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Crotonaldehyde exposure in U.S. tobacco smokers and nonsmokers: NHANES 2005–2006 and 2011–2012

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ABSTRACT

Introduction: Crotonaldehyde is an α,β-unsaturated carbonyl compound that is a potent eye, respiratory, and skin irritant. Crotonaldehyde is a major constituent of tobacco smoke and its exposure can be quantified using its urinary metabolite N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMM). A large-scale biomonitoring study is needed to determine HPMM levels, as a measure of crotonaldehyde exposure, in the general U.S. population.

Materials and methods: Urine samples were obtained as part of the National Health and Nutrition Examination Survey 2005–2006 and 2011–2012 from participants who were at least six-years-old (N = 4692). Samples were analyzed for HPMM using ultra performance liquid chromatography - tandem mass spectrometry. Exclusive tobacco smokers were distinguished from non- tobacco users through a combination of self-reporting and serum cotinine data.

Results: Detection rate of HPMM among eligible samples was 99.9%. Sample-weighted, median urinary HPMM levels for smokers and non-users were 1.61 and 0.313 mg/g creatinine, respectively. Multivariable regression analysis among smokers showed that HPMM was positively associated with serum cotinine, after controlling for survey year, urinary creatinine, age, sex, race, poverty level, body mass index, pre-exam fasting time, and food intake. Other significant predictors of urinary HPMM include sex (female > male), age (children > non-user adults), race (non-Hispanic Blacks < non-Hispanic Whites).

Conclusions: This study characterizes U.S. population exposure to crotonaldehyde and confirms that tobacco smoke is a major exposure source. Urinary HPMM levels were significantly higher among exclusive combusted tobacco users compared to non-users, and serum cotinine and cigarettes per day were significant predictors of increased urinary HPMM. This study also found that sex, age, ethnicity, pre-exam fasting time, and fruit consumption are related to urinary HPMM levels.

1. Introduction

Crotonaldehyde (2-butenal), an α,β-unsaturated carbonyl compound, is a colorless liquid with a pungent odor. It exists as the *cis* and the *trans* isomers; commercial crotonaldehyde consists of > 95% *trans* isomer (IARC, 1995). It is mainly used in the manufacturing of sorbic acid and n-butanol. It is a potent eye, respiratory, and skin irritant (Coenraads et al., 1975). The occupational short term exposure limit (STEL) for crotonaldehyde is 0.3 ppm according to the American Conference of Governmental Industrial Hygienists (ACGIH, 2015).

Crotonaldehyde reacts with deoxyguanosine in DNA to generate 1,N^2-propanodeoxyguanosine adducts that may lead to genetic mutations (Chung et al., 1984). These adducts have been found in human lung tissues (Zhang et al., 2006). In rats, crotonaldehyde forms non-neoplastic and neoplastic liver lesions including hepatocellular carcinomas (Chung and Hecht, 1986). However, no human data associates carcinogenicity with crotonaldehyde exposure; thus the International Agency for Research on Cancer classifies the compound as group 3, not classifiable as to its carcinogenicity in human (IARC, 1995). In contrast, the U.S. Environmental Protection Agency (EPA) lists crotonaldehyde as a possible human carcinogen (group C) based on limited animal data and supporting genotoxicity data (EPA, 1991).

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A major source of crotonaldehyde exposure is cigarette smoke (Counts et al., 2004). The amount of the compound in cigarette smoke varies from 1 to 53 μg per cigarette, depending on the machine smoking protocol used for measurement and the cigarette brand filter ventilation (Pazo et al., 2016). Crotonaldehyde is also found in smokeless tobacco, engine exhaust, and wood combustion (Destaillats et al., 2002; IARC, 1995; Masiol and Harrison, 2014; Stepanov et al., 2008). Crotonaldehyde occurs naturally in many foods (Feron et al., 1991; Kensler et al., 2012), such as fruits (e.g., apples, guavas, grapes, strawberries and tomatoes), vegetables (e.g., cabbage, cauliflower, Brussels sprouts, carrots and celery leaves), dairy products (e.g., bread, cheese and milk), animal proteins (e.g., meat and fish), alcoholic beverages (e.g., beer and wine), heated cooking oils, and chips. Additionally, endogenous lipid peroxidation could result in crotonaldehyde exposures in humans (Nair et al., 2007; Niki, 2009; Voulgaridou et al., 2011). Crotonaldehyde can also form in vivo as a metabolite of N-nitrosopyrrolidine and 1,3-butadiene (Elfarra et al., 1991; Wang et al., 1988).

Crotonaldehyde is metabolized primarily to N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMM) and to a lesser extent, 2-carboxy-1-methylethylmercapturic acid, both of which are excreted via the urine in rats (Gray and Barnsley, 1971). The identification of HPMM as a major crotonaldehyde metabolite is supported by the HPMM structural homologue, N-acetyl-S-(3-hydroxypropyl)-L-cysteine (HPMA), being identified as a primary metabolite of crotonaldehyde's three carbon structural homologue acrolein (Parent et al., 1998). Urinary HPMM levels are proportional to crotonaldehyde exposure (Carmella et al., 2013), and it is a useful biomarker for smoking-related exposure (Scherer et al., 2007). Cigarette smokers have higher urinary HPMM compared to non-smokers (Pluym et al., 2015; Scherer et al., 2007). Carmella et al. also demonstrated that urinary HPMM decreases significantly in the first three days after a smoker ceases smoking (Carmella et al., 2009).

Although there are studies on crotonaldehyde exposure among smokers, there are no large-scale biomonitoring studies assessing exposure in the general population. Moreover, the effect of diet on crotonaldehyde exposure has not been assessed systematically. These gaps prompted us to examine crotonaldehyde exposure in a representative sample of the U.S. population. In this study, we measured HPMM concentrations in urine samples provided by participants in the 2005–06 and 2011–12 cycles of the National Health and Nutrition Examination Survey (NHANES). Multivariable regression models were used to determine the influence of demographic variables (e.g., age, sex, and race) on HPMM concentrations, as well as the effects of certain lifestyle factors, such as obesity, tobacco use, and diet. Thus, this biomonitoring study characterizes crotonaldehyde exposure in the U.S. population and explores different exposure sources and modifiers.

2. Material and methods

2.1. Study design

NHANES is a population-based survey designed to assess the health and nutritional status of adults and children in the United States (https://www.cdc.gov/nchs/nhanes/index.htm). The survey is based on cross-sectional observation of a complex, multistage probability sample representative of the civilian, non-institutionalized U.S. population. The survey collects questionnaire data, physical examination data, and biological samples. NHANES is conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The study protocol was reviewed and approved by a CDC institutional review board, and informed written consent is obtained from all study participants before they participate in the study.

Spot urine samples were collected from participants in two NHANES survey cycles—a one-half subsample of participants ≥ 12 years old from NHANES 2005–2006 and a one-third subsample of participants ≥ 6 years old from NHANES 2011–2012—and were measured for HPMM to determine crotonaldehyde exposure.

2.2. Chemical analysis

The collected urine samples were stored at −70 °C until analysis. Urinary HPMM concentrations were measured using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) according to a published procedure (Alwis et al., 2012). Briefly, urine samples were analyzed at 1:10 dilution (a mixture of 50 μL urine, 25 μL $^{2}H_{3}$-HPMM internal standard, and 425 μL 15 mM ammonium acetate, pH 6.8). Liquid chromatography was performed using an ACQUITY UPLC HSS T3 Column, 1.8 μm, 2.1 mm × 150 mm, with mobile phases containing 15 mM ammonium acetate, pH 6.8 (solvent A) and acetonitrile (solvent B). The eluate was ionized using ESI technique. The mass spectrometer was operated in scheduled multiple reaction monitoring (SMRM) mode for negative ions; mass-to-charge (m/z) transitions were monitored at 234→105 for HPMM and 237→105 for the internal standard, $^{2}H_{3}$-HPMM. Urinary HPMM concentrations were calculated from a linear calibration curve obtained by plotting the relative response factor (ratio of the peak area of native analyte to the peak area of the corresponding internal standard) as a function of the native standard concentration. The limit of detection (LOD) in urine was 2.0 ng/mL for HPMM (Alwis et al., 2012).

2.3. Statistical analysis

The crotonaldehyde metabolite HPMM was measured in spot urine samples collected from 5815 participants in the one-third environmental subsample of NHANES 2005–2006 and 2011–2012. Many of these study participants were likely exposed to crotonaldehyde as a component of tobacco smoke; therefore we categorized tobacco smoke exposure based on a combination of questionnaire and serum cotinine data (Pirkle et al., 1996). Study participants were identified as exclusive users of combusted tobacco products (named “exclusive combusted tobacco users” or “exclusive tobacco smokers”) if they had serum cotinine > 10 ng/mL and responded “yes” to question SMQ680 (tobacco or nicotine use within 5 days prior to NHANES physical examination), “yes” to at least one of SMQ690A–SMQ690C (cigarettes, pipes, cigars), and “no” to all of SMQ690D–SMQ690F (smokeless tobacco and nicotine delivery products). Participants were identified as non-users of tobacco products if they answered “no” to either SMQ680 or SMD020 (smoked 100 cigarettes in life), or answered “never smoked cigarettes regularly” to SMD030 (age started smoking regularly). Non-users were confirmed by a serum cotinine measurement ≤10 ng/mL. Alternatively, participants missing responses for SMQ680, SMD020, and SMD030 were classified as non-users if they had serum cotinine ≤10 ng/mL. Participants were excluded from analysis because of missing serum cotinine data (N = 284), for not having answered SMQ680 (230 participants), or missing data for other variables used in the regression models (N = 609), leaving 4692 study participants eligible for statistical analysis.

Reported results met the accuracy and precision specifications of the quality control/quality assurance program of the CDC National Center for Environmental Health, Division of Laboratory Sciences (Caudill et al., 2008). Measurements below the limit-of-detection (LOD) were imputed with the quotient of the LOD divided by the square root of two (Hornung and Reed, 1990).

Because NHANES participants are recruited through a multistage sampling design, it is necessary to account for this complex design to estimate variances properly and to produce unbiased, nationally representative statistics. Robust estimation may be accomplished by applying survey sample weights to each participant's data and using Taylor series linearization to produce variance estimates. We used this estimation approach as it was implemented in the DESCRIPT subroutine of the statistical software package SUDAAN®, Version 11.0.0 (Research