Perchlorate and Tobacco Updates from CDC's National Biomonitoring Program

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Centers for Disease Control and Prevention

Biomonitoring California Scientific Guidance Panel Meeting

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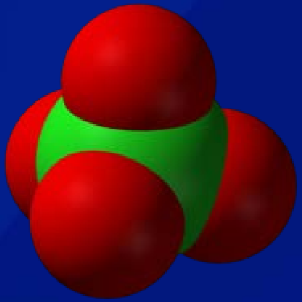
Perchlorate



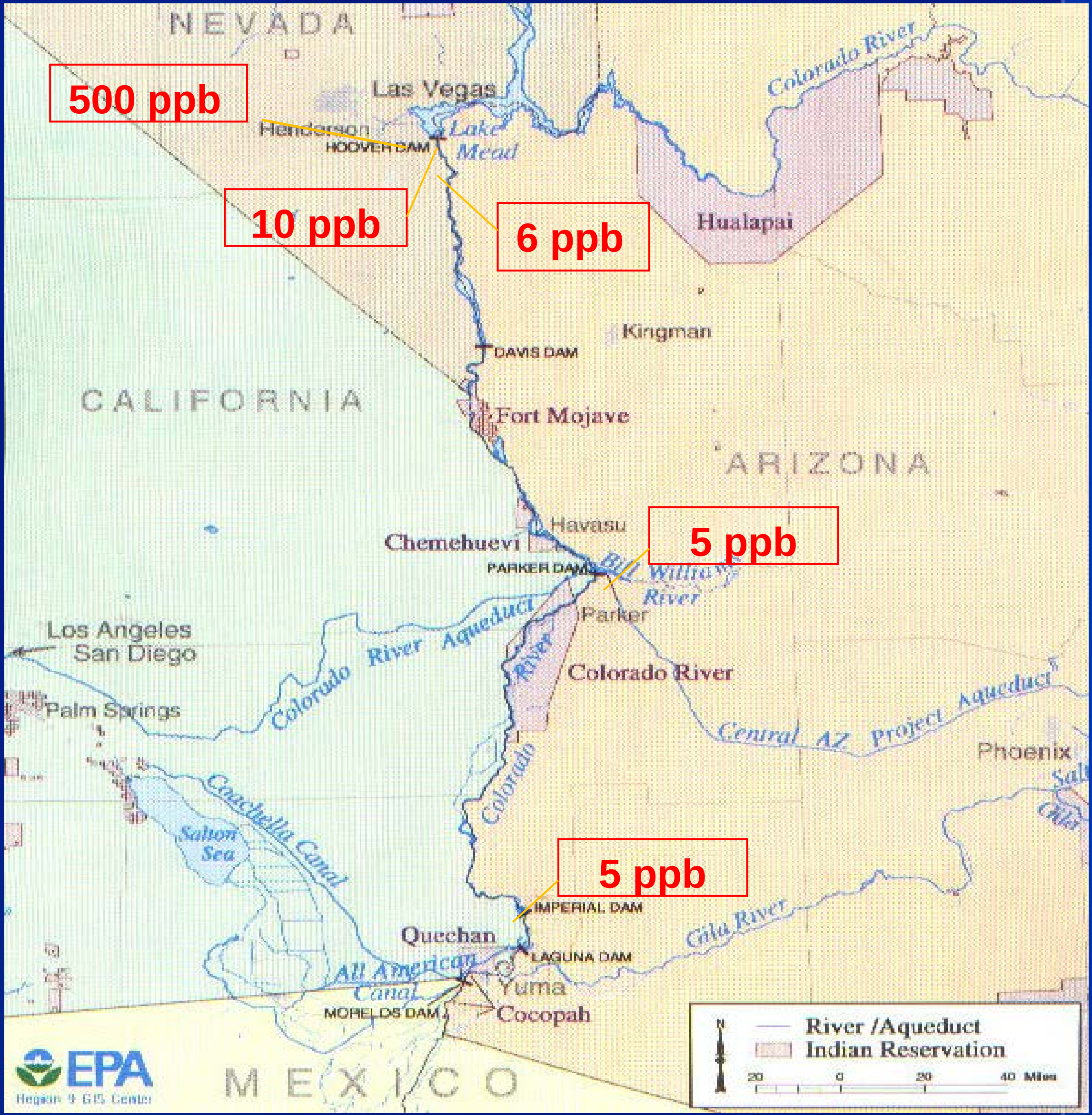
- Component of propellant for rockets and missiles
- Explosives, fireworks, road flares, dyes, leather tanning, matches
- Can form naturally in atmosphere

Perchlorate in the Environment

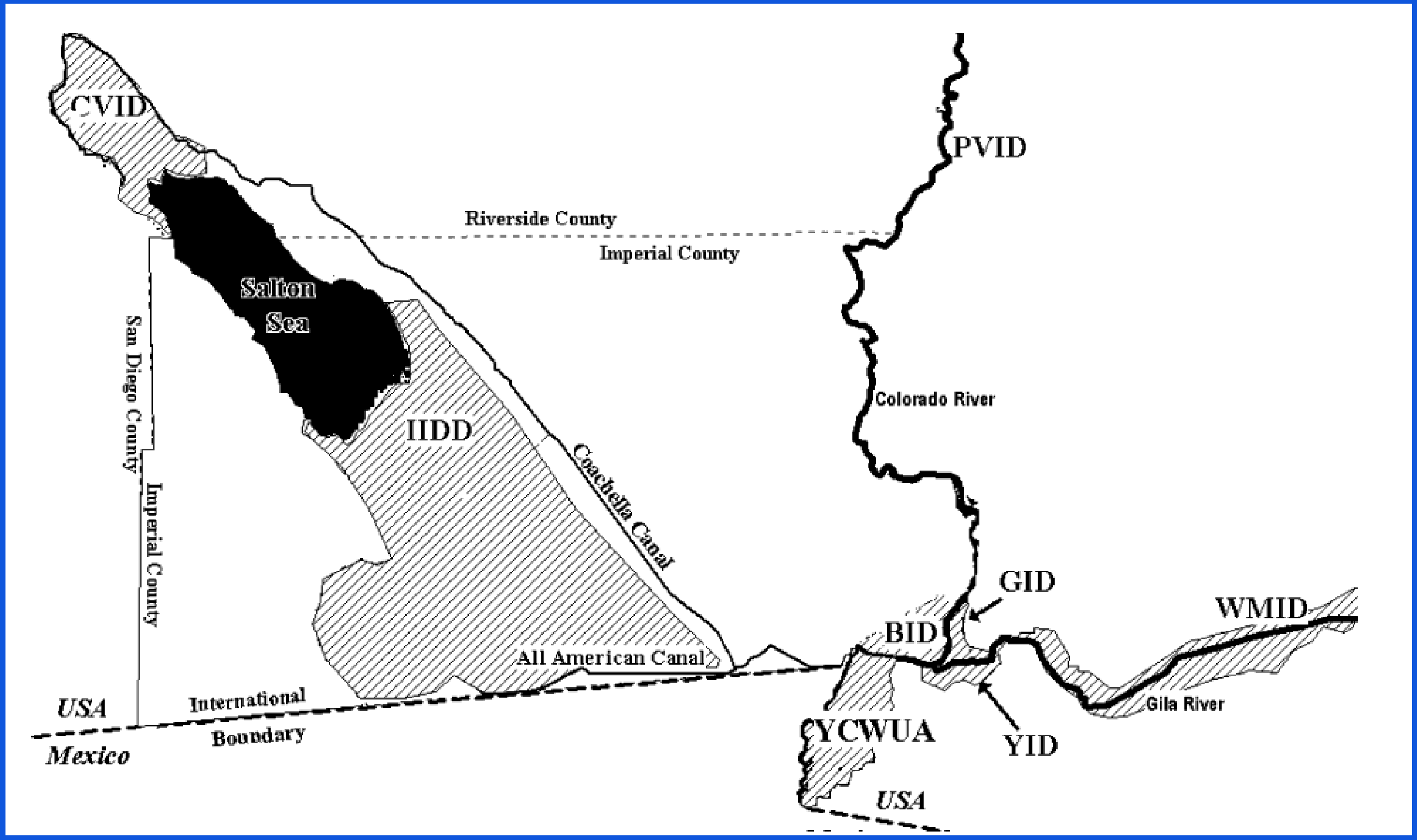
- Manmade leaching from industrial sites
  - Perchlorate freely soluble in water
  - Ground water contamination plumes
  - Lower Colorado River
- Natural atmospheric formation/deposition
  - Trace levels (ng/L in precipitation)
  - Can accumulate in arid regions
  - Chilean fertilizer was ~0.15% perchlorate, applied to crops
- Formation in sodium hypochlorite and in some water distribution systems

Perchlorate Contamination in Colorado River (2002)

- In 1997, 450 kg/day entered Lake Mead
- Measurable entire 300 miles to Mexico
- Used by Las Vegas, Los Angeles, San Diego, Phoenix; water supply for approx. 25 million people
- Treatment reduced ClO_4 to 1 ppb in 2012



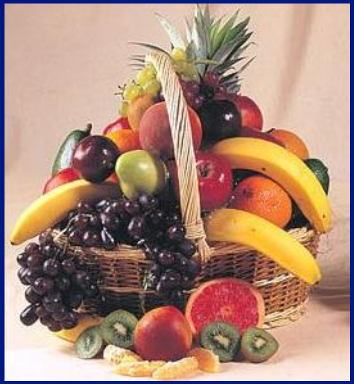
Major Agricultural Districts Grow Food Crops Using Lower Colorado River Water



Source: Sanchez et al. *J. Agric. Food Chem.*, 53 (13), 2005. 5479 – 5486

Potential sources for human exposure

- Direct consumption of contaminated water
- Crops grown with contaminated water, fertilizer or soil
  - Food crops
  - Forage crops

Perchlorate Inhibits of Iodide Uptake at Sodium-Iodide Symporter (NIS)

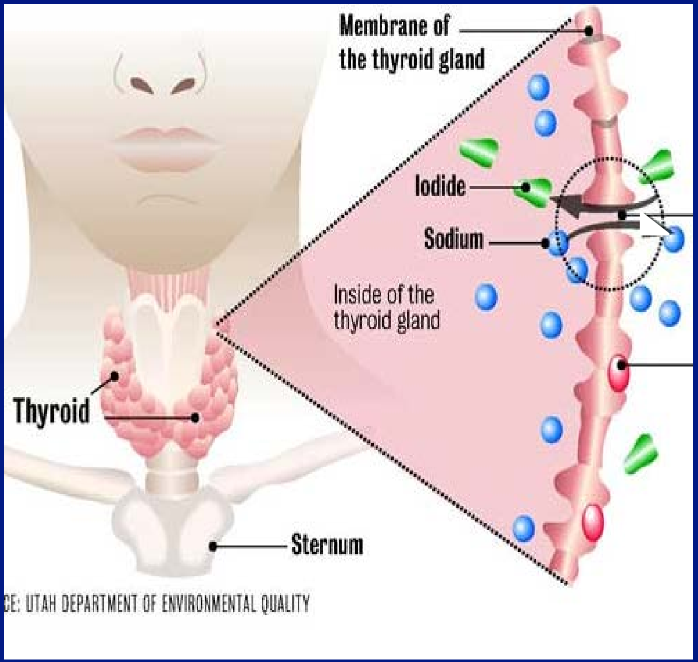
NIS function

- Active transport of I^- and ClO_4^- across cell membrane using sodium ion gradient

NIS Inhibitors: ClO_4^-, SCN^-, NO_3^-

ClO_4^- is transported by NIS 30-fold more avidly than iodide

NIS and Pendrin transport ClO_4^- across cell membranes



Tran et al. (2008); Dohan et al. (2008); Attanasio et al. (2011)

Perchlorate Biomonitoring Update

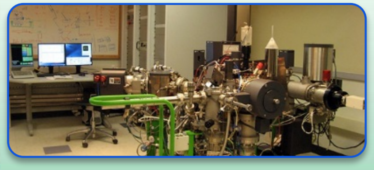
- Sources of perchlorate exposure: synthetic vs. natural
- Trends in perchlorate exposure for the U.S. population (urinary perchlorate from NHANES)

Study to Characterize Perchlorate Exposure From Synthetic vs. Natural Sources

- Perchlorate formed synthetically is subtly different from perchlorate formed naturally
- Analysis of chlorine isotopes ^{35}Cl, ^{36}Cl, and ^{37}Cl requires >0.1 mg perchlorate (~30 L of typical U.S. resident urine)
- Study design:
  - Isolate perchlorate from urine collected from residents of Atlanta (U.S.) and Taltal (Chile)
  - Compare urinary perchlorate chlorine isotope pattern with isotope patterns from reference perchlorate (synthetic and naturally-formed)

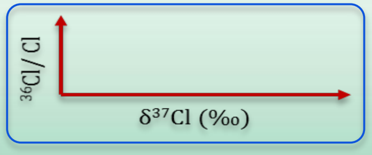
Identification of Perchlorate Exposure Sources

Extract Perchlorate from complex aqueous matrix and recrystallize as $TPAClO_4$



Stable Cl isotope Analysis ($\delta^{37}Cl$) by Secondary Ion Mass Spectrometry (SIMS)

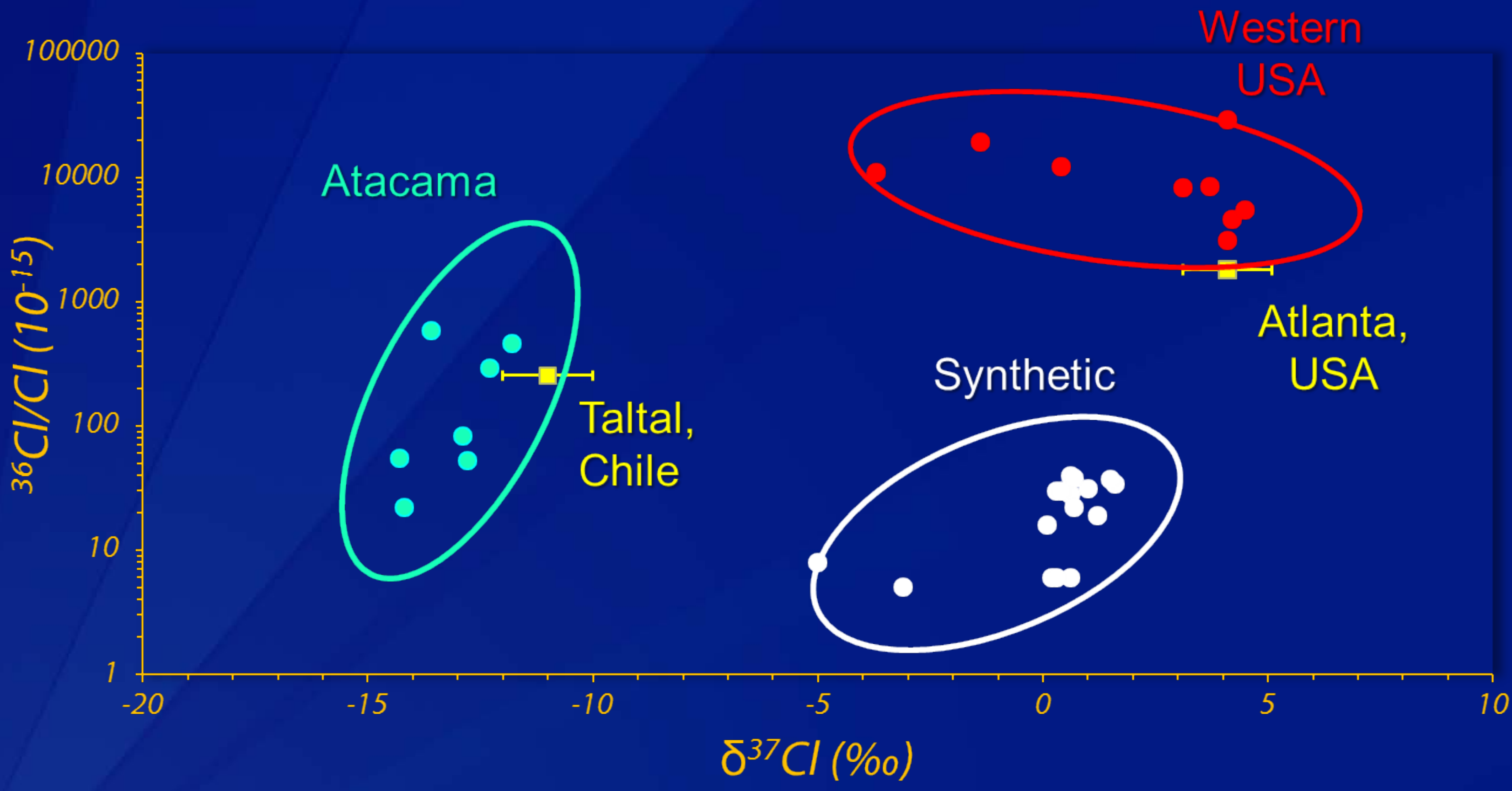
^{36}Cl Abundance Analysis by Accelerator Mass Spectrometry (AMS)



Comparative Isotopic Pattern: $^{36}Cl/Cl$ *vs* $\delta^{37}Cl$ (‰)

1. Hatzinger PB, Bohlke JK, Sturchio NC, Gu B. U.S. Department of Defense: Washington, DC, 2011.
2. Godon A, Jendrzejewski N, Eggenkamp HGM, Banks DA, Ader M, Coleman ML et al. Chem Geol 2004; 207: 1–12.
3. Jackson WA, Bohlke JK, Gu B, Hatzinger PB, Sturchio NC. Environ Sci Technol 2010; 44: 4869–4876.
4. Sturchio NC, Caffee M, Beloso AD Jr., Heraty LJ, Bohlke JK, Hatzinger PB et al. Environ Sci Technol 2009; 43: 6934–6938.

Distinguishing Among Perchlorate Exposure Sources



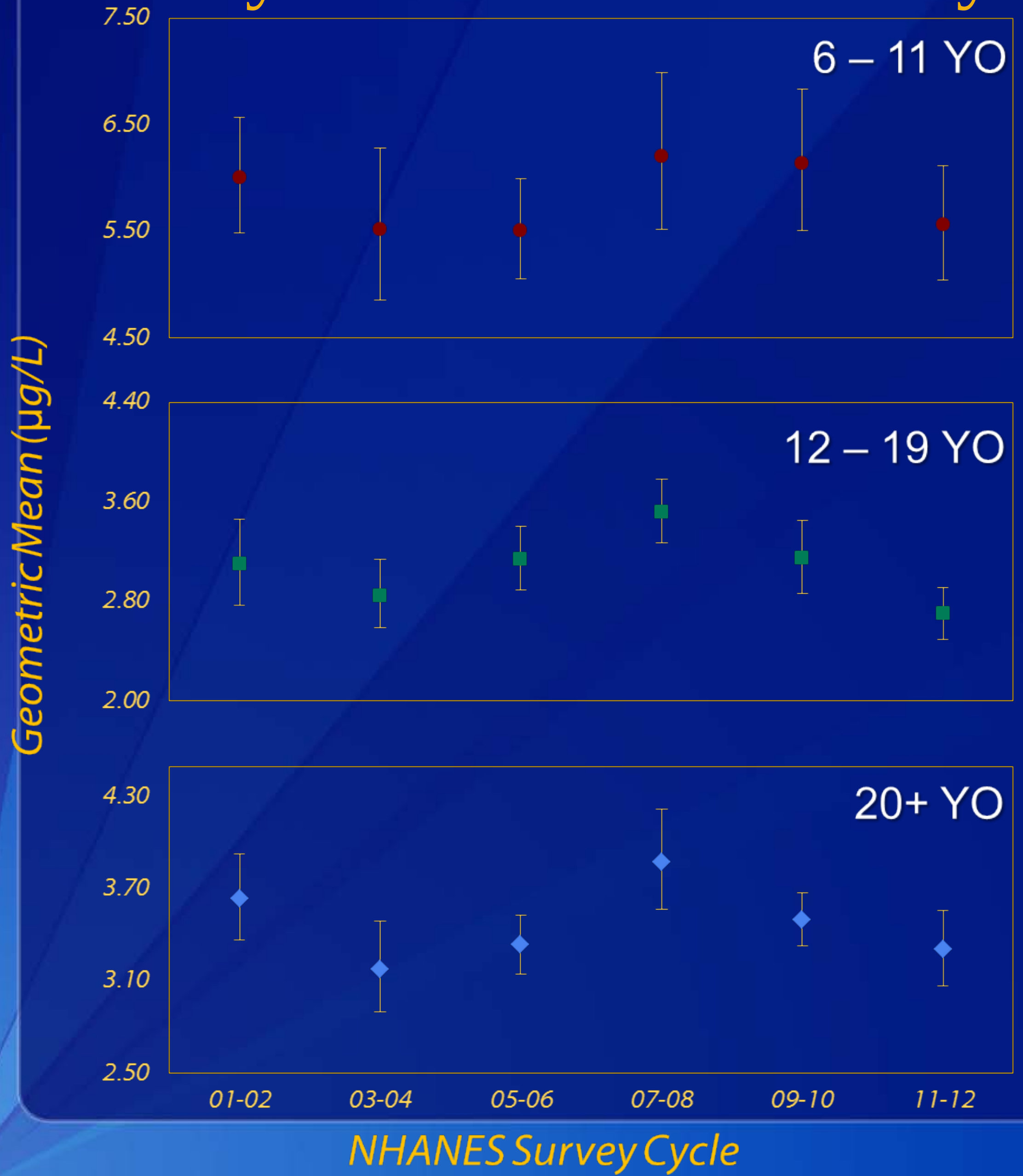
Conclusions

- Chlorine isotopes patterns differ among perchlorate formed by various natural and synthetic processes
- Perchlorate isolated from urine collected from Atlanta residents resembles naturally-formed perchlorate that is found in the Western U.S.
- Perchlorate isolated from urine collected from Taltal (Chile) residents resembles naturally-formed perchlorate that is found in Northern Chile
- Next step is to compare NHANES urine pool with perchlorate worker urine pool

Perchlorate Trend Analysis 2001-2012

- Have urinary perchlorate concentrations decreased since 2001 as states limit drinking water perchlorate?
  - No Federal MCL
  - No regulation of food perchlorate levels
  - Reduced perchlorate in the Lower Colorado River
- Urinary perchlorate measured in NHANES across 12 years using IC-MS/MS method
- Population weighted results stratified by age

Urinary Perchlorate Relatively Constant 2001-2012



- Perchlorate exposure in the U.S. has not changed significantly over the 12 year period studied
- Higher urinary perchlorate levels in children compared to other age groups likely results from consuming more perchlorate-rich foods per kg of body weight

Biomarkers of Exposure to Tobacco and Smoke

Biomarkers of Exposure to Tobacco and Smoke

- Who has been exposed to smoke?
- How intensely are people using which tobacco products?
- How much has each person been exposed in comparison with toxicological benchmarks?
- What are the exposure consequences of different tobacco product use?
- Do interventions reduce exposure?
- Are study participants forthcoming about their tobacco use?

Current Tobacco-Related Biomarker Methods at CDC

- Cotinine in serum (2)
- Nicotine metabolites and tobacco alkaloids in urine (11)
- TSNAs in urine (4)
- VOCs in blood (54)
- Aldehyde adducts in serum (15)
- Aldehyde/VOC metabolites in urine (29)
- Aromatic amines in urine (13)
- Heterocyclic amines in urine (9)
- Volatile nitrosamines in urine (7)
- PAHs in urine (11)
- Toxic anions in urine (4)
- Toxic metals in urine and blood (10)

Cotinine is the leading biomarker of tobacco exposure

(Wildox 1979, Benowitz 1983, Jarvis 1985, Jarvis 1987)

Comparison of biochemical markers of tobacco smoke consumption

Marker	Specificity	Sensitivity	Limitations	Cost	Advantages
Carbon Monoxide	Good	Good short term	Time of sampling, Variable absorption and elimination	Inexpensive	Direct toxin, Noninvasive (expired CO)
Thiocyanate	Good	Good long term	Dietary interference, Qualitative measure	Moderate	Noninvasive (saliva)
Nicotine	Excellent	Excellent short term	Time of sampling, Variable elimination rate	Expensive	Direct measurement of reinforcer
Cotinine	Excellent	Excellent intermediate term	Variable (?) elimination rate	Moderate	Measurement of nicotine consumption

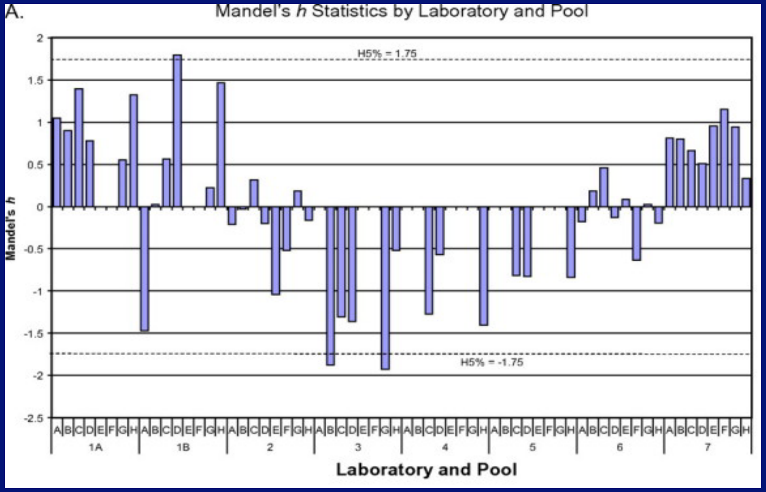
*Reprinted from Benowitz 1983

CDC Serum Cotinine Assay

Year	1992 - 2002	2002 - 2013	2013 -
Analyte	Cotinine	Cotinine	Cotinine and Trans-3'-Hydroxycotinine
Pre-screening	ELISA	ELISA	None
Sample Preparation	Manual Liquid-liquid Extraction	Manual SPE Column Extraction	Automated SPE Plate Extraction
Samples/Day	100	100	384
Time to prep 100 samples	12 hours	5 hours	2 hours
Sample Volume	500 uL	500 uL	200 uL
LC	Hewlett-Packard 1090L	Shimadzu 10A Agilent Injector	Shimadzu 20A
Mass Spec	API 3	API 4000	API 6500
Cost/Sample	>$200	$115	$60

Harmonization of Methods

- Common accuracy basis
- NIST reference materials
  - CRMs
  - SRMs
- PhenX, other open exchange of methodological info
- Round robin sample exchanges

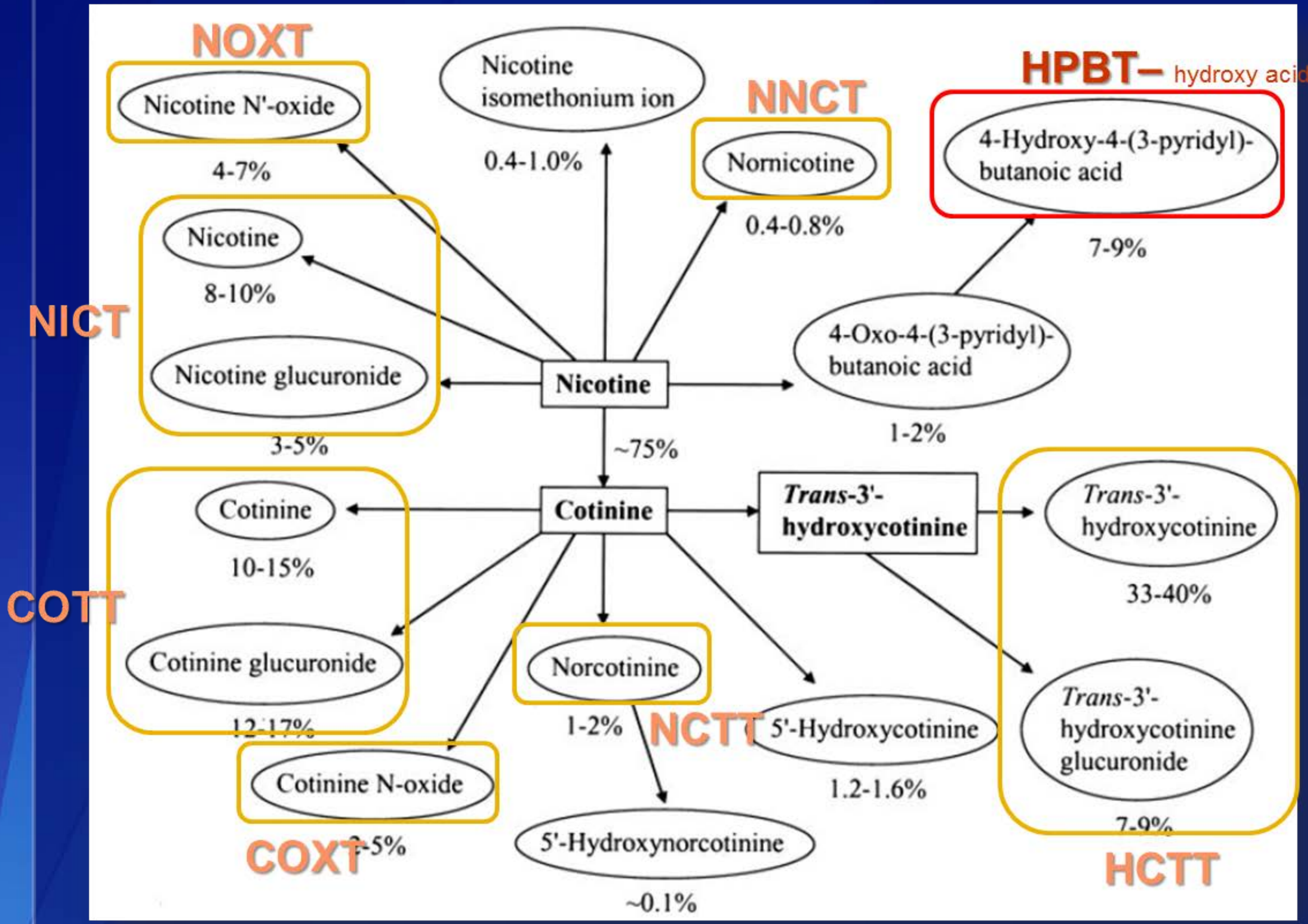


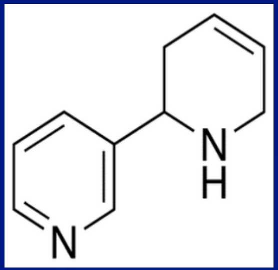
Bernert, et al Nicotine Tob Res. 2009 Dec;11(12):1458-66. doi: 10.1093/ntr/ntp161. Epub 2009 Nov 23.

Nicotine Biomarkers Crucial for Assessing Tobacco Exposure

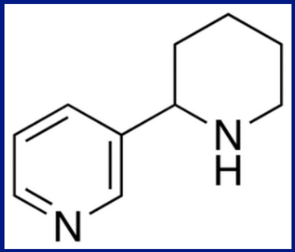
- Serum Cotinine: gold standard/historical data
- Urine Nicotine Metabolites
  - Measures nicotine and 7 metabolites (mass balance, ~95% of nicotine in urine)
  - Functional assessment of nicotine metabolism, with implications for addiction and exposure
  - Measure minor alkaloids to monitor compliance to nicotine replacement therapy (NRT)
  - Need to adjust for dilution caused by variable hydration

Measuring Nicotine Equivalents





(R,S)-Anatabine
ANTT



(R,S)-Anabasine
ANBT

Hukkanen et al. 2005

Importance of combustion biomarkers for differentiating nicotine sources

- Acrylonitrile metabolite CYMA
- PAHs
- Aromatic amines
- Heterocyclic aromatic amines
- 2,5-Dimethylfuran
- Other VOCs, including aldehydes

Harmful VOCs in mainstream cigarette smoke

- 4 of the 5 top carcinogens in tobacco smoke are VOCs
  - 1,3-Butadiene
  - Acrylonitrile
  - Acetaldehyde
  - Benzene
- Acrolein , Acetaldehyde, and Acrylonitrile are among the most potent respiratory irritators
  (*Fowles & Dybing, Tobacco Control 2003*)

- "…cigarette smoking is a primary source of benzene, toluene and styrene and an important source of ethylbenzene and xylene exposure for the U.S. population…"
  (*Chambers, et. al. Environ. Int. 2011*)

Challenges in measuring HPHC VOCs in smoke and human matrices

Aldehydes:
- Acetaldehyde
- Acrolein
- Butanal
- Crotonaldehyde

Ketones:
- Methyl vinyl ketone
- 2,3-Butanedione
- 2-Butanone
- 2-Pentanone
- 3-Pentanone

Unsaturated hydrocarbons:
- Vinyl chloride
- 1,3-Butadiene
- Furan
- 2,5-Dimethylfuran
- Vinyl acetate
- Acrylonitrile

Monoaromatics:
- Benzene
- Toluene
- Ethylbenzene
- *m/p*-Xylene
- *o*-Xylene
- Styrene
- 3-Ethyltoluene
- Pyridine
- Nitrobenzene

Nitro:
- Nitromethane
- 2-Nitropropane

Broad boiling point range
- *Vinyl chloride -13.4 °C*
- *Nitrobenzene 210.9 °C*

Broad range of polarities (Henry's Constant)
- *Vinyl Chloride* $\frac{1}{k_H} = K_{H_2O/air} = 0.51$
- *Nitrobenzene* $\frac{1}{k_H} = K_{H_2O/air} = 741$

Reactive and non-reactive compounds
- *Some compounds can polymerize if not stored properly*

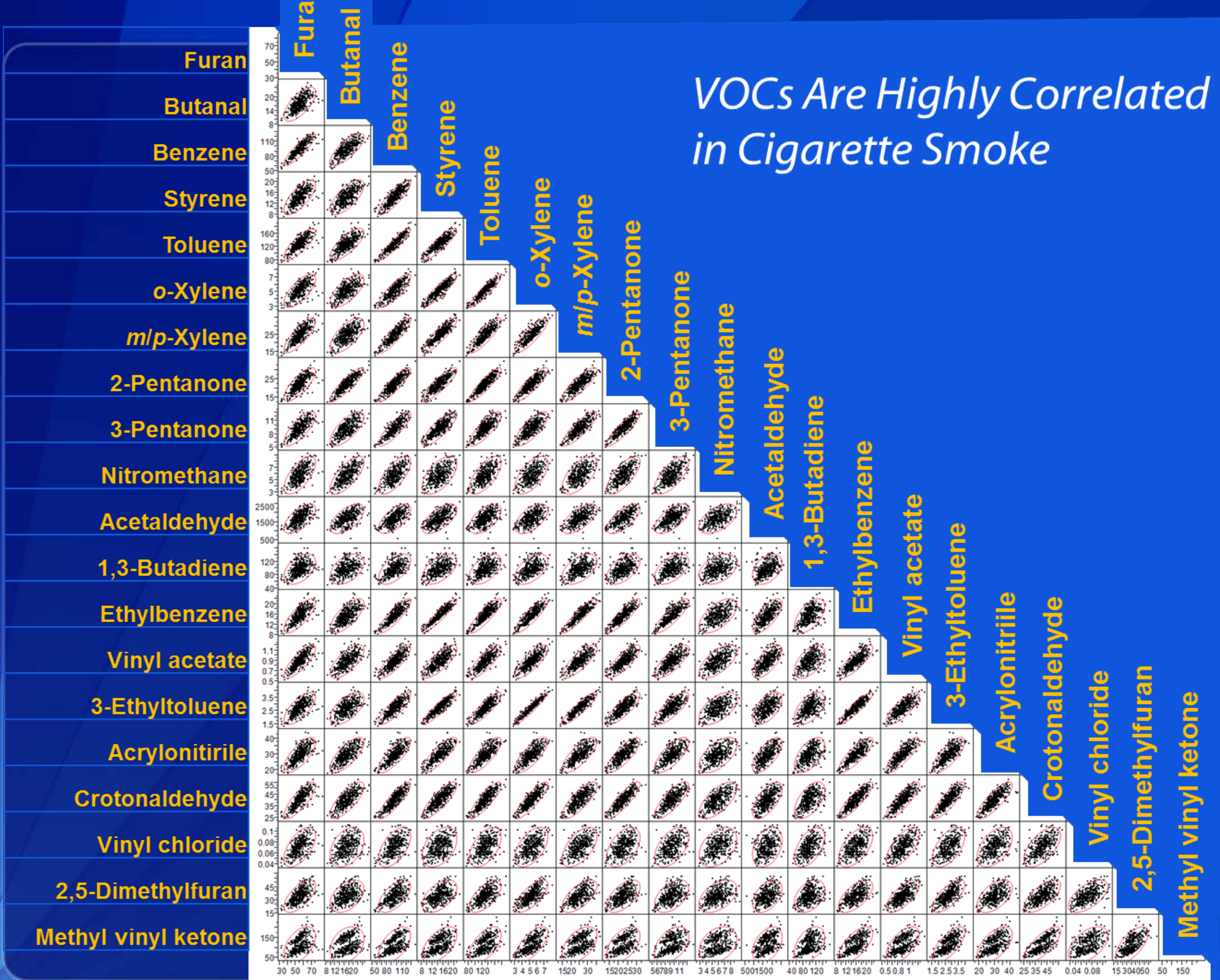
Contamination of solvents and labware

Monoaromatic VOCs are highly correlated in blood collected from smokers

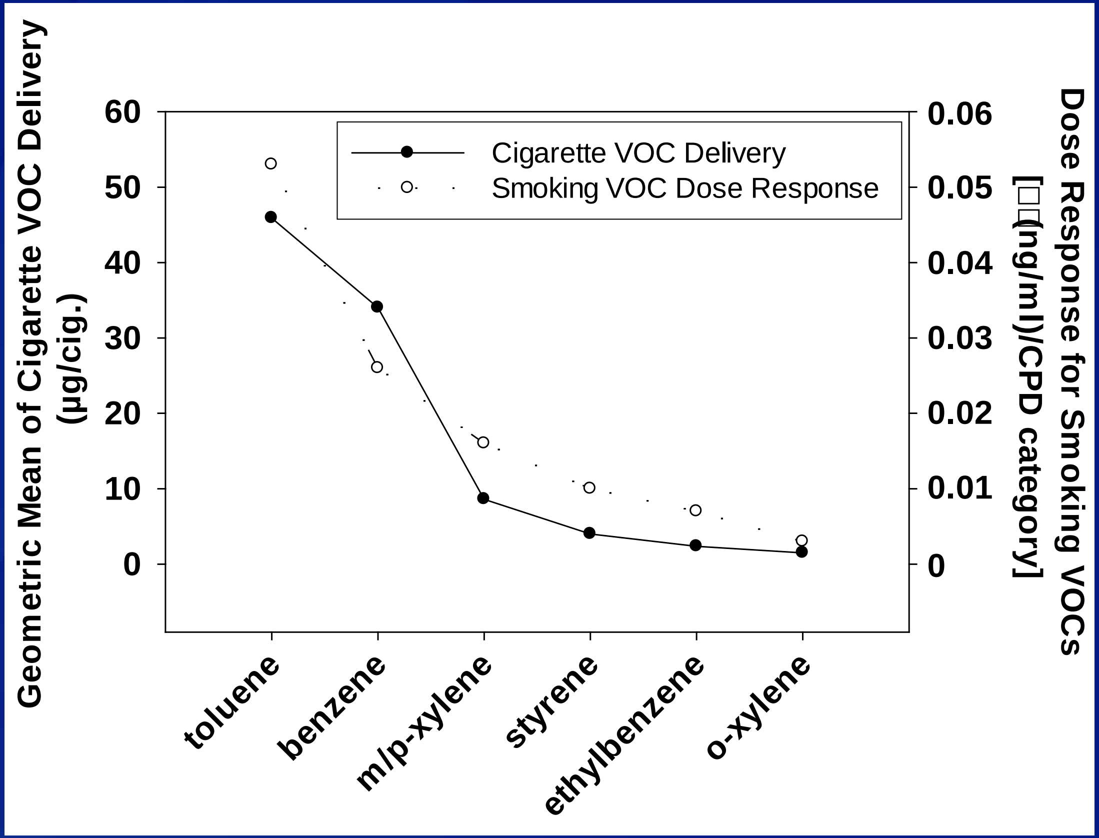
	cotinine	2,5-dimethylfuran	benzene	toluene	ethylbenzene	*o*-xylene	*m*/*p*-xylene	styrene	1,4-dichlorobenzene[e]	total samples	weighted % above LOD
Cotinine[b]	1.00									290 287 287	100
2,5-dimethylfuran[b] excluding outliers[c] excluding < LOD[d]	0.60 0.60 0.60	1.00								290 287 287	100
benzene[b] excluding outliers[c] excluding < LOD[d]	0.49 0.53 0.53	0.87 0.89 0.89	1.00							289 286 286	100
toluene[b] excluding outliers[c] excluding < LOD[d]	0.55 0.56 0.56	0.86 0.88 0.88	0.87 0.89 0.89	1.00						285 282 282	100
ethylbenzene[b] excluding outliers[c] excluding < LOD[d]	0.40 0.45 0.45	0.71 0.76 0.77	0.76 0.80 0.81	0.83 0.88 0.88	1.00					278 275 272	98.9
o-xylene[b] excluding outliers[c] excluding < LOD[d]	0.27 0.30 0.21	0.41 0.46 0.41	0.45 0.48 0.43	0.54 0.58 0.52	0.82 0.80 0.80	1.00				289 286 168	61.2
m/*p*-xylene[b] excluding outliers[c] excluding < LOD[d]	0.35 0.38 0.38	0.57 0.62 0.62	0.61 0.64 0.64	0.72 0.76 0.76	0.93 0.92 0.91	0.83 0.81 0.86	1.00			287 284 284	100
styrene[b] excluding outliers[c] excluding < LOD[d]	0.49 0.52 0.38	0.77 0.79 0.73	0.80 0.80 0.77	0.81 0.82 0.80	0.74 0.77 0.77	0.48 0.51 0.54	0.64 0.67 0.68	1.00		290 287 255	88.0
1,4-dichlorobenzene[b, e] excluding outliers[c] excluding < LOD[d]	0.00 0.01 -0.04	0.04 0.04 0.07	0.09 0.09 0.11	0.08 0.09 0.07	0.08 0.10 0.06	0.04 0.06 0.06	0.07 0.09 0.09	0.12 0.12 0.02	1.00	279 276 150	46.1

Chambers et al: Environ Int. 2011 Nov;37(8):1321-8.

VOCs Are Highly Correlated in Cigarette Smoke



Pattern of monoaromatic VOCs is similar in cigarette smoke and smoker's blood



Chambers et al: Environ Int. 2011 Nov;37(8):1321-8. doi: 10.1016/j.envint.2011.05.016

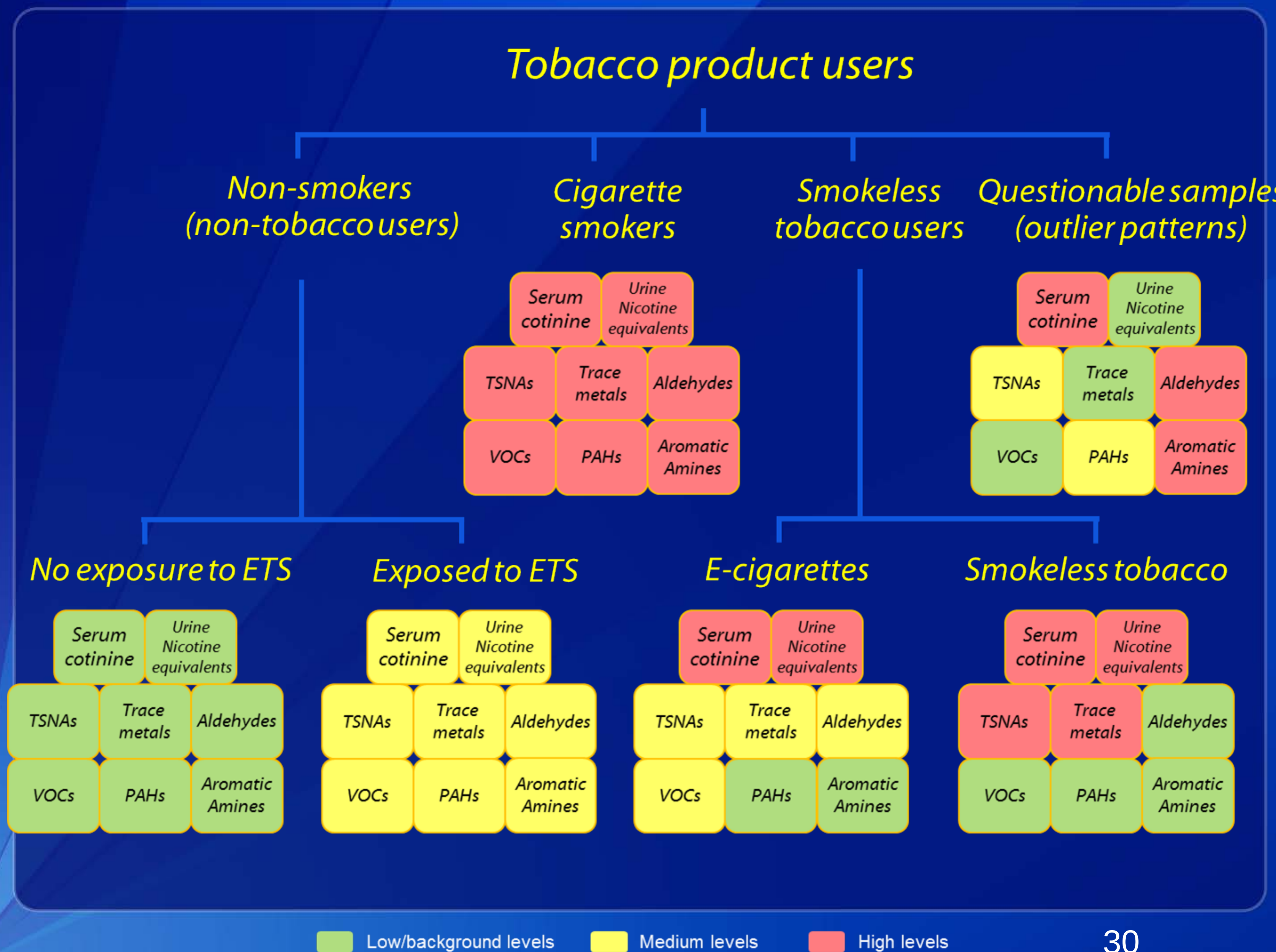
Tobacco Smoke Exposure in the U.S. Population

National Health and Nutrition Examination Survey (NHANES)

Summary of Tobacco-Related Biomarkers in NHANES 2011-2016

assay	sample	2011-2012	2013-2014	2015-2016
serum cotinine	all 3+	x	x	x
urine nicotine metabolites and minor alkaloids	1/3 subset 6+; all adult smokers		x	x
serum aldehydes	1/3 subset 12+; all adult smokers		x	x
urine aromatic amines	1/3 subset 6+; all adult smokers		x	x
urine NNAL/TSNAs	all 3+	x	x	
urine NNAL/TSNAs	1/3 subset 6+; all adult smokers			x
urine heterocyclic amines	1/3 subset 6+; all adult smokers		x	
urine volatile nitrosamines	1/3 subset 6+; all adult smokers		x	
blood VOCs	1/3 subset 6+; all adult smokers	x	x	x
urine VOC metabolites	1/3 subset 6+; all adult smokers	x	x	x
urine thiocyanate	1/3 subset 6+; all adult smokers	x	x	x
urine metals	1/3 subset 6+; all adult smokers	x	x	x
urine arsenic	1/3 subset 6+; all adult smokers	x	x	x
urine hydroxyPAHs	1/3 subset 6+; all adult smokers	x	x	x

*Environmental subsets are ages 3+ after 2012

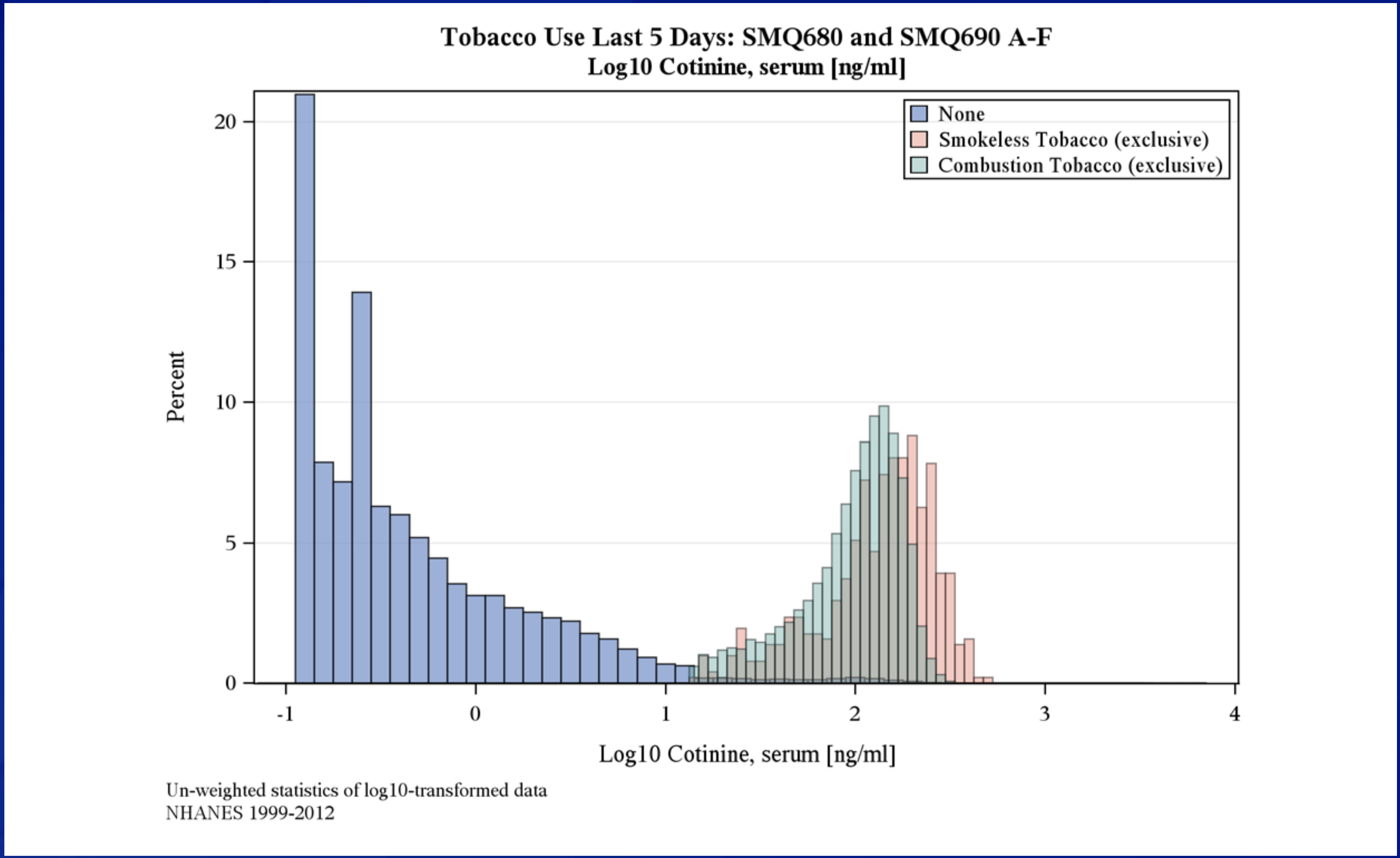


Tobacco product users
Non-smokers (non-tobacco users)
Cigarette smokers
Smokeless tobacco users
Questionable samples (outlier patterns)
Serum cotinine
Urine Nicotine equivalents
TSNAs
Trace metals
Aldehydes
VOCs
PAHs
Aromatic Amines
No exposure to ETS
Exposed to ETS
E-cigarettes
Smokeless tobacco
Low/background levels
Medium levels
High levels

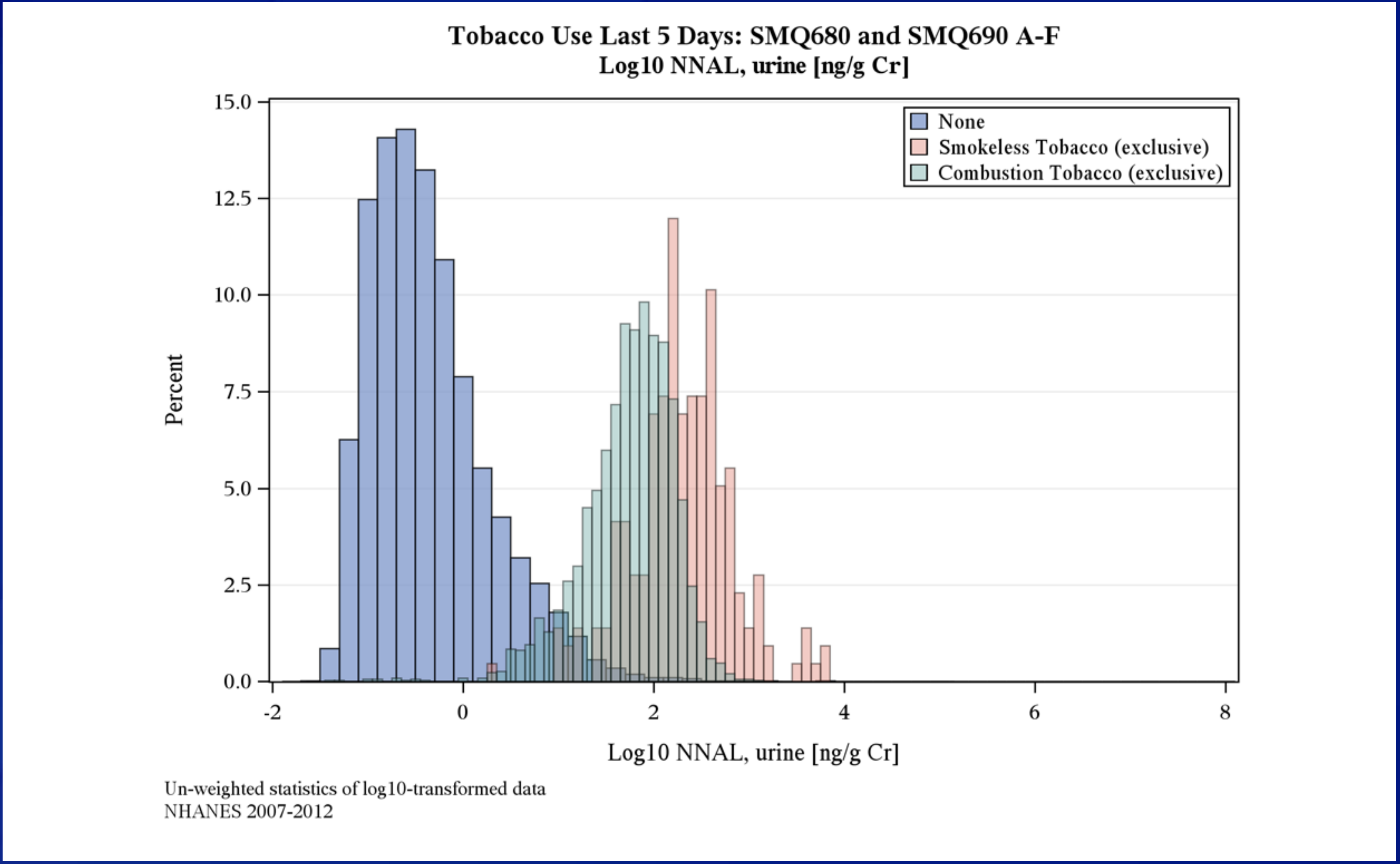
Tobacco-Related Exposure Patterns in NHANES 1999 – 2012

- Download all publicly available NHANES data
  - N ranges from ~6,700 – 56,000 individuals
- Categorize use based on smoking questionnaire (SMQ680 and SMQ690)
  - Never user
  - exclusive smokeless tobacco
  - exclusive combusted tobacco
- Plot histogram of log10-transformed data

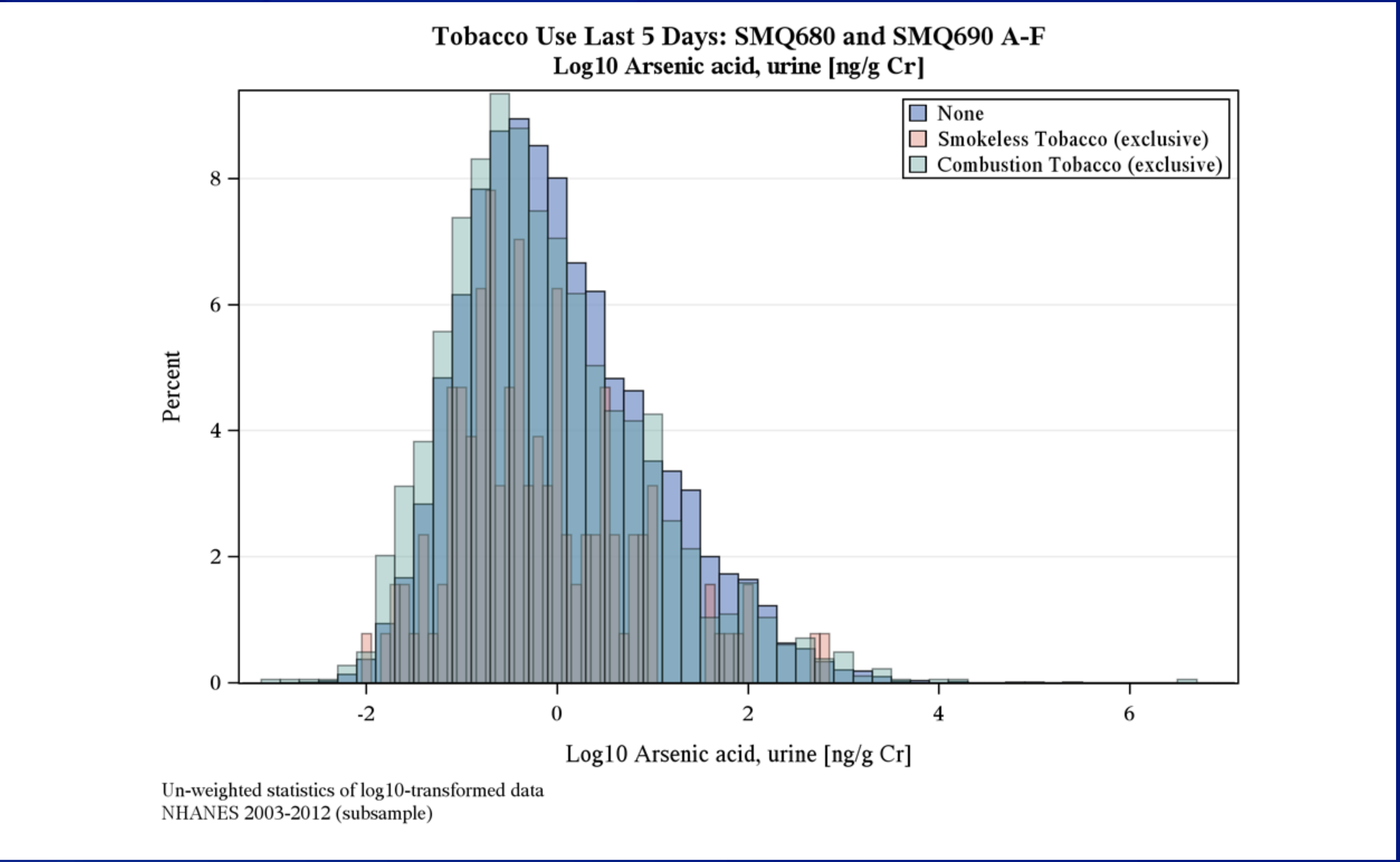
Serum cotinine higher in tobacco users



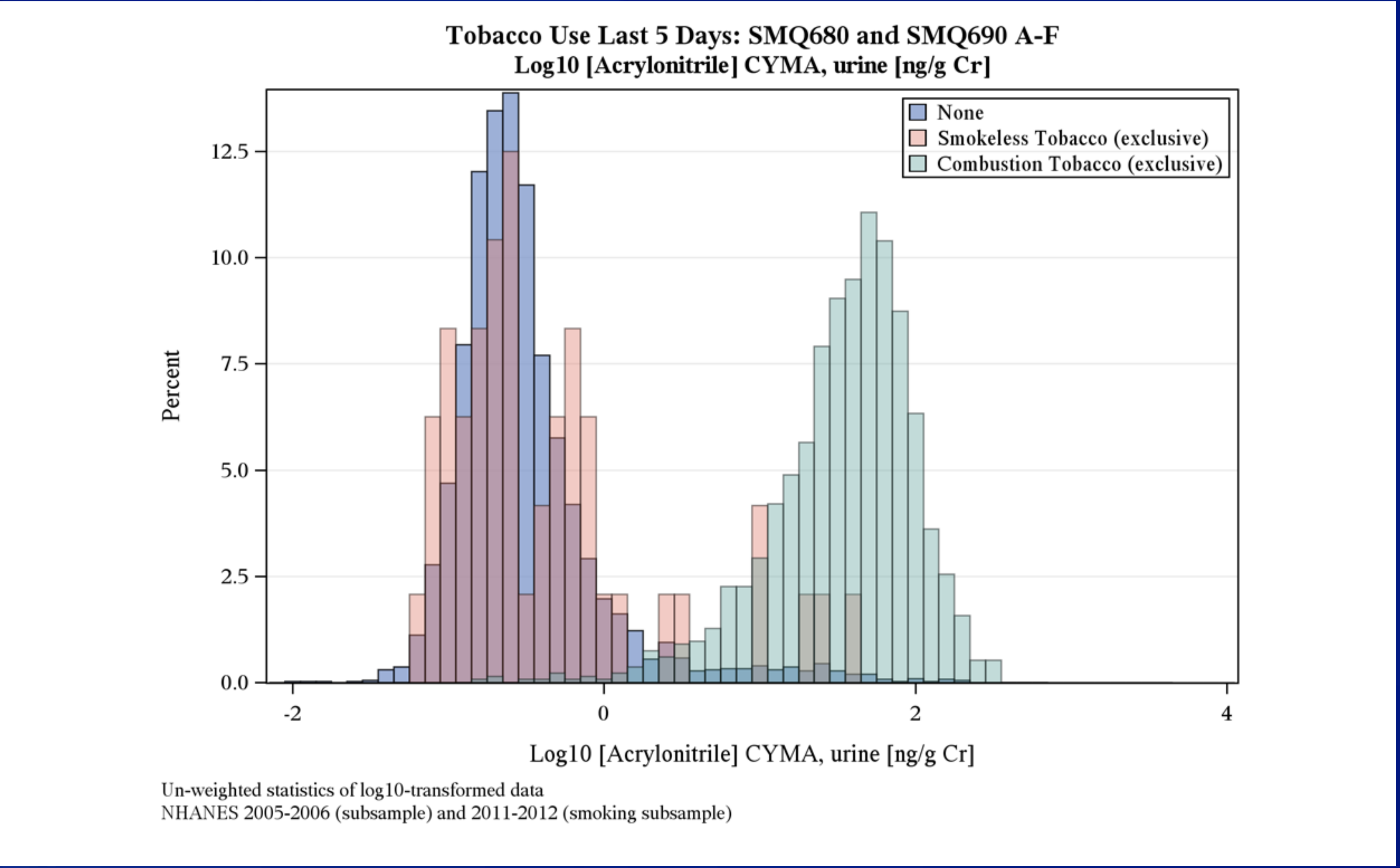
Urine NNAL higher in tobacco users



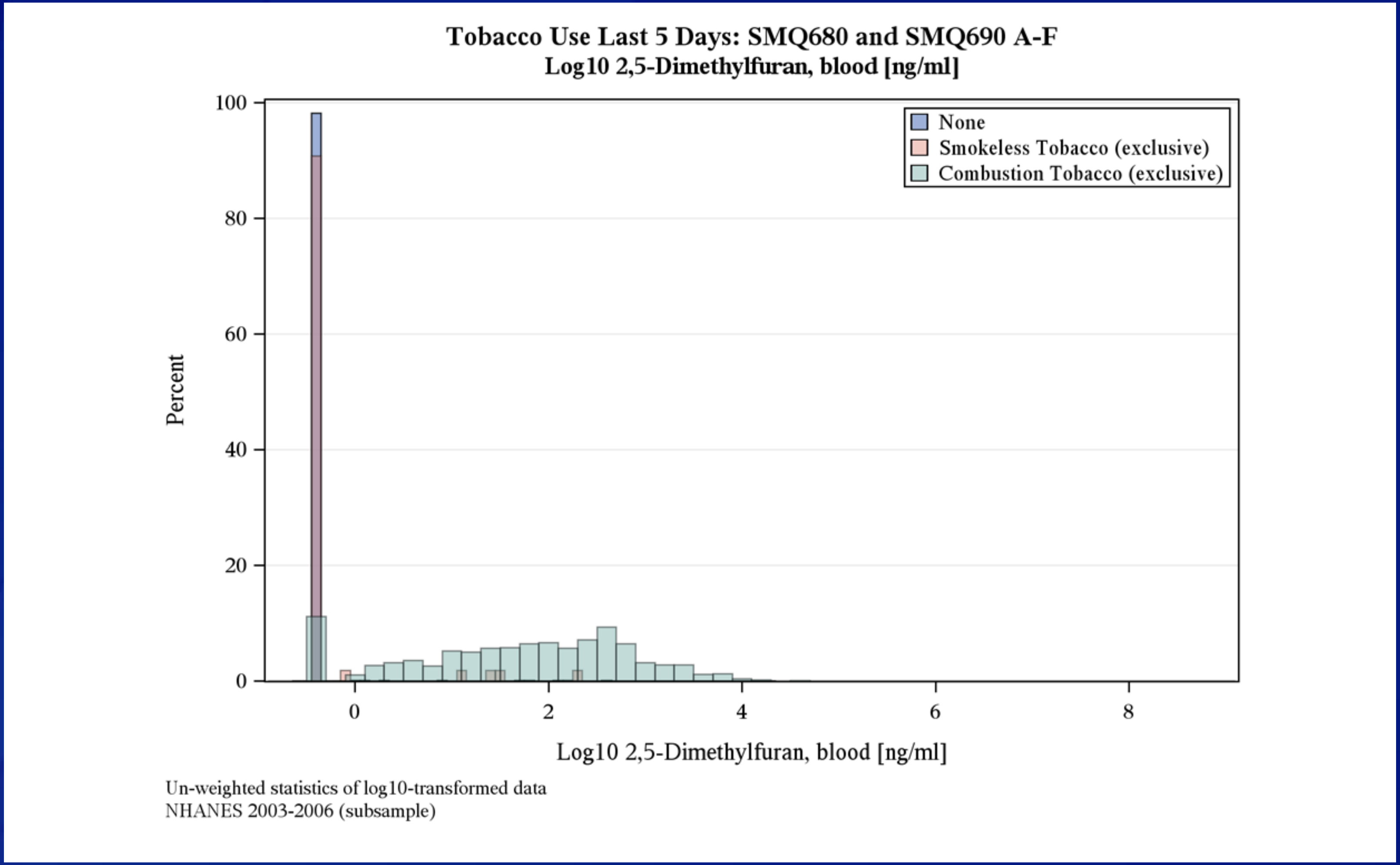
Urine arsenic levels unrelated to tobacco use



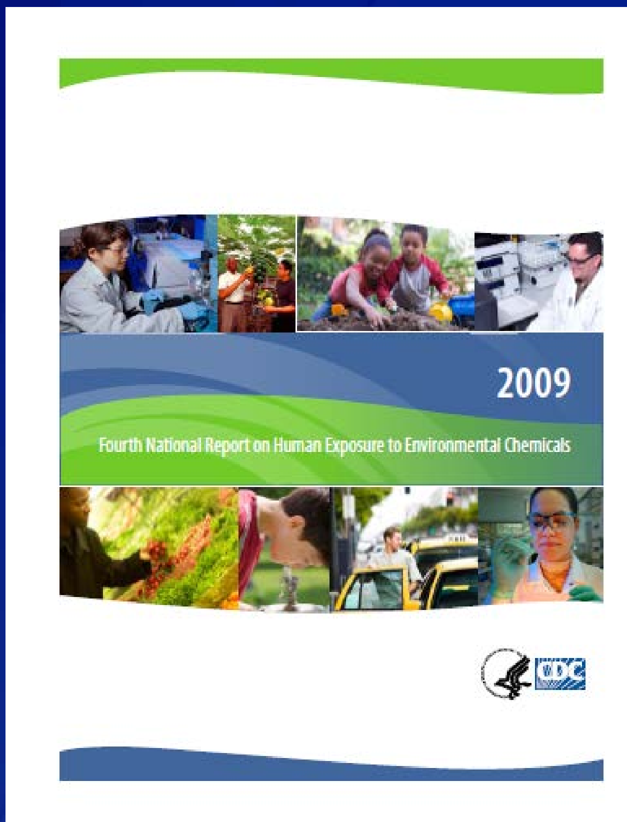
Urine CYMA higher in tobacco smokers



Blood 2,5-dimethylfuran higher in tobacco smokers



CDC National Exposure Report includes smoking categorization



Urinary N-Acetyl-S-(2-cyanoethyl)-L-cysteine

Metabolite of Acrylonitrile

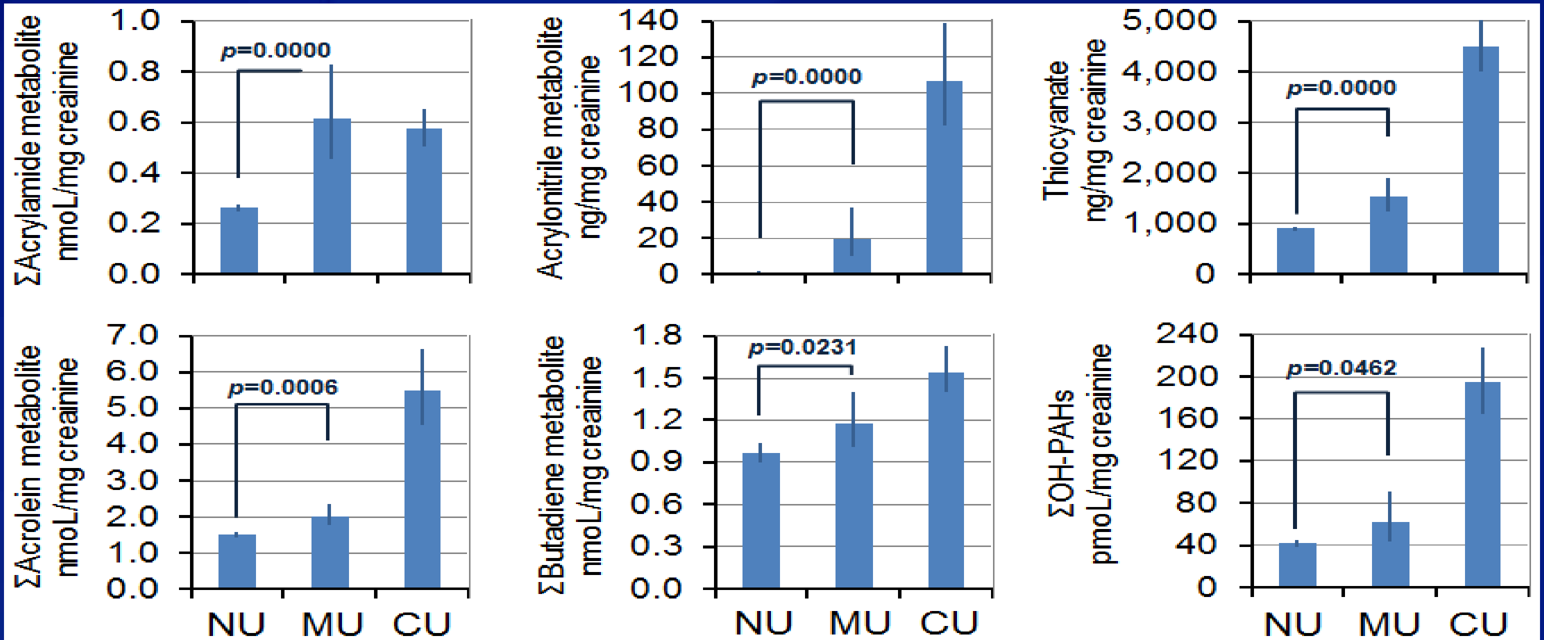
Geometric mean and selected percentiles of urine concentrations (in µg/L) for the U.S. adult population from the National Health and Nutrition Examination Survey by smoking status.

	Survey years	Geometric mean (95% conf. interval)	Selected percentiles (95% confidence interval) 50th	75th	90th	95th	Sample size
Cigarette Smokers							
Total	11-12	**122** (106-141)	**140** (119-166)	**277** (240-310)	**504** (439-580)	**717** (513-827)	889
Age group							
20-49 years	11-12	**114** (90.4-143)	**132** (105-172)	**265** (214-306)	**515** (372-717)	**717** (467-888)	532
50 years and older	11-12	**137** (118-159)	**146** (125-184)	**306** (232-344)	**481** (384-699)	**705** (439-846)	357
Gender							
Males	11-12	**123** (98.8-153)	**156** (131-183)	**306** (261-355)	**552** (463-745)	**780** (608-858)	536
Females	11-12	**121** (104-140)	**128** (116-146)	**240** (201-277)	**403** (289-652)	**652** (344-786)	353
Nonsmokers							
Total	11-12	**1.50** (1.35-1.67)	**1.33** (1.22-1.45)	**2.35** (2.16-2.52)	**4.81** (3.79-5.80)	**12.1** (7.05-18.2)	1308
Age group							
20-49 years	11-12	**1.67** (1.38-2.02)	**1.45** (1.25-1.65)	**2.49** (2.23-3.00)	**5.84** (4.64-10.3)	**13.4** (7.05-79.9)	655
50 years and older	11-12	**1.34** (1.18-1.53)	**1.23** (1.07-1.36)	**2.19** (1.83-2.44)	**3.65** (3.03-4.36)	**6.84** (3.89-10.6)	653
Gender							
Males	11-12	**1.90** (1.65-2.20)	**1.64** (1.44-1.85)	**2.96** (2.53-3.24)	**7.05** (4.06-12.4)	**14.7** (8.19-58.5)	625
Females	11-12	**1.24** (1.06-1.45)	**1.19** (1.02-1.26)	**1.90** (1.74-2.09)	**3.55** (2.95-4.36)	**6.84** (3.97-10.8)	683

Tobacco product users who did not smoke cigarettes were excluded. See Adult Cigarette Smokers and Nonsmokers: Analysis of Select Chemicals in a Special Sample.
Limit of detection (LOD, see Data Analysis section) for Survey year 11-12 is 0.5.

http://www.x.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf

Marijuana Smoke Exposure in NHANES



Adjusted geometric means of urinary concentrations of PAH and VOC metabolites (creatinine corrected) among nonusers (NU), marijuana users (MU) and cigarette users (CU). Error bars indicate 95% confidence intervals. Wei et al (2016).

Marijuana smoke contains similar chemicals as tobacco smoke (Moir et al. 2008)

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- The dedicated staff of the Tobacco and Volatiles Branch
- Funding from CDC and FDA Center for Tobacco Products

Analytical measurement of biomarkers of exposure to harmful and addictive chemicals is crucial for characterizing and countering the epidemic of disease caused by tobacco use

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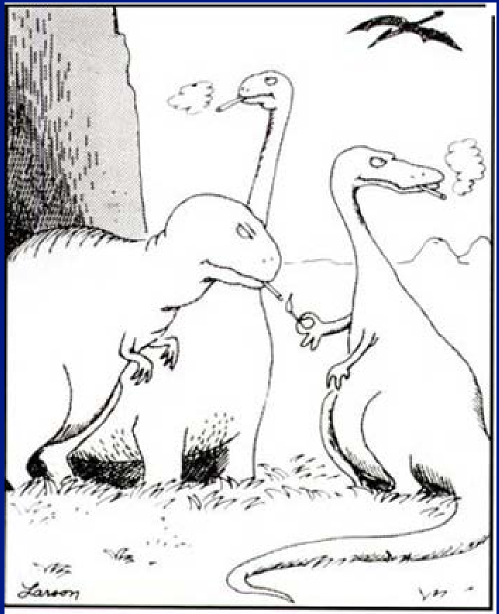

The real reason dinosaurs became extinct

- Division of Laboratory Sciences
- National Center for