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# Urinary antimony and leukocyte telomere length: An analysis of NHANES 1999–2002

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ABSTRACT

Telomeres are repetitive DNA sequences (TTAGGG) at the end of chromosomes. Cells with critically short telomeres enter replicative senescence and apoptosis. Several *in vitro* studies report that antimony causes cell apoptosis in human leukocyte cell lines. The goal of this analysis was to investigate whether there is an association between antimony exposure and leukocyte telomere length (LTL) among US adults aged 20 and older based on the National Health and Nutrition Examination Survey (NHANES) 1999–2002. We used multivariate linear regression to analyze the association of urinary antimony with LTL. LTL was log-natural transformed and the results were re-transformed and presented as percent differences. After adjustment for potential confounders, individuals in the 3rd and 4th quartiles of urinary antimony had statistically significantly shorter LTL (−4.78%, 95% CI: −8.42, −0.90; and −6.11%, 95% CI: −11.04, −1.00, respectively) compared to the lowest referent quartile, with evidence of a dose-response relationship (p-value for trend =0.03). Shorter LTL with antimony was driven by middle aged (40–59 years) and older (60–85 years) adult groups. The association may be biologically plausible because of reported oxidative stress and apoptosis effects of antimony on blood cells, effects known to shorten telomere length.

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## 1. Introduction

The enzyme telomerase reverse transcriptase (TERT) is a cellular ribonucleoprotein with reverse transcriptase activity responsible for the maintenance of telomere length (Aubert and Lansdorp, 2008). The main function of TERT is the addition of the hexamaric guanine-rich repetitive sequence (5′-TTAGGG-3′) to the chromosomal ends to compensate for the progressive loss of telomeric sequence, thereby maintaining chromosomal integrity and genetic stability (Aubert and Lansdorp, 2008). Germ cells, certain white blood cells (*e.g.*, lymphocyte), and cancer cells have active telomerase complexes, which make them relatively long-lived compared to other cell types (Finkel et al., 2007). In contrast, all other somatic cell types have a more finite life span that is regulated at the cell level; telomere erosion leads to loss of approximately 50–200 base pairs (bps) of telomeric DNA at each cell division until replicative senescence, a state of irreversible cell growth arrest and apoptosis, is reached (Chiu and Harley, 1997). The process of telomere-shortening can be accelerated by increased oxidative stress (von Zglinicki, 2002). Independent of aging, telomere shortening is also associated with several health outcomes such as cardiovascular diseases (Haycock et al., 2014), atherosclerosis (Samani et al., 2001), hypertension (Demissie et al., 2006), diabetes (Zee et al., 2010), and mortality (Weischer et al., 2012).

Antimony is a silvery white metal that is commonly found within the Earth's crust; it exists in either a trivalent or pentavalent state (ATSDR (Agency for Toxic Substances and Disease Registry), 1992). Antimony is a persistent, bio-accumulative, and toxic chemical. Exposure to and toxicity from antimony may arise due to occupational exposure, domestic use, or when it is used as a medical therapy (Sundar and Chakravarty, 2010). Antimony is used to treat parasitic diseases such as leishmaniasis and schistosomiasis. Antimony is also used as a fire-retardant for plastics, and in textiles, rubber, adhesives, pigments in paints, lead storage batteries, pipe metals, semiconductors, and pewter (ATSDR (Agency for Toxic Substances and Disease Registry), 1992). Antimony is released into air and water from coal-burning power plants, incinerators, mines, and industrial facilities. In urban areas, the primary sources of exposure are likely to be vehicle exhaust and industrial emissions. The general population is constantly exposed to low levels of antimony in the environment through food, air, and drinking water (ATSDR (Agency for Toxic Substances and Disease Registry), 1992). People may also be exposed by skin contact with soil, water and other substances that contain antimony (ATSDR (Agency for Toxic Substances and Disease Registry), 1992).

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The absorption, distribution, and excretion of antimony can vary depending on its oxidation state, with urinary excretion appearing to be greater for pentavalent antimony compounds than for trivalent compounds (Elinder and Friberg, 1986). An elimination half-life of approximately 95 h has been estimated after occupational exposures (Kentner et al., 1995). Human health effects from antimony at low environmental doses are not well characterized, although respiratory and cardiovascular effects have been reported (ATSDR (Agency for Toxic Substances and Disease Registry), 1992; Sundar and Chakravarty, 2010). Studies have shown that most of the antimony that enters the body concentrates in the liver, lungs, intestines, and spleen (ATSDR (Agency for Toxic Substances and Disease Registry), 1992). It also rapidly accumulates in blood cells following *in vivo* administration (Winship, 1987). Several *in vitro* studies report that antimony generates reactive oxygen species (ROS) and activates caspase-3 leading to cell apoptosis in human leukocyte cell lines (Lecureur et al., 2002a, 2002b; Losler et al., 2009; Wyllie and Fairlamb, 2006).

In this study, we conducted a cross-sectional analysis to examine the association of urinary antimony concentrations with leukocyte telomere length (LTL) in a representative sample of US adults (20 years of age and older) who participated in the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2002.

## 2. Methods

### 2.1. Study population

NHANES is a cross-sectional, nationally representative survey of the non-institutionalized civilian population of the United States conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention (NCHS, CDC). Beginning in 1999, the survey has been conducted continuously and released in 2-year cycles. For our study we merged the publicly available files for NHANES cycles 1999–2000 and 2001–2002 using the NCHS recommendations (Johnson et al., 2013). The survey employs a multistage stratified probability sample based on selected counties, blocks, households, and persons within households.

NCHS-trained professionals conducted interviews in participants' homes, and extensive physical examinations, including blood and urine collection, were conducted at mobile exam centers (MECs). All procedures were approved by the NCHS Research Ethics Review Board (Protocol #98-12 http://www.cdc.gov/nchs/nhanes/irba98.htm), and all participants provided written informed consent. The response rates for all of the examined person were 76.3% for NHANES 1999–2000 and 79.6% for NHANES 2001–2002 (http://www.cdc.gov/nchs/data/nhanes/analytic_guidelines_11_12.pdf).

### 2.2. Leukocyte telomere length (LTL) measurements

Aliquots of purified DNA, isolated from whole blood, were provided by NCHS. The LTL assay was performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco, using the quantitative polymerase chain reaction. Briefly, the method measures the ratio of telomere length (T) relative to standard (S) single-copy gene reference DNA, known as the T/S ratio (Cawthon, 2002; Lin et al., 2010). Each sample was assayed three times on three different days. The samples were assayed in duplicate wells, resulting in six data points. Control DNA values were used to normalize between-run variability. Runs with more than four control DNA values falling outside 2.5 standard deviations from the mean for all assay runs were excluded from further analysis ( $<6\%$ of runs). For each sample, potential outliers were identified and excluded from the calculations ( $<2\%$ of samples). The CDC conducted a quality control review before linking the LTL data to the NHANES public-use data files. The formula $3274+ 2413^{*}(T/S)$ was used to convert T/S ratio to base pairs (bps) (http://wwwn.cdc.gov/Nchs/Nhanes/2001-2002/TELO_B.htm).

### 2.3. Urinary biomarkers

Urinary antimony and urinary lead levels were measured by inductively coupled plasma-mass spectrometry using a multielement analytical technique at the CDC, National Center for Environmental Health (NCEH), Division of Laboratory Sciences (DLS). The level of detection (LOD) for urinary antimony and urinary lead were 0.04 and 0.10 ng/mL, respectively. Urinary concentrations of antimony and lead below the LOD were assigned the LOD divided by the square root of 2, as recommended by NCHS (Johnson et al., 2013). Only 5% of individuals in the population had non-detectable levels of urinary antimony. Urinary antimony was categorized as weighted quartiles based on the distribution of urinary levels among the study population, resulting in approximately the same number of participants within each quartile.

Urinary lead (as natural log-transformed variable) was entered into the models because it has been associated with antimony exposures (ATSDR (Agency for Toxic Substances and Disease Registry) 1992; Sundar and Chakravarty, 2010). To account for variation in dilution in spot urinary samples, urinary creatinine was entered into the analyses as an independent variable in all the models as suggested (Barr et al., 2005). Urinary creatinine was determined using a Jaffe rate reaction measured with a CX3 analyzer and was entered into the model as a natural log-transformed variable.

### 2.4. Statistical analysis

LTL was not normally distributed, thus it was natural log-transformed. Analyses were performed using the weights from the urinary heavy metals subsample as recommended by NCHS and were calculated according to NHANES guidelines (Johnson et al., 2013). SAS-Callable SUDAAN 10 (Research Triangle Institute, Research Triangle Park, NC) was used to account for the NHANES complex sampling design. All tests were two sided, and $p \leq 0.05$ was the level of significance. We ran three models: Model 1 was adjusted for urinary creatinine, age (years) and age squared; Model 2 was further adjusted for sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or Others), education (less than high-school, high school graduate, or some college and above), alcohol consumption, self-reported smoking status (current, former, or never smoker), serum cotinine (natural log-transformed), and body weight status (underweight/normal, overweight, or obese); and, Model 3 was further adjusted for urinary lead (natural log-transformed).

Preliminary analyses found a significant interaction term between antimony and age-groups ($p=0.03$); therefore, we performed analyses by age-group stratification. The following groups were used: young adults (ages 20–39 years), middle aged adults (age 40–59 years), and older adults (age 60 and older). A sensitivity analysis was conducted using adjustment for lymphocyte and granulocyte proportions in whole blood because telomere length in the leukocyte population differs by cell subset (Weng et al., 2001). Since our dependent variable LTL was log-transformed, the results were re-transformed by exponentiation of the $\beta$ coefficients and presented as percent differences estimated by comparing each of the upper three quartiles to the lowest quartile using the formula $100^{*}(e^{\beta}-1)$; statistical tests for linear trends