ABSTRACT

COMPARISON OF ACRYLAMIDE EXPOSURE BIOMARKERS IN CHILDREN AND ADOLESCENTS USING NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) 2003-04 AND 2015-16

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Acrylamide (AA) is an important industrial chemical of occupational concern due to its neurotoxicity and probable carcinogenicity; it is also a tobacco burning product and thus contributes to health concerns in smokers. More recently it was discovered to be thermally generated in the cooking of starch-rich foods, creating a potentially wider public health concern. Children and adolescents are a particularly vulnerable group because they consume more acrylamide-rich foods compared to adults. In addition, they are still going through important developmental stages. This study examines AA and its metabolite glycidamide (GA) using hemoglobin adduct biomarkers (HbAA and HbGA respectively) from the U.S. children (6-11) and adolescents' (12-19) National Health and Nutrition Examination Survey's 2003-04 (n=2814) and the 2015-16 (n=697). The study investigated changes in exposure over time and examined the contribution of potential modifiers including smoking status, race/ethnicity, poverty-to-income ratio, sex, and age. All HbAA and HbGA are reported as pmoL adduct per gram Hb (pmol/G Hb).

Overall, HbAA biomarkers significantly ($p<0.0001$) declined from 2003-04, GMs (95% CI) (57.9 [55.7, 60.1] pmol/G Hb) versus (42.8 [41.4, 44.2] pmol/G Hb) in 2015-16 for all ages, with similar

reductions observed in the individual children and the adolescent groups. Smokers had a higher burden of HbAA biomarkers than non-smokers, and with a significant reduction in numbers of smokers from 2003-04 to 2015-16, this likely contributes to the reduction in overall exposure. When non-smokers only were examined, a significant ($p<0.0001$) decrease in HbAA was still observed, from 2003-04 GMs (95% CI) (53.4 [52.0, 54.9] pmol/G Hb) versus (41.2 [40.2, 42.2] pmol/G Hb) in 2015-16, suggesting an additional contribution of changes in AA levels in food or frequency of high-risk food consumption. Similar statistically significant reductions were seen for both children and adolescent groups separately.

HbGA is a marker of AA biotransformation to GA, which is a more mutagenic metabolite of AA. The ratio is of HbAA:HbGA is a phenotypic marker of mutagenic risk. In non-smokers, there was a significant ($p=0.001$) difference in the HbAA:HbGA ratio in children GMs (95% CI) (0.8 [0.8, 0.8] pmol/G Hb) at 2003-04 and (0.9 [0.9, 1.0] pmol/G Hb) at 2015-16 versus adolescents (1.0 [1.0, 1.1] pmol/G Hb) at 2003-04 and (1.1 [1.0, 1.2] pmol/G Hb) at 2015-16, respectively, suggesting children may be at greater risk to the mutational effects of AA exposure compared to adolescents. In multivariate regression analysis of non-smokers only, age and race significantly contributed to the HbAA biomarker levels, with higher HbAA in younger age groups and in non-Hispanic black participants, highlighting a disparity in exposure pattern.

Overall, AA exposure seems to have reduced from 2003-04 to 2015-16; the reduction is driven by both changes in smoking but also diet. The young and non-Hispanic black participants remain at highest risk of exposure and potential health effects from exposure to AA.

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By

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Dedication

This thesis is dedicated to the children and youth of our world, especially those who are vulnerable and in need of a voice.

Acknowledgements

I would like to acknowledge Dr. Turner, who has been my close advisor and has mentored me throughout my graduate program. I would also like to thank Dr. Kadry, Dr. Dallal, and Dr. Cruz-Cano, who have been very patient with me and have helped guide me along my academic journey.

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List of Abbreviations

AA	Acrylamide
AAMA	N-acetyl-S-(2-carbamoylethyl)-l-cysteine
GA	Glycidamide
GAMA	*N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-cysteine
GM	Geometric mean
HbAA	Hemoglobin-acrylamide adduct
HbGA	Hemoglobin-glycidamide adduct
MA	Mexican Americans
NHA	Non-Hispanic Asians
NHB	Non-Hispanic Blacks
NHANES	National Health and Nutrition Examination Survey
NHW	Non-Hispanic Whites
PIR	Poverty-to-income ratio
SC	Survey cycle
SHS	Second-hand smoke
WHO	World Health Organization

Chapter 1: Introduction & Objectives

1.1 Introduction

Acrylamide (AA) is an industrial chemical, that is additionally found in tobacco smoke and produced by some carbohydrate-rich foods during cooking (Hagmar et al., 2005; Urban et al., 2006; Becalski et al., 2003; U.S. EPA, 2010, Mojska et al., 2007; Stadler et al., 2002; Tareke et al., 2002). AA is a proven human neurotoxin, is carcinogenic in animal models, and is a suggested human carcinogen; thus, is both an occupational hazard from industry, and a public health concern related to behavior and diet (Hagmar et al., 2005; Urban et al., 2006; WHO, 2011).

An age-dependent relationship in levels of AA in blood and urine biomarkers for the U.S. population has been reported in U.S children (Vesper et al., 2010; Tran et al., 2010; Duke et al., 2017); and adults (Vesper et al., 2013; Jain et al., 2015a) with AA exposure generally decreasing with increasing age due to food consumption patterns and differences in biotransformation processes. However, education about AA sources in the diet combined with reductions in tobacco use may have modulated overall AA exposure among U.S. children and adolescents over the past decade, which could be examined using AA exposure biomarkers, such as AA-hemoglobin (HbAA) and metabolite adduct glycidamide (GA)-hemoglobin (HbGA) (WHO, 2011; Abt et al., 2019; Barber et al., 2001; Cornelius, 2020; CDC, 2023a).

In this study AA and GA adducts in blood of U.S. children (6-11) and adolescents (12-19) were analyzed by comparing the first National Health and Nutrition Examination Survey (NHANES) survey cycle (SC), 2003-04, to the most recent NHANES SC, 2015-16. This study will provide data on exposure levels of HbAA and HbGA in U.S. children and adolescents over time and examine whether smoking or diet is driving the observed changes. The blood biomarker cotinine will be used to identify smokers for both children and adolescents in the study. Potential social determinants of these AA biomarkers that will be examined include sex, race/ethnicity, and poverty to income ratio (PIR). Changes in HbAA and HbGA biomarkers will additionally be examined in smokers and in non- smokers to assess differences in the two SCs.

The objective of this thesis is to study AA exposure by smoking status across children and adolescents using NHANES measures of HbAA and HbGA for both the 2003-04 SC and the 2015-16 SC. It is hypothesized that AA exposure in children and adolescents has changed from 2003-04 to 2015-16 due to public health interventions to reduce AA concentration levels in food and to modify smoking exposures. To test this hypothesis, this thesis has three aims:

1.2 Aims

Aim 1. Provide descriptive data on acrylamide exposure using blood biomarkers (HbAA, HbGA, and HbAA:HbGA ratio), as well as cotinine, and children's and

adolescents' demographic variables (sex, race/ethnicity, and poverty-to-income ratio [PIR]) based on both NHANES 2003-04 and NHANES 2015-16 SCs.

Aim 2. Determine if there is a difference in HbAA, HbGA, and HbAA:HbGA ratio in smoking and non-smoking children and adolescents between NHANES surveys 2003-04 and 2015-16.

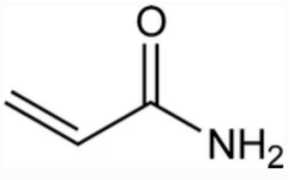
Aim 3. Examine the determinants of each AA biomarker, by survey, in non-smokers.

Chapter 2: Background

2.1 What is AA

AA is a colorless, odorless, hydrophilic crystalline industrial compound with a chemical formula of C_3H_5NO and a molecular weight of 71.08 grams per mol (g/mol) (Figure 1) (Lewis, 2007; HSDB 2009; Kroschwitz, et al., 2004). AA has a melting point of 84.5^oC and a boiling point of 136^oC (Smith et a., 1996). AA is semi-volatile and can react violently when melted (Lewis, 2007). It also has high mobility in soil and groundwater, and does not bioaccumulate (U.S. EPA, 1994). Due to its polymerization properties, AA is commonly used to produce polyacrylamide, for use of wastewater treatment, paper pulp production, cosmetic use, and gel electrophoresis (Siyam, 2001; Amended, 2005; Yeng et al., 2004; Raymond & Weintraub et al., 1959). Occupational exposures generally occur through inhalation or skin contact, and in the U.S., OSHA has set an airborne permissible level to be an average of 300 $\mu g/m^3$ over an 8-hour work shift and NIOSH has set the airborne exposure limit of 30 $\mu g/m^3$ over a 10-hour work shift (OSHA, 2022). This exposure level limit also considers dermal absorption route. Outside of potential occupational exposures, food and tobacco smoke inhalation are estimated to be the main sources of AA exposure for the general population (Kenwood et al., 2022; Virk-Baker et al., 2014).

Figure 1. Chemical structure of Acrylamide

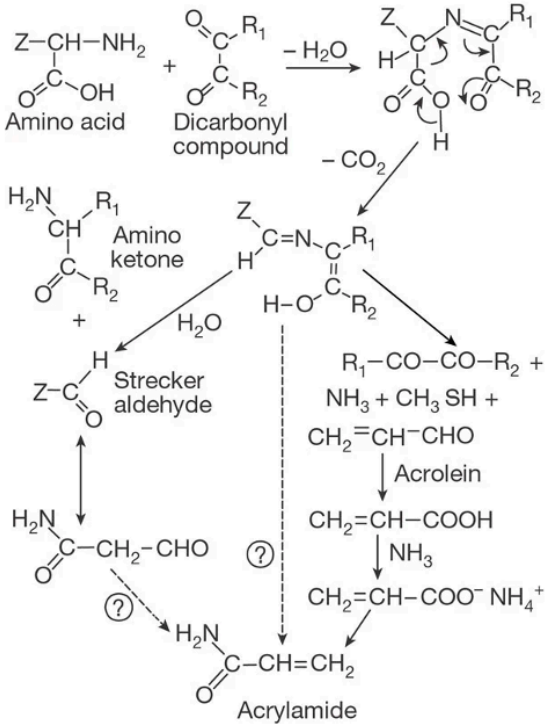


Note. Reprinted from Krska et al. (2012). *Analytical and Bioanalytical Chemistry, 402(1), 139–162*

2.2 Formation of AA in food and tobacco

AA was first reported in food by the Swedish National Food Administration and the Stockholm University in Sweden (Tareke et al. 2000, 2002; Rosén & Hellenäs et al., 2002). When naturally occurring asparagine-rich foods, such as potatoes, asparagus, coffee, almonds, cereals, breads, and crackers, are heated above 120 ^{o}C in conditions with humidity below 30%, such as frying, roasting, and baking, they react with reducing sugars like fructose and glucose to produce AA (Stadler et al., 2002; Becalski et al., 2003; Amrein et al., 2005; Lantz et al., 2006; Mojska et al., 2007; Ferrer-Aguirre et al., 2016; U.S. FDA, 2022a; U.S. FDA 2022b). As seen in Figure 2, this chemical reaction is known as the Maillard reaction, which is an important culinary process that contributes to the flavor, aroma, and color of food (Mottram et al., 2002; Stadler et al., 2002; Abt et al., 2019). Furthermore, Becalski et al. (2011) studied the presence of AA in simulated plum juice and found that the chemical compound formed even under drying conditions, which was defined as being below 100^{o}C for 24 hours. Together, these findings demonstrate the complexity of AA formation from temperature and environmental conditions in certain foods.

Figure 2. Acrylamide formation in food via the Maillard Reaction



Note. Reprinted from Mottram et al. (2002). Nature, 419(6906), 448–9.

For dietary exposure, the current major food sources of AA are pretzels, which contain an average 470 µg/kg, followed by fried potatoes (390 µg/kg), teething biscuits (380 µg/kg), corn/tortilla chips (360 µg/kg), and crackers (340 µg/kg) (U.S. FDA, 2022b). These foods are consistently consumed more frequently by children compared to adults, therefore potentially exacerbating the health effects of AA and its metabolite GA.

AA is a well-known contaminant of tobacco smoke and occurs during the combustion reaction; cigarette smoke exposure yields range between 1.1 and 2.34 µg/cigarette (Smith et al., 2000). Smoking as a source of AA exposure for children and

adolescents due to direct inhalation, as well as secondhand smoking contributes to AA exposure. This data outline just how influential smoking is on AA exposure and how important it is for researchers to measure AA exposure by cotinine levels. Additionally, AA can also form in smokeless tobacco products (STP); Mcadam et al. (2015) reported AA in Swedish loose and portion snus, US snus, chewing tobacco, moist snuff, dry sniff, soft pellet, hard pellet, and plug. The study found that average levels of acrylamide by type of STP were not significantly different ($p > 0.05$) except for US snus which had, on average, greater levels but with a wide range of individual levels depending on the manufacturer. This finding reveals how manufacturing processes are crucial for AA reduction. The authors concluded that consumption of STPs is minimal compared with exposure from food consumption or cigarette smoke.

2.3 Toxicokinetics of AA

Absorption

AA is a low molecular weight organic compound that is readily absorbed into the body through ingestion, inhalation, and dermal exposure (Ramsey et al., 1984; Kadry et al., 1999; Sumner et al., 2003; Kroschwitz et al, 2004; Lewis, 2007). Orally administered, AA is quickly and extensively absorbed from the gastrointestinal tract; Miller et al. (1982) reported that 90% of acrylamide was recovered from tissues and bodily fluids of Fischer-344 rats after orally administering 10,000 μg/kg body weight of AA.

Ramsey et al. (1984) studied rats' dermal absorption of AA and observed that 25% of 2,000 or 50,000 μg/kg bw doses of AA was absorbed within 24 hours. This study exemplifies how skin acts as a partial barrier against AA absorption. Additionally, Frantz et al. (1985) found that for dermal absorption of AA in Fischer 344 rats, 26% of a 1% aqueous solution was absorbed within 24 hours, with 35% remaining on the skin for potential further absorption.

In some occupational settings, there is a risk for vapor inhalation of AA. Additionally, significant AA absorption also occurs through smoking. Sumner et al. (2003) studied the biotransformation rate of AA via various exposure routes, including vapor inhalation and found that immediately or 24 hours following the 6-hour inhalation exposure to 2900 ug/kg of AA, rat blood cells had the highest relative level (~0.1 μmol/g tissue) of radioactivity compared with all other tissues. Plasma levels were higher immediately following exposure termination (0.03 μmol/g tissue) and reduced 24 hours later (0.004 μmol/g tissue). This study provides valuable insight into how AA is readily absorbed via inhalation route of exposure. This study also found that 56% of the inhaled dose in rats remained in the body 24 hours following exposure termination.

Fennel et al. (2005) studied the dermal and oral absorption rates of AA in humans; AA was administered orally in an aqueous solution (single dose of 500, 1,000, or 3,000 μg/kg bw by gavage) or dermally (3 doses of 3,000 μg/kg bw) to sterile male volunteers. The authors concluded that dermal uptake was approximately 6.6% of that

observed with oral uptake, exemplifying how AA absorption differs on exposure route and is more prominent in oral exposure compared to dermal.

Distribution

AA is widely distributed to all tissues, including the fetus of pregnant animals (Ikeda, 1985; Schettgen, 2004; Calleman et al.,1996) This is of concern due to the sensitive nature of neuronal development during this period of growth. Sumner et al., (2003) found that the rank order of relative (μg/g tissue) radioactivity in rats immediately following exposure was blood, testes, skin, liver, kidneys, brain, spleen, lungs, and epididymis (2003). After 24 hours, the ranking was blood, skin, spleen, lung, liver, kidney, brain, testes, epididymis, and fat. AA can also cross the blood-brain barrier (Ikeda 1985; Manson et al., 2005); in in utero, infancy and young children, the tight junctions of the brain are not fully formed, thus the effects of AA may be magnified. AA has also been detected in umbilical cord blood and breast milk in humans (Sörgel et al., 2002; Manson et al., 2005). These findings outline how vulnerable children are to the effects of AA.

Metabolism

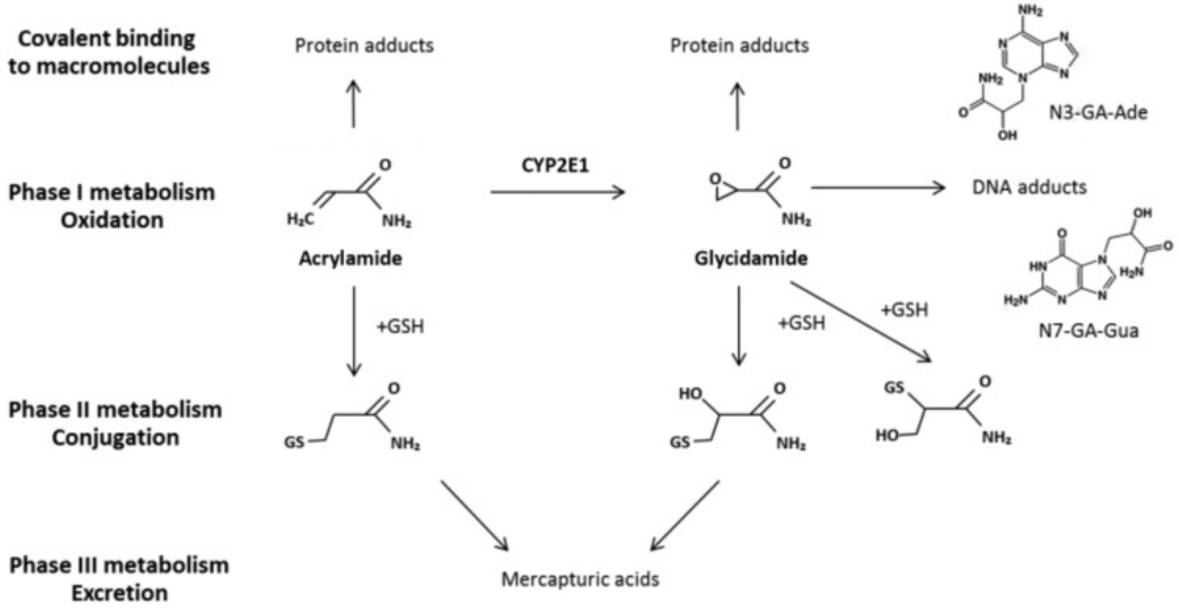
As seen in Figure 3, AA can undergo phase one and phase two metabolism and is primarily metabolized by two pathways: saturable epoxidation by cytochrome P450 and conjugation with GSH either nonenzymatically or catalyzed by glutathione-S-transferase (GST) (Dixit et al., 1980; Sumner et al., 1992). During saturable epoxidation by cytochrome P450, the highly reactive GA is produced, that is known

for its DNA adduct formation, specifically it can bind to deoxyguanosine and deoxyadenosine (Kotova et al., 2011). For this reason, measuring GA is also of interest in this study. The percentage of AA that is converted to GA varies from species to species due to differences in genetic polymorphic enzymes (Settels al., 2008; Paulsson et al., 2005; Duale et al., 2009; Vesper et al., 2010; Arribas & Morales, 2012). GA and metabolites derived from it can account for 33 to 59% of the total metabolites excreted in rat and mouse urine, respectively, within 24 hours (Toxicology, 2010). This is important from a public health perspective because of GA's high reactivity and its ability to cause DNA mutation, which is associated with cancer. Thus, analyzing the HbAA:HbGA ratio allows for a measurement of the rate of biotransformation.

Furthermore, during conjugation by glutathione-S-transferase (GST), one of the common urinary metabolites produced is the mercapturic acid N-acetyl-S-(2-carbamoylethyl)-l-cysteine (AAMA) (Sweeney, 2010). GA is also commonly conjugated with GSH by GST to produce another mercapturic acid called *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-cysteine (GAMA) (Fennel et al., 2005; Sweeney 2010). Both compounds are then excreted through urine.

Figure 3.

Metabolism of Acrylamide.



Note. Reprinted from Katen & Roman, 2015, Mutation Research, 777, 91–100.

Nicotine's Effects on AA biotransformation

Nicotine, a chemical compound notably found in tobacco products, may impact the function of certain CYP450 genes, and thus, affect the ability for an individual to metabolize AA (Lee et al., 2009). This is important to consider for this study because children and adolescents may be exposed to cigarette smoking either directly or indirectly. Nicotine appears to affect the function of CYP2E1; Lee et al. (2009) studied nicotine exposure on the metabolism of chlorpyrifos in male Sprague-Dawley rats and found that the total P450s were not altered by nicotine treatment, but marker substrate activities for CYP2E1 were increased 24 hours after a single treatment. These findings suggest that nicotine could affect AA metabolism as it uses the same P450s. Ferguson et al. (2013) studied the effects of nicotine treatment and its effects

on brain activity in African green monkeys and found that nicotine induced CYP2E1 activity, in particular brain regions, which may contribute to altered drug efficacy, neurotoxicity, and metabolic tolerance. However, in a human study conducted by Hukkanen et al. (2010), CYP2E1 was not affected by nicotine. These differences in studies indicate variation among nicotine metabolism by species and/or dose and thus should be cautiously interpreted.

Excretion

AAMA and GAMA are the more commonly excreted mercapturic acid metabolites of AA; approximately 50% of the ingested dose of AA is eliminated in the form of AA-MA (Boettcher et al., 2006; Fennell et al., 2005). Furthermore, previous studies in mice and rats estimated that 40-70% of AA is excreted in urine within 24 hours (Hashimoto and Aldridge, 1970; Miller *et al*., 1982; Sumner et al*.,* 1992).; Sumner et al., 2003).

2.4 AA Biomarkers for measurement

Because they are commonly found in urine, biomarkers of exposure used for measuring AA include urinary biomarkers, such as the mercapturic acids AAMA and GAMA. This method is often preferred because it is less costly and invasive, but it only reflects short term exposure (Ruenz et al., 2016; WHO, 2011). These urine biomarkers were not used for this study; rather blood biomarkers were preferred because they capture a more integrated measure of typical exposure over months.

The hemoglobin adducts of acrylamide (HbAA) and glycidamide (HbGA) are useful and frequently utilized measurements for long-term exposure assessment (up to 120 days) (Fennel et al., 1992). However, because the same level of hemoglobin adducts can be produced by a single exposure or a repeated exposure over an extended period if the cumulative exposure is the same, this poses a limitation on the reliability of using these biomarkers for dose-response modeling, where the amount and frequency of AA exposure may more variable (WHO, 2011). This may be a concern for acute high dose exposure of AA, such as in occupational settings. However, for children and adolescents this is not of great concern, given that they are mostly exposed to AA in low chronic amounts from diet, and in some cases, smoking.

2.5 Health Outcomes

Genotoxicity and Mutagenicity

AA is an effective clastogen but a weak mutagen, whereas GA is a clastogen and a mutagen, which has been demonstrated by the Ames test (Hashimoto and Tanii, 1985; WHO, 2011). The most common mutagenicity effects associated with AA exposure are DNA adduct formation, such as N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-guanine), N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-adenine), N1-(2-carboxy-2-hydroxyethyl)deoxyadenosine (N1-GA-dA) and N6-(2-carboxy-2-hydroxyethyl)deoxyadenosine (N6-GA-dA), the latter being a rearrangement product of the N1-GA-dA adduct (Gamboa da Costa et al., 2003; Segerbäck *et al.*, 1995; Kotova et al., 2011; Hansen et al., 2018; Benford, 2022)

Carcinogenicity

The Environmental Protection Agency (EPA) has classified AA as a B2 probable human carcinogen; this occurs via the mutagenic mode of action where adducts are formed in DNA, which can alter the function of these integral structures (Acrylamide, 2010). Several forms of cancers have been identified as potentially being associated with AA exposure. For example, Johnson et al. (1986) found an increase in tumor incidences in female mice CNS, oral cavity, uterus, and pituitary and male adrenals at doses above 2000 μg/ kg bw/day. However, this far exceeds general exposure for the U.S. population.

Neurotoxicity

Neurological effects, such as ataxia and skeletal muscle weakness of AA have been observed in monkeys with repeated exposure as low as 500 μg/kg bw/day (McCollister et al., 1964). Liu et al. (2022) recently reported an association between chronic AA exposure at a dose of 5000 μg/ kg bw/day for 12 months and an increased risk in Sprague Dawley rats developing Parkinson's disease. Neurological symptoms range from gait abnormalities, hands and feet numbness to muscle weakness and ataxia (Mariano et al., 2013). The Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Committee concluded that while adverse neurological effects are unlikely at the estimated average exposure, AA can exacerbate morphological changes in nerves for individuals with high exposure (2011). Neurotoxicity involves mostly the peripheral nervous system, but also affects the central nervous system (Mariano et al., 2013).

Reproductive Toxicity

Although the WHO reported no reproductive effects of AA are known in humans, there are published peer-reviewed studies that have reported growing evidence for reproductive effects (2011). For example, Ma et al. (2011) treated weaning male rats with various doses (0, 5000, 15000 or 30000 μg /kg/day) to study reproductive effects and found a decrease in luteinizing hormone and an increase in testosterone and follicle-stimulating hormone.

Camacho et al. (2012) studied the effects of AA on male Fischer 344 rats and found a decrease in testosterone at a dose of 50000 μg/kg bw/day significantly altered the histology of Leydig and germ cells. Furthermore, Chu et al. (2020) used the NHANES 2003-2004 SC to study the association between AA exposure and sex hormones in U.S. males. The authors concluded that a positive association was observed between AA exposure and several sex hormones in men, such as inhibin B and sex hormone binding globulin, and HbGA was positively associated with Anti-Mullerian Hormone. However, no relationship was observed between AA exposure and other sex hormones, such as testosterone, estradiol, and androstenedione glucuronide.

Developmental Toxicity

Additionally, Park et al. (2021) used zebrafish to study the relationship between AA and developmental effects at doses of 10,000, 30,000, 100,000, or 300,000 μg/L and found that AA caused developmental effects, such as yolk retention, scoliosis, swim

bladder deficiency, and curvature of the body. Significant developmental effects, such as body weight decrease have also been observed in Fischer 344 rats at doses as low as 1000 μg/kg bw/day (Garey et al., 2005). Furthermore, Huang et al. (2018b) also found that AA causes severe cardiac deficits in the developmental stages of zebrafish via disordered expression of cardiac genes and a reduction in myocardial and endocardial cells, which led to developmental disruptions of the ventricle and atrium, along with a lack of differentiation of the atrioventricular canal. Additionally, there is also research showing a connection with acrylamide uptake from food during pregnancy and low birth weight (Mojska et al., 2020); Duarte-Salles et al. (2013) studied the effects of AA intake of pregnant women and found that intake was negatively associated with fetal growth.

Other Health Effects

Although not specific to children and adolescents, several studies have also investigated other potential health effects of AA exposure, such as lipid level alteration, cardiovascular disease, chronic obstructive pulmonary disease, allergies, and depressive symptoms. Using macrophages, dermal cells and zebrafish, Kim et al. (2015) investigated the physiological effects of AA on lipoprotein metabolism and found that it had an effect that resulted in exacerbation of atherosclerosis. Furthermore, Cheang et al. (2021) studied lipid levels and AA among adults over 20 years of age using NHANES 2013-2016 SC. The study reported having found an association between AA and atherosclerotic lipid changes, such as a positive correlation with triglycerides, and a negative association between low density

lipoprotein-cholesterol. The authors determined that AA and its metabolites may be associated with abnormal lipogenesis and fatty acid bioconversion pathways.

In an epidemiological study using NHANES 2003-06 SCs, Zhang et al. (2018) analyzed the associations between HbAA. HbGA, and HbAA:HbGA ratios for adults ≥ 20 years of age and their association with cardiovascular disease (CVD). The authors reported that in people exposed to environmental tobacco smoke, HbGA and HbAA:HbGA ratios were significantly and inversely associated with the prevalence of total CVD after adjusting for several covariates. They concluded that AA biomarkers are significantly associated with CVD in smokers and those exposed to environmental tobacco smoke but not in nonsmokers. Huang et al. (2018a) also used 2003-06 NHANES data to measure the relationship between HbAA, HbGA, and HbAA:HbGA and CVD mortality among U.S. adults ages ≥ 25 years of age and found that HbAA and HbAA:HbGA were inversely associated with all-cause mortality and CVD mortality.

Using the NHANES 2013-16 SC, Li et al. (2021) studied the association between chronic obstructive pulmonary disease and AA exposure in individuals ages 20-80 years old and reported that even after adjusting for serum cotinine levels, the HbAA:HbGA ratio significantly increased the risk for COPD. However, it is unclear how AA ingestion is associated with lung health. Guo et al. (2017) used NHANES 2005-2006 SC to study AA exposure and its relationship to allergy-related outcomes in children (≤ 18 years of age) and adults (≥18 years of age) and observed an

association between increased likelihood of eczema and HbAA levels. Additionally, HbGA levels were associated with an increased likelihood of itchy rash and eczema.

2.6 Mitigation Efforts for AA Exposure

Several efforts have been internationally implemented to educate consumers and manufacturers about how to reduce AA exposure; this has been accomplished by both food industries and government authorities (WHO, 2011; U.S. FDA, 2016). Practices that can be modified that may influence glucose, fructose, and asparagine concentration and thus affect AA formation include variety selection/genetic modification, production practices and environmental conditions (e.g., soil temperature, water availability), storage conditions (e.g., temperature), and processing practices (Rosen et al., 2018). Additionally, some food additives, such as acids, amino acids, divalent cations, and food antioxidants may also help reduce AA concentration in food (WHO, 2011).

Efforts to decrease cigarette smoking in the U.S. population have been successful thus far; Cornelius et al. (2020) reported that tobacco smoking has decreased from 20.9% in 2005 to 12.5% in 2020. This may have a positive effect on reducing child and adolescent exposure to both the harmful effects of direct and indirect (e.g., SHS) tobacco exposure, and thus reduce AA exposure overall.

2.7 Health Equity

Social determinants of health (SDH) are the conditions in which people are born, grow, work, live, and age, and the wider set of forces and systems shaping the conditions of daily life, that impact quality of life outcomes and health outcomes (WHO, 2023). An individual's health can be influenced by the demographic variables of interest for this thesis, such as age, sex, race/ethnicity, and PIR through discrimination, policies implemented, and health inequity. Thus far, research on these demographic variables and their association with AA exposure is mostly limited to adults in the U.S. Nonetheless, the current research available provides valuable insight into potential health disparities and is discussed extensively throughout this thesis. Additionally, this thesis expands the current scope of knowledge for this area of research by interacting demographic variables with child and adolescent AA and GA exposure.

2.8 Demographic Variables

Age and its association to AA exposure

Several studies indicate that children are at greater risk for higher AA exposure than adults because on average children consume more french fries, cookies, and cereals, and these foods are known to be high in AA (Cengiz & Gündüz, 2013; Von Mühlendahl & Otto, 2003). Difference in exposure by age, as well as food choices outlines the importance these factors have on exposure and thus support the need to investigate AA exposure among children and adolescents.

Using NHANES 2003-04 SC for people ≥3 years of age, Vesper et al. (2010) concluded that for non-smokers the covariate-adjusted geometric mean (GM) (95% CI) for HbAA and HbGA levels were highest for children 3-11 years of age (54.5 [52.4-56.8] pmol/ G Hb; 73.9 [71.3-76.6] pmol/ G Hb respectively) and lowest in adults ≥ 60 years HbGA (46.2 [44.3-48.2] pmol/ G Hb; 41.8 [38.7-45.2] pmol/ G Hb respectively). The covariate adjusted GMs (95% CI) for HbAA:HbGA ratios were also highest among children 3-11 years of age (1.62 [1.39-1.87] pmol/ G Hb), whereas adults ≥ 60 years old (0.63 [0.51-0.78] pmol/ G Hb) had the lowest levels. These findings may be due to differences in AA metabolism in these age groups; metabolism and clearance of drugs in children are higher compared to adults because children have a greater liver-to-body weight ratio and more blood flow to the liver compared to adults (Gibbs et al., 1997).

Also, using 2003-04 NHANES SC for individuals ≥ three to 85 years of age, Duke et al. (2017) reported that children (3-12 years old) had the second highest HbAA levels and had significantly higher ($p<0.001$) HbGA when compared to adolescents, adults, and seniors. Also, HbAA:HbGA ratio for children was significantly higher ($p<0.001$) than for adolescents, adults, and seniors. These findings suggest potential differences in exposure and in biotransformation processes, with children having greater exposure to AA.

Sex and its association to AA exposure

Most recent AA studies interacted with sex have indicated potential genetic differences among males and females. However, this topic is still developing, and more studies are needed. Thus far, only one study was identified that has investigated the interaction between AA exposure and sex for U.S. children (6-11 years old): Jain et al. (2015b) found that girls had slightly higher GMs (95% CI) for both AAMA and GAMA compared to boys. However, the findings were not statistically significant (AAMA, p=0.92; GAMA,p=0.68). More research is needed in this field to conclude. For adults, Vesper et al. (2010) found that the GM (95% CI) for HbAA was higher in men (51.7 [49.6-53.8] pmol/ G Hb) compared to women (50.3 [49.1-51.5] pmol/ G Hb), but the GM (95% CI) for HbGA and HbAA:HbGA were higher in women (53.4 [48.2-52.1] pmol/ G Hb; 1.07 [1.03-1.13] pmol/ G Hb respectively) compared to men (50.1 [50.7-56.3] pmol/ G Hb; 0.97 (0.94-1.02) pmol/ G Hb respectively). Vesper et al. (2013) collected data on sex and found that men had statistically significant higher GMs (95% CI) for HbAA (65.9 [61.5, 70.5] pmol/ G Hb) compared to women (58.9 [55.7, 62.2] pmol/ G Hb) ($p<0.0001$). Differences in these two studies may be due to age. However, no research has been published to explore if age affects differences in AA exposure by sex.

Furthermore, using NHANES 2003-04 SC, Duke et al. (2017) reported that men had higher GMs (95% CI) for HbAA levels (65.4 [61.6-69.4)] pmol/ G Hb) compared to women (59.9 [56.6-63.4] pmol/ G Hb) ($p<0.001$). Additionally, the GMs (95% CI) for HbAA:HbGA ratio was significantly higher for women (0.99 [0.95-1.04] pmol/ G

Hb) compared to men (0.91 [0.88-0.95] pmol/ G Hb) ($p<0.001$). Similarly, Huang et al. (2018a) also found that men had statistically significant higher levels of HbAA, HbGA, and HbAA:HbGA. Zhang et al. (2018) reported a statistically significant trend for sex, with males having higher HbAA and females having higher HbGA, and HbAA:HbGA ratios. These literature findings indicate a potential for AA exposure differences by sex. However, no known differences of AA metabolism have been established before for sex and more research is needed to conclude whether there are significant differences by sex. This thesis will contribute to this area of AA research by studying HbAA and HbGA exposure and interacting it with sex and race/ethnicity.

Race/ethnicity and its association to AA exposure

Thus far, research on AA exposure and its association with race/ethnicity is still emerging. This is an important variable to consider for the U.S. because of its diverse cultural make up. Shifting racial/ethnic demographics may affect AA exposure outcomes for the U.S. population due to potential genetic differences among racial/ethnic groups, as well as variation in consumption of staple food groups and in cooking methodologies that are influenced by culture (Preston et al., 2009; Vesper et al., 2010).

Differences in AA exposure among racial/ethnic groups were observed by Vesper et al. (2013), who found that Non-Hispanic Blacks (NHB) had statistically significantly higher levels of GMs (95% CI) for HbAA (66.5 [58.0, 76.2] pmol/ G Hb) compared to NHW (63.1 [59.2, 67.3] pmol/ G Hb) and MA (62.7 [59.3, 66.3] pmol/ G Hb)

($p<0.0001$). The authors concluded that differences in biomarkers by race/ethnicity may be indicative of differences in polymorphisms of the genes for cytochrome P450 (CYP450) 2E1 (CYP2E1) or glutathione S-transferase, which are involved in phase II detoxification. Such differences by race/ethnicity have been reported before (Paulsson et al., 2005). Duke et al. (2017) also found significant differences in GMs (95% CI) for HbGA, with NHW (61.0 [56.7–65.6] pmol/ G Hb) having the highest exposure level compared to NHB (53.2 [50.3–56.4] pmol/ G Hb), Hispanic (62.1 [59.4–65.0] pmol/ G Hb), and Other (49.7 [42.1–58.7] pmol/ G Hb) ($p=0.005$). GMs (95% CI) for HbAA:HbGA were also significant with Hispanics having the highest ratio (1.07 [1.00–1.15] pmol/ G Hb) compared to NHW (0.96 [0.92–1.00] pmol/ G Hb), other (0.90 [0.83–0.98] pmol/ G Hb), and NHB (0.82 [0.74–0.91] pmol/ G Hb).

Huang, et al. (2018a) reported a statistically significant finding that Non-Hispanic Whites (NHW) had the highest HbGA, followed by MA, NHB, and lastly, others. One explanation for the observed differences may be that food consumption patterns of carbohydrate-rich foods vary among cultures (Vesper et al., 2010). However, more dietary research is needed to conclude how AA may vary racial/ethnic groups, and this thesis will contribute to further understanding how race/ethnicity influence AA exposure by examining exposure among smokers vs. non-smokers.

Socioeconomic status and its association to AA exposure

The socioeconomic status (SES) variable is of interest because it is known to contribute to food insecurity; lower-income families make food-choices based off

affordability rather than nutritional value or quality (Pechey & Monsivais, 2016). Additionally, those with higher SES live in more affluent communities, and have more access to supermarkets and grocery stores and/or transportation; thus, equipping them with more food options compared to those with lower SES (USDA, 2009). However, no dietary study was found that examines AA-rich food selection and SES.

Furthermore, those with lower SES have higher rates of smoking, putting them at greater risk for AA exposure (Garrett et al., 2019). Multiple studies have interacted SES with AA exposure: Duke et al. (2017) reported that those with higher income had significantly lower GMs (95% CI) for HbAA ($p=0.006$) and HbGA ($p=0.008$) (61.0 [58.4–63.8] pmol/ G Hb; 58.4 [55.7–61.2] pmol/ G Hb respectively) compared to those with lower income (69.1 [62.5–76.5] pmol/ G Hb; 65.9 [60.1–72.2] pmol/ G Hb respectively). Similarly, Vesper et al. (2013), Huang et al. (2018 a) and Zhang et al. (2018) also reported similar trends. These studies demonstrate the significance that income may play on AA exposure through behavioral and social factors.

2.9 Gaps in Knowledge

This thesis will fill in the gap in knowledge regarding U.S. child and adolescent exposure using the most up-to-date SC 2015-16 and comparing it to the 2003-04 NHANES SCs, which to our knowledge has not been done before. Additionally, this thesis will contribute to the progression of research in AA exposure among children and adolescents and how demographic variables may influence the outcome.

Chapter 3: Methods

3.1 Sampling

NHANES is a complex sampling, multistage, probability design conducted every year by the CDC's National Centers for Health Statistics that conducts household screening and collects biospecimens from non-incarcerated civilian residents from the general U.S. population, including U.S. territories (CDC, 2023b). Oversampling was conducted for subgroups of specific public health interest to increase the reliability and precision of estimates of health status indicators for these groups. For more information about how NHANES conducted sampling, see (CDC, n.d.).

3.2 Biomonitoring Data

Blood samples from individuals at or over one year of age were collected by trained medical technologists and/or phlebotomists (CDC, n.d.).

3.3 Study Population

The study population consists of individuals ages six through 19 for both SCs. Six years of age was the minimum age for this study because it was the youngest age that NHANES collected data for HbAA and HbGA specimens from the 2015-16 SC. Additionally, participants in this study were only included if they had at least HbAA or HbGA reported. Figures 4 and 5 depict the sample selection of both SCs. To obtain the sample size, SAS Studio® running SAS 9.4 was used.

Figure 4. Flow Diagram Depicting the Eligibility of the 2003-04 Survey Cycle

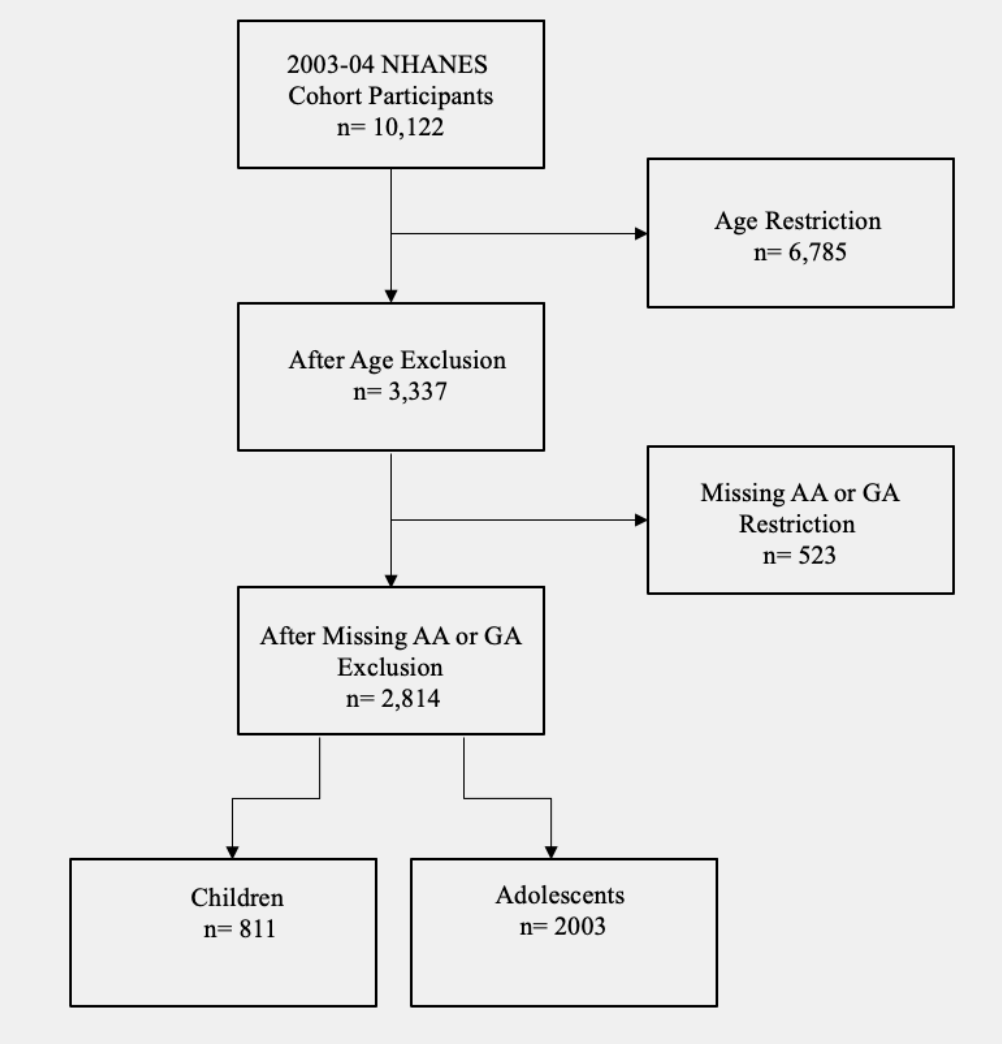
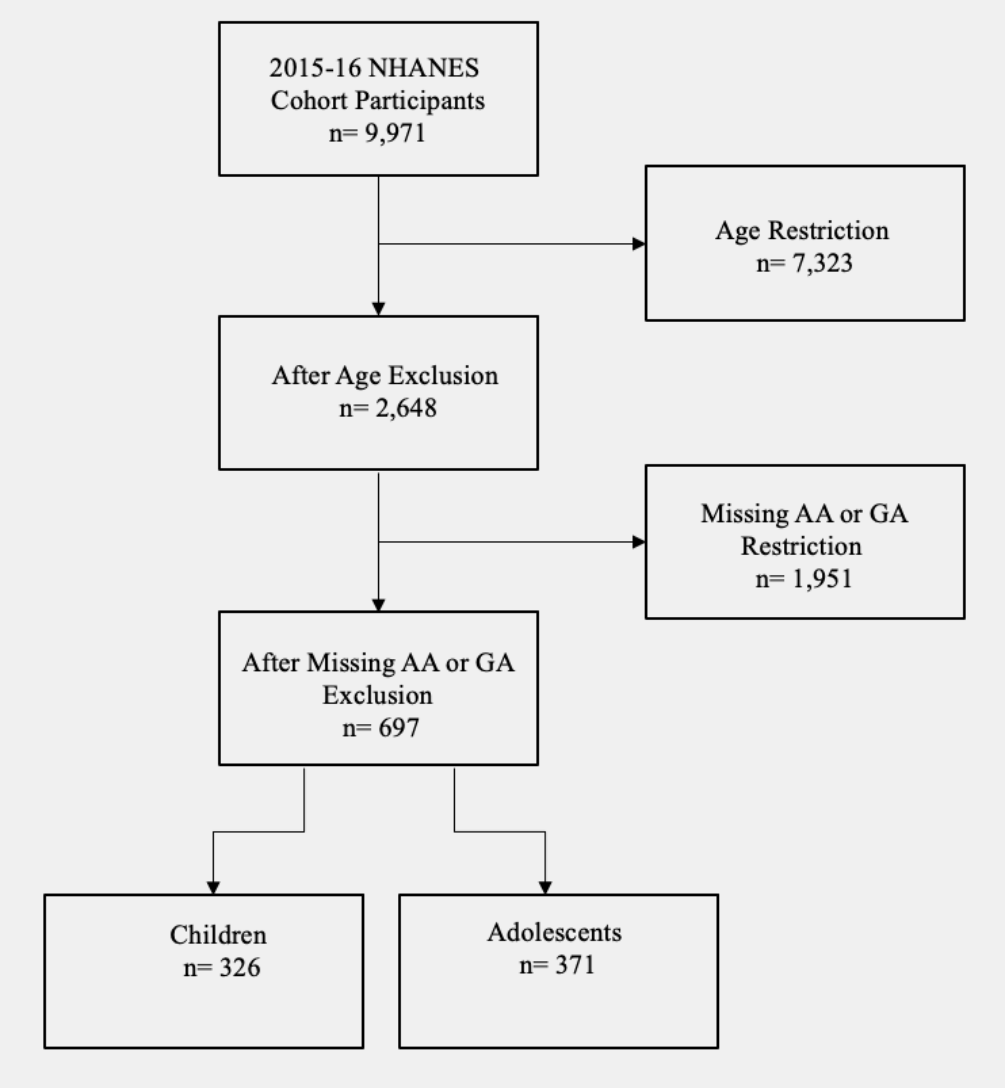


Figure 5. Flow Diagram Depicting the Eligibility of the 2015-16 Survey Cycle



Missing values for blood biomarkers and demographic variables, as provided by NHANES for each SC, were represented as a "." As seen in figure 4, after subsetting by age, the total sample size for 2003-04 is 3337. Missing values for each blood biomarker for this sample size are as follows: HbAA- 679; HbGA- 622; and cotinine- 450. For 2015-16, the total sample size is 2648 after also sub setting by age (Figure

5). The missing values for blood biomarkers in this sample size are as follows: HbAA- 1960; HbGA- 2004; cotinine- 542. For age, sex and race/ethnicity, there were no missing values.

For missing values of PIR, an unknown category was created for Tables 7 and 10, as well as for later use in the regression analyses. NHANES conducted imputations to correct for any true missingness for AA, GA, and cotinine that had a value below the LOD (CDC, n.d.). Thus, this research implicitly assumed that any other values that were missing at random did not create additional bias in the analysis.

3.4 Limits of Detection

For the biomarkers of interest, NHANES reported limits of detection (LOD) as follows: For the 2003-04 SC, HbAA's LOD was reported as 3.00 pmol/G Hb. HbGA's LOD was reported as 4.00 pmol/G Hb and cotinine's (blood biomarker) LOD as 0.015 ng/mL for serum (CDC, n.d.). For 2015-16 SC, HbAA's LOD was reported as 3.90 pmol/G Hb. HbGA's LOD was reported as 4.90 pmol/G Hb and cotinine's LOD as 0.015 ng/mL (CDC, n.d.). To maintain consistency, the highest value from both SCs was used as the LOD. For HbAA, participants with values less than 3.90 pmol/g Hb were assigned a below LOD imputed value using the formula provided by NHANES: $LOD/\sqrt{2}$. For HbGA, participants with values less than 4.90 pmol/g Hb were assigned a below LOD imputed value using the formula provided by NHANES: $LOD/\sqrt{2}$. For cotinine, participants with values less than 0.015 ng/mL, they were also designated as having values below the LOD. They were assigned an

imputed value that was generated using the formula provided by NHANES: LOD/$\sqrt{2}$. A summary of biomarkers below the LOD and the percentage below the limit f can be found in Table 1.

Table 1. Number of Samples Below the Limit of Detection by Survey Cycle

SC	Below LOD (n)	% Below LOD
0304 AA	4	0.1
1516 AA	0	0
0304 GA	76	2.3
1516 GA	0	0
0304 Cotinine	419	15
1516 Cotinine	231	34

3.5 IRB and Special Procedures

Since NHANES conducted and collected the data, no IRB approval was needed for this thesis. Nonetheless, NHANES study protocols were approved by the National Center for Health Statistics research ethics review board; 2003-2004 SC abided by protocol #98-12 and 2015-2016 SC followed protocol # 2011-17 (CDC, n.d.). Furthermore, NHANES abides by federal law requirements: Public Health Service Act, Privacy Act of 1974, and the Confidential Information Protection and Statistical Efficiency Act (CDC, n.d.).

Chapter 4: Data Handling

4.1 Aim 1

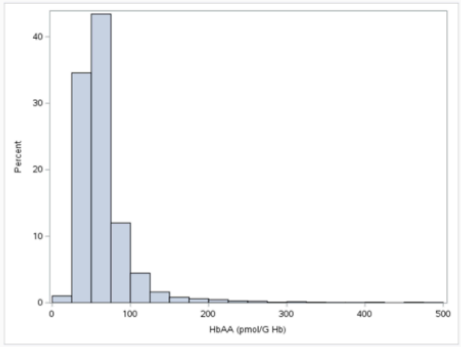
Aim 1: To provide descriptive data on acrylamide exposure using serum biomarkers (HbAA, HbGA, and HbAA:HbGA ratio), plus cotinine, and demographic variables, in children and adolescents based on both NHANES 2003-04 and NHANES 2015-16. To initially explore the distribution of the population in the SCs, frequency counts were run (Table 2 and 3). Continuous demographic variables selected for inclusion in this analysis were age and PIR. Age was classified into two groups: children (6-11) and adolescents (12-19). Poverty was measured as a continuous ratio variable and classified into three categories: below poverty (cutoff), above poverty (cutoff), and unknown poverty (missing values).

Histograms and log-transformed histograms were also produced for continuous variables of blood biomarker data (HbAA, HbGA, and cotinine) to assess their normality and thus determine whether a log-transformation was required ahead of statistical analysis (Figures 6-8). To further explore the distributions of the continuous variables (HbAA, HbGA, HbAA:HbGA ratio, cotinine, age, and PIR), untransformed unweighted means with standard deviations and quartiles, along with weighted means with standard errors, were generated for both SCs (Table 2 and 3). Subsequently, a log-transformation was used for HbAA, HbGA, and cotinine due to the data's lack of normality. All further analysis utilized this log-transformed data. The weighted GMs with standard errors were also generated for all continuous log-transformed variables

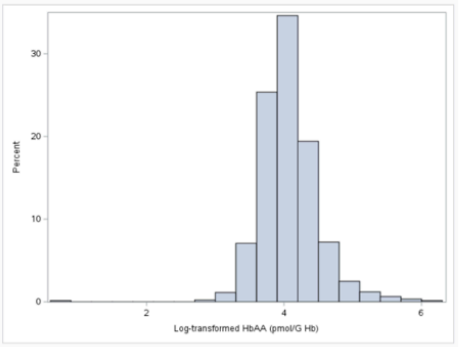
in both SCs. The log-transformed data was adjusted for both data clustering and weighting.

Figure 6. Histograms of HbAA Distribution

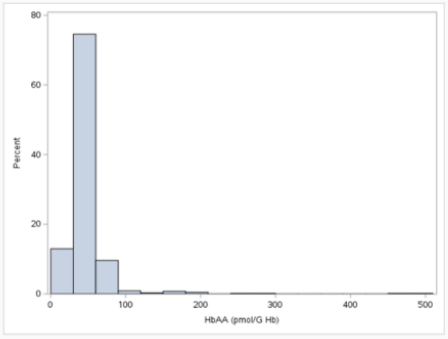
(A) HbAA for 2003-04 Survey Cycle



(B) Log-transformed HbAA for 2003-04 Survey cycle



(C) HbAA for 2015-16 Survey Cycle



(D) Log-transformed HbAA for 2015-16 Survey cycle

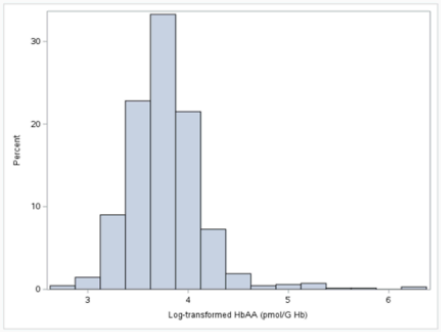
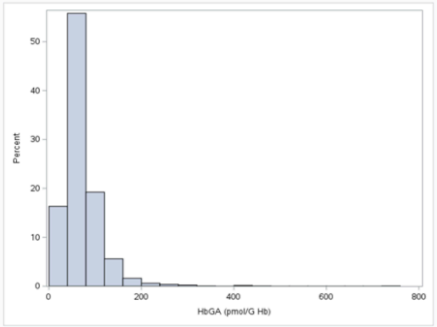
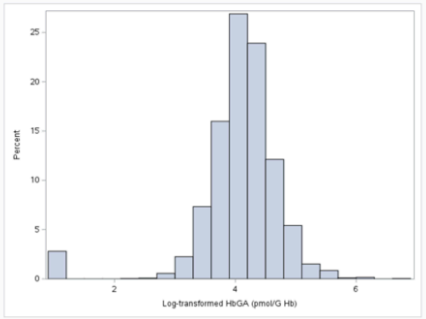


Figure 7. Histograms of HbGA Distribution

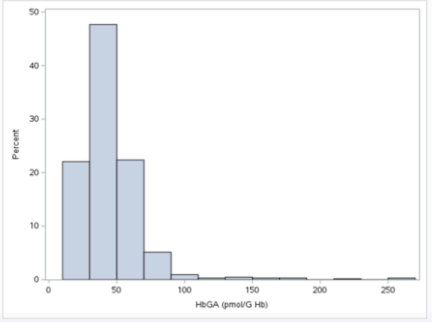
(A) HbGA for 2003-04 Survey Cycle



(B) Log-transformed HbGA for 2003-04 Survey cycle



(C) HbGA for 2015-16 Survey Cycle



(D) Log-transformed HbGA for 2015-16 Survey cycle

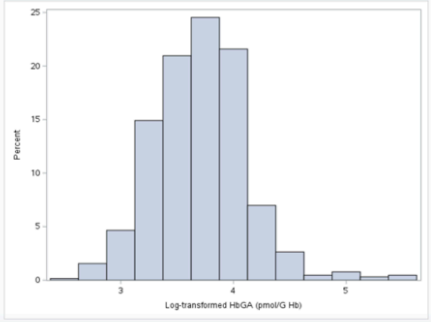
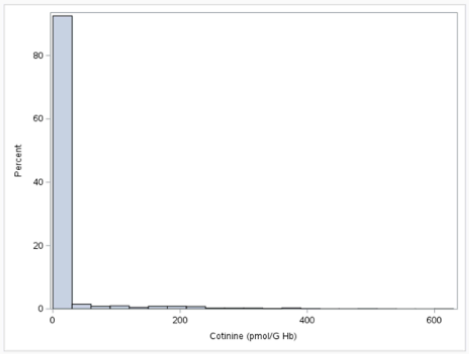
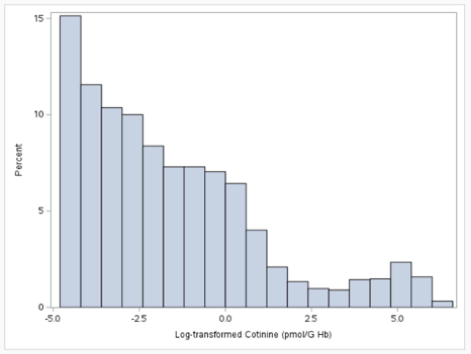


Figure 8. Histograms of Cotinine Distribution

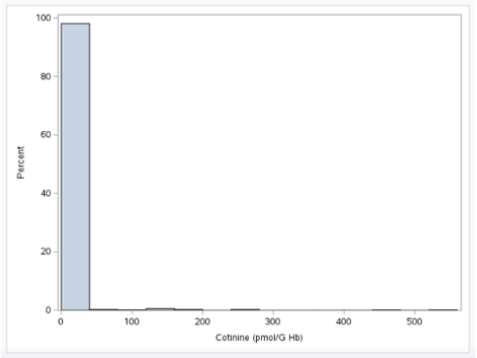
(A) Cotinine for 2003-04 Survey Cycle



(B) Log-transformed Cotinine for 2003-04 Survey cycle



(C) Cotinine for 2015-16 Survey Cycle



(D) Log-transformed Cotinine for 2015-16 Survey cycle

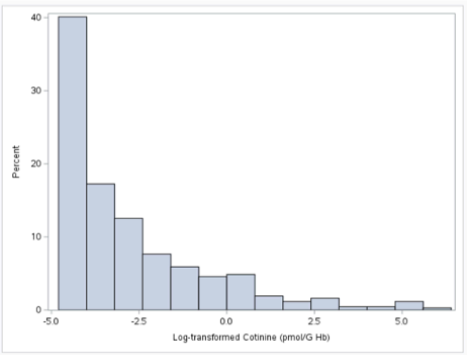


Table 2. 2003-04 Survey Cycle Descriptive Statistics of Blood Biomarker Data and Continuous Demographic Variable for Combined Ages

	HbAA	HbGA	HbAA:HbGA	Cotinine	Age	PIR
Sample Size (N)	2658	2715	2559	2769	2814	2692
Unweighted Mean	64.9	70.3	1.5	13.3	13.5	1.9
SD	38.1	42.8	N/A	53.8	3.8	1.5
Weighted Mean	63.3	72.5	1.3	14.3	12.8	2.4
SE	1.4	1.9	N/A	2	0.2	0.1
Weighted Geometric Mean (95% CI)	57.9 (55.7, 60.1)	62.2 (59.2, 65.5)	1 (1.0, 1.0)	0.2 (0.1, 0.3)	N/A[a]	N/A[a]
Quartile						
Minimum	2.8	3.5	0.2	0.001	6	0
Q1	45.2	46.8	0.8	0.02	11	0.7
Median	56.6	63	0.9	0.1	14	1.4
Q3	71.5	83.3	1.2	0.8	17	2.8
Maximum	499	756	92.2	623	19	5

HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
PIR: Poverty-to-income ratio
HbAA and HbGA are measured in pmoL/G Hb
Cotinine is measured in ng/mL
[a]Geometric means were only generated for blood biomarkers
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 3. 2015-16 Survey Cycle Descriptive Statistics of Blood Biomarker Data and Continuous Demographic Variable for Combined Ages

	HbAA	HbGA	HbAA:HbGA	Cotinine	Age	PIR
Sample Size (N*)	688	644	635	678	697	629
Unweighted Mean	47.5	44.9	1.1	4.6	12.1	2.1
SD	32.9	24.5	N/A	34.5	4	1.5
Weighted Mean	46.7	44.8	1.1	4.5	12.5	2.5
SE	1.6	1.2	N/A	1.9	0.2	0.2
Weighted Geometric Mean (95% CI)	42.8 (41.4, 44.2)	40.8 (39.2, 42.4)	1.1 (1.0, 1.1)	0.1 (0.04, 0.1)	N/A[a]	N/A[a]
Quartile						
Minimum	14.9	12	0.5	0.01	6	0
Q1	34.4	31.3	0.9	0.01	9	0.9
Median	42.1	40.2	1.1	0.03	12	1.7
Q3	51.5	52.3	1.3	0.1	15	3.1
Maximum	485	264	2.4	554	19	5

HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
PIR: Poverty-to-income ratio
HbAA and HbGA are measured in pmoL/G Hb
Cotinine is measured in ng/mL
[a]Geometric means were only generated for blood biomarkers
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Additionally, to explore whether significant differences were observable for the weighted GMs (95% CI) by race/ethnicity, income, sex, and age group, ANOVA F-statistics were produced for both SCs (Tables 4 and 7). Participant's race/ethnicity was classified into NHB, NHW, Hispanics (Mexican Americans and other Hispanics), and Others (Asians and multi-racial individuals). Sex was defined as male or female in both SCs, and the same age categories were used as before.

A Bonferroni post-hoc test was also conducted for race/ethnicity and income for 2003-04 SC (Tables 5 and 6) and race/ethnicity for 2015-16 SC (Tables 8) to determine what groups were significantly different from each other. For sex and age group, unpaired t-tests were implemented to analyze for significant differences between the groups in both SCs (Tables 4 and 7).

Table 4. 2003-04 Survey Cycle Geometric Means (95% Confidence Intervals) of Blood Biomarker Data and Continuous Demographic Variables

	HbAA	P-value	HbGA	P-value	HbAA:HbGA	P-value
Ethnicity						
Hispanic	55.8 (53.0, 58.7)	0.02	64.5 (60.3, 69.0)	<0.0001	0.09 (0.8, 1.0)	<0.0001
NHW	58.8 (56.6, 61.0)		64.7 (61.4, 68.3)		0.9 (0.9, 1.0)	
NHB	58 (54.4, 61.8)		53.5 (50.8, 56.3)		1.1 (1.0, 1.2)	
Other	54.6 (46.1, 64.6)		55.4 (46.6, 65.9)		1 (0.9,1.2)	
Income						
Above Poverty	57 (55.4, 58.7)	0.0007	62 (59.1, 65.0)	0.14	0.9 (0.9, 1.0)	0.46
Below Poverty	60.8 (56.3, 65.7)		64 (58.3, 70.1)		1 (0.9, 1.0)	
Unknown Poverty	54.8 (48.0, 62.6)		56.3 (47.0, 67.4)		1 (0.9, 1.1)	
Sex						
Male	57.9 (55.1, 60.9)	0.84	61.1 (57.8, 64.7)	0.11	1 (0.9, 1.0)	0.07
Female	57.8 (55.9, 59.7)		63.5 (60.1, 67.2)		0.9 (0.9,1.0)	
Age Group						
Children (6-11)	58.6 (56.2, 61.1)	0.2	74.2 (70.6, 78.1)	<0.0001	0.8 (0.8, 0.8)	<0.0001
Adolescents (12-19)	57.4 (54.6, 60.4)		55.7 (51.6, 60.0)		1.1 (1.0, 1.1)	

NHW: Non-Hispanic Whites
NHB: Non-Hispanic Blacks
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
P-values are generated from an ANOVA F-statistic

Table 5. 2003-04 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences Among Race/ethnicity

			HbAA	HbGA	HbAA:HbGA
Race					
	Hispanic	NHW	-0.05	-0.004	-0.04
	Hispanic	NHB	-0.04	0.19**	-0.22**
	Hispanic	Others	0.02	0.15	-0.14
	NHW	NHB	0.01	0.19**	-0.18**
	NHW	Others	0.07	0.16	-0.09
	NHB	Others	0.06	-0.04*	0.09

NHW: Non-Hispanic Whites
NHB: Non-Hispanic Blacks
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
*,** for statistical significance at 0.05, 0.01 respectively
Estimates are differences in log means
HbAA and HbGA are measured in pmoL/ G Hb
Tests are based on Bonferroni-corrected p-values

Table 6. 2003-04 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences Among Income Status

		HbAA
Poverty Status		
Above Poverty	Below Poverty	-0.06*
Above Poverty	Unknown Poverty	0.04
Below Poverty	Unknown Poverty	0.1

HbAA: Hemoglobin-acrylamide adduct
* Significant at 0.01 level
Estimates are differences in log means
HbAA and HbGA are measured in pmoL/ G Hb
Tests are based on Bonferroni-corrected p-values

Table 7. 2015-16 Survey Cycle Geometric Means (95% Confidence Intervals) of Blood Biomarker Data by Demographics for All Ages

	HbAA	P-value	HbGA	P-value	HbAA:HbGA	P-value
Ethnicity						
Hispanic	42.9 (40.9, 44.9)	0.13	40.88 (38.39, 43.54)	0.22	1.05 (1.02, 1.08)	<0.0001
NHW	41.7 (39.4, 44.1)		44.5 (38.2, 45.0)		1 (1.0, 1.1)	
NHB	46.2 (41.9, 51.0)		37.4 (33.4, 41.9)		1.2 (1.2, 1.3)	
Other	43.5 (39.0, 48.6)		41.6 (36.1, 47.8)		1.1 (1.0, 1.1)	
Income						
Above Poverty	42.3 (40.7, 44.0)	0.47	40.8 (38.9, 42.9)	0.88	1.1 (1.0, 1.1)	0.27
Below Poverty	44.1 (41.5, 46.9)		41 (38.1, 44.0)		1.1 (1.0, 1.1)	
Unknown Poverty	43.9 (38.83, 49.7)		39.6 (32.4, 48.24		1.1 (1.0, 1.2)	
Sex						
Male	44.1 (41.3, 47.0)	0.03	42.4 (39.2, 45.7)	0.02	1.1 (1.0, 1.1)	0.4
Female	41.4 (40.1, 42.8)		39.2 (37.6, 40.8)		1.1 (1.0, 1.1)	
Age Group						
Children (6-11)	43.2 (41.8, 44.5)	0.58	46.6 (44.9, 48.4)	<0.0001	0.9 (0.9, 1.0)	<0.001
Adolescents (12-19)	42.5 (40.3, 44.8)		37.3 (34.8, 39.9)		1.2 (1.1, 1.2)	

NHW: Non-Hispanic Whites
NHB: Non-Hispanic Blacks
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
P-values are generated from an ANOVA F-statistic

Table 8. 2015-16 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences Among Race/ethnicity

			HbAA:HbGA
Race			
	Hispanic	NHW	0.02
	Hispanic	NHB	-0.16*
	Hispanic	Others	-0.01
	NHW	NHB	-0.18*
	NHW	Others	-0.03
	NHB	Others	0.15*

NHW: Non-Hispanic Whites
NHB: Non-Hispanic Blacks
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
Estimates are differences in log means
HbAA and HbGA are measured in pmoL/ G Hb
* Significant at 0.01 level
Tests are based on Bonferroni-corrected p-values

4.2 Aim 2

Aim 2: To determine if there is a difference in acrylamide serum biomarkers (HbAA, HbGA, and HbAA:HbGA ratio) in children and adolescents by smoking status between 2003-04 and 2015-16 SCs.

Smoking exposure was defined as follows: smoking (cotinine ≥ 10 ng/ mL), SHS (cotinine ≥ 1 and <10 ng/ mL) and non-smoking (cotinine<1 ng/ mL). As seen in Tables 9 and 10, weighted means, geometric means (95% CI) and sample sizes were generated by age for each SC and classified by smoking status for combined age groups. ANOVAs and post-hoc tests were then run to determine significant differences among the smoking groups and the findings were reported in Tables 11 and 12. The same analyses were also conducted for children separately, and adolescents separately (Tables 13-18). However, for children, only non-smokers and SHS were evaluated in both SCs, given that there were only four participants in the 2003-04 SC and only one participant in the 2015-16 SC (Table 13 and 14).

Furthermore, unpaired t-test were conducted to test significant changes among the SCs by HbAA, HbGA, and HbAA:HbGA separately for each age category and are reported in Tables 19-24.

Table 9. 2003-04 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and By Combined Age Groups

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA*	116.9	7.7	101.6 (88.6, 116.6)	243
HbGA*	105.3	7.1	92.6 (81.3,105.6)	252
HbAA:HbGA*	1.3	0.1	1.1 (1.1, 1.2)	237
SHS				
HbAA	64.6	2.5	60.9 (56.2, 66.1)	362
HbGA	76.1	3.5	66.8 (58.8, 75.9)	367
HbAA:HbGA	1.4	0.4	0.9 (0.8, 1.1)	352
Non-smoking				
HbAA	56.5	0.8	53.4 (52.0, 54.9)	2015
HbGA	67.4	1.6	58.3 (55.9, 60.8)	2051
HbAA:HbGA	1.3	0.1	0.9 (0.9, 2.0)	1932

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
*At least one smoking group is statistically significant different ($p<0.0001$) from the other
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 10. 2015-16 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and By Combined Age Groups

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA*	111.9	30.6	77.9 (47.6, 127.5)	26
HbGA*	79.9	19.6	59.6 (38.7, 91.7)	27
HbAA:HbGA*	1.3	0	1.3 (1.2, 1.4)	26
SHS				
HbAA	61.9	12	52.1 (39.6, 68.6)	52
HbGA	51.7	8	44.0 (35.0, 55.3)	53
HbAA:HbGA	1.2	0.1	1.2 (1.1, 1.3)	51
Non-smoking				
HbAA	43	0.5	41.2 (40.2, 42.2)	591
HbGA	42.3	0.7	39.6 (38.2, 41.0)	550
HbAA:HbGA	1.1	0	1.1 (1.0, 1.1)	544

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct

HbAA and HbGA are measured in pmoL/ G Hb
*At least one smoking group is statistically significantly different ($p<0.0001$) from the other
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 11. 2003-04 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences Among Smoking Status for Combined Age Groups

			HbAA	HbGA	HbAA:HbGA
Smoking Status					
	SHS	NS	0.13*	0.14*	0.01
	SHS	Smoking	-0.51*	-0.33*	-0.16*
	NS	Smoking	-0.64*	-0.46*	-0.18*

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
*for statistical significance at 0.01
Estimates are differences in log means

Table 12. 2015-16 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences for Smoking Status and by Combined Age Groups

			HbAA	HbGA	HbAA:HbGA
Smoking Status					
	SHS	NS	0.24*	0.11	0.13*
	SHS	Smoking	-0.40*	-0.30*	-0.09
	NS	Smoking	-0.64*	-0.41*	-0.22*

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
* for statistical significance at 0.01
Estimates are differences in log means
Tests are based on Bonferroni-corrected p-values

Table 13. 2003-04 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and by Children Only

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA**	ND[a]	ND[a]	ND[a]	ND[a]
HbGA*	ND[a]	ND[a]	ND[a]	ND[a]
HbAA:HbGA	ND[a]	ND[a]	ND[a]	ND[a]
SHS				
HbAA	67.6	3.4	64.6 (58.2, 71.7)	115
HbGA	87	4.7	81.2 (72.5, 90.9)	116
HbAA:HbGA	0.9	0	0.8 (0.8, 0.9)	112
Non-smoking				
HbAA	60.1	1.4	57.1 (54.7, 59.5)	633
HbGA	79.3	2.3	72.6 (69.2, 76.2)	644
HbAA:HbGA	1	0.1	0.8 (0.8, 0.8)	609

[a]*n=4*
SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
* At least one smoking group is statistically significantly different (P<0.05) from the other
**At least one smoking group is statistically significantly different (p<0.0001) from the other
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 14. 2015-16 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and by Children Only

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA*	ND[a]	ND[a]	ND[a]	ND[a]
HbGA	ND[a]	ND[a]	ND[a]	ND[a]
HbAA:HbGA*	ND[a]	ND[a]	ND[a]	ND[a]
SHS				
HbAA	46.7	1.7	45.8 (42.7, 49.2)	23
HbGA	47.6	3.4	45.3 (38.8, 52.9)	23
HbAA:HbGA	1.1	0.1	1.0 (0.9, 1.2)	23
Non-smoking				
HbAA	44.7	0.8	43.2 (42.0, 44.5)	285
HbGA	48.8	0.9	46.7 (45.1, 48.5)	269
HbAA:HbGA	1	0	0.9 (0.9, 1.0)	266

[a]*n=1*
SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
* At least one smoking group is statistically significantly different (P<0.05) from the other

N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 15. 2003-04 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and by Adolescents Only

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA*	118.3	8.2	102.6 (88.7, 118.6)	240
HbGA*	105.7	7.6	93.3 (81.0, 107.4)	248
HbAA:HbGA	1.3	0.1	1.1 (1.1, 1.2)	234
SHS				
HbAA	62.1	2.7	58.1 (53.2, 63.5)	247
HbGA	67.2	4.2	56.9 (47.3, 68.4)	251
HbAA:HbGA	1.8	0.6	1.1 (0.9, 1.3)	240
Non-smoking				
HbAA	53.9	0.9	51.0 (49.4, 52.5)	1382
HbGA	58.9	2.2	49.8 (46.6, 53.2)	1407
HbAA:HbGA	1.6	0.2	1.0 (1.0, 1.1)	1323

SHS: Secondhand smoking
HbAA and HbGA are measured in pmoL/ G Hb
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
*At least one smoking group is statistically significant different ($p<0.0001$) from the other
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 16. 2003-04 Survey Cycle Estimates for Post-hoc Test to Determine Significant Differences Among Smoking Status by Adolescents Only

			HbAA	HbGA
Smoking Status				
	SHS	NS	0.13*	0.13*
	SHS	Smoking	-0.57*	-0.49*
	NS	Smoking	-0.70*	-0.63*

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/G Hb
* for statistical significance at 0.01
Estimates are differences in log means
Tests are based on Bonferroni-corrected p-values

Table 17. 2015-16 Survey Cycle Weighted Descriptive Statistics of Blood Biomarker Data for Smoking Status and by Adolescents Only

	Weighted Mean	SE	Weighted Geometric Mean (95% CI)	Sample Size (N)
Smoking				
HbAA**	113.4	31.5	78.7 (47.2, 131.0)	25
HbGA**	80.9	20.2	60.4 (38.6, 94.5)	26
HbAA:HbGA*	1.3	0	1.3 (1.2, 1.3)	25
SHS				
HbAA	68.7	16.6	55.2 (37.6, 81.0)	29
HbGA	53.5	11.1	43.5 (31.5, 60.1)	30
HbAA:HbGA	1.3	0.1	1.3 (1.2, 1.4)	28
Non-smoking				
HbAA	41.8	0.8	39.8 (38.2, 41.4)	306
HbGA	37.7	1.1	35.2 (33.0, 37.4)	281
HbAA:HbGA	1.2	0	1.1 (1.1, 1.2)	278

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb
*At least one smoking group is statistically significantly different ($p<0.05$) from the other
**At least one smoking group is statistically significantly different ($p<0.0001$) from the other
N* All data was reported separately for HbAA and HbGA and for the ratio only where both values were present

Table 18. 2015-16 Survey Cycle Estimates For Post-hoc Test to Determine Significant Differences Among Smoking Status and By Adolescents Only

			HbAA	HbGA
Smoking Status				
	SHS	NS	0.33**	0.21
	SHS	Smoking	-0.35**	-0.33*
	NS	Smoking	-0.68**	-0.54**

SHS: Secondhand smoking
HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
*,** for statistical significance at 0.05, 0.01 respectively
Estimates are differences in log means
HbAA and HbGA are measured in pmoL/ G Hb
Tests are based on Bonferroni-corrected p-values

Table 19. Summary Of Unpaired T-tests Comparing HbAA Across Survey Cycles for All Smoking Categories and Separated by Age Groups

		Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
All Smoking Categories by Age Group				
Combined Age Groups				
		2003-04	57.9 (55.7, 60.1)	2658
		2015-16	42.8 (41.4, 44.2)	688
	T-Statistic	22.72		
	P-Value	<0.0001		
Children				
		2003-04	58.6 (56.2, 61.1)	769
		2015-16	43.2 (41.8, 44.5)	323
	T-Statistic	17.6		
	P-value	<0.0001		
Adolescents				
		2003-04	57.4 (54.6, 60.4)	1889
		2015-16	42.5 (40.3, 44.78)	365
	T-Statistic	16.3		
	P-value	<0.0001		

HbAA: Hemoglobin-acrylamide adduct
HbAA is measured in pmoL/ G Hb

Table 20. Summary of Unpaired T-tests Comparing HbGA Across Survey Cycles for all Smoking Categories and Separated by Age Groups

		Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
All Smoking Categories by Age Group				
Combined Age Groups				
		2003-04	62.24 (59.18, 65.45)	2715
		2015-16	40.76 (39.15, 42.43)	644
	T-Statistic	22.29		
	P-value	<0.0001		
Children				
		2003-04	74.2 (70.6, 78.1)	784
		2015-16	46.6 (44.9, 48.4)	303
	T-Statistic	19.8		
	P-value	<0.0001		
Adolescents				
		2003-04	55.7 (51.6, 60.0)	1931
		2015-16	37.3 (34.8, 39.8)	341
	T-Statistic	15.55		
	P-value	<0.0001		

HbGA: Hemoglobin-glycidamide adduct
HbGA is measured in pmoL/ G Hb

Table 21. Summary Unpaired T-tests Comparing HbAA:HbGA Across Survey Cycles for all Smoking Categories and Separated by Age Groups

	Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
All Smoking Categories by Age Group			
Combined Age Groups			
	2003-04	1.1 (1.1, 1.2)	2559
	2015-16	1.3 (1.2, 1.4)	635
T-Statistic	-8.03		
P-value	<0.0001		
Children			
	2003-04	0.8 (0.8, 0.8)	742
	2015-16	0.9 (0.9, 1.0)	300
T-Statistic	-8.38		
P-value	<0.0001		
Adolescents			
	2003-04	1.1 (1.0, 1.1)	1817
	2015-16	1.2 (1.1, 1.2)	335
T-Statistic	-4.92		
P-value	<0.0001		

HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb

Table 22. Summary of Unpaired T-tests Comparing HbAA Across Survey Cycles for Nonsmoking Status and Separated by Age Groups

	Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
Nonsmoking Status by Age Group			
Combined Age Groups			
	2003-04	53.4 (52.0, 54.9)	2015
	2015-16	41.2 (40.2, 42.2)	591
T-Statistic	21.3		
P-value	<0.0001		
Children			
	2003-04	57.1 (54.7, 59.5)	633
	2015-16	43.2 (42.0, 44.5)	285
T-Statistic	14.8		
P-value	<0.0001		
Adolescents			
	2003-04	51.0 (49.4, 52.5)	1382
	2015-16	43.2 (42.0, 44.5)	306
T-Statistic	15.6		
P-value	<0.0001		

HbAA: Hemoglobin-acrylamide adduct
HbAA is measured in pmoL/ G Hb

Table 23. Summary Unpaired T-tests Comparing HbGA Across Survey Cycles for Nonsmoking Status and Separated by Age Groups

		Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
All Smoking Categories by Age Group				
Combined Age Groups				
		2003-04	58.3 (55.9, 60.8)	2051
		2015-16	39.6 (38.2, 41.0)	550
	T-Statistic	18.82		
	P-value	<0.0001		
Children				
		2003-04	72.6 (69.2, 76.2)	644
		2015-16	46.7 (45.1, 48.4)	269
	T-Statistic	17.27		
	P-value	<0.0001		
Adolescents				
		2003-04	49.8 (46.6, 53.2)	1407
		2015-16	35.2 (33.0, 37.4)	281
	T-Statistic	12.63		
	P-value	<0.0001		

HbGA: Hemoglobin-glycidamide adduct
HbGA is measured in pmoL/ Hb

Table 24. Summary Unpaired T-tests Comparing HbAA:HbGA Across Survey Cycles by Nonsmoking Status and Separated by Age Groups

		Survey Cycle	Geometric Means (95% CI)	Sample Size (N)
Nonsmoking Status by Age Group				
Combined Age Groups				
		2003-04	0.9 (0.9, 1.0)	1932
		2015-16	1.0 (1.0, 1.1)	544
	T-Statistic	-7.27		
	P-value	<0.0001		
Children				
		2003-04	0.8 (0.8, 0.8)	609
		2015-16	0.9 (0.9, 1.0)	266
	T-Statistic	-7.24		
	P-value	<0.0001		
Adolescents				
		2003-04	1.0 (1.0, 1.1)	1323
		2015-16	1.1 (1.1, 1.2)	278
	T-Statistic	-4.24		
	P-value	<0.0001		

HbAA: Hemoglobin-acrylamide adduct
HbGA: Hemoglobin-glycidamide adduct
HbAA and HbGA are measured in pmoL/ G Hb

4.3 Aim 3

Aim 3: To examine the determinants of each acrylamide biomarkers, in each survey separately, in non-smokers only.

Univariate regressions were conducted for HbAA, HbGA, and HbAA:HbGA for each demographic variable separately, in each survey cycle separately, and for non-smokers only. All age groups were analyzed together, and then separated by age group (children only vs. adolescents only). Variables examined include race, sex, and PIR. The tables can be found in the appendices. A multivariate regression model was then built, and results are presented in Table 25-27.

Table 25. Nonsmoking Multivariate Regressions for HbAA Exposure by Age Categories and Demographic Controls by Year

Cohorts	Significant Variables	Nonsignificant variables	N	R^2
0304 All Ages	Age (-0.02**)	Sex, Ethnicity, PIR	2015	0.04
0304 Children	Age (-0.02*)	Sex, Ethnicity, PIR	633	0.01
0304 Adolescents	Age (-0.01*)	Sex, Ethnicity, PIR	1382	0.02
1516 All Ages	Age (-0.01*)	Sex, Ethnicity, PIR	591	0.03
1516 Children	NHB (0.12*)	Age, Sex, PIR	285	0.05
1516 Adolescents		Age, Sex, Ethnicity, PIR	306	0.02

NHB: Non-Hispanic Blacks
PIR: Poverty-to-income ratio
Reference groups were defined as NHW, male, and above* poverty
NHW: Non-Hispanic Whites
Regression Coefficients presented with standard errors in parentheses
*, ** for statistical significance at 0.05, 0.01 respectively

Table 26. Nonsmoking Multivariate Regressions for HbGA Exposure by Age Categories and Demographic Controls by Year

Cohorts	Significant Variables	Nonsignificant variables	N	R^2
0304 all Ages	Age (-0.05**), Sex (0.08*), NHB (-0.16**), Others (-0.18*)	PIR	2015	0.09
0304 Children	Age (-0.03**), NHB (-0.23**)	Sex, PIR	644	0.06
0304 Adolescents	Sex (0.17**), NHB (-0.11*), Others (-0.27)	Age, PIR	1407	0.03
1516 All Ages	Age (-0.04**), NHB (-0.17**)	Sex, PIR	550	0.18
1516 Children	Age (-0.04**), NHB (-0.13*)	Sex, PIR	269	0.09
1516 Adolescents	NHB (-0.16*)	Age, Sex, PIR	281	0.05

NHB: Non-Hispanic Blacks
PIR: Poverty-to-income ratio
Reference groups were defined as NHW, male, and above* poverty
NHW: Non-Hispanic Whites
Reference groups were defined as NHW, male, unknown poverty
Regression Coefficients presented with standard errors in parentheses
*, ** for statistical significance at 0.05, 0.01 respectively

Table 27. Nonsmoking Multivariate Regressions for HbAA:HbGA Exposure by Age Categories and Demographic Controls by Year

Cohorts	Significant Variables	Nonsignificant variables	N	R^2
0304 All Ages	Age (0.03**), NHB (0.16**), Others (0.15*)	Sex, PIR	1932	0.06
0304 Children	NHB (0.22**)	Age, Sex, PIR	609	0.06
0304 Adolescents	Sex (-0.12*), NHB (0.11*)	Age, PIR	1323	0.02
1516 All Ages	Age (0.03**), NHB (0.17**)	Sex, PIR	544	0.21
1516 Children	Age (0.03**), Sex (0.10**), Hispanic (0.09**), NHB (0.24**), Others (0.07*), Above Poverty (0.07**)		266	0.21
1516 Adolescents	NHB (0.12*)	Age, Sex, PIR	278	0.06

PIR: Poverty-to-income ratio
Reference groups were defined as NHW, male, and above* poverty
NHW: Non-Hispanic Whites
Reference groups were defined as NHW, male, unknown poverty
Regression Coefficients presented with standard errors in parentheses
*, ** for statistical significance at 0.05, 0.01 respectively

Chapter 5: Results and Discussion

5.1 Aim 1

This aim is providing simple descriptive data of the two SCs, and subsequent aims will go into more statistical analyses. For combined age groups, GMs (95% CI) values for HbAA, HbGA, and cotinine were lower in 2015-16 SC (42.8 [41.4, 44.2] pmol/G Hb, 40.8 [39.2, 42.4] pmol/G Hb, 0.1 [0.04, 0.1] pmol/G Hb respectively) compared to the 2003-04 SC (57.9 [55.7, 60.1] pmol/G Hb, 62.2 [59.2, 65.5] pmol/G Hb, 0.2 [0.1, 0.3] pmol/G Hb respectively), suggesting a decline in AA and smoke exposure (Tables 2 and 3). These findings are consistent with published research that smoking and AA exposure from select foods have decreased for the U.S. population (WHO, 2011; U.S. FDA, 2016; Abt et al., 2019; Cornelius, 2020; CDC, 2023a). The GMs (95% CI) for HbAA:HbGA ratio increased by 10 % in 2015-16 compared to 2003-04, suggesting that there was probably an increase in HbAA to HbGA. However, these observed changes may be due to the variability in biotransformation of AA among individuals in the two studies.

Statistically significant differences in the GMs (95% CI) for HbAA ($p=0.02$), HbGA ($p<0.0001$), and HbAA:HbGA ($p<0.0001$) for the 2003-04 SC were observed by race/ethnicity (Table 4). A Bonferroni post-hoc test for this SC revealed that Hispanics had 19% higher HbGA concentration, and a 22% lower HbAA:HbGA ratio than NHB ($p\leq0.01$) (Table 5). NHW had 19% greater HbGA and a 18% lower HbAA:HbGA than NHB ($p\leq0.01$). Lastly, NHB had 4% less HbGA than Others

($p \leq 0.05$). For 2015-16, only significant differences in race/ethnicity were found for the HbAA:HbGA ratio ($p < 0.0001$) (Table 7). Furthermore, a Bonferroni post-hoc test revealed differences among racial groups; Hispanics had 16% less HbAA:HbGA than NHB ($p \leq 0.01$). NHW had 18% less HbAA:HbGA than NHB ($p \leq 0.01$) (Table 8). Additionally, NHB had 15% more HbAA:HbGA than Others ($p \leq 0.01$).

Differences in HbAA for race have been similarly reported in previous research and may be explained by food consumption patterns influenced by culture, and differences in HbGA and HbAA:HbGA may be indicative of variability in polymorphisms of genes for CYP2E1 or of the genes for glutathione S-transferase, which are involved in phase II detoxification of AA and GA (Garte et al., 2001; Paullson et al., 2005; Vesper et al., 2010).

For 2003-04, only HbAA was significantly different for income categories ($p = 0.0007$) (Table 4); upon running the Bonferroni post-hoc test, those above poverty had 6% less HbAA than those below poverty ($p \leq 0.01$) (Table 6). Potential differences in AA exposure by PIR may be explained by consumption patterns that are driven by SES; low-income households consume less refined grains and less fruits, vegetables, and wholegrains (Pechey et al., 2016). Additionally, smoking is also driven by SES, with lower income individuals having higher rates of smoking (Garrett et al., 2019). For this thesis, the 2015-16 SC did not reveal a statistically significant difference between PIR classifications for HbAA, HbGA or

HbAA:HbGA, and therefore this variable may not significantly influence AA outcomes for this study population (Table 7).

In the 2003-04 SC no significant differences were found by sex (Table 4). However, for the 2015-16 SC, HbAA GMs (95% CI) values for males (44.1[41.3, 47.0] pmol/G Hb) were significantly higher compared to females (41.4 [40.1, 42.8] pmol/G Hb) (p=0.03) (Table 10). Furthermore, HbGA GM (95% CI) values were also significantly higher for males (42.4 [39.2, 45.7] pmol/G Hb)) compared to females (39.2 [37.6, 40.8] pmol/G Hb) (p=0.02) (Table 7). Although no particular research has determined that health disparities or genetic variations exist among AA exposure by sex, there have been multiple studies that have found differences in AA and GA exposure for this variable; specifically men tending to have higher AA exposure and women having higher GA and HbAA:HbGA (Vesper at al., 2010; Duke al., 2017; Huang et al., 2018a; Guo et al., 2017; Li et al., 2021). However, there is variability among these studies and therefore more research is needed.

For age group, a t-test was also conducted for both SCs: For the 2003-04, children had significantly higher HbGA GMs (95% CI) (74.2 [70.6, 78.1] pmol/G Hb) than adolescents (55.7[51.6, 60.0] pmol/G Hb) (p <.0001) (Table 4). HbAA:HbGA ratios was also significantly higher in 2003-04 SC for adolescents (1.1 [1.0, 1.1] pmol/G Hb) compared to children (0.8 [0.8, 0.8] pmol/G Hb) (p<0.0001). For 2015-16 SC, children (46.6 [44.9, 48.4] pmol/G Hb) had significantly higher HbGA levels than adolescents (37.3 [34.8, 39.9] pmol/G Hb) (p<0.0001) (Table 7). Additionally,

HbAA:HbGA ratios were also significantly higher in this SC for adolescents (1.2 [1.1, 1.2] pmol/G Hb) compared to children (0.9 [0.9, 1.0] pmol/G Hb) ($p<0.001$). These findings suggest that children are producing more GA than adolescents, and this could be due to a greater capacity to use the GA-forming pathway in children compared to adolescents. This is concerning because of GA's more pronounced DNA reactivity and adduct-forming abilities compared to AA (Barber et al., 2001).

5.2 Aim 2

As seen in Tables 9 and 10, for combined age groups, the HbAA GMs (95% CI) decreased in smoking, SHS, and non-smoking in 2015-16 (77.9 [47.6, 127.5] pmol/G Hb, 52.1 [39.6, 68.6] pmol/G Hb, 41.2 [40.2, 42.2] pmol/G Hb respectively) compared to 2003-04 (101.6 [88.6, 116.6] pmol/G Hb, 60.9 [56.2, 66.1] pmol/G Hb, 53.4 [52.0, 54.9] respectively). The HbGA GM (95% CI) also decreased in smoking, SHS, and non-smoking in 2015-16 (59.6 [38.7, 91.7] pmol/G Hb, 44.0 [35.0, 55.3] pmol/G Hb, 39.6 [38.2, 41.0] pmol/G Hb respectively) compared to 2003-04 (92.6 [81.3,105.6], 66.8 [58.8, 75.9], 58.3 [55.9, 60.8] pmol/G Hb respectively). These findings are also consistent with previous research discussed in this thesis suggesting that AA has decreased because of a decrease in smoking, as well as consumption of AA-rich foods (WHO, 2011; U.S. FDA, 2016; Abt et al., 2019; Cornelius, 2020; CDC, 2023a). Furthermore, there appeared to be an increase in the HbAA:HbGA ratio GMs (95% CI) for smoking, SHS, and non-smoking in 2015-16 (1.3 [1.2, 1.4] pmol/G Hb, 1.2 [1.1, 1.3] pmol/G Hb, 1.1 [1.0, 1.1] pmol/G Hb respectively) compared to 2003-04 (1.1 [1.1, 1.2] pmol/G Hb, 0.9 [0.8, 1.1] pmol/G Hb, 0.9 [0.9,

2.0] pmol/G Hb respectively) suggesting more HbAA is produced in the body than HbGA in the most recent SC compared to 2003-04. Currently, there is no research to suggest biotransformation of AA could be changing so more studies are needed to conclude.

Additionally, Tables 9 and 10, show that at least one smoking group was significantly different for HbAA, HbGA, and HbAA:HbGA in both SCs ($p<0.001$); in 2003-04, SHS had 13% more HbAA and 14% more HbGA than non-smokers; SHS had 51% less HbAA, 33% less HbGA, and 16% less HbAA:HbGA than smokers; Non-smokers had 64% less HbAA, 46% less HbGA, and 18% less HbAA:HbGA than smokers (Table 11). For 2015-16, SHS had 24% more HbAA, and 13% more HbAA:HbGA than NS; SHS had 40% less HbAA, 30% less HbAA, and 9% less HbAA:HbGA than smokers; NS had 64% less HbAA, 41% less HbGA, and 22% less HbAA:HbGA than smokers (Table 12). These differences in AA exposure by smoking status showcase the importance of reducing smoke exposure to also decrease AA exposure and the harmful effects associated with this chemical.

For children, the GMs (95% CI) of HbAA and HbGA for SHS and non-smoking were 45.8 [42.7, 49.2] pmol/G Hb, 45.3 [38.8, 52.9] pmol/G Hb respectively for 2015-16 compared to 64.6 [58.2, 71.7] pmol/G Hb, 81.2 [72.5, 90.9] pmol/G Hb respectively in 2003-04 (Tables 13 and 14), suggesting a decline, which is also consistent with literature that AA has decreased due to both smoking and diet (WHO, 2011; Abt et al., 2019; U.S. FDA, 2016; Cornelius, 2020; CDC, 2023a). However, there was a

slight increase in the HbAA:HbGA ratio GMs (95% CI) for SHS and NS groups in 2015-16 (1.0 [0.9, 1.2] pmol/G Hb, 0.9 (0.9, 1.0) pmol/G Hb respectively) compared to 2003-04 (0.8 [0.8, 0.9] pmol/G Hb, 0.8 [0.8, 0.8] pmol/G Hb respectively).

Additionally, an unpaired t-test revealed a significant difference in SHS and non-smoking categories for HbAA ($p<0.0001$) and HbGA ($p<0.05$), but not for HbAA:HbGA in the 2003-04 SC (Table 13). In the 2015-16 SC, there was only a statistically significant difference among SHS and NS for HbAA ($p<0.05$), but not for HbGA, and HbAA:HbGA (Table 14). These findings suggest that the method used to classify participants by smoking status is not as pronounced for this study population and findings should be carefully interpreted.

For the adolescent's smoking category, there was a decrease in the GMs (95% CI) of HbAA and HbGA in 2015-16 (78.7 [47.2, 131.0] pmol/G Hb, 60.4 [38.6, 94.5] pmol/G Hb respectively) compared to 2003-04 (102.6 [88.7, 118.6] pmol/G Hb, 93.3 [81.0, 107.4] pmol/G Hb respectively) (Table 17 and 15 respectively). HbAA and HbGA GMs (95% CI) also decreased in 2015-16 for SHS (55.2 [37.6, 81.0] pmol/G Hb, 43.5 [31.5, 60.1] pmol/G Hb respectively) compared to 2003-04 (58.1[53.2, 63.5] pmol/G Hb, 56.9 [47.3, 68.4] pmol/G Hb respectively). Lastly, HbAA and HbGA GMs (95% CI) decreased in 2015-16 for NS (39.8 [38.2, 41.4] pmol/G Hb, 35.2 [33.0, 37.4] pmol/G Hb respectively) compared to 2003-04 SC (51.0 [49.4, 52.5] pmol/G Hb, 49.8 [46.6, 53.2] pmol/G Hb respectively).

Furthermore, for the 2003-04 SC, an ANOVA revealed a significant difference in at least one smoking category for both HbAA and HbGA ($p<0.0001$) (Table 15). A Bonferroni post-hoc test found that for the 2003-04 SC, SHS had 13% more HbAA and HbGA than NS (Table 16); SHS had 57% less HbAA and 49% less HbGA than smokers; and NS had 70% less HbAA and 63% less HbGA than smokers. These findings were expected given that smoking is known to increase AA exposure.

For the 2015-16 SC, an ANOVA also confirmed significant differences in at least one smoking category for both HbAA and HbGA ($p<0.0001$) (Table 17); SHS had 33% more HbAA than NS; SHS had 35% less HbAA and 33% less HbGA than smokers; and NS had 68% less HbAA and 54% less HbGA than smokers (Table 18).

Tables 19 and 20 confirmed a significant decrease of HbAA and HbGA for all smoking categories in combined age groups, children separately, and adolescents separately in 2015-16 SC compared to 2003-04 SC via an unpaired t-test ($p<0.0001$). These findings are consistent with current research suggesting that AA exposure has decreased in the U.S. population due to a decrease in concentration of exposure from select food sources and a decrease in smoking (WHO, 2011; U.S. FDA, 2016; Abt et al., 2019; Cornelius, 2020; CDC, 2023a). For NS, a significant decrease was also observed for HbAA and HbGA in the 2015-16 unpaired t-test compared to 2003-04 ($p<0.0001$) (Tables 22 and 23). These findings also support the current research that AA has been decreasing due to dietary changes for the U.S. population due to robust campaigns aimed at food manufacturers and consumers to prevent the development of

AA in foods (Abt et al., 2019; U.S. FDA, 2016; Cornelius, 2020). Additionally, a statistically significant increase in HbAA:HbGA ($p<0.0001$) was also observed for all smoking and NS categories in combined age groups, children separately, and adolescents separately in 2015-16 compared to the 2003-04 SC ($p<0.001$) (Table 21 and 23). These findings call for more research as no studies have been published to explore and suggest emerging biological changes in humans for AA biotransformation.

5.3 Aim 3

For the univariate regression analysis, HbAA was not greatly influenced by the demographic variables of interest (Tables A1-A3). For HbGA, there were more demographic variables that appeared to significantly influence exposure. However, the r^2 values were relatively low, even among statistically significant variables. As seen in Table 25, the most common significant variable among nonsmokers' HbAA exposure for both SCs was age; for all ages in 2003-04 SC, HbAA decreased by 2% as age increased ($p\leq0.01$); for 2003-04 children in 2003-04 SC, HbAA decreased by 2% as age increased ($p\leq0.05$); for 2003-04 adolescents, HbAA decreased by 1% as age increased ($p\leq0.05$); for all ages in 2015-16, HbAA decreased by 1% as age increased ($p\leq0.05$). For HbGA, a similar trend was observed (Table 26); for all ages in 2003-04 SC, HbGA decreased by 5% as age increased ($p\leq0.01$); for children in 2003-04 SC, HbGA decreased by 3% as age increased ($p\leq0.01$); for all ages in 2015-16 SC, HbGA decreased by 4% as age increased ($p\leq0.01$); for children HbGA decreased by 4% as age increased ($p\leq0.01$). The HbAA:HbGA ratio in 2003-04 SC

increased by 3% ($p \leq 0.01$) with increasing age for all ages; for 2015-16 SC, it also increased by 3% with increasing age for all ages and children ($p \leq 0.01$). Age appears to have the most influence on AA exposure outcomes; this finding is also aligned with previous research that children have higher exposure to AA through diet and when expressed on a bodyweight basis (Gibbs et al., 1997; Cengiz & Gündüz, 2013; Von Mühlendahl & Otto, 2003). However, the relationship between age and biomarker levels is commonly significant but weak in this study, given the r^2 values.

Race/ethnicity was another variable that appeared to, at times, influence exposure, particularly for NHB participants; in 2015-16 SC, NHB children had 12% more AA than NHW ($p \leq 0.01$) (Table 25). For HbGA, NHB had 16% less than NHW in 2003-04 SC ($p \leq 0.01$) (Table 26); NHB children in 2003-04 SC had 23% less HbGA than NHW ($p \leq 0.01$); NHB adolescents had 11% less HbGA than NHW ($p \leq 0.05$); for all ages in 2015-16 SC, NHB had 4% less HbGA than NHW ($p \leq 0.01$); NHB children had 4% less HbGA than NHW ($p \leq 0.05$) in 2015-16 SC; and NHB adolescents had 16% less HbGA than NHW ($p \leq 0.05$). For HbAA:HbGA, NHB appeared to commonly have higher ratios than NHW (Table 27). In the 2003-04 SC, NHB had 16% higher HbAA:HbGA ratios than NHW for all ages ($p \leq 0.01$); NHB children also had 22% higher HbAA:HbGA ratios than NHW ($p \leq 0.01$); NHB adolescents also had 11% higher HbAA:HbGA ratios than NHW ($p \leq 0.05$); NHB had 17% higher HbAA:HbGA ratios than NHW ($p \leq 0.01$) for 2015-16 SC all ages; and lastly, NHB children had 24% higher HbAA:HbGA ratios than NHW ($p \leq 0.01$) in the 2015-16 SC. These findings suggest potential differences in biotransformation of AA by

race/ethnicity, which have been studied before; the frequency of GSTM1 null individuals is higher in Caucasians and Asians (42–60%) than in Africans (16–36%) (Garte et al., 2001). The GSTM1 enzyme metabolizes epoxides, and is expressed in many organs, such as the liver, testis, adrenals, and white blood cells (Paullson et al., 2005).

Overall, demographic variables do not appear to significantly influence HbAA exposure, except for age, where younger ages appear to have more AA exposure compared to older ages, even when controlling for smoking. However, HbAA exposure appears to be driven mostly by diet and smoking habits. The variability in findings for HbGA and HbAA:HbGA among groups are consistent with previous research studies that suggest interindividual differences, rather than demographics, drives exposure outcomes. In conclusion, AA exposure in the U.S. population has decreased from 2015-16 compared to 2003-04, and this can be attributed to a decrease in smoking and education about AA in food since its discovery in food in 2002.

5.4 Overall Conclusions

Overall, combined age groups, children, and adolescents experienced a decline in HbAA GMs (95% CI) in 2015-16 (42.8 [41.4, 44.2] pmol/G Hb; 43.2 [41.8, 44.5] pmol/G Hb; 42.5 [40.3, 44.78] pmol/G Hb respectively) compared to 2003-04 (57.9 [55.7, 60.1] pmol/G Hb; 58.6 [56.2, 61.1] pmol/G Hb; 57.4 [54.6, 60.4] pmol/G Hb respectively) ($p<0.0001$). These findings are consistent with current research suggesting that AA exposure has decreased in the U.S. population due to a decrease

in concentration of exposure from select food sources and a decrease in smoking. For the non-smoking groups, a significant decrease in HbAA GM (95% CI) was also observed for combined age groups, children, and adolescents in 2015-16 (41.2 [40.2, 42.2] pmol/G Hb; 43.2 [42.0, 44.5] pmol/G Hb; 43.2 [42.0, 44.5] pmol/G Hb; compared to 2003-04 (53.4 [52.0, 54.9] pmol/G Hb; 57.1 [54.7, 59.5] pmol/G Hb; 51.0 [49.4, 52.5] pmol/G Hb) ($p<0.0001$). These findings also support the current research that AA has been decreasing due to dietary changes for the U.S. population.

Additionally, a statistically significant increase in HbAA:HbGA ($p<0.0001$) was also observed for all smoking and NS categories in combined age groups, children separately, and adolescents separately in 2015-16 compared to the 2003-04 SC ($p<0.001$). No studies have been published to explore and suggest emerging biological changes in AA biotransformation, therefore more research is needed. However, there was a significant difference ($p=0.001$) in non-smoking children (0.8 [0.8, 0.8] pmol/G Hb at 2003-04 and (0.9 [0.9, 1.0] pmol/G Hb at 2015-16 versus adolescents (1.0 [1.0, 1.1] pmol/G Hb at 2003-04 and (1.1 [1.0, 1.2] pmol/G Hb at 2015-16, respectively, suggesting children may be at greater risk to health effects associated with AA exposure compared to adolescents. Furthermore, higher levels of HbAA and HbGA were observed in children compared to adolescents at both time points, despite smoking being far less frequent, highlighting the role of diet. In a multivariate regression analysis of non-smokers only, age and race significantly contributed to the HbAA biomarker levels, with higher HbAA in younger age groups and in non-Hispanic black populations, highlighting a disparity in exposure pattern.

In conclusion, AA exposure appears to have reduced from 2003-04 to 2015-16; the reduction is driven by both changes in smoking and diet. The young and non-Hispanic blacks remain at highest risk of exposure and potential health effects from exposure to AA.

5.5 Strengths and Weaknesses

This thesis contributed to AA research by updating current literature and filling in gaps in knowledge regarding the two SC comparisons. This was accomplished by using the most up-to-date data SC to analyze if AA exposure levels have changed for children and adolescents since the chemical's discovery in foods in 2002. This is critical because these findings measured the success of reducing AA exposure among this vulnerable population, along with determining areas of improvement. These findings may guide public health researchers and government agencies in future studies, policy making, and targeted outreach efforts for populations at highest risk. Additionally, the use of blood biomarkers (HbAA, HbGA, and cotinine) were a strength of this study because HbAA and HbGA measure long-term exposure, and cotinine is an objective marker of smoking status.

Weaknesses of this paper include the small sample sizes from the 2015-2016 SC that only surveyed a small population of people for the AA biomarkers, although NHANES provided a special survey weight to allow for the calculation of population estimates. Additionally, this thesis has omitted variable bias caused by the lack of

dietary data, occupational, and environmental exposure data, which can all serve as confounders and/or effect modifiers.

Another weakness of this paper is the lack of a robust imputation methodology, which would require a detailed understanding of the paradata associated with the survey. The strategy implemented in this study assumes that the missingness in AA and GA values is random and therefore does not bias the estimation. However, a more detailed look at the missing values would be necessary to make this claim with certainty. As discussed in the study, non-response bias from missing data is an important source of survey error and should be carefully considered (NHANES, 2018).

Lastly, collapsing Mexican Americans with Other Hispanics to create the Hispanic category introduces bias in that this category is very broad; Mexican Americans and Other Hispanics' food consumption patterns vary greatly. Cuisine for these racial/ethnic groups is influenced by regional origin, as well as other racial/ethnic backgrounds, such as African, European, Indigenous, and Asian heritage, and/or as a blend of two or more of these races. Additionally, the "Other" category presented by NHANES, which comprises Asians and multiracial individuals, who are also of a diverse ethnic background, is too broad as well; lack of distinctions among these groups decreases the reliability of the results. Lastly, the classification of PIR is problematic because the below and above poverty categories are too broad. Researchers may benefit from creating more subcategories.

Appendices

Table A1. Nonsmoking Univariate Regressions for HbAA Exposure by Age Categories and by Year

Cohorts	Significant	Nonsignificant	N
AA 0304 All Ages			
Gender		0.2382	2015
Race/ethnicity		0.6642	2015
Age	0.0001		2015
Poverty		0.1477	2015
AA 0304 Children			
Gender		0.7764	633
Race/ethnicity		0.7912	633
Age	0.0071		633
Poverty		0.9320	633
AA 0304 Adolescents			
Gender		0.1340	1382
Race/ethnicity		0.6309	1382
Age		0.0698	1382
Poverty		0.2196	1382
AA 1516 All Ages			
Gender		0.4557	591
Race/ethnicity		0.4649	591
Age	0.0110		591
Poverty		0.9866	591
AA 1516 Children			
Gender		0.3141	285
Race/ethnicity		0.4674	285
Age		0.1019	285
Poverty		0.2966	285
AA 1516 Adolescents			
Gender		0.0804	306
Race/ethnicity		0.6575	306
Age		0.4285	306
Poverty		0.5743	306

Table A2. Nonsmoking Univariate Regressions for HbGA Exposure by Age Categories and by Year

Cohorts	Significant	Nonsignificant	N
GA 0304 All Ages			
Gender	0.0378		2051
Race/ethnicity	0.0004		2051
Age	<0.0001		2051
Poverty		0.1525	2051
GA 0304 Children			
Gender		0.2148	644
Race/ethnicity	0.003		644
Age	0.0094		644
Poverty		0.2586	644
GA 0304 Adolescents			
Gender	0.0047		1407
Race/ethnicity	0.0263		1407
Age		0.4023	1407
Poverty		0.3814	1407
GA 1516 All Ages			
Gender		0.2752	550
Race/ethnicity		0.619	550
Age	<.0001		550
Poverty		0.6989	550
GA 1516 Children			
Gender		0.2112	269
Race/ethnicity		0.9326	269
Age	0.0004		269
Poverty		0.2227	269
GA 1516 Adolescents			
Gender		0.6059	281
Race/ethnicity		0.7322	281
Age		0.2497	281
Poverty		0.2046	281

Table A3. Nonsmoking Univariate Regressions for HbAA:HbGA Exposure by Age Categories and by Year

Cohorts	Significant	Nonsignificant	N
AA:GA 0304 All Ages			
Gender		0.1995	1932
Race/ethnicity	0.0009		1932
Age	<.0001		1932
Poverty		0.2562	1932
AA:GA 0304 Children			
Gender		0.2555	609
Race/ethnicity	0.0063		609
Age		0.0667	609
Poverty		0.3948	609
AA:GA 0304 Adolescents			
Gender	0.0198		1323
Race/ethnicity	0.0133		1323
Age		0.587	1323
Poverty		0.6502	1323
AA 1516 All Ages			
Gender		0.1839	544
Race/ethnicity	0.0035		544
Age	<.0001		544
Poverty		0.6756	544
AA:GA 1516 Children			
Gender	0.0064		266
Race/ethnicity		0.054	266
Age	0.0044		266
Poverty		0.9571	266
AA:GA 1516 Adolescents			
Gender		0.5226	278
Race/ethnicity		0.0619	278
Age		0.3127	278
Poverty	0.0226		278

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