Utility of Biomarkers in Assessing Exposure to Cigarette Smoke Constituents in Adult Smokers

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Altria Client Services Inc.

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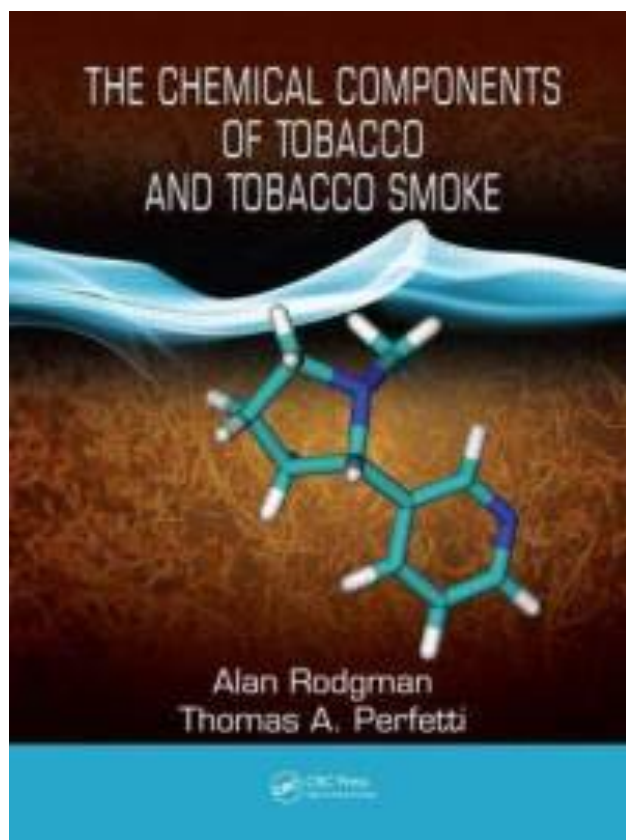Altria Client Services

Outline

- Introduction

- Categorization of Biomarkers of Exposure
  - Tobacco Specific
  - Non-tobacco Specific

- Factors Influencing Biomarker of Exposure

- Characterization of Some Biomarkers of Exposure

- Alternative Methods of Estimating Exposure
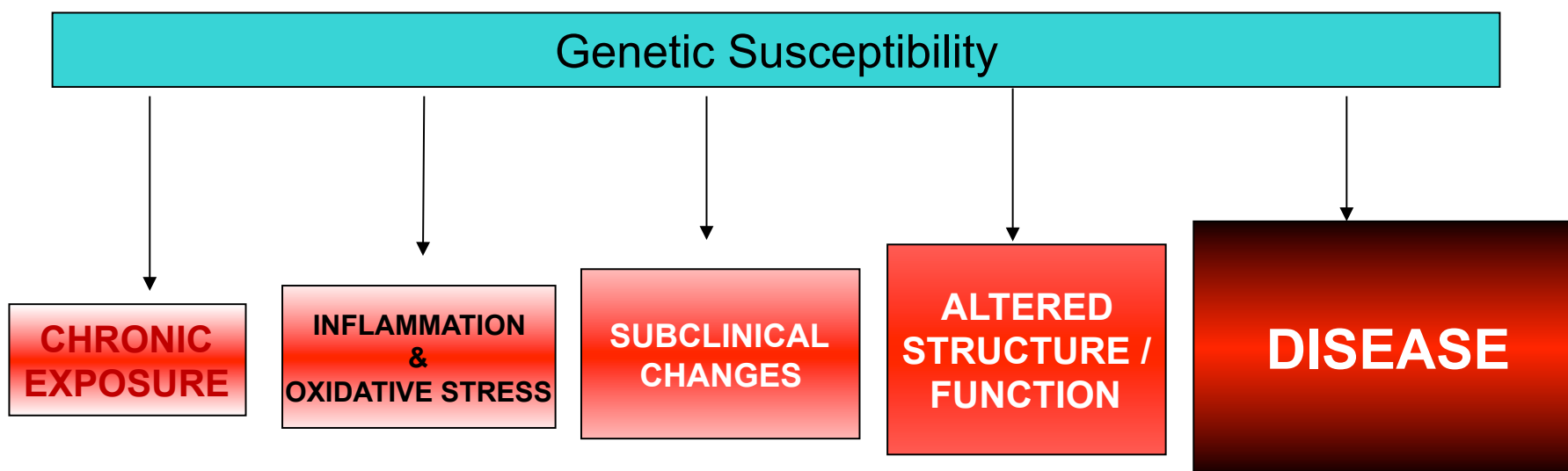
- Conclusions

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Introduction

THE CHEMICAL COMPONENTS OF TOBACCO AND TOBACCO SMOKE

Alan Rodgman
Thomas A. Perfetti

- There are thousands of constituents in cigarette smoke, which makes assessment of total systemic exposure to cigarette smoke challenging.

- The role of specific constituents in causing smoking related disease is still not well established

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Exposure is the First Step towards Potential Biological Outcome from Smoking

Genetic Susceptibility

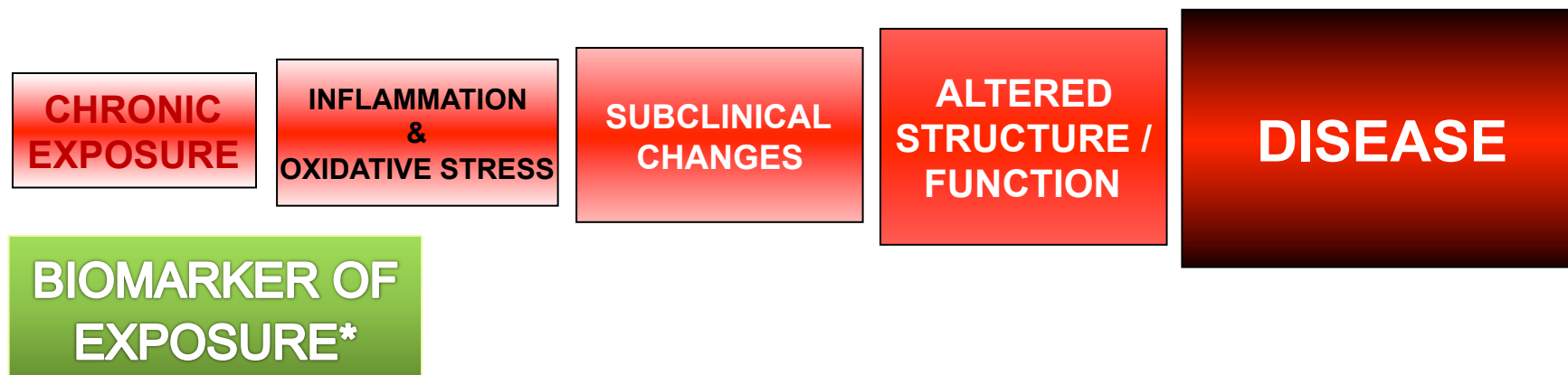| CHRONIC EXPOSURE | INFLAMMATION & OXIDATIVE STRESS | SUBCLINICAL CHANGES | ALTERED STRUCTURE / FUNCTION | DISEASE |

Adapted from: National Research Council. 1987. Biological markers in environmental health research. Environmental Health Perspectives 74:1-191.

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Biomarkers of Exposure

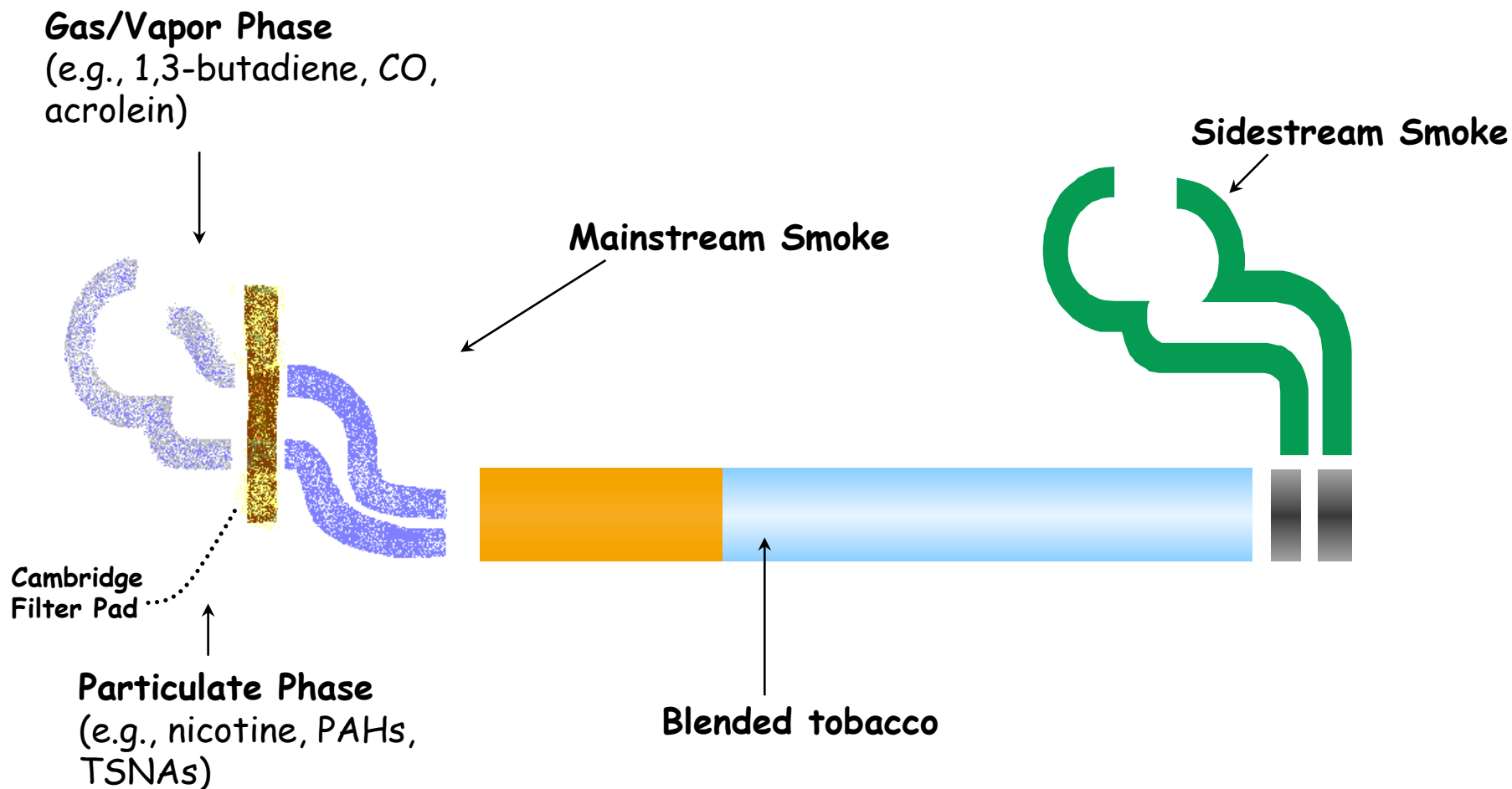| CHRONIC EXPOSURE | INFLAMMATION & OXIDATIVE STRESS | SUBCLINICAL CHANGES | ALTERED STRUCTURE / FUNCTION | DISEASE |

BIOMARKER OF EXPOSURE*

A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose

*Clearing the smoke: Assessing the science base for tobacco harm reduction. Stratton K, Shetty P, Wallace R and Bondurant S. Eds. (2001)   Institute of Medicine Publication, National Academy Press Washington DC
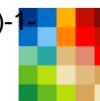
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Cigarette Smoke is a Complex Mixture

Gas/Vapor Phase
(e.g., 1,3-butadiene, CO, acrolein)

Sidestream Smoke

Mainstream Smoke

Cambridge Filter Pad

Particulate Phase
(e.g., nicotine, PAHs, TSNAs)

Blended tobacco

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Biomarkers of Exposure

Biomarker of Exposure	Smoke Constituent	Sample Matrix
Carboxyhemoglobin	Carbon monoxide	Blood
3-hydroxypropylmercapturic acid (3-HPMA)	Acrolein	Urine
Monohydroxybutenylmercapturic acid (MHBMA) and dihydroxybutylmercapturic acid (DHBMA)	1,3-Butadiene	Urine
S-phenylmercapturic acid	Benzene	Urine
3-hydroxy-1-methylpropylmercapturic acid 2-carboxy1-1-methylethylmercapturic acid	Crotonaldehyde	Urine
4-Aminobiphenyl adducts, 4-Aminobiphenyl	Aminobiphenyl	Blood / Urine
o-toluidine, 2-aminonaphthalene	Aromatic amines	Urine
Nicotine, cotinine, trans-3'-hydroxycotinine and their glucuronide conjugates[A]	Nicotine (T)	Urine
Nicotine, Cotinine, trans-3'-hydroxycotinine	Nicotine (T)	Plasma
Total NNAL[B]	NNK[C] (T)	Urine
Total NNN[D]	NNN (T)	Urine
Total 1-hydroxypyrene	Pyrene	Urine
3-hydroxy-benzo[a]pyrene	Benzo[a]pyrene	Urine

Gas / Gas-Vapor Phase; Particulate Phase

(T) Tobacco specific

[A]The molar sum of nicotine and its five major metabolites were expressed as nicotine equivalents (NE) [B] 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; [C] 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone ; [D]N'-nitrosonornicotine

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Philip Morris USA Study
Total Exposure Study -TES

- The TES was a stratified, multi-center, cross-sectional study that had 3585 evaluable adult smokers and 1077 evaluable non-smokers from 31 states (39 investigative sites) across the U.S.

States with sites

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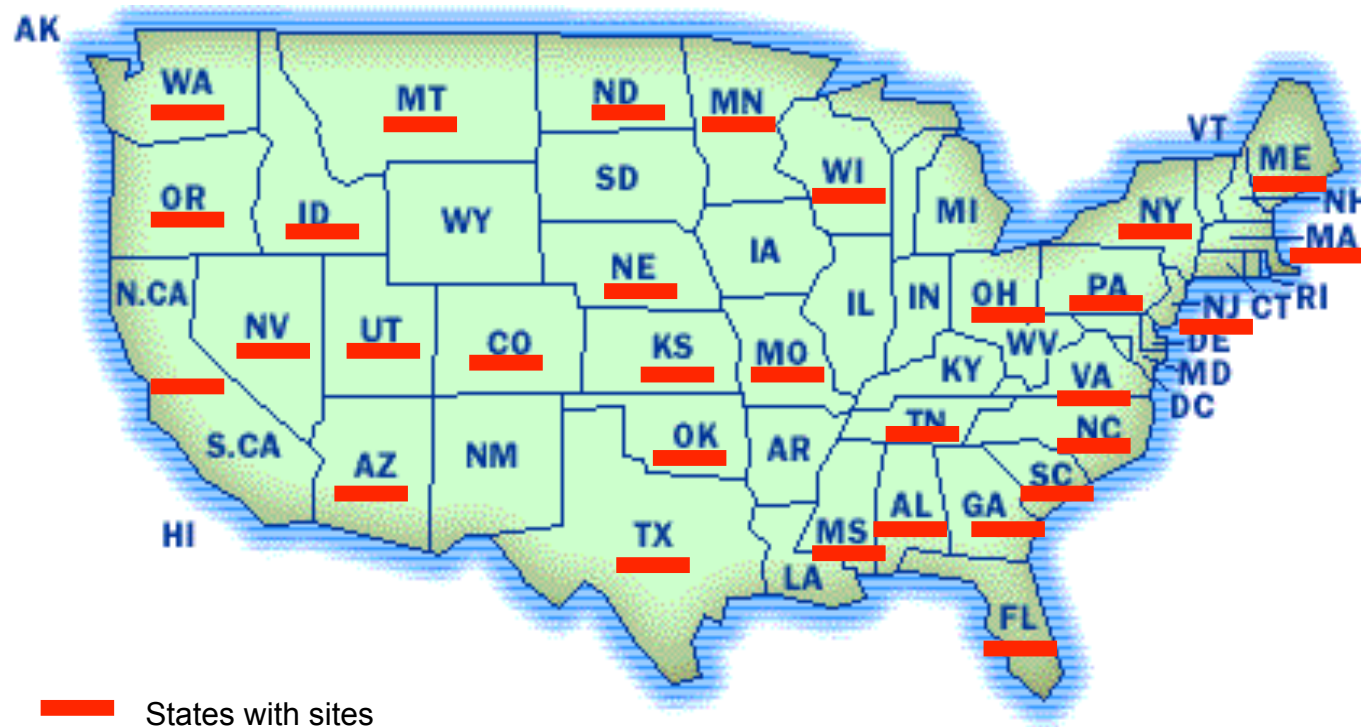
Clinical Models to Assess Exposure

- **Switching Studies**
  - **Short Term**
    - Randomized
    - Parallel Group
    - Confined to the Clinic
    - Appropriate Comparators
    - Daily Investigations
      - Biomarkers of exposure
        - 24 hour urine and precisely timed blood measurements
      - Behavioral Measurements

Adult Smokers Baseline measures while smoking own cigarette	R A N D O M I Z A T I O N	Continue smoking Own Cigarette
		Switch to Test Product
		Stop Smoking

Day -1 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8

Primary Objective of Short Term Studies – Exposure Assessment

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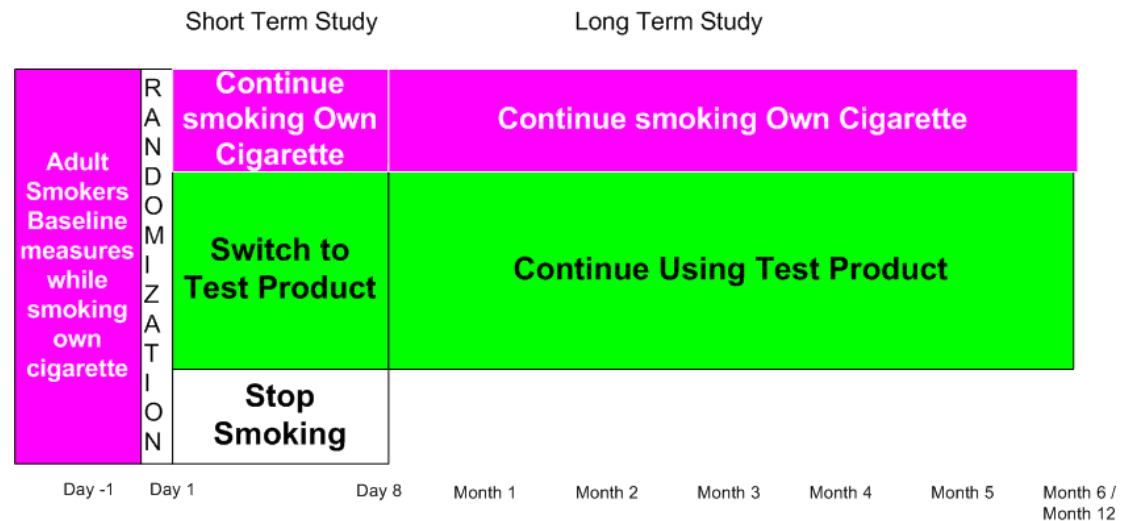Altria Client Services

Clinical Models to Assess Exposure

- **Switching Studies**
  - **Long Term**
    - Randomized
    - Parallel Group
    - Ambulatory
    - *Ad Libitum* Product use
    - Appropriate Comparators
    - Monthly Investigations
      - Biomarkers of exposure
      - Biomarkers of potential harm
        - 24 hour urine and blood measurements
      - Behavioral Measurements

Primary Objective of Long Term Studies – To assess biomarkers of exposure and potential harm under ambulatory product use behavior

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Categorization of Biomarkers of Exposure Tobacco Specific Biomarkers

Biomarker		Adult Smoker (AS)	Non-Smoker (NS)	Ratio AS/NS
Nicotine Equivalents (mg/24h)	Mean ± SD	13.1 ± 7.89	0.120 ± 1.22 ~78% BLLOQ	109.2
Serum Cotinine (ng/mL)	Mean ± SD	184 ± 106	4.05 ± 21.3 ~98% BLLOQ	45.4
Total NNAL (ng/24h)	Mean ± SD	433 ± 306	10.8 ± 68.5 ~88% BLLOQ	40.1
NNN (ng/24h)	Mean ± SD	24.1 ± 29.41	1.0 ± 6.91 ~94% BLLOQ	24.1

Data from the Total Exposure Study; N=3585 AS; N1077 NS for all biomarkers except NNN (n=371 for AS and N=179 for NS); BLLOQ=Below Lower Limit of Quantification; Roethig et al Nicotine Tob Res. 2009 Oct;11(10):1216-25.

Biomarkers of Exposure to tobacco specific constituents provide clear discrimination between AS and NS; however, secondhand exposure or misclassification should be considered

Categorization of Biomarkers of Exposure Non-Tobacco Specific Biomarkers

Biomarker		Adult Smoker (AS)	Non-Smoker (NS)	Ratio $\frac{AS}{NS}$
S-PMA (µg/24h)	Mean ± SD	6.5 ± 4.93	0.6 ± 0.47	11.03
Urine mutagenicity (revertants/24h)	Median	26549	3283	7.42
4-ABP (ng/24h)	Mean ± SD	20.5 ± 11.05	3.1 ± 1.84	6.59
3-OH B(a)P (pg/24h)	Mean ± SD	218.7 ± 307.09	104.2 ± 163.28	2.10
o-toluidine (ng/24h)	Mean ± SD	414.1 ± 221.17	346.8 ± 1083.5	1.19

N=200 for each group from the Total Exposure Study; some biomarkers had slightly different sample sizes
Sarkar M *et al* manuscript under review , Cancer Epidemiology Biomarkers and Prevention.

Although statistically significantly different between NS & AS, other sources of exposure may confound the interpretation from such "non-tobacco specific biomarkers"

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- **Factors Influencing Biomarker of Exposure**

- Characterization of Some Biomarkers of Exposure

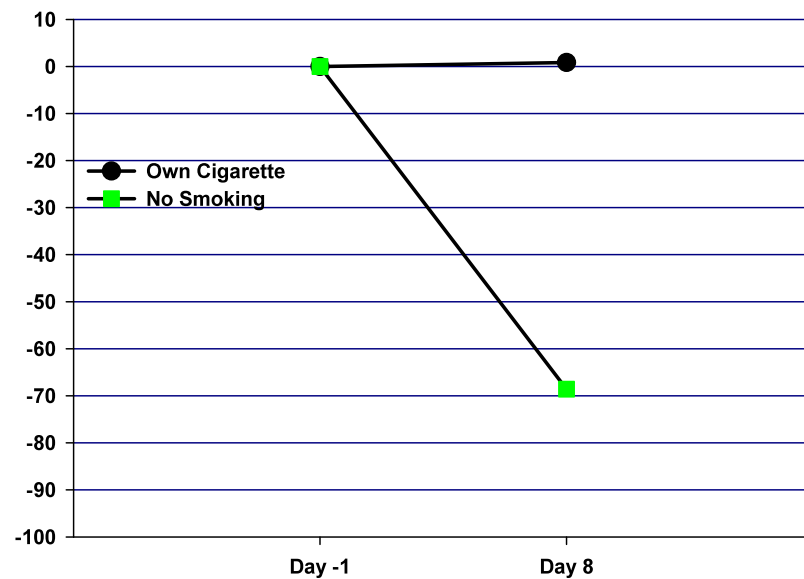- Alternative Methods of Estimating Exposure

- Conclusions

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Factors influencing Biomarkers of Exposure – Half-Life

Own Cigarette
Test Product
No smoking
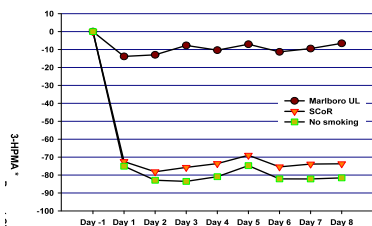
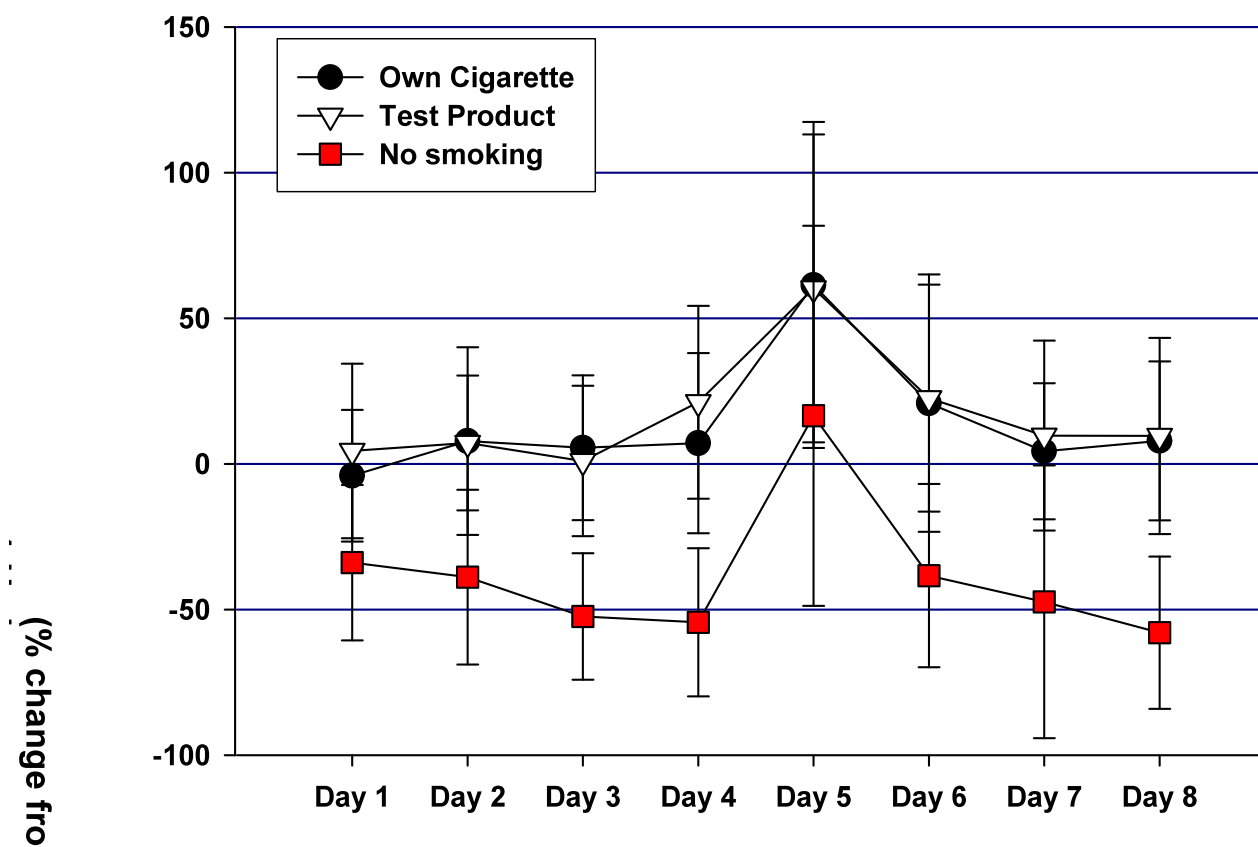**Own Cigarette**
No Smoking

3-HPMA=3-hydroxypropyl mercapturic acid

Total NNAL=4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide conjugates

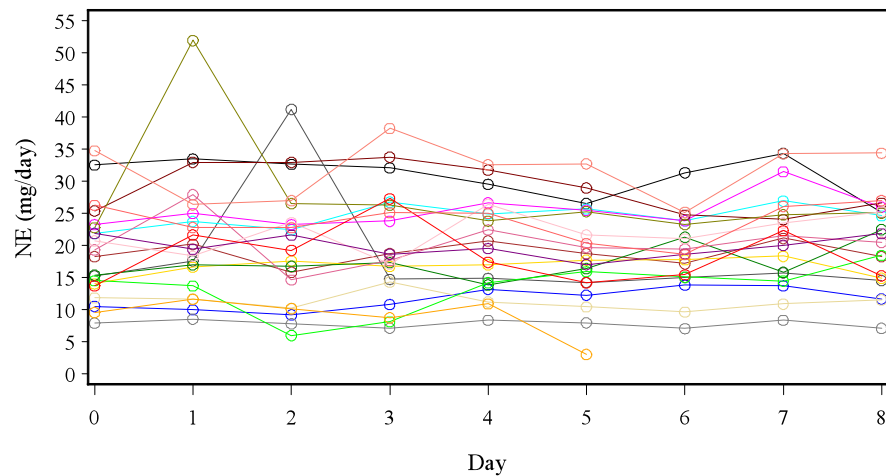Data, shown as Median values, adapted from Sarkar MA *et al* Nicotine Tob Res. 2008 Dec;10(12):1761-72.

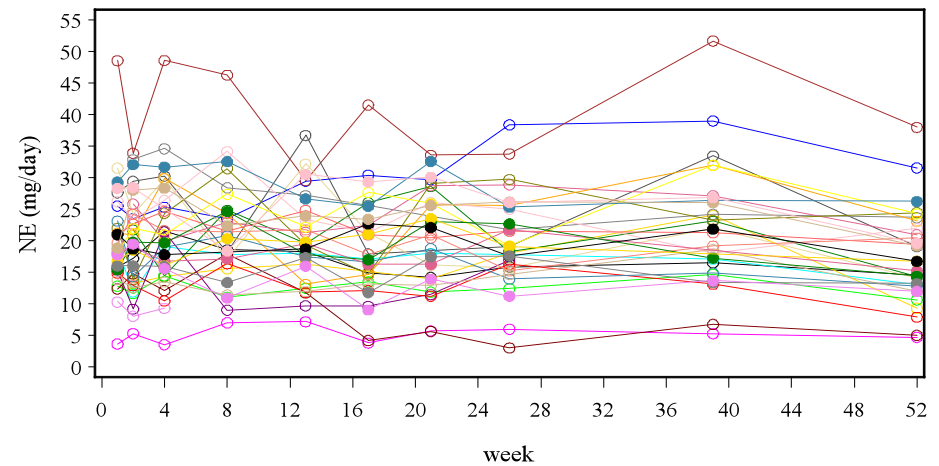# Factors influencing Biomarkers of Exposure – Specificity

- Own Cigarette
- Test Product
- No smoking

(% change fro

Data, shown as Mean ± SD values, adapted from Sarkar MA *et al* Nicotine Tob Res. 2008 Dec;10(12):1761-72.

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Factors influencing Biomarkers of Exposure – Variability
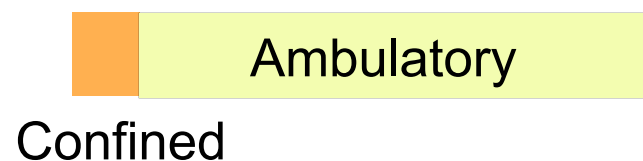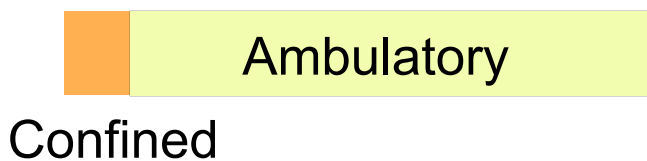
Short-Term Study – *Confined Setting*

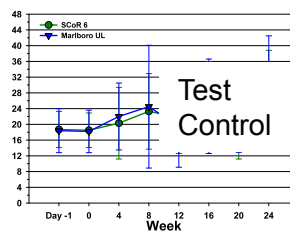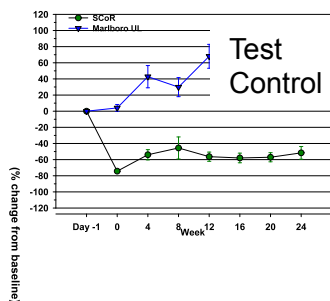## Long-Term Study – *Ambulatory Setting*

Data, shown for individual subjects; NE=Nicotine Equivalents; Liang *et al* Manuscript under review

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Factors influencing Biomarkers of Exposure – Study Conduct Setting

3-HPMA=3-hydroxypropyl mercapturic acid

Data, shown as Mean $\pm$ SD values, adapted from Sarkar MA *et al* Nicotine Tob Res. 2008 Dec;10(12):1761-72.

Factors influencing Biomarkers of Exposure – Study Conduct Setting

Short-term (Confined Clinic)

Long-term (*Ad libitum*)

Cross-sectional **(***Ad libitum***)**
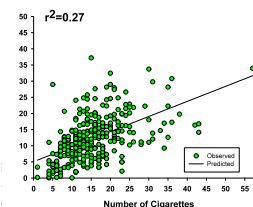
Difficult to ascertain protocol adherence

Nicotine Equivalents = Nicotine and five of its metabolites expressed as molar equivalents of nicotine measured in 24 hour urine
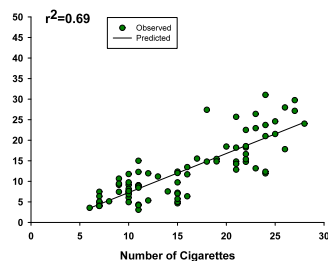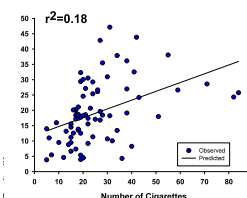
Data, shown as Mean $\pm$ SD values, adapted from Sarkar MA *et al* Nicotine Tob Res. 2008 Dec;10(12):1761-72. and Roethig *et al* Nicotine Tob. Res. 2009 Oct;11(10):1216-25.

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Characterization of Nicotine Exposure[1]

Pearson Correlation Coefficient (r) values for the relationship between Nicotine Equivalents and biomarkers of exposure

Cigarette Type[2]	Particulate Phase			Gas-Vapor Phase			
	Total NNAL	Total 1-OHP	Plasma Cotinine[3]	3-HPMA	S-PMA	MHBMA	COHb[3]
CC6	0.67	0.52	0.75	0.84	0.65	0.62	0.76
CC11	0.50	0.51	0.67	0.80	0.56	0.47	0.62
CC15	0.72	0.54	0.43	0.69	0.61		0.53

[2]Different cigarette types reflect different products with varying machine measured tar yields, CC6= 6 mg tar; CC11=11 mg tar and CC15 = 15 mg tar as measured by the Cambridge Filter Method.
[3] Collected at 19:00 daily.
$p < .0001$ for all the correlation significance testing. [1]Wang J, Liang Q, Mendes P, Sarkar M. Biomarkers. **16**:144-54, 2011

24H urinary Nicotine Equivalents is a good surrogate of overall smoke exposure

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Characterization of urinary excretion of NNN*

Biomarker	Independent variable	p-value	r
NNN	Num of cig	<.0001	0.21
	NE	<.0001	0.27
	Total NNAL	<.0001	0.29

24H urinary excretion of NNN does not appear to be a representative biomarker of the particulate phase from cigarette smoke

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Characteristics of Biomarkers of Exposure to Polycyclic Aromatic Hydrocarbons

Biomarker	Independent variable	p-value	r
3-OH BaP	Num of cig	0.7559	0.02
	NE	0.0001	0.19
	1-hydroxy pyrene	<0.0001	0.79

24H urinary excretion of 3-hydroxy benzo(a)pyrene does not appear to be a representative biomarker of exposure

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Characterization of Nicotine Exposure[1]

Pearson Correlation Coefficient (r) values for the relationship between Nicotine Equivalents and biomarkers of exposure

Cigarette Type[2]	Particulate Phase			Gas-Vapor Phase			
	Total NNAL	Total 1-OHP	Plasma Cotinine[3]	3-HPMA	S-PMA	MHBMA	COHb[3]
CC6	0.67	0.52	0.75	0.84	0.65	0.62	0.76
CC11	0.50	0.51	0.67	0.80	0.56	0.47	0.62
CC15	0.72	0.54	0.43	0.69	0.61		0.53

[2]Different cigarette types reflect different products with varying machine measured tar yields,
CC6= 6 mg tar; CC11=11 mg tar and CC15 = 15 mg tar as measured by the Cambridge Filter Method.
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24H urinary Nicotine Equivalents is a good surrogate of overall smoke exposure

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Characteristics of Biomarkers of Exposure to 4-aminobiphenyl

Biomarker	Independent variable	p-value	r
4-Aminobiphenyl	Num of cig	<.0001	0.44
	NE	<.0001	0.64
	4-ABP Hb Adducts	<.0001	0.31

24H urinary excretion of 4-aminobiphenyl and its hemoglobin adducts serve unique purposes in assessing cigarette smoke exposure

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Characteristics of Biomarkers of Exposure to Aromatic Amines

Biomarker	Independent variable	p-value	r
2-aminonaphthalene	Num of cig	<.0001	0.55
	NE	<.0001	0.73
o-toluidine	Num of cig	0.0006	0.24
	NE	<.0001	0.30

24H urinary excretion of *o*-toluidine does not appear to be a representative biomarker of aromatic amines from cigarette smoke

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Relationship of Urine Mutagenicity with Biomarkers of Exposure

Parameter	T Value	P Value	Partial R-square
Intercept	-1.63	0.1056	
2-Aminonaphthalene (ng/24h)	10.67	<.0001	0.5631
o-Toluidine (ng/24h)	2.22	0.0285	0.0116

The association of 2-aminonaphthalene with urine mutagenicity suggests that this biomarker of exposure may have some biological relevance

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Alternative Methods to Estimate Exposure: Mouth Level Exposure (MLE)

- MLE is an unobtrusive technique based on measurements made on cigarettes actually smoked in an ambulatory environment.

- The retained smoke particulates and nicotine in the mouth-end filter section of a smoked cigarette are measured.

- However this method does not appear to represent actual exposure.

Percent change in mean daily exposure: mouth level exposure (MLE) compared to biomarker of exposure (BOE)

	MLE	BOE
Nicotine	-15.7	-34.1 (NE)
NNK	-34.1	-21.3 (NNAL)
Acrolein	-2.7	-22.9 (3-HPMA)

Data adapted from Shepperd *et al* Regul. Toxicol. Pharmacol. 2011, shown as % reduction in constituent or biomarker when switching to a test product

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Conclusions

- Several factors, in addition to smoking behavior, influence biomarkers of exposure

- Some of the biomarkers have poor discriminatory power (e.g. *o*-toluidine) and would not be suitable for estimating exposure in adult smokers.

- Nicotine equivalents should be considered as a surrogate biomarker of total cigarette smoke exposure.

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