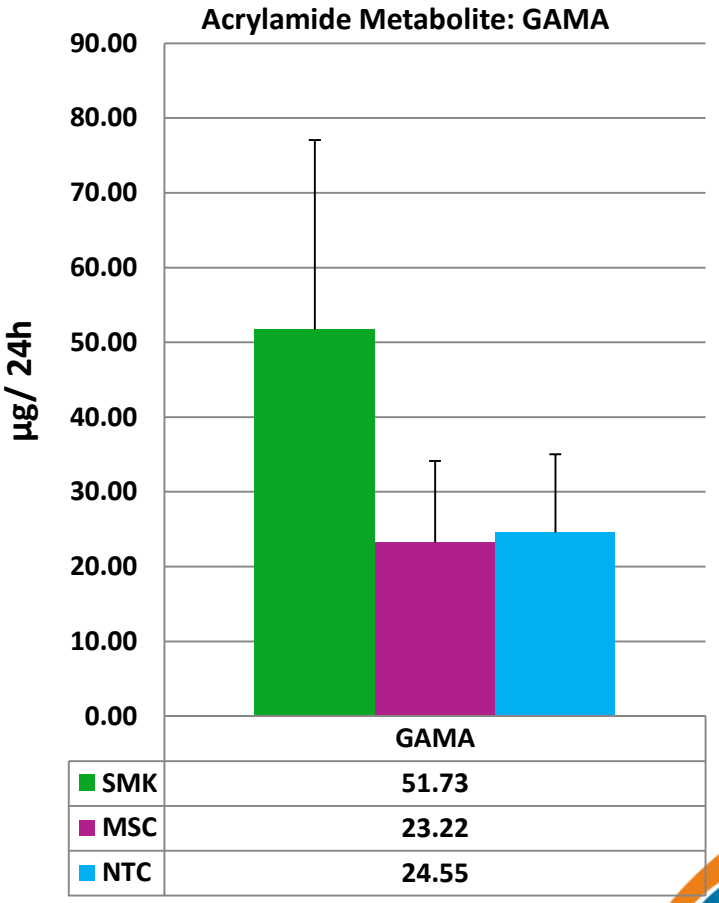
	3-aminobiphenyl	4-aminobiphenyl
SMK	11.09	23.03
MSC	1.57	4.57
NTC	1.19	5.54

Urine Mercapturic Acid Metabolites

SMK > MSC & NTC

No difference detected between MSC & NTC

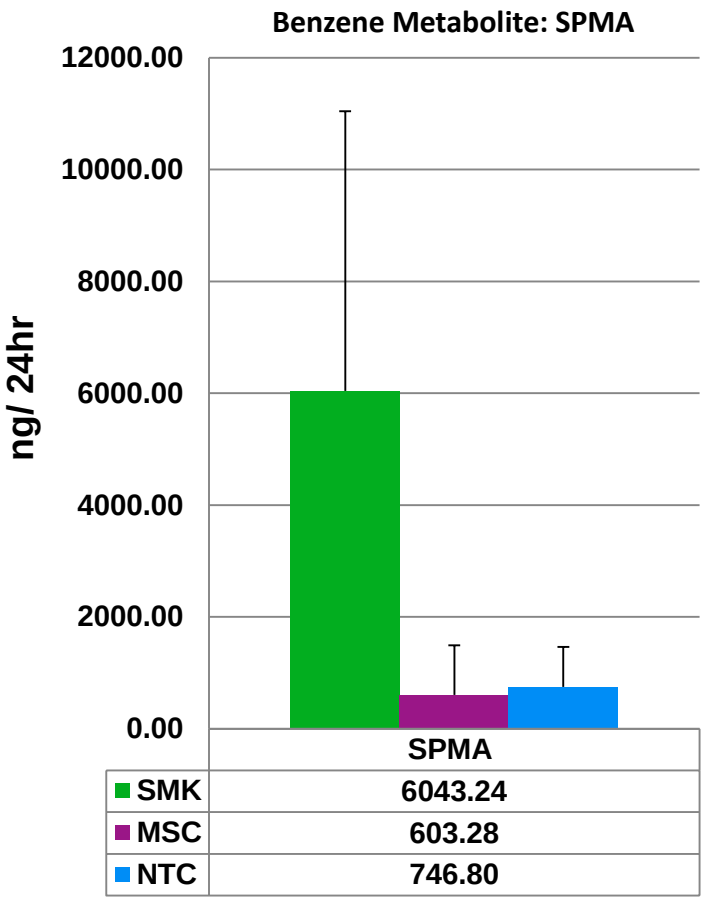


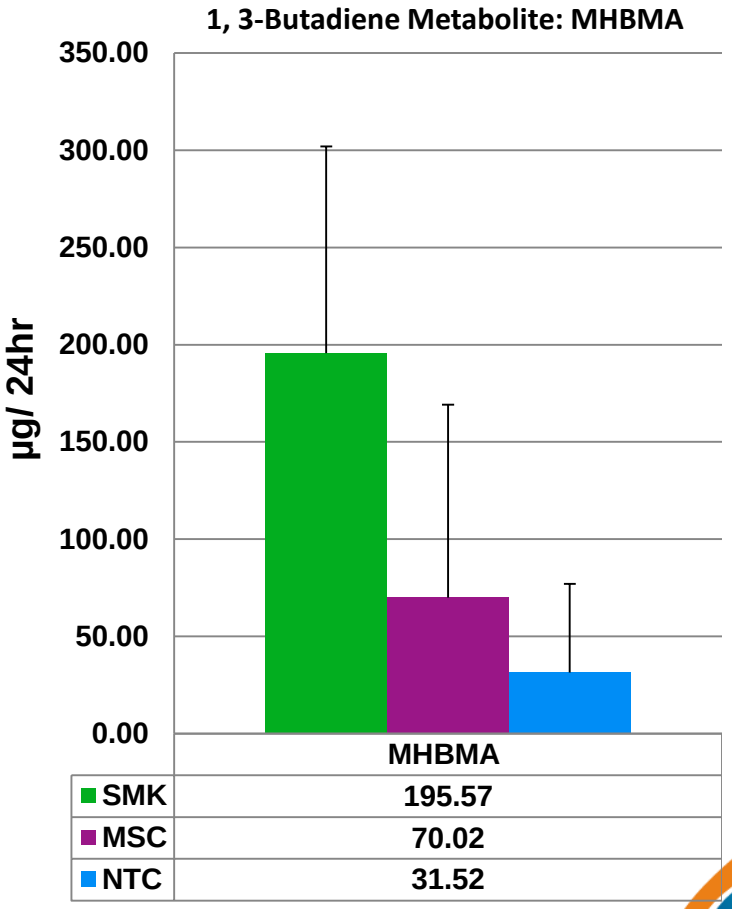


Urine Mercapturic Acid Metabolites

SMK > MSC & NTC

No difference detected between MSC & NTC

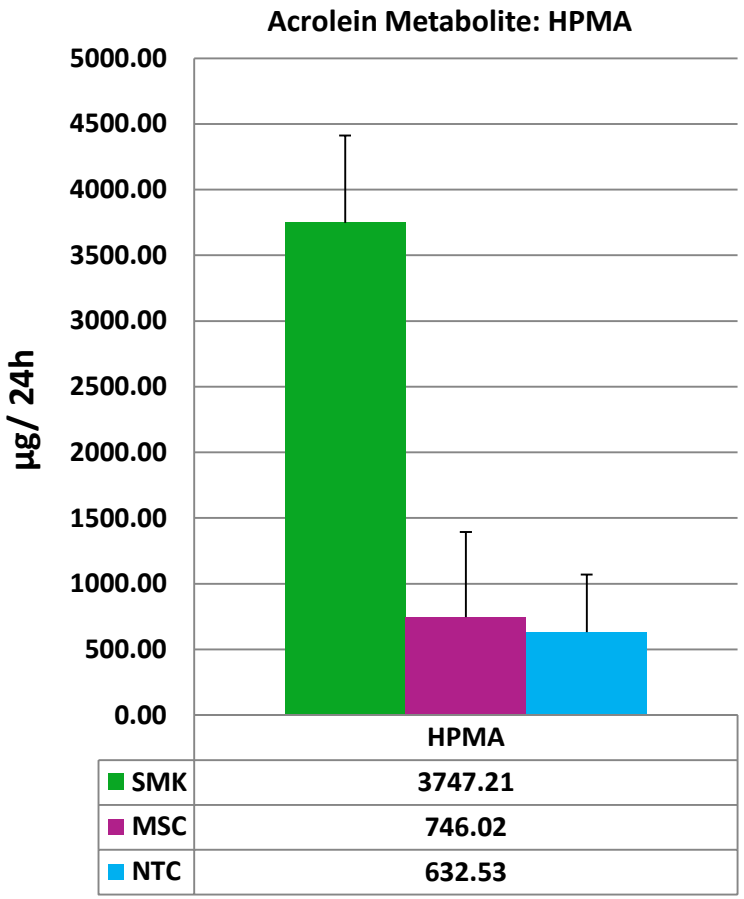


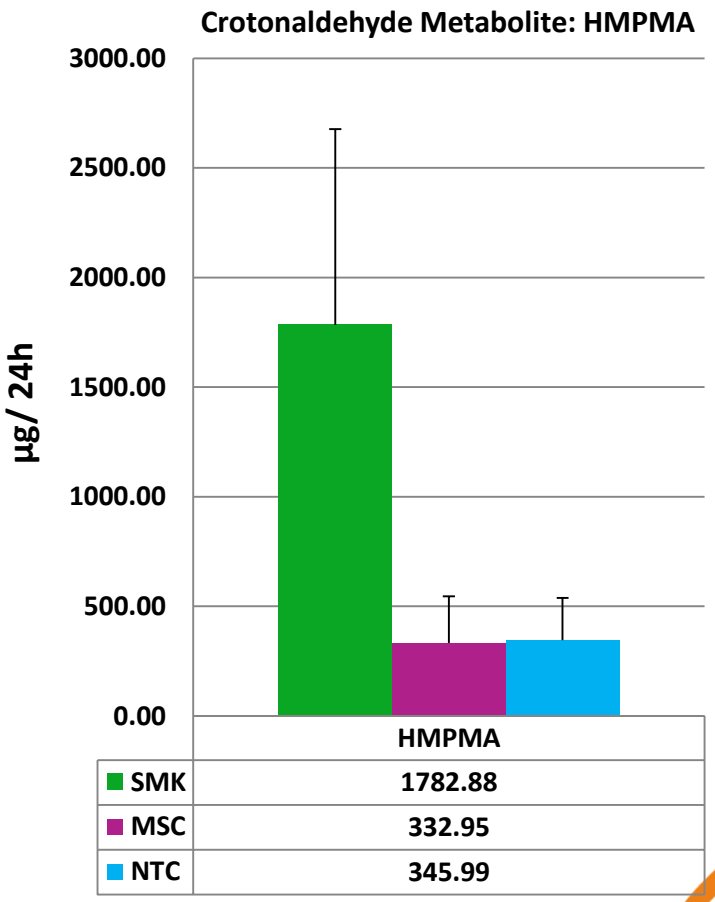


Urine Mercapturic Acid Metabolites

SMK > MSC & NTC

No difference detected between MSC & NTC



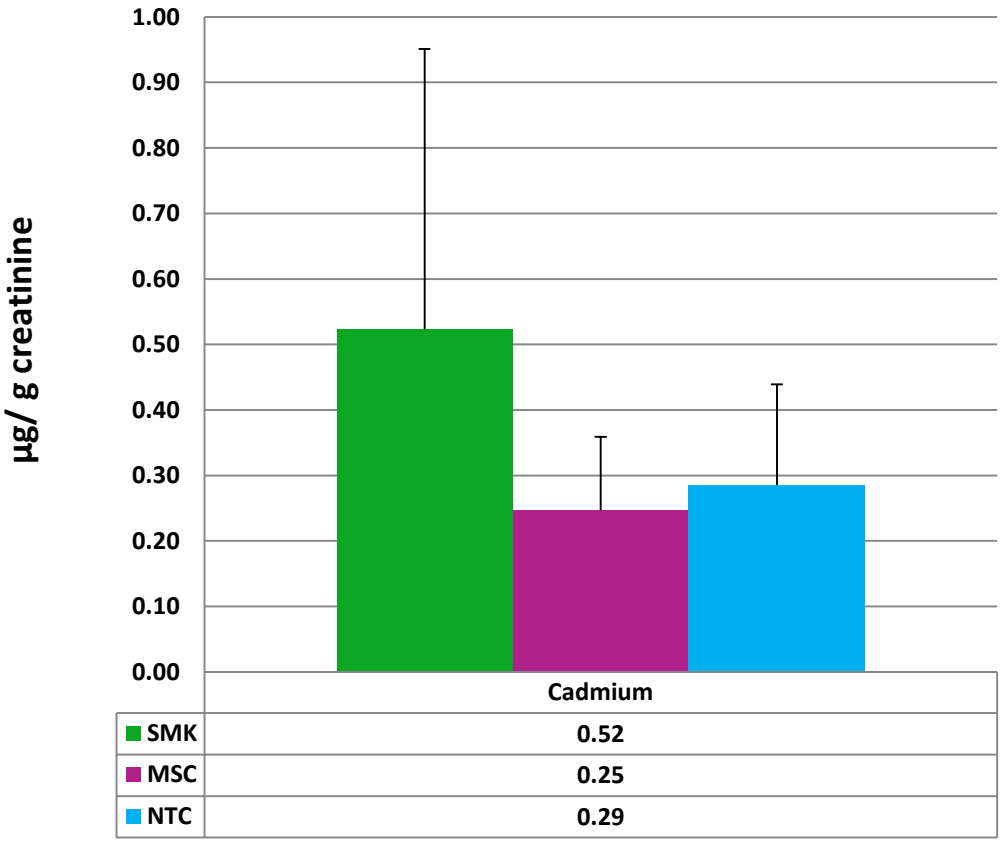


First Urine Void – Trace Metals

Cadmium – only difference observed

SMK > MSC & NTC

No difference detected between MSC & NTC



	Cadmium
SMK	0.52
MSC	0.25
NTC	0.29

Summary and Conclusions

Blood Biomarkers		
Nicotine	SMK & MSC > NTC	No difference between SMK and MSC
Cotinine	MSC > SMK > NTC	
COHb	SMK > MSC & NTC	No difference between MSC and NTC
SCN	SMK > NTC > MSC	
Urine Biomarkers		
NICEq-T	MSC > SMK	NTC always had lowest levels
TSNAs		
PAHs	SMK > MSC & NTC	No differences between MSC and NTC
AAs		
SPMA, HPMA, HMPMA, MHBMA, AAMA, GAMA		
SCN		

Summary and Conclusions (continued)

- MSC exhibit greater exposure to nicotine (and its metabolites) compared to Smokers.
- MSC exhibit greater exposure to all four TSNAs compared to Smokers.
- Compared with Smokers, MSC have markedly reduced levels of combustion-related biomarkers, resembling those of NTC.
- Conclusions from this study demonstrate that MSC show significantly reduced exposure to harmful tobacco combustion-related toxicants compared to cigarette smokers. This is consistent with previous RJRT study results and the literature.
- Our data suggest that tobacco products may be placed on a relative risk continuum with cigarettes being the most harmful.

Acknowledgements

Study Partners

- **Clinical Study Site**
  - High Point Clinical Trials Center, High Point NC (formerly Mendenhall Clinical Research Center)
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  - EDC, eTMF – PharmaVigilant Inc., Westborough MA
  - Data management – Comprehensive Clinical Development, Miramar FL
  - Statistical analysis, CSR writing – Celerion, Lincoln NE
- **Analytical Laboratories**
  - Analytisch-Biologisches Forschungslabor, München Germany
  - Kronos Science Laboratory, Phoenix AZ
  - Labstat, Kitchener, Ontario
  - Pacific Biomarkers, Inc., Seattle WA
  - Rules Based Medicine, Austin TX
  - Solstas Lab Partners, Greensboro NC
- **Medical Monitor**
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- **Clinical Study Monitor**
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