America's Children and the Environment, Third Edition

DRAFT Indicators

Biomonitoring: Cotinine

EPA is preparing the third edition of *America's Children and the Environment* (ACE3), following the previous editions published in December 2000 and February 2003. ACE is EPA's compilation of children's environmental health indicators and related information, drawing on the best national data sources available for characterizing important aspects of the relationship between environmental contaminants and children's health. ACE includes four sections: Environments and Contaminants, Biomonitoring, Health, and Special Features.

EPA has prepared draft indicator documents for ACE3 representing 23 children's environmental health topics and presenting a total of 42 proposed children's environmental health indicators. This document presents the draft text, indicators, and documentation for the cotinine topic in the Biomonitoring section.

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Cotinine

Environmental tobacco smoke (ETS) is a mixture of particles and gases that are generated by the high-temperature combustion of tobacco, paper, and additives.[1] There are at least 250 chemicals in ETS that are known to be toxic or carcinogenic, including carbon monoxide, ammonia, formaldehyde, and hydrogen cyanide.[1,2] In 1992, EPA classified ETS as a known human carcinogen.[3] Children can be exposed to ETS in their homes or in places where people are allowed to smoke, such as some restaurants in some locations throughout the United States.

According to the U.S. Surgeon General, there is no safe level of exposure to ETS, and breathing even a small amount can be harmful to human health.[1] Children and infants who are exposed to ETS have an increased risk for a number of adverse health outcomes, including lower respiratory infections, bronchitis, pneumonia, impaired lung function, middle ear infection, and fluid in the middle ear.[1,3-5] Exposure to ETS is a known cause of sudden infant death syndrome (SIDS).[1,5] ETS can play a role in the development and exacerbation of asthma and other wheeze illnesses, particularly for children under 6 years of age.[1,6-11]

Young children appear to be more susceptible to the respiratory effects of ETS than are older children.[3,9] It is also possible that early-life exposures to ETS may lead to adverse health effects in adulthood. Exposure to ETS in childhood has been found to be associated with early emphysema in adulthood among nonsmokers.[12] The California Environmental Protection Agency has concluded that there is sufficient evidence to attribute a causal association between the exposure of girls to ETS and increased incidence of breast cancer later in life.[13]

The exposure of a pregnant woman to ETS can also be harmful to her developing fetus. Exposure of pregnant women to ETS has been linked to a reduction in birth weight and increased risk of low birth weight, fetal mortality, preterm delivery, and spontaneous abortion.[1,14-20] Research suggests that the combination of prenatal and postnatal exposure to ETS may lead to some childhood cancers.[1] A review study found that prenatal exposure to ETS is associated with impaired lung function and increased risk of developing asthma.[21] Additionally, the exposure of pregnant women to ETS has been associated with significantly lower cognitive development in their children.[22]

Cotinine is considered the best biomarker of exposure to tobacco smoke for both active smokers and those exposed to ETS.[23] The following indicators present the concentrations of cotinine measured in the blood serum of children ages 3 to 17 years and women ages 16 to 49 years as an indicator of exposure to ETS.

Indicator B5: Cotinine in nonsmoking children ages 3 to 17 years: Median and 95th percentile concentrations in blood serum, 1988–2008

Indicator B6: Cotinine in nonsmoking women ages 16 to 49 years: Median and 95th percentile concentrations in blood serum, 1988–2008

Overview

Indicators B5 and B6 present concentrations of cotinine in the blood of U.S. children ages 3 to 17 years and women ages 16 to 49 years. Cotinine is a marker of exposure to environmental tobacco smoke. The data are from a national survey that collects blood specimens from a representative sample of the population, and then measures the concentration of cotinine in the blood serum. Indicators B5 and B6 show the change in blood cotinine levels over time. The focus is on both children and women of child-bearing age because environmental tobacco smoke exposure in both population groups has been associated with adverse health outcomes for children.

Environmental Tobacco Smoke (ETS) and Cotinine

Nicotine is a distinctive component of tobacco that is found in large amounts in tobacco smoke, including ETS. Once nicotine enters the body, it is rapidly broken down into other chemicals. Cotinine is a primary breakdown product of nicotine, and has a substantially longer half-life compared with nicotine. This characteristic makes cotinine a better indicator than nicotine of an individual's exposure to ETS.[24-26] Cotinine can be measured in blood serum, saliva, hair, and urine. Measuring cotinine in blood is preferred because the level of cotinine in the blood stays relatively stable.

Measurement of cotinine in blood serum is a marker for exposure to ETS in the previous 1 to 2 days.[27] Some studies have shown that, given the same exposure to tobacco smoke, cotinine levels may differ by race/ethnicity and sex, and there are genetic differences in the rate at which cotinine is removed from the body.[1,28-32]

NHANES

Data for these indicators are from the National Health and Nutrition Examination Survey (NHANES). NHANES is a nationally representative survey designed to assess the health and nutritional status of the civilian noninstitutionalized U.S. population, conducted by the Centers for Disease Control and Prevention (CDC). Interviews and physical examinations are conducted with approximately 5,000 people each year. CDC's National Center for Environmental Health measures concentrations of environmental chemicals in blood and urine samples collected from NHANES participants.[23] Concentrations of cotinine in blood serum have been measured in a representative subset of NHANES participants ages 4 years and older for the 1988–1991 and 1991–1994 survey cycles, and then for ages 3 years and older beginning with the 1999–2000 survey cycle. NHANES data from 1988–2008 are used in Indicator B5 for children ages 3 to 17 years and Indicator B6 for women ages 16 to 49 years. NHANES does not provide cotinine measurements for children under the age of 3 years, who may be especially sensitive to the effects of ETS exposure.

Birthrate Adjustment

This indicator uses measurements of cotinine in blood serum of women ages 16 to 49 years to represent the distribution of ETS exposures to women who are pregnant or may become pregnant. However, women of different ages have a different likelihood of giving birth. For example, in 2005–2006, women aged 27 had a 12% probability of giving birth, and women aged 37 had a 5% probability of giving birth.[33] A birthrate-adjusted distribution of women's blood serum levels is used in calculating this indicator, meaning that the data are weighted using the age-specific probability of a woman giving birth.[34]

Non-Smokers

These indicators present cotinine levels for non-tobacco-users only. Children and women who were smokers, as indicated by a relatively high serum cotinine level, were excluded from these statistics. For these analyses, individuals with a serum cotinine level greater than 10 nanograms of cotinine per milliliter of serum (ng/mL) are considered active smokers.[23] Active smokers will almost always have serum cotinine levels above 10 ng/mL, and sometimes those levels will be higher than 500 ng/mL.[27,35] Nonsmokers who are exposed to typical levels of ETS have serum cotinine levels of less than 1 nanogram per milliliter (ng/mL), whereas those nonsmokers with heavy exposure to ETS will have serum cotinine levels between 1 and 10 ng/mL.

Data Presented in the Indicators

Indicator B5 presents the median (50th percentile) and 95th percentile of blood serum cotinine levels over time for children ages 3 to 17 years, and Indicator B6 presents the same for women of child-bearing age. The median is the value in the middle of the distribution of blood serum cotinine levels: half of the individuals have blood serum cotinine levels greater than the median, and half have levels below the median. The median can be thought of as representing a typical exposure. The 95th percentile is a value representing the upper range of blood serum cotinine levels: 5% of individuals have levels greater than the 95th percentile. This value therefore can be thought of as representing a relatively high exposure among individuals, but not a maximum level.

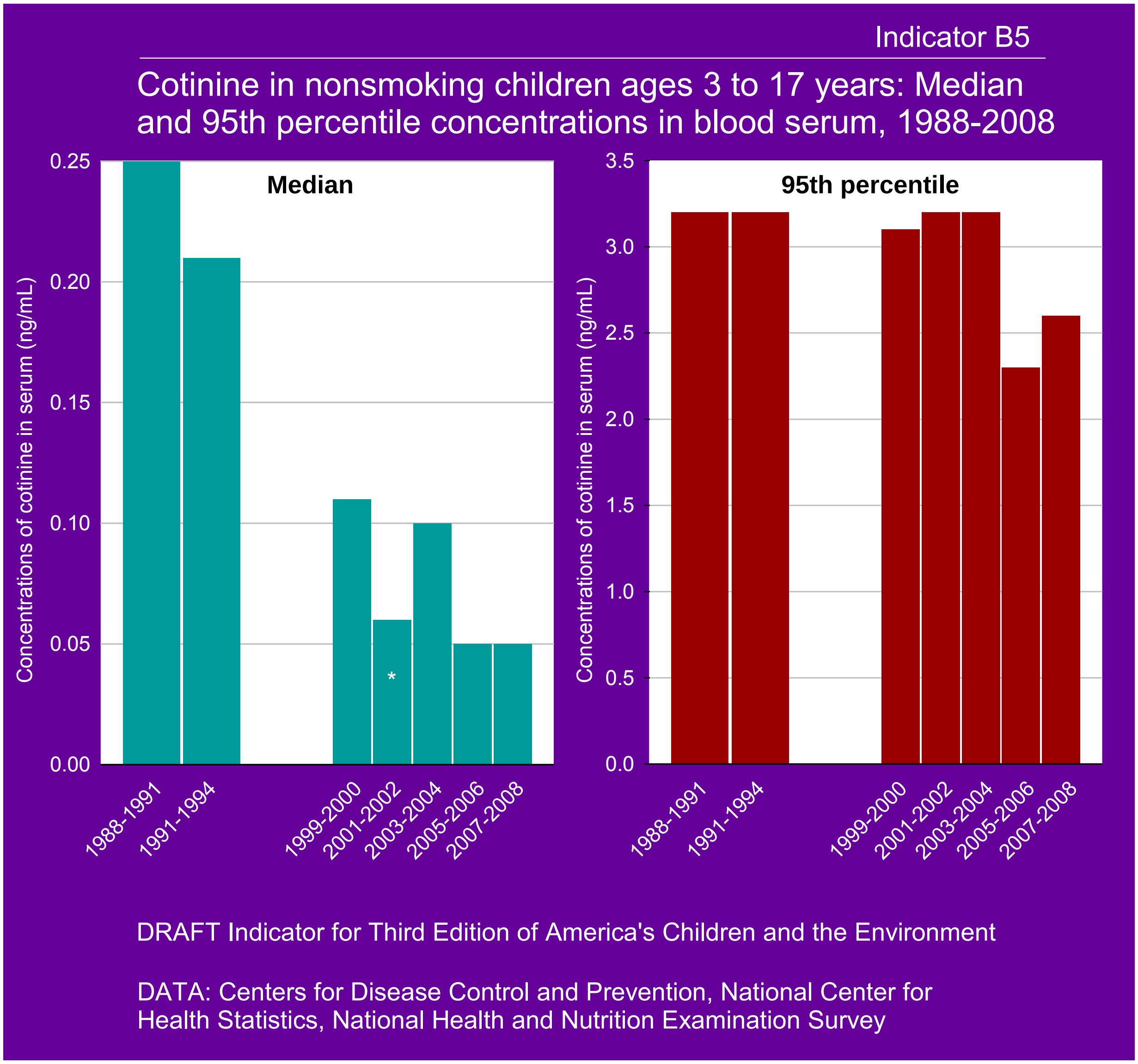
Although the sensitivity of measurement techniques has improved over the years spanned by Indicators B5 and B6, allowing increased detection of lower serum cotinine levels over time, these improvements do not affect the comparability of the median or 95th percentiles over time since the majority of children and women have had detectable levels of cotinine in each NHANES cycle.

Additional information showing how blood serum levels of cotinine vary by race/ethnicity and family income is presented in the supplemental data tables for these indicators.

Statistical Testing

Statistical analysis has been applied to the biomonitoring indicators to determine whether any changes in chemical concentrations over time, or any differences in chemical concentrations between demographic groups, are statistically significant. These analyses use a 5% significance level ($p \leq 0.05$), meaning that a conclusion of statistical significance is made only when there is no more than a 5% chance that the observed change over time or difference between demographic groups occurred randomly. It should be noted that when statistical testing is conducted for differences among multiple demographic groups (e.g., considering both race/ethnicity and income level), the large number of comparisons involved increases the probability that some differences identified as statistically significant may actually have occurred randomly.

A finding of statistical significance for a biomonitoring indicator depends not only on the numerical difference in the value of a reported statistic between two groups, but also on the number of observations in the survey, the amount of variability among the observations, and various aspects of the survey design. For example, if two groups have different median levels of a chemical in blood or urine, the statistical test is more likely to detect a difference when samples have been obtained from a larger number of people in those groups. Similarly, if there is low variability in levels of the chemical within each group, then a difference between groups is more likely to be detected. A finding that there is or is not a statistically significant difference in exposure levels between two groups or in exposure levels over time does not necessarily suggest any interpretation regarding the health implications of those differences.



* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

- In 2007–2008, the median level of cotinine measured in nonsmoking children ages 3 to 17 years was 79% lower than it was in 1988–1991. This decrease was statistically significant.

- Cotinine values at the 95th percentile showed a smaller, but still statistically significant, relative decline (18%) from 1988–1991 to 2007–2008.

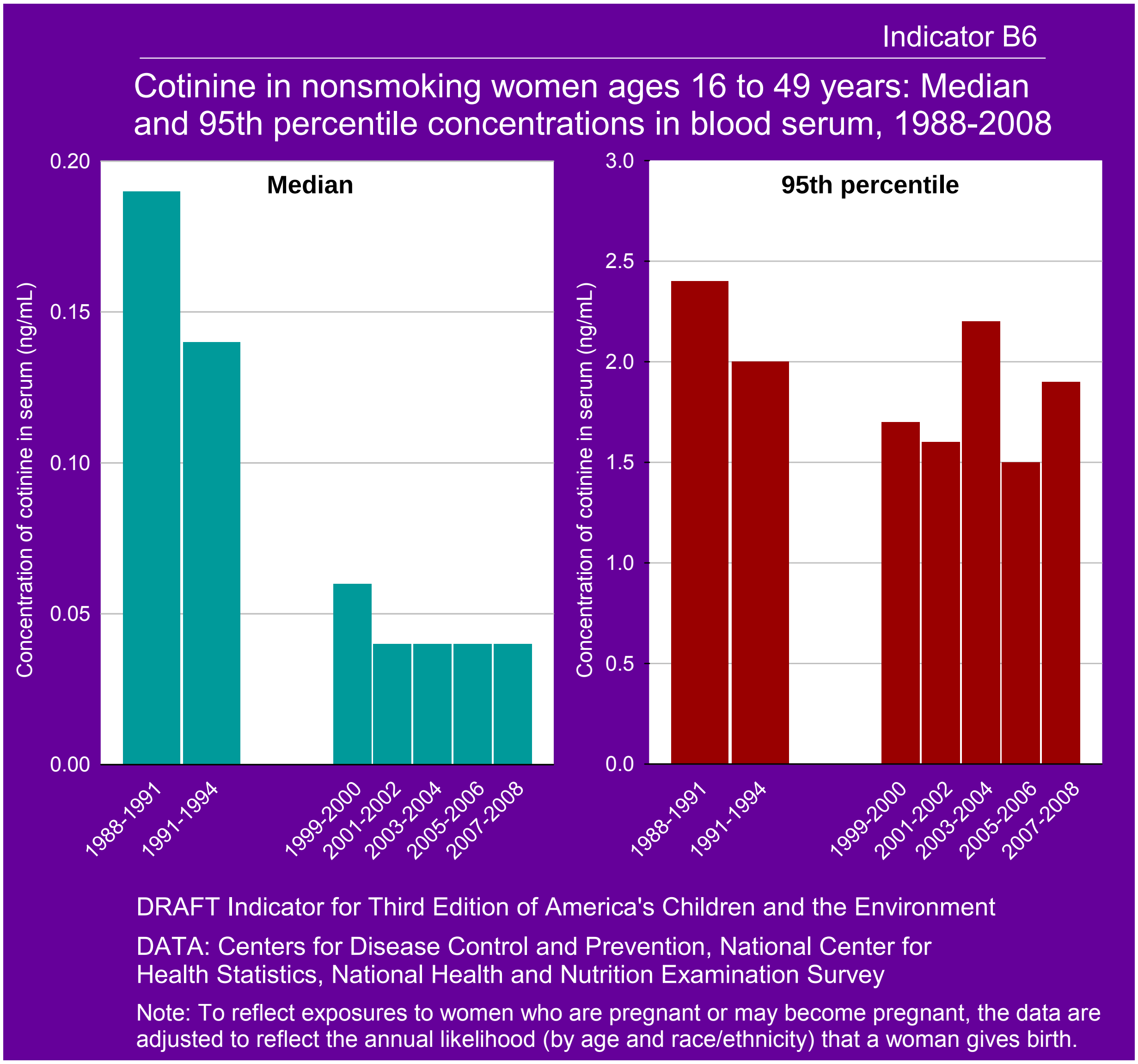
- Children at the 95th percentile of cotinine levels have much higher levels than those at the median. In 1988–1991, the 95th percentile cotinine level (3.2 ng/mL) was 13 times the median level (0.25 ng/mL; in 2007–2008, the 95th percentile cotinine level (2.6 ng/mL) was 52 times the median level (0.05 ng/mL).

- Eighty-seven percent of nonsmoking children ages 4 to 17 years had detectable levels (at or above 0.05 ng/mL) of cotinine in 1988–1991. Fifty-one percent of nonsmoking children ages 3 to 17 years had levels at or above 0.05 ng/mL of cotinine in 2007–2008, although improvements in laboratory methods have made it possible to detect cotinine at lower concentrations starting with the 2001–2002 survey cycle (data not shown).

- The reduction in children's cotinine levels is in part likely attributable to a decline in the percentage of adults who smoke. In 2009, an estimated 20.6% of adults were current smokers, down from 25.0% in 1993 (data not shown).[36,37]

- In 2005–2008, median concentrations of cotinine in blood for nonsmokers were approximately 0.1 ng/mL for Black non-Hispanic children, 0.05 ng/mL for White non-Hispanic children, and 0.03 ng/mL for Mexican-American children (see Table B5a).
    - Statistical note: The differences between race/ethnicity groups were statistically significant and remained so after accounting for other demographic differences (i.e., differences in income or age profile).

- In 2005–2008, the median concentration of cotinine in blood serum for nonsmoking children living below the poverty level (0.18 ng/mL) was about 5 times the median for nonsmoking children living at or above the poverty level (0.04 ng/mL). (See Table B5a.)
    - Statistical note: The differences between income groups were statistically significant after accounting for other demographic differences (i.e., differences in sex, race/ethnicity or age profile).



- In 2007–2008, the median (50th percentile) level of cotinine measured in nonsmoking women of childbearing age was 80% lower than it was in 1988–1991. This decrease was statistically significant.

- Cotinine values at the 95th percentile, representing the most highly exposed 5% of nonsmoking women, showed a smaller relative decline (19%) from 1988–1991 to 2007–2008, although this decline was not statistically significant.

- Women at the 95th percentile cotinine levels have much higher levels than those at the median. In 1988–1991, the 95th percentile cotinine level (2.3 ng/mL) was 11 times the median level (0.21 ng/mL); in 2007–2008, the 95th percentile cotinine level (1.9 ng/mL) was 47 times the median level (0.04 ng/mL) (see Table B6).

- The reduction in nonsmoking women's cotinine levels is in part likely attributable to a decline in the percentage of adults who smoke. In 2009, an estimated 20.6% of adults were current smokers, down from 25.0% in 1993 (data not shown).[36,37]

Data Tables

Table B5: Cotinine in nonsmoking children ages 3 to 17 years: Median and 95th percentile concentrations in blood serum, 1988-2008

	Concentration of cotinine in serum (ng/mL)						
	1988-1991	1991-1994	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008
Median	0.25	0.21	0.11	0.06*	0.10	0.05	0.05
95th percentile	3.2	3.2	3.1	3.2	3.2	2.3	2.6

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTE: Based on children ages 3 to 17 years with cotinine ≤ 10 ng/mL (ages 4 to 17 years for 1988-1991 and 1991-1994).

* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

Table B5a. Cotinine in nonsmoking children ages 3 to 17 years: Median concentrations in blood serum, by race/ethnicity and family income, 2005-2008

	Median concentration of cotinine in serum (ng/mL)					
				≥ Poverty (Detail)		
Race / Ethnicity	All Incomes	< Poverty Level	≥ Poverty Level	100-200% of Poverty Level	> 200% of Poverty Level	Unknown Income
All Races/Ethnicities	0.05	0.18	0.04	0.10	0.03	0.04
White non-Hispanic	0.05	NA**	0.05	0.16	0.03	NA**
Black non-Hispanic	0.12	0.45	0.06	0.15	0.04	NA**
Mexican-American	0.03	0.04	0.02	0.02	0.02	0.02
Other†	0.04	0.14*	0.03	0.04	0.03	NA**

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTE: Based on children ages 3 to 17 years with cotinine < 10 ng/mL.

† "Other" includes Asian non-Hispanic; Native American non-Hispanic; Hispanic other than Mexican-American; those reporting multi-racial; and those with a missing value for race/ethnicity.

* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

** The estimate is not reported because it has large uncertainty: the relative standard error, RSE, is at least 40% (RSE = standard error divided by the estimate).

Table B5b. Cotinine in nonsmoking children ages 3 to 17 years: 95th percentile concentrations in blood serum, by race/ethnicity and family income, 2005-2008

	95th percentile concentration of cotinine in serum (ng/mL)					
				≥ Poverty (Detail)		
Race / Ethnicity	**All Incomes**	**< Poverty Level**	**≥ Poverty Level**	**100-200% of Poverty Level**	**> 200% of Poverty Level**	**Unknown Income**
All Races/Ethnicities	2.5	4.1	2.1	3.5	1.2	1.5*
White non-Hispanic	2.7	4.9	2.3	3.8	1.3	1.5*
Black non-Hispanic	2.7	3.4	1.9	2.6	1.1	3.0
Mexican-American	0.83*	NA**	0.7*	1.3*	0.54*	0.6
Other†	1.9	4.1	1.4	NA**	NA**	NA**

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTE: Based on children ages 3 to 17 years with cotinine < 10 ng/mL.

† "Other" includes Asian non-Hispanic; Native American non-Hispanic; Hispanic other than Mexican-American; those reporting multi-racial; and those with a missing value for race/ethnicity.

* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

** The estimate is not reported because it has large uncertainty: the relative standard error, RSE, is at least 40% (RSE = standard error divided by the estimate).

Table B5c: Cotinine in nonsmoking children ages 3 to 17 years: Median and 95th percentile concentrations in blood serum, by age group, 2005-2008

	Concentration of cotinine in serum (ng/mL)				
	All ages	Ages 3 to <6 years	Ages 6 to <11 years	Ages 11 to <16 years	Ages 16 to <18 years
Median	0.05	0.06	0.06	0.04	0.04
95th percentile	2.5	2.8	2.7	2.4	2.6

Table B6: Cotinine in nonsmoking women ages 16 to 49 years: Median and 95th percentile concentrations in blood serum, 1988-2008

	Concentration of cotinine in serum (ng/mL)						
	1988-1991	1991-1994	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008
Median	0.21	0.15	0.06	0.04	0.04	0.04	0.04
95th percentile	2.3	2.1	1.7	1.6	2.2	1.5	1.9

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTES:

- Based on women ages 16 to 49 years with cotinine ≤ 10 ng/mL.
- The distribution of the data for women ages 16 to 49 years is adjusted for the likelihood that a woman of a particular age and race/ethnicity gives birth in a particular year. The intent of this adjustment is to approximate the distribution of exposure to pregnant women. Results will therefore differ from a characterization of exposure to adult women without consideration of birthrates.

Table B6a. Cotinine in nonsmoking women ages 16 to 49 years: Median concentrations in blood serum, by race/ethnicity and family income, 2005–2008

	Median concentration of cotinine in serum (ng/mL)					
				≥ Poverty (Detail)		
Race / Ethnicity	**All Incomes**	**< Poverty Level**	**≥ Poverty Level**	**100-200% of Poverty Level**	**> 200% of Poverty Level**	**Unknown Income**
All Races/Ethnicities	0.04	0.08	0.03	0.05	0.03	NA**

	Median concentration of cotinine in serum (ng/mL)					
White non-Hispanic	0.04	0.16*	0.03	0.06	0.03	NA**
Black non-Hispanic	0.10	0.14	0.08	0.12	0.06	NA**
Mexican-American	0.02	0.04	0.02	0.02*	0.02	NA**
Other†	0.03	0.06*	0.03	0.04	0.03	NA**

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTES:

- Based on women ages 16 to 49 years with cotinine $\leq$ 10 ng/mL.

- The distribution of the data for women ages 16 to 49 years is adjusted for the likelihood that a woman of a particular age and race/ethnicity gives birth in a particular year. The intent of this adjustment is to approximate the distribution of exposure to pregnant women. Results will therefore differ from a characterization of exposure to adult women without consideration of birthrates.

† "Other" includes Asian non-Hispanic; Native American non-Hispanic; Hispanic other than Mexican-American; those reporting multi-racial; and those with a missing value for race/ethnicity.

* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

** The estimate is not reported because it has large uncertainty: the relative standard error, RSE, is at least 40% (RSE = standard error divided by the estimate).

Table B6b. Cotinine in nonsmoking women ages 16 to 49 years: 95th percentile concentrations in blood serum, by race/ethnicity and family income, 2005–2008

	95th percentile concentration of cotinine in serum (ng/mL)					
Race / Ethnicity	All Incomes	< Poverty Level	≥ Poverty Level	≥ Poverty (Detail)		Unknown Income
				100-200% of Poverty Level	> 200% of Poverty Level	
All Races/Ethnicities	1.6	2.4	1.5	1.8	1.3	1.6*
White non-Hispanic	1.4	1.6	1.4	NA**	1.3	NA**
Black non-Hispanic	2.5	7.0	2.0	NA**	1.9*	2.9
Mexican-American	NA**	1.6*	NA**	NA**	0.4	NA**
Other†	NA**	NA**	NA**	NA**	NA**	NA**

DATA: Centers for Disease Control and Prevention, National Center for Health Statistics, National Health and Nutrition Examination Survey

NOTES:

- Based on women ages 16 to 49 years with cotinine ≤ 10 ng/mL.
- The distribution of the data for women ages 16 to 49 years is adjusted for the likelihood that a woman of a particular age and race/ethnicity gives birth in a particular year. The intent of this adjustment is to approximate the distribution of exposure to pregnant women. Results will therefore differ from a characterization of exposure to adult women without consideration of birthrates.

† "Other" includes Asian non-Hispanic; Native American non-Hispanic; Hispanic other than Mexican-American; those reporting multi-racial; and those with a missing value for race/ethnicity.

* The estimate should be interpreted with caution because the standard error of the estimate is relatively large: the relative standard error, RSE, is at least 30% but is less than 40% (RSE = standard error divided by the estimate).

** The estimate is not reported because it has large uncertainty: the relative standard error, RSE, is at least 40% (RSE = standard error divided by the estimate).

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Metadata

Metadata for	**National Health and Nutrition Examination Survey (NHANES)**
Brief description of the data set	The National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States, using a combination of interviews, physical examinations, and laboratory analysis of biological specimens.
Who provides the data set?	Centers for Disease Control and Prevention, National Center for Health Statistics.
How are the data gathered?	Laboratory data are obtained by analysis of blood and urine samples collected from survey participants at NHANES Mobile Examination Centers. Health status is assessed by physical examination. Demographic and other survey data regarding health status, nutrition, and health-related behaviors are collected by personal interview, either by self-reporting or, for children under 16 and some others, as reported by an informant.
What documentation is available describing data collection procedures?	See http://www.cdc.gov/nchs/nhanes.htm for detailed survey and laboratory documentation by survey period.
What types of data relevant for children's environmental health indicators are available from this database?	Concentrations of environmental chemicals in urine, blood, and serum. Body measurements. Health status, as assessed by physical examination, laboratory measurements, and interview responses. Demographic information.
What is the spatial representation of the database (national or other)?	NHANES sampling procedures provide nationally representative data. Analysis of data for any other geographic area (region, state, etc.) is possible only by special arrangement with the NCHS Research Data Center, and such analyses may not be representative of the specified area.
Are raw data (individual measurements or survey responses) available?	Individual laboratory measurements and survey responses are generally available. Individual survey responses for some questions are not publicly released.
How are database files obtained?	http://www.cdc.gov/nchs/nhanes.htm
Are there any known data quality or data analysis concerns?	Some environmental chemicals have large percentages of values below the detection limit. Data gathered by interview, including demographic information, and responses regarding health status, nutrition, and health-related behaviors are self-reported, or (for individuals age 16 years and younger) reported by an adult informant.

Metadata for	**National Health and Nutrition Examination Survey (NHANES)**
What documentation is available describing QA procedures?	http://www.cdc.gov/nchs/nhanes.htm includes detailed documentation on laboratory and other QA procedures. Data quality information is available at http://www.cdc.gov/nchs/about/policy/quality.htm.
For what years are data available?	Some data elements were collected in predecessors to NHANES beginning in 1959; collection of data on environmental chemicals began with measurement of blood lead in NHANES II, 1976-1980. The range of years for measurement of environmental chemicals varies; apart from lead and cotinine (initiated in NHANES III), measurement of environmental chemicals began with 1999-2000 or later NHANES.
What is the frequency of data collection?	Data are collected on continuous basis, but are grouped into NHANES cycles: NHANES II (1976-1980); NHANES III phase 1 (1988-1991); NHANES III phase 2 (1991-1994); and continuous two-year cycles beginning with 1999-2000 and continuing to the present.
What is the frequency of data release?	Data are released in two-year cycles (e.g. 1999-2000); particular data sets from a two-year NHANES cycle are released as available.
Are the data comparable across time and space?	Detection limits can vary across time, affecting some comparisons. Some contaminants are not measured in every NHANES cycle. Within any NHANES two-year cycle, data are generally collected and analyzed in the same manner for all sampling locations.
Can the data be stratified by race/ethnicity, income, and location (region, state, county or other geographic unit)?	Data are collected to be representative of the U.S. population based on age, sex, and race/ethnicity. The public release files allow stratification by these and other demographic variables, including family income range and poverty income ratio. Data cannot be stratified geographically except by special arrangement with the NCHS Research Data Center.

Methods

Indicator

B5. Cotinine in nonsmoking children ages 3 to 17 years: Median and 95th percentile concentrations in blood serum, 1988-2008

B6. Cotinine in nonsmoking women ages 16 to 49 years: Median and 95th percentile concentrations in blood serum, 1988-2008

Summary

Since the 1970s, the National Center for Health Statistics, a division of the Centers for Disease Control and Prevention, has conducted the National Health and Nutrition Examination Surveys (NHANES), a series of U.S. national surveys of the health and nutrition status of the noninstitutionalized civilian population. The National Center for Environmental Health at CDC measures environmental chemicals in blood and urine samples collected from NHANES participants.[i] Indicator B5 uses serum cotinine measurements in nonsmoking children ages 3 to 17 years (ages 4 to 17 for 1988-1994). Indicator B6 uses serum cotinine measurements in nonsmoking women ages 16 to 49 years. For these analyses, individuals with a serum cotinine level greater than 10 nanograms of cotinine per milliliter of serum (ng/mL) are considered active smokers, and so were excluded from the results. The NHANES 1988-1991 and 1991-1994 survey cycles included serum cotinine data for ages 4 years and over. The NHANES 1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008 survey cycles included serum cotinine data for ages 3 years and over.

Indicator B5 gives the median and 95th percentile concentrations of the serum cotinine for nonsmoking children ages 3 to 17 (ages 4 to 17 for 1988-1994). The median is the estimated concentration such that 50% of all noninstitutionalized civilian nonsmoking children ages 3 to 17 have serum cotinine concentrations below this level. The 95th percentile is the estimated concentration such that 95% of all noninstitutionalized civilian nonsmoking children ages 3 to 17 have serum cotinine concentrations below this level.

Indicator B6 gives the median and 95th percentile concentrations of the serum cotinine for nonsmoking women ages 16 to 49. The median is the estimated concentration such that 50% of all noninstitutionalized civilian nonsmoking women ages 16 to 49 during the survey period have serum cotinine concentrations below this level. The 95th percentile is the estimated concentration such that 95% of all noninstitutionalized civilian nonsmoking women ages 16 to 49 during the survey period have serum cotinine concentrations below this level. These estimates for women of child-bearing age were adjusted by age-specific birthrates to estimate the median and 95th percentile pre-natal exposure. Tables B5a and B5b give the median and 95th percentiles of serum

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[i] Centers for Disease Control and Prevention. 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Atlanta, GA. Available at: www.cdc.gov/exposurereport.

cotinine for nonsmoking children ages 3 to 17 years for 2005-2008, stratified by race/ethnicity and family income. Tables B6a and B6b give the median and 95th percentiles of serum cotinine for nonsmoking women ages 16 to 49 years for 2005-2008, stratified by race/ethnicity and family income. The survey data were weighted to account for the complex multi-stage, stratified, clustered sampling design.

Data Summary

Indicator	B5. Cotinine in nonsmoking children ages 3 to 17 years: Median and 95th percentile concentrations in blood serum, 1988-2008						
Time Period	1988-2008						
Data	Serum cotinine in children ages 3 to 17						
Years	1988-1991	1991-1994	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008
Limits of Detection (ng/mL)*	0.05	0.05	0.05	0.05 or 0.015	0.015	0.015	0.015
Number of Non-missing Values**	2,672	3,237	2,591	2,955	2,651	2,635	2,093
Number of Missing Values	1,308	503	771	689	580	782	662
Number of Values Above 10 ng/mL	129	115	159	142	148	130	64
Percentage Below Limit of Detection***	13	18	37	26	18	22	20

*The Limit of Detection (LOD) is defined as the level at which the measurement has a 95% probability of being greater than zero.
**Non-missing values include those below the analytical LOD, which are reported as LOD/√2, and exclude values above 10 ng/mL.
***This percentage is survey-weighted using the NHANES survey weights for the given period and is for the percentage among children of ages 3 to 17 with cotinine at or below 10 ng/mL.

Indicator	B6. Cotinine in nonsmoking women ages 16 to 49 years: Median and 95th percentile concentrations in blood serum, 1988-2008						
Time Period	1988-2008						
Data	Serum cotinine in women ages 16 to 39						
Years	1988-1991	1991-1994	1999-2000	2001-2002	2003-2004	2005-2006	2007-2008
Limits of Detection (ng/mL)*	0.05	0.05	0.05	0.05 or 0.015	0.015	0.015	0.015
Number of Non-missing Values**	1,784	2,286	1,324	1,490	1,315	1,489	1,206
Number of Missing Values	235	169	290	231	204	220	175
Number of Values Above 10 ng/mL	670	772	330	419	381	376	368
Percentage Below Limit of Detection***	12	18	49	32	24	28	27

*The Limit of Detection (LOD) is defined as the level at which the measurement has a 95% probability of being greater than zero.
**Non-missing values include those below the analytical LOD, which are reported as LOD/√2, and exclude values above 10 ng/mL.
***This percentage is survey-weighted using the NHANES survey weights for the given period and is for the percentage among children of ages 3 to 17 with cotinine at or below 10 ng/mL.

Overview of Data Files

The following files are needed to calculate this indicator. The files together with the survey documentation and SAS programs for reading in the data are available at the NHANES website: http://www.cdc.gov/nchs/nhanes.htm.

- NHANES III: Second Laboratory file LAB2.DAT. This text file contains the measured serum cotinine (COP), age in months (MXPAXTMR), sex (HSSEX), NHANES III Phase (SDPPHASE), pseudo-stratum (SDPSTRA1 for Phase 1 and SDPSTRA2 for Phase 2), pseudo-PSU (SDPPSU1 for Phase1 and SDPPSU2 for Phase 2), and the survey weights (WTPFEX1 for Phase I and WTPFEX2 for Phase 2).

- NHANES 1999-2000: Demographic file demo.xpt. Laboratory file lab06.xpt. The demographic file demo.xpt is a SAS transport file that contains the subject identifier (SEQN), age (RIDAGEYR), sex (RIAGENDR), the two-year laboratory survey weight (WTMEC2YR), the pseudo-stratum (SDMVSTRA), and the pseudo-PSU (SDMVPSU). The laboratory file lab06.xpt contains SEQN and the serum cotinine (LBXCOT). The two files are merged using the common variable SEQN.

- NHANES 2001-2002: Demographic file demo_b.xpt. Laboratory file l06_b.xpt. The demographic file demo_b.xpt is a SAS transport file that contains the subject identifier (SEQN), age (RIDAGEYR), sex (RIAGENDR), the two-year laboratory survey weight (WTMEC2YR), the pseudo-stratum (SDMVSTRA), and the pseudo-PSU (SDMVPSU). The laboratory file l06_b.xpt contains SEQN, the serum cotinine (LBXCOT), and the cotinine non-detect comment code (LBDCOTLC). The two files are merged using the common variable SEQN.

- NHANES 2003-2004: Demographic file demo_c.xpt. Laboratory file l06cot_c.xpt. The demographic file demo_c.xpt is a SAS transport file that contains the subject identifier (SEQN), age (RIDAGEYR), sex (RIAGENDR), the laboratory survey weight (WTMEC2YR), the pseudo-stratum (SDMVSTRA), and the pseudo-PSU (SDMVPSU). The laboratory file l06cot_c.xpt contains SEQN and the serum cotinine (LBXCOT). The two files are merged using the common variable SEQN.

- NHANES 2005-2006: Demographic file demo_d.xpt. Laboratory file cot_d.xpt. The demographic file demo_d.xpt is a SAS transport file that contains the subject identifier (SEQN), age (RIDAGEYR), sex (RIAGENDR), race/ethnicity (RIDRETH1), the poverty income ratio (INFMPIR), the laboratory survey weight (WTMEC2YR), the pseudo-stratum (SDMVSTRA), and the pseudo-PSU (SDMVPSU). The laboratory file cot_d.xpt contains SEQN and the serum cotinine (LBXCOT). The two files are merged using the common variable SEQN.

- NHANES 2007-2008: Demographic file demo_e.xpt. Laboratory file cotnal_e.xpt. The demographic file demo_e.xpt is a SAS transport file that contains the subject identifier (SEQN), age (RIDAGEYR), sex (RIAGENDR), race/ethnicity (RIDRETH1), the poverty income ratio (INFMPIR), the laboratory survey weight (WTMEC2YR), the pseudo-stratum (SDMVSTRA), and the pseudo-PSU (SDMVPSU). The laboratory file cotnal_e.xpt contains SEQN and the serum cotinine (LBXCOT). The two files are merged using the common variable SEQN.

National Health and Nutrition Examination Surveys (NHANES)

Since the 1970s, the National Center for Health Statistics, a division of the Centers for Disease Control and Prevention, has conducted the National Health and Nutrition Examination Surveys (NHANES), a series of U.S. national surveys of the health and nutrition status of the noninstitutionalized civilian population. The National Center for Environmental Health at CDC measures environmental chemicals in blood and urine samples collected from NHANES participants. Indicator B5 uses serum cotinine measurements in children ages 4 to 17 from NHANES 1988-1991 and 1991-1994, and uses serum cotinine measurements in children ages 3 to 17 from NHANES 1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008. Indicator B6 uses serum cotinine measurements in women ages 16 to 49 from NHANES 1988-1991, 1991-1994, 1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008. The NHANES data were obtained from the NHANES website: http://www.cdc.gov/nchs/nhanes.htm. Following the CDC recommended approach, values below the analytical limit of detection (LOD) were replaced by $LOD/\sqrt{2}$.[ii]

The NHANES use a complex multi-stage, stratified, clustered sampling design. Certain demographic groups were deliberately over-sampled, including Mexican-Americans and Blacks. Oversampling is performed to increase the reliability and precision of estimates of health status indicators for these population subgroups. The publicly released data includes survey weights to adjust for the over-sampling, non-response, and non-coverage. The statistical analyses used the laboratory survey weights (WTPFEX1 for 1988-1991, WTPFEX2 for 1991-1994, and WTMEC2YR for 1999 and later) to re-adjust the serum cotinine data to represent the national population.

Age-Specific Birthrates

In addition to the NHANES survey weights, for Indicator B6, the data for women of child-bearing age (ages 16 to 49) were also weighted by the birthrate for women of the given age and race/ethnicity to estimate pre-natal exposures. Thus the overall weight in each two year period is the product of the NHANES survey weight and the total number of births in the two calendar years for the given age and race/ethnicity, divided by twice the corresponding population of

[ii] See Hornung RW, Reed LD. 1990. Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene* 5:46–51.

women at the midpoint of the two year period.[iii] For the years 2007-2008, the natality and total population data used to compute the birthrate adjustments are not currently publicly available. For those two years the birthrate adjustments were estimated from the 2005-2006 data.

Race/Ethnicity and Family Income

For Tables B5a, B5b, B6a, and B6b, the percentiles were calculated for demographic strata defined by the combination of race/ethnicity and family income.

The family income was characterized based on the INDFMPIR variable, which is the ratio of the family income to the poverty level. The National Center for Health Statistics used the U.S. Census Bureau Current Population Survey to define the family units, and the family income for the respondent was obtained during the interview. The U.S. Census Bureau defines annual poverty level money thresholds varying by family size and composition. The poverty income ratio (PIR) is the family income divided by the poverty level for that family. Family income was stratified into the following groups:

- Below Poverty Level: PIR < 1
- Between 100% and 200% of Poverty Level: 1 ≤ PIR ≤ 2
- Above 200% of Poverty Level: PIR > 2
- Above Poverty Level: PIR ≥ 1 (combines the previous two groups)
- Unknown Income: PIR is missing

Race/ethnicity was characterized using the RIDRETH1 variable. The possible values of this variable are:

- 1. Mexican American
- 2. Other Hispanic
- 3. Non-Hispanic White
- 4. Non-Hispanic Black
- 5. Other Race – Including Multi-racial
- "." Missing

Category 5 includes: all Non-Hispanic single race responses other than White or Black; and multi-racial responses.

For these indicators, the RIDRETH1 categories 2, 5, and missing were combined into a single "Other" category. This produced the following categories:

- White non-Hispanic: RIDRETH1 = 3
- Black non-Hispanic: RIDRETH1 = 4
- Mexican-American: RIDRETH1 = 1

[iii] Axelrad, D.A., Cohen, J. 2010. Calculating summary statistics for population chemical biomonitoring in women of childbearing age with adjustment for age-specific natality. *Environmental Research* 111 (1) 149-155.

- Other: RIDRETH1 = 2 or 5 or missing

The "Other" category includes Asian non-Hispanic, Native American non-Hispanic, Hispanic other than Mexican-American, those reporting multi-racial, and those with a missing value for race/ethnicity.

Calculation of Indicator

Indicator B5 is the median and 95th percentile for serum cotinine in children of ages 3 to 17 years (ages 4 to 17 years for 1988-1994). The median is the estimated concentration such that 50% of all noninstitutionalized civilian nonsmoking children ages 3 to 17 years have serum cotinine concentrations below this level. The 95th percentile is the estimated concentration such that 95% of all noninstitutionalized civilian nonsmoking children ages 3 to 17 years have serum cotinine concentrations below this level. Indicator B6 is the median and 95th percentile for serum cotinine in women of ages 16 to 49 years. The median is the estimated concentration such that 50% of all noninstitutionalized civilian nonsmoking women ages 16 to 49 years during the survey period have serum cotinine concentrations below this level. The 95th percentile is the estimated concentration such that 95% of all noninstitutionalized civilian nonsmoking women ages 16 to 49 years during the survey period have serum cotinine concentrations below this level. Tables B5a and B5b give the median and 95th percentiles of serum cotinine for nonsmoking children ages 3 to 17 years for 2005-2008, stratified by race/ethnicity and family income. Tables B6a and B6b give the median and 95th percentiles of serum cotinine for nonsmoking women ages 16 to 49 years for 2005-2008, stratified by race/ethnicity and family income. To adjust the NHANES data to represent pre-natal exposures, the data for each woman surveyed was multiplied by the estimated number of births per woman of the given age and race/ethnicity.

To simply demonstrate the calculations, we will use the NHANES 2007-2008 serum cotinine values for women ages 16 to 49 years of all race/ethnicities and all incomes as an example for Indicator B6. The calculations for Indicator B5 use the same calculations applied to the serum cotinine data for children ages 3 to 17, except that the birthrate adjustment is not applied.

We begin with all the non-missing NHANES 2007-2008 serum cotinine values for women ages 16 to 49 years. First, we exclude all serum cotinine values above 10 ng/mL to give the cotinine values for nonsmoking women. Each sampled woman has an associated annual survey weight that estimates the annual number of U.S. women represented by that sampled woman. The annual survey weight for each woman is WTMEC2YR. Each sampled woman also has an associated birthrate giving the numbers of annual births per woman of the given age, race, and ethnicity. The product of the annual survey weight and the birthrate estimates the annual number of U.S. births represented by that sampled woman, which we will refer to as the adjusted survey weight. For example, the lowest serum cotinine measurement for a nonsmoking woman between 16 and 49 years of age is 0.011 ng/mL with an annual survey weight of 91,000, a birthrate of 0.08, and thus an adjusted survey weight of 7,200, and so represents 7,200 births. The total of the adjusted survey weights for the sampled nonsmoking women equals 2.93 million, the total number of annual U.S. births to women ages 16 to 49 years. The second lowest measurement is also 0.011 ng/mL with an adjusted survey weight of 10,500, and so represents another 10,500

U.S. births. The highest measurement for nonsmoking women was 9.89 ng/mL, with an adjusted survey weight of 1,600, and so represents another 1,600 U.S. births.

To calculate the median, we can use the adjusted survey weights to expand the data to the entire U.S. population of births to women ages 16 to 49. We have 7,200 values of 0.011 ng/mL from the lowest measurement, 10,500 values of 0.011 ng/mL from the second lowest measurement, and so on, up to 1,600 values of 9.89 ng/mL from the highest measurement. Arranging these 2.9 million values in increasing order, the 1.45 millionth value is 0.04 ng/mL. Since half of the values are below 0.04 and half of the values are above 0.04, the median equals 0.04 ng/mL. To calculate the 95th percentile, note that 95% of 2.9 million equals 2.76 million. The 2.76 millionth value is 1.9 ng/mL. Since 95% of the values are below 1.9, the 95th percentile equals 1.9 ng/mL.

For 2007-2008, there were a total of 1,749 women participants of ages 16 to 49 in the NHANES survey. Of these 1,749 women, 368 had cotinine values above 10 ng/mL and 175 had missing cotinine measurements. These calculations assume that the 1,574 (1,749 minus 175) sampled women with valid serum cotinine data are representative of women giving birth without valid serum cotinine data. The calculations also assume that the sampled women are representative of women that actually gave birth in 2007-2008, since NHANES information on pregnancy and births was not incorporated into the analysis.

<u>Equations</u>

These percentile calculations can also be given as the following mathematical equations, which are based on the default percentile calculation formulas from Statistical Analysis System (SAS) software. Exclude all missing serum cotinine values and all serum cotinine values above 10 ng/mL. Suppose there are n women of ages 16 to 49 years with valid serum cotinine values at or below 10 ng/mL. Arrange the serum cotinine concentrations in increasing order (including tied values) so that the lowest concentration is x(1) with an adjusted survey weight of w(1), the second lowest concentration is x(2) with an adjusted survey weight of w(2), …, and the highest concentration is x(n) with an adjusted survey weight of w(n).

1. Sum all the adjusted survey weights to get the total weight W:

$$W = \Sigma[1 \le i \le n]\ w(i)$$

2. Find the largest number i so that the total of the weights for the i lowest values is less than or equal to W/2.

$$\Sigma[j \le i]\ w(j) \le W/2 < \Sigma[j \le i + 1]\ w(j)$$

3. Calculate the median using the results of the second step. We either have

$$\Sigma[j \le i]\ w(j) = W/2 < \Sigma[j \le i + 1]\ w(j)$$

or

$$\Sigma[j \le i]\, w(j) < W/2 < \Sigma[j \le i + 1]\, w(j)$$

In the first case we define the median as the average of the i'th and i + 1'th values:

$$\text{Median} = [x(i) + x(i + 1)]/2 \text{ if } \Sigma[j \le i]\, w(j) = W/2$$

In the second case we define the median as the i + 1'th value:

$$\text{Median} = x(i + 1) \text{ if } \Sigma[j \le i]\, w(j) < W/2$$

(The estimated median does not depend upon how the tied values of x(j) are ordered).

A similar calculation applies to the 95th percentile. The first step, to calculate the sum of the weights, W, is the same. In the second step, find the largest number i so that the total of the weights for the i lowest values is less than or equal to 0.95W.

$$\Sigma[j \le i]\, w(j) \le 0.95W < \Sigma[j \le i + 1]\, w(j)$$

In the third step we calculate the 95th percentile using the results of the second step. We either have

$$\Sigma[j \le i]\, w(j) = 0.95W < \Sigma[j \le i + 1]\, w(j)$$

or

$$\Sigma[j \le i]\, w(j) < 0.95W < \Sigma[j \le i + 1]\, w(j)$$

In the first case we define the 95th percentile as the average of the i'th and i + 1'th values:

$$95^{th} \text{ Percentile} = [x(i) + x(i + 1)]/2 \text{ if } \Sigma[j \le i]\, w(j) = 0.95W$$

In the second case we define the 95th percentile as the i + 1'th value:

$$95^{th} \text{ Percentile} = x(i + 1) \text{ if } \Sigma[j \le i]\, w(j) < 0.95W$$

<u>Relative Standard Error</u>

The uncertainties of the median and 95th percentile values were calculated using a revised version of the CDC method given in CDC 2005,[iv] Appendix C, and the SAS® program provided by CDC. The method uses the Clopper-Pearson binomial confidence intervals adapted for complex surveys by Korn and Graubard (see Korn and Graubard, 1999,[v] p. 65). The following

[iv] CDC Third National Report on Human Exposure to Environmental Chemicals. 2005
[v] Korn E. L., Graubard B. I. 1999. *Analysis of Health Surveys.* Wiley.

text is a revised version of the Appendix C. For the birthrate adjusted calculations for women ages 16 to 49, the sample weight is adjusted by multiplying by the age-specific birthrate.

Step 1: Use SAS® Proc Univariate to obtain a point estimate P_{SAS} of the percentile value. Use the Weight option to assign the exact correct sample weight for each chemical result.

Step 2: Use SUDAAN® Proc Descript with Taylor Linearization DESIGN = WR (i.e., sampling with replacement) and the proper sampling weight to estimate the proportion (p) of subjects with results less than and not equal to the percentile estimate P_{SAS} obtained in Step 1 and to obtain the standard error (se_p) associated with this proportion estimate. Compute the degrees-of-freedom adjusted effective sample size

$$n_{df} = (t_{num}/t_{denom})^2 \, p(1 - p)/(se_p{}^2)$$

where t_{num} and t_{denom} are 0.975 critical values of the Student's t distribution with degrees of freedom equal to the sample size minus 1 and the number of PSUs minus the number of strata, respectively. Note: the degrees of freedom for t_{denom} can vary with the demographic sub-group of interest.

Step 3: After obtaining an estimate of p (i.e., the proportion obtained in Step 2), compute the Clopper-Pearson 95% confidence interval ($P_L(x,n_{df})$, $P_U(x,n_{df})$) as follows:

$$P_L(x,n_{df}) = v_1F_{v1,v2}\,(0.025)/(v_2 + v_1F_{v1,v2}(0.025))$$
$$P_U(x,n_{df}) = v_3F_{v3,v4}\,(0.975)/(v_4 + v_3F_{v3,v4}(0.975))$$

where x is equal to p times n_{df}, $v_1 = 2x$, $v_2 = 2(n_{df} - x + 1)$, $v_3 = 2(x + 1)$, $v_4 = 2(n_{df} - x)$, and $F_{d1,d2}(\beta)$ is the β quantile of an F distribution with d1 and d2 degrees of freedom. (Note: If n_{df} is greater than the actual sample size or if p is equal to zero, then the actual sample size should be used.) This step will produce a lower and an upper limit for the estimated proportion obtained in Step 2.

Step 4: Use SAS Proc Univariate (again using the Weight option to assign weights) to determine the chemical percentile values P_{CDC}, L_{CDC} and U_{CDC} that correspond to the proportion p obtained in Step 2 and its lower and upper limits obtained in Step 3. Do not round the values of p and the lower and upper limits. For example, if p = 0.4832, then P_{CDC} is the 48.32'th percentile value of the chemical. The alternative percentile estimates P_{CDC} and P_{SAS} are not necessarily equal.

Step 5: Use the confidence interval from Step 4 to estimate the standard error of the estimated percentile P_{CDC}:

$$\text{Standard Error } (P_{CDC}) = (U_{CDC} - L_{CDC}) / (2t_{denom})$$

Step 6: Use the estimated percentile P_{CDC} and the standard error from Step 4 to estimate the relative standard error of the estimated percentile P_{CDC}:

$$\text{Relative Standard Error (\%)} = [\text{Standard Error } (P_{CDC}) / P_{CDC}] \times 100\ \%$$

The tabulated estimated percentile is the value of P_{SAS} given in Step 1. The relative standard error is given in Step 6, using P_{CDC} and its standard error.

The relative standard error depends upon the survey design. For this purpose, the public release version of NHANES includes the variables SDMVSTRA and SDMVPSU, which are the Masked Variance Unit pseudo-stratum and pseudo-primary sampling unit (pseudo-PSU). For approximate variance estimation, the survey design can be approximated as being a stratified random sample with replacement of the pseudo-PSUs from each pseudo-stratum; the true stratum and PSU variables are not provided in the public release version to protect confidentiality.

Percentiles with a relative standard error less than 30% were treated as being reliable and were tabulated. Percentiles with a relative standard error greater than or equal to 30% but less than 40% were treated as being unstable; these values were tabulated but were flagged to be interpreted with caution. Percentiles with a relative standard error greater than or equal to 40%, or without an estimated relative standard error, were treated as being unreliable; these values were not tabulated and were flagged as having a large uncertainty.

Questions and Comments

Questions regarding these methods, and suggestions to improve the description of the methods, are welcome. Please use the "Contact Us" link at the bottom of any page in the America's Children and the Environment website.

Statistical Comparisons

Statistical analyses of the percentiles were used to determine whether the differences between percentiles for different demographic groups were statistically significant. For these analyses, the percentiles and their standard errors were calculated for each combination of age group, sex (in the cases of children), income group (below poverty, at or above poverty, unknown income), and race/ethnicity group using the method described in the "Relative Standard Error" section. In the notation of that section, the percentile and standard error are the values of P_{CDC} and Standard Error (P_{CDC}), respectively. These calculated standard errors account for the survey weighting and design and, for women, for the age-specific birthrate.

Using a weighted linear regression model, the percentile was assumed to be the sum of explanatory terms for age, sex, income and/or race/ethnicity and a random error term; the error terms were assumed to be approximately independent and normally distributed with a mean of zero and a variance equal to the square of the standard error. Using this model, the difference in the value of a percentile between different demographic groups is statistically significant if the difference between the corresponding sums of explanatory terms is statistically significantly different from zero. A p-value at or below 0.05 implies that the difference is statistically significant at the 5% significance level. No adjustment is made for multiple comparisons.

For each type of comparison, we present unadjusted and adjusted analyses. The unadjusted analyses directly compare a percentile between different demographic groups. The adjusted analyses add other demographic explanatory variables to the statistical model and use the statistical model to account for the possible confounding effects of these other demographic variables. For example, the unadjusted race/ethnicity comparisons use and compare the percentiles between different race/ethnicity pairs. The adjusted race/ethnicity comparisons use the percentiles for each age/sex/income/race/ethnicity combination. The adjusted analyses add age, sex, and income terms to the statistical model and compare the percentiles between different race/ethnicity pairs after accounting for the effects of the other demographic variables. For example, if White non-Hispanics tend to have higher family incomes than Black non-Hispanics, and if the body burden depends strongly on family income only, then the unadjusted differences between these two race/ethnicity groups would be significant but the adjusted difference (taking into account income) would not be significant.

Comparisons between pairs of race/ethnicity groups are shown in Tables 1 and 2 for nonsmoking children ages 3 to 17 years and in Tables 4 and 5 for nonsmoking women ages 16 to 49 years. In Tables 1 and 4, for the unadjusted "All incomes" comparisons, the only explanatory variables are terms for each race/ethnicity group. For these unadjusted comparisons, the statistical tests compare the percentiles for each pair of race/ethnicity groups. For the adjusted "All incomes (adjusted for age, sex, income)" comparisons, the explanatory variables are terms for each race/ethnicity group together with terms for each age, sex, and income group. For these adjusted comparisons, the statistical test compares the pair of race/ethnicity groups after accounting for any differences in the age, sex, and income distributions between the race/ethnicity groups. The adjustment for sex is applicable only for children, and thus appears only in Tables 1 and 2.

In Tables 1 and 4, for the unadjusted "Below Poverty Level" and "At or Above Poverty Level" comparisons, the only explanatory variables are terms for each of the 12 race/ethnicity/income combinations (combinations of four race/ethnicity groups and three income groups). For example, in row 1, the p-value for "Below Poverty Level" compares White non-Hispanics below the poverty level with Black non-Hispanics below the poverty level. The same set of explanatory variables are used in Tables 2 and 5 for the unadjusted comparisons between one race/ethnicity group below the poverty level and the same or another race/ethnicity group at or above the poverty level. The corresponding adjusted analyses include extra explanatory variables for age and sex, so that race/ethnicity/income groups are compared after accounting for any differences due to age or sex.

Additional comparisons are shown in Table 3 for nonsmoking children ages 3 to 17 years and in Table 6 for nonsmoking women ages 16 to 49 years. The AGAINST = "income" unadjusted p-value compares the body burdens for those below poverty level with those at or above poverty level, using the explanatory variables for the three income groups (below poverty, at or above poverty, unknown income). The adjusted p-value includes adjustment terms for age, sex (for children), and race/ethnicity in the model. The AGAINST = "yearnum" p-value examines whether the linear trend in the body burden is statistically significant (using the percentiles for each NHANES period regressed against the midpoint of that period); the adjusted model for trend adjusts for demographic changes in the populations from year to year by including terms for age, sex, income, and race/ethnicity.

For women, the age groups used were 16-19, 20-24, 25-29, 30-39, and 40-49. For children, the age groups used were 3-5, 6-10, 11-15, and 16-17.

For more details on these statistical analyses, see the memorandum by Cohen (2010).[vi]

Table 1. Statistical significance tests comparing the percentiles of cotinine in nonsmoking children ages 3 to 17 years, between pairs of race/ethnicity groups, for 2005-2008.

				P-VALUES					
Variable	**Percentile**	**RACE1**	**RACE2**	**All incomes**	**All incomes (adjusted for age, sex, income)**	**Below Poverty Level**	**Below Poverty Level (adjusted for age, sex)**	**At or Above Poverty Level**	**At or Above Poverty Level (adjusted for age, sex)**
cotinine	50	White non-Hispanic	Black non-Hispanic	0.006	< 0.0005	0.584	0.674	0.097	0.006
cotinine	50	White non-Hispanic	Mexican-American	0.006	< 0.0005	0.153	0.016	< 0.0005	< 0.0005
cotinine	50	White non-Hispanic	Other	0.203	< 0.0005	0.366	0.123	0.018	0.009
cotinine	50	Black non-Hispanic	Mexican-American	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
cotinine	50	Black non-Hispanic	Other	< 0.0005	< 0.0005	0.002	< 0.0005	< 0.0005	< 0.0005

[vi] Cohen, J. 2010. *Selected statistical methods for testing for trends and comparing years or demographic groups in ACE NHIS and NHANES indicators.* Memorandum submitted to Dan Axelrad, EPA, 21 March, 2010.

				P-VALUES					
Variable	**Percentile**	**RACE1**	**RACE2**	**All incomes**	**All incomes (adjusted for age, sex, income)**	**Below Poverty Level**	**Below Poverty Level (adjusted for age, sex)**	**At or Above Poverty Level**	**At or Above Poverty Level (adjusted for age, sex)**
cotinine	50	Mexican-American	Other	0.017	0.617	0.018	0.068	0.055	0.045
cotinine	95	White non-Hispanic	Black non-Hispanic	0.870	0.238	0.075	0.255	0.227	0.194
cotinine	95	White non-Hispanic	Mexican-American	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
cotinine	95	White non-Hispanic	Other	0.015	< 0.0005	0.454	0.108	0.013	< 0.0005
cotinine	95	Black non-Hispanic	Mexican-American	< 0.0005	< 0.0005	0.006	< 0.0005	0.007	< 0.0005
cotinine	95	Black non-Hispanic	Other	0.083	< 0.0005	0.306	0.507	0.289	< 0.0005
cotinine	95	Mexican-American	Other	0.006	0.998	< 0.0005	< 0.0005	0.104	0.017

Table 2. Statistical significance tests comparing the percentiles of cotinine in nonsmoking children ages 3 to 17 years, between pairs of race/ethnicity/income groups at different income levels, for 2005-2008.

				P-VALUES	
Variable	**Percentile**	**RACEINC1**	**RACEINC2**	**Unadjusted**	**Adjusted (for age, sex)**
cotinine	50	White non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.166	0.020
cotinine	50	White non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.188	0.029
cotinine	50	White non-Hispanic, < PL	Mexican-American, ≥ PL	0.134	0.012
cotinine	50	White non-Hispanic, < PL	Other, ≥ PL	0.145	0.015
cotinine	50	Black non-Hispanic, < PL	White non-Hispanic, ≥ PL	< 0.0005	< 0.0005
cotinine	50	Black non-Hispanic, < PL	Black non-Hispanic, ≥ PL	< 0.0005	< 0.0005
cotinine	50	Black non-Hispanic, < PL	Mexican-American, ≥ PL	< 0.0005	< 0.0005
cotinine	50	Black non-Hispanic, < PL	Other, ≥ PL	< 0.0005	< 0.0005
cotinine	50	Mexican-American, < PL	White non-Hispanic, ≥ PL	0.261	0.044
cotinine	50	Mexican-American, < PL	Black non-Hispanic, ≥ PL	0.009	< 0.0005
cotinine	50	Mexican-American, < PL	Mexican-American, ≥ PL	0.029	< 0.0005
cotinine	50	Mexican-American, < PL	Other, ≥ PL	0.381	0.373
cotinine	50	Other, < PL	White non-Hispanic, ≥ PL	0.031	0.101
cotinine	50	Other, < PL	Black non-Hispanic, ≥ PL	0.067	0.202
cotinine	50	Other, < PL	Mexican-American, ≥ PL	0.007	0.039
cotinine	50	Other, < PL	Other, ≥ PL	0.012	0.057
cotinine	95	White non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.001	< 0.0005
cotinine	95	White non-Hispanic, < PL	Black non-Hispanic, ≥ PL	< 0.0005	< 0.0005
cotinine	95	White non-Hispanic, < PL	Mexican-American, ≥ PL	< 0.0005	< 0.0005
cotinine	95	White non-Hispanic, < PL	Other, ≥ PL	< 0.0005	< 0.0005
cotinine	95	Black non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.104	0.001
cotinine	95	Black non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.022	< 0.0005
cotinine	95	Black non-Hispanic, < PL	Mexican-American, ≥ PL	< 0.0005	< 0.0005

				P-VALUES	
Variable	Percentile	RACEINC1	RACEINC2	Unadjusted	Adjusted (for age, sex)
cotinine	95	Black non-Hispanic, < PL	Other, ≥ PL	0.002	< 0.0005
cotinine	95	Mexican-American, < PL	White non-Hispanic, ≥ PL	0.055	< 0.0005
cotinine	95	Mexican-American, < PL	Black non-Hispanic, ≥ PL	0.275	< 0.0005
cotinine	95	Mexican-American, < PL	Mexican-American, ≥ PL	0.544	0.294
cotinine	95	Mexican-American, < PL	Other, ≥ PL	0.694	< 0.0005
cotinine	95	Other, < PL	White non-Hispanic, ≥ PL	0.008	0.066
cotinine	95	Other, < PL	Black non-Hispanic, ≥ PL	0.001	0.009
cotinine	95	Other, < PL	Mexican-American, ≥ PL	< 0.0005	< 0.0005
cotinine	95	Other, < PL	Other, ≥ PL	< 0.0005	< 0.0005

Table 3. Other statistical significance tests comparing the percentiles of cotinine in nonsmoking children ages 3 to 17 years, for 2005-2008 (trends for 1988-2008).

					P-VALUES	
Variable	Percentile	From	To	Against	Unadjusted	Adjusted*
cotinine	50	2005	2008	income	0.001	< 0.0005
cotinine	50	1988	2008	yearnum	< 0.0005	< 0.0005
cotinine	95	2005	2008	income	< 0.0005	< 0.0005
cotinine	95	1988	2008	yearnum	0.007	< 0.0005

* For AGAINST = "income," the p-values are adjusted for age, sex, and race/ethnicity.
For AGAINST = "yearnum," the p-values are adjusted for age, sex, race/ethnicity, and income.

Table 4. Statistical significance tests comparing the percentiles of cotinine in nonsmoking women ages 16 to 49 years, between pairs of race/ethnicity groups, for 2005-2008.

				P-VALUES					
Variable	Percentile	RACE1	RACE2	All incomes	All incomes (adjusted for age, income)	Below Poverty Level	Below Poverty Level (adjusted for age)	At or Above Poverty Level	At or Above Poverty Level (adjusted for age)
cotinine	50	White non-Hispanic	Black non-Hispanic	< 0.0005	0.006	0.838	0.339	< 0.0005	0.013
cotinine	50	White non-Hispanic	Mexican-American	0.023	< 0.0005	0.014	0.274	0.006	< 0.0005
cotinine	50	White non-Hispanic	Other	0.734	0.589	0.087	0.279	0.740	0.746
cotinine	50	Black non-Hispanic	Mexican-American	< 0.0005	< 0.0005	< 0.0005	0.086	< 0.0005	< 0.0005
cotinine	50	Black non-Hispanic	Other	< 0.0005	0.004	0.029	0.337	< 0.0005	0.013
cotinine	50	Mexican-American	Other	0.040	0.003	0.239	0.963	0.014	0.002
cotinine	95	White non-Hispanic	Black non-Hispanic	0.003	0.461	0.005	< 0.0005	0.243	0.337
cotinine	95	White non-Hispanic	Mexican-American	0.402	< 0.0005	0.967	0.024	0.465	0.538
cotinine	95	White non-Hispanic	Other	0.793	< 0.0005	0.615	0.001	0.853	0.382
cotinine	95	Black non-	Mexican-	0.005	< 0.0005	0.007	0.034	0.134	0.685

Variable	Percentile	RACE1	RACE2	P-VALUES All incomes	All incomes (adjusted for age, income)	Below Poverty Level	Below Poverty Level (adjusted for age)	At or Above Poverty Level	At or Above Poverty Level (adjusted for age)
		Hispanic	American						
cotinine	95	Black non-Hispanic	Other	0.675	< 0.0005	0.134	0.031	0.860	0.046
cotinine	95	Mexican-American	Other	0.615	< 0.0005	0.634	0.146	0.654	0.073

Table 5. Statistical significance tests comparing the percentiles of cotinine in nonsmoking women ages 16 to 49 years, between pairs of race/ethnicity/income groups at different income levels, for 2005-2008.

Variable	Percentile	RACEINC1	RACEINC2	P-VALUES Unadjusted	Adjusted (for age)
cotinine	50	White non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.011	0.271
cotinine	50	White non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.134	0.296
cotinine	50	White non-Hispanic, < PL	Mexican-American, ≥ PL	0.005	0.251
cotinine	50	White non-Hispanic, < PL	Other, ≥ PL	0.010	0.269
cotinine	50	Black non-Hispanic, < PL	White non-Hispanic, ≥ PL	< 0.0005	0.068
cotinine	50	Black non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.040	0.272
cotinine	50	Black non-Hispanic, < PL	Mexican-American, ≥ PL	< 0.0005	0.015
cotinine	50	Black non-Hispanic, < PL	Other, ≥ PL	< 0.0005	0.061
cotinine	50	Mexican-American, < PL	White non-Hispanic, ≥ PL	0.677	0.807
cotinine	50	Mexican-American, < PL	Black non-Hispanic, ≥ PL	< 0.0005	0.082
cotinine	50	Mexican-American, < PL	Mexican-American, ≥ PL	0.007	0.009
cotinine	50	Mexican-American, < PL	Other, ≥ PL	0.477	0.657
cotinine	50	Other, < PL	White non-Hispanic, ≥ PL	0.185	0.926
cotinine	50	Other, < PL	Black non-Hispanic, ≥ PL	0.483	0.704
cotinine	50	Other, < PL	Mexican-American, ≥ PL	0.054	0.607
cotinine	50	Other, < PL	Other, ≥ PL	0.157	0.891
cotinine	95	White non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.684	0.032
cotinine	95	White non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.439	0.156
cotinine	95	White non-Hispanic, < PL	Mexican-American, ≥ PL	0.329	0.076
cotinine	95	White non-Hispanic, < PL	Other, ≥ PL	0.940	0.003
cotinine	95	Black non-Hispanic, < PL	White non-Hispanic, ≥ PL	0.004	0.030
cotinine	95	Black non-Hispanic, < PL	Black non-Hispanic, ≥ PL	0.011	0.001
cotinine	95	Black non-Hispanic, < PL	Mexican-American, ≥ PL	0.002	0.001
cotinine	95	Black non-Hispanic, < PL	Other, ≥ PL	0.044	0.011
cotinine	95	Mexican-American, < PL	White non-Hispanic, ≥ PL	0.745	0.909
cotinine	95	Mexican-American, < PL	Black non-Hispanic, ≥ PL	0.578	0.276
cotinine	95	Mexican-American, < PL	Mexican-American, ≥ PL	0.403	0.451
cotinine	95	Mexican-American, < PL	Other, ≥ PL	0.954	0.443
cotinine	95	Other, < PL	White non-Hispanic, ≥ PL	0.551	0.120
cotinine	95	Other, < PL	Black non-Hispanic, ≥ PL	0.774	0.006
cotinine	95	Other, < PL	Mexican-American, ≥ PL	0.420	0.011

				P-VALUES	
Variable	**Percentile**	**RACEINC1**	**RACEINC2**	**Unadjusted**	**Adjusted (for age)**
cotinine	95	Other, < PL	Other, ≥ PL	0.732	0.291

Table 6. Other statistical significance tests comparing the percentiles of cotinine in nonsmoking women ages 16 to 49 years, for 2005-2008 (trends for 1988-2008).

					P-VALUES	
Variable	**Percentile**	**From**	**To**	**Against**	**Unadjusted**	**Adjusted***
cotinine	50	2005	2008	income	0.012	0.009
cotinine	50	1988	2008	yearnum	< 0.0005	< 0.0005
cotinine	95	2005	2008	income	0.141	0.150
cotinine	95	1988	2008	yearnum	0.058	0.354

*For AGAINST = ”income,” the p-values are adjusted for age and race/ethnicity.
For AGAINST = “yearnum,” the p-values are adjusted for age, race/ethnicity, and income.