SELECTION OF BIOMARKERS OF EXPOSURE FOR A POPULATION STUDY OF U.S. ADULT SMOKERS TO CIGARETTE SMOKE

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Why Measure Cigarette Smoke Exposure?

- Development of reduced harm cigarettes is a priority
- Measurements of actual human exposure are required components for reduced harm assessments
- Machine-derived data may not reflect actual human exposure

What is Cigarette Smoke?

- Aerosol composed of ~4,000 constituents in either gas/vapor phase or particulate phase; constituents include air, water, examples of most organic functional groups; oxides of nitrogen, and metals
- Mainstream (MS) smoke: smoke drawn during puffing through unlit end into mouth
- Sidestream (SS) smoke: smoke arising from lit end between puffs
- Environmental tobacco smoke (ETS): mixture of aged SS and exhaled MS, disseminated in air

How are Humans Exposed to Cigarette Smoke?

- Smoker exposed to MS, SS and ETS
- Non-smoker may be exposed to SS and ETS
- Determination of internal (systemic) exposure to smoker and non-smoker is difficult for MS, SS, and ETS due to differences in human smoking patterns and ventilation conditions

What Determines Smoke Exposure?

- Determined by smoker, can be learned from questionnaires: *cigarette brand style, number of cigarettes smoked*
- Determined by smoker, can be approximated by smoking machine: *puff interval, puff frequency, puff volume, puff duration, flow rate during puffing, butt length, pressure drop during puff, ventilation hole blocking*
- Determined by smoker, not experimentally accessible: *mouth spill, depth of inhalation, duration of inhalation*

What Were the Criteria for Biomarker Selection?

- "Unique or nearly unique" to tobacco smoke, "so that other sources are minor in comparison"
  Benowitz, N.L. *Environmental Health Perspectives*, **1999**, 107(Suppl. 2), 349 – 355
- Representative of particulate and gas phase
- Representative of health-relevant constituents
- Reliable analytical methods available ("easily detectable")
  Benowitz, ibid.
- Sampling to acquire material for analysis only minimally invasive
- Constituent metabolism understood

Assessment of Biomarker Validity

- Analytical validation
  - Consistent with U.S. Food and Drug Administration Guidance for Industry on Bioanalytical Methods Validation
- Biological validation
  - Pilot study of 60 adult male and female smokers of 3.9 - 6.0 mg tar yield (U.S. Federal Trade Commission methods) and 60 adult male and female non-smokers with repetitive sampling over a 6-week period. See Poster # for details

Summary

- The following biomarkers of exposure were selected:
  - Acetonitrile in exhalate and/or blood
  - Carbon monoxide in exhalate and/or blood
  - Nicotine and five metabolites in 24-h urine
  - Hemoglobin adducts of 3- and 4-aminobiphenyl
  - Metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in 24-h urine
- Data or suggestions are invited as input into these selections

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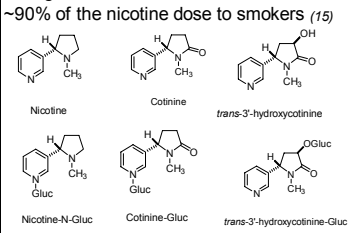
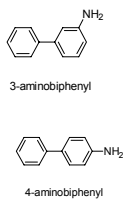
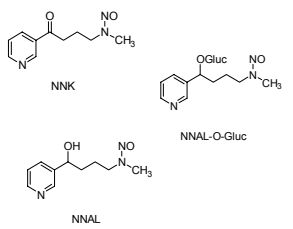
BIOMARKER → / SELECTION CRITERIA ↓	*Acetonitrile*	*Carbon Monoxide*	*Nicotine and Metabolites*	*Hb Adducts of 3- and 4-Aminobiphenyl (3-ABP and 4-ABP)*	*NNK Metabolites*
Unique or nearly unique to tobacco smoke	44 - 140 ug/cigt in mainstream smoke *(2)*	Mainstream smoke levels of 1.8 -13.7 mg/cigt *(7)*; but confounders include vehicular exhaust and home heating systems	Mainstream smoke yields of 0.07 - 1.92 mg/cigt *(12)*	MS yields of 3-ABP 2.7 - 5.0 ng/cigt and 4-ABP 2.4 - 4.6 ng/cigt *(16)*	Mainstream NNK yield ca. 84 ng/cigt *(20)*
Represents both gas/vapor and particulate phases of smoke	gas phase	gas phase	particulate phase	particulate phase	particulate phase
Represents health-relevant constituents		Diminished oxygen-transport capacity of hemoglobin		4-ABP classified by IARC as carcinogenic to humans (Group I)	NNK classified by IARC as possibly carcinogenic to humans (Group IIB)
Biological half-life	24 h for acetonitrile$_{ex}$ *(3)*; 32 h for acetontitrile$_{blood}$ (elimination) *(4)*	CO_{ex}: 2 - 3 h *(8)*; COHb: ~3 h (awake) and ~6.9 h (sleeping) *(9)*	nicotine: 11 h *(13)* (elimination) cotinine: 19.5 h *(13)* (elimination) 3'-OH-cotinine: 6.4 h *(25)* (elimination) 3'-OH-cotinine-Gluc: 7.2 h *(25)* (elimination)	4-ABP: 7 - 9 weeks *(18)*	distribution half-life 3 - 4 days; elimination half-life 40 -45 days *(22)*
Smoker/non-smoker ratios	~11:1 for acetonitrile$_{ex}$ *(3)*; not detected in blood of non-smokers *(5)*	CO_{ex}:~8:1 *(10)*; COHb: ~5:1 *(11)*	nicotine: 136:1 *(15)* cotinine: 302:1 *(15)*	Hb adduct of 4-ABP = 3:1 - 5:1; Hb adduct of 3-ABP = 8:1 *(17)*	NNAL: between 100:1 and 1000:1 *(21)*
Reliable analytical methods available	Proton transfer mass spectrometry of exhalate sample *(6)*; GC/FID for blood determinations *(4)*	CO_{ex}: electrochemical gas sensor COHb: spectrophotometry	LC/MS *(14)*	GC/MS of derivatized amines *(19)*	GC/TEA (Thermal Energy Analyzer) of derivatized TSNA *(23)*
Sampling to acquire material for analysis only minimally invasive	Breath collection at clinical site for exhalate; venous blood draw	Breath collection at clinical site for CO_{ex}; venous blood draw for COHb	24 h urine	Venous blood	24 h urine
Constituent metabolism understood	Detected unchanged in exhalate and blood *(4, 6)*	Exhaled unchanged; forms adduct with hemoglobin	Nicotine, cotinine, trans-3'-hydroxycotinine and their glucuronides in a 24- h urine reflect ~90% of the nicotine dose to smokers *(15)*  Nicotine; Cotinine; *trans*-3'-hydroxycotinine; Nicotine-N-Gluc; Cotinine-Gluc; *trans*-3'-hydroxycotinine-Gluc	Form hemoglobin adducts *(19)*  3-aminobiphenyl; 4-aminobiphenyl	NNAL primary metabolite resulting from carbonyl reduction *(24)*  NNK; NNAL-O-Gluc; NNAL

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