Occupational Chemical Exposure Assessment in a Brain Cancer Case-Control Study

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THESIS

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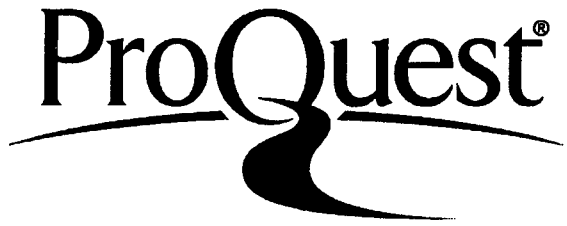

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This thesis is dedicated to the memory of my parents, Paul B. and Helen B. Shimek who brought me into this world and to my sister, Helen, for her continuous support.

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LIST OF ABBREVIATIONS

%	Percent
√	Square root
µg	Microgram
µm	Micrometer
AA	Acrylamide
AA-Val	N-(2-carbamoylethyl)-valine
AA-Hb	N-(2-carbamoylethyl)-valine
ACGIH	American Conference of Governmental Industrial Hygienists
ANC	Animal Neurocarcinogen
Arg	Arginine
ATSDR	Agency for Toxic Substances and Disease Registry
B6C3F1	Mouse strain
BD	1,3-Butadiene
BD-diol	3-Butene-1,2-diol
BLS	U.S. Bureau of Labor Statistics
CAS	Chemical Abstract Service
CBTRUS	Central Brain Tumor Registry of the United States
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CPD	Carcinogenic Potency Database
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cancer Slope Factor
CYP 2E1	Cytochrome P450 2E1
DEB	1,2,3,4-Diepoxybutane
DNA	Deoxyribonucleic acid
DOL	U.S. Department of Labor
EB	1,2-Exoxy-3-butene
EB-diol	1,2-Epoxy-3,4-butanediol
EH	Epoxide hydrolase
EIF	Exposure Intensity Factor
EMF	Electromagnetic frequencies
EPA	Environmental Protection Agency
EPHX1	Microsomal epoxide hydrolase
GA	Glycidamide
GA-Hb	N-(2-carbamoyl-2-hydroxyethyl)-valine
GA-Val	N-(2-carbamoyl-2-hydroxyethyl)-valine
GI	Gastrointestinal
GSH	Glutathione-S-transferase
GSTM1	Glutathione S-transferase Mu 1
GSTT1	Glutathione S-transferase Theta 1
GSTP1	Glutathione S-transferase Pi 1

Hb	Hemoglobin
HB-Val	N-(2-hydroxy-3-buten-1-yl)-valine
HepG2	Hepatocellular carcinoma human pepetual cell line
His	Histidine
HPLC	High Performance Liquid Chromatography
hprt	Hypoxanthine-guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
IISRP	International Institute of Synthetic Rubber Workers
in	Inch
IRB	Institution Review Board
IRIS	Integrated Risk Information System
JEM	Job Exposure Matrix
K	Kappa
lbs	pounds
LOAEL	Lowest Observed Adverse Effect Level
m^2	square meter
MS	Mass Spectroscopy
mg/kg/day	Milligram per kilogram per day
mg/m^3	Milligram per cubic meter
N	Number
NCI	National Cancer Institute
NHANES	National
NIOSH	National Institute for Occupational Safety and Health
nmol/g globin	Nanomole per gram globin
NOES	National Occupational Exposure Survey
NOHS	National Occupational Health Survey
NPL	National Priority List
NTP	National Toxicology Program
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
p	Probability
PAH	Polycyclic Aromatic Hydrocarbon
PEL	Permissible Exposure Limit
pmol/g globin	Picomole per gram globin
ppm	Part per million
Pyr-Val	*N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine
REL	Recommended Exposure Limit
RTECS	Registry of Toxic Effects of Chemical Substances
SD	Standard Deviation
SIC	Standard Industry Classification
SMR	Standard Mortality Ratio

SOC	Standard Occupational Classification
SPH	School of Public Health
SPORE	Specialized Programs of Research Excellence
TD_{50}	Toxic Dose to fifty percent of test animals
THB-Val	2,3,4-trihydroxybutyl-valine
TLV-TWA	Threshold Limit Value - Time Weighted Average
Tyr	Tyrosine
tk	Thymidine kinase
UIC	Univeristy of Illinois at Chicago
UNC	Univerity of North Carolina at Chapel Hill
Val	Valine

SUMMARY

This dissertation titled *"Occupational Chemical Exposure Assessment in a Brain Cancer Case-Control Study"* documents three projects exploring the association between the potential exposure to known of suspected animal neurocarcinogens (ANCs) found in the workplace and the incidence of brain cancer, specifically, glioma.

The data used in this study were obtained from a case-control study of glioma. This study was divided into two phases, a pilot study and the main study. Pilot study data were used to complete the first project. Study participants in the pilot study provided hand written self-reported work histories. Each of the study participant's jobs was coded for industry using the Standard Industry Classification System (SIC) and occupation using the Standard Occupation Classification System (SOC) based on the work history.

Members of the research group completed an extensive literature search and compiled a list of more than 40 known or suspected animal neurocarcinogens. This list was narrowed to 18 chemicals found in the workplace as identified by the National Institute for Occupational Safety and Health (NIOSH) during the completion of the National Occupational Exposure Survey (NOES), 1981-83. The NOES was similarly coded for industry and occupation and the work histories compared to the NOES to develop a cumulative exposure score. Cumulative exposure scores were generated for each study participant based on the 18 chemicals, their work history and an exposure intensity factor based on occupation within the industry of their jobs.

The results of the pilot study data analyses demonstrated that a cumulative exposure score could be developed from the work histories; however, all industries and/or occupations were not surveyed by the NOES and only 41% of all jobs could be scored for potential exposure. The association between the cumulative exposure score and case/control status was examined by calculation of odds ratios for matched pair analyses using all controls, only sibling controls and only friend controls and a frequency matched pair, as well as unmatched cases and controls. Odds ratios indicated a non-significant association with reduced odds between cases and controls.

Analyses of individual industries, occupations and chemical (ever versus never exposed) showed slightly elevated odds ratios.

The second project utilized main study data. Study participants were asked a series of questions concerning the industries and occupations where they had worked, which were targeted to evaluate potential exposure to specific ANCs. From the responses to the questions provided by study participants, potential exposure to acrylamide, 1,3-butadiene and polycyclic aromatic hydrocarbons were evaluated for cases and controls.

Odds ratios were again calculated to evaluate the association between case/control status and industry, occupation or chemical. These results indicated statistically significant reduced risk of glioma based on potential workplace exposure to these chemicals.

The third project was a biomarker study. Hemoglobin adducts are biomarkers that indicate that an exposure to a specific chemical has occurred. In this study, a subset of the control population of either the pilot or main studies provided blood samples which were analyzed to detect exposure to 1,3-butadiene and acrylamide. The control population was used to avoid any interference from treatments the cases may have received. The study was intended to show an association between potential exposure to the chemicals as indicated by the SIC code of their current job and the reported hemoglobin adduct concentrations. However, the study was small and a majority of participants were either retired or working in an industry not surveyed by NIOSH. Therefore, there either was no potential exposure or the exposure could not be determined.

Hemoglobin adduct results were also compared to available demographic, smoking and diet variables. When comparing study participants who resided in Southern states to those living in Northern states the difference between the geometric means of the hemoglobin adducts for the *pyr*-Val were higher and statistically significant. Statistically significant differences between smokers and non-smokers and ever smokers and never smokers were also statistically significant for the acrylamide adduct results.

Study participants reported the frequency of consumption of several foods as part of the diet history questionnaire. The resulting data were combined for analyses of the various question responses and were suggestive of an association but not statistically significant for most of the geometric mean comparisons. A comparison of the responses to the question regarding the frequency of consumption of corn, wheat and millet products indicated a statistically significant difference in the geometric means for the glycidamide adduct based on the frequency of consumption.

Hemoglobin adduct results were not associated with the potential for exposure to either acrylamide or 1,3-butadiene. The study does report the first human detection of the *pyr*-Val hemoglobin adduct to 1,3-butadiene. The epoxide that forms the *pyr*-Val hemoglobin adduct is the most mutagenic of the three possible epoxides formed by 1,3-butadiene metabolism. Acrylamide adduct formation was comparable to the results recently published in the Fourth National Report on Human Exposures to Environmental Chemicals.

Collectively, these three projects demonstrate the need for improved exposure assessment methods including the sharing of available qualitative data, such as personal sampling data, to better predict potential exposures to ANCs. Collaboration between epidemiology and exposure assessment fields with additional quantitative data such as biomarker data will continue to refine the exposure assessment process.

1.0 BACKGROUND

1.1 The National Occupational Exposure Survey

The National Occupational Exposure Survey (NOES) (National Occupational Exposure Survey, 1991) was conducted by the National Institute for Occupational Safety and Health (NIOSH) during 1981-1983 with the intent of providing an understanding of the potential exposure to chemical, physical and biological hazards faced by workers and related health and safety workplace conditions. The NOES was a follow-up to the 1972-1974 National Occupational Hazard Survey (NOHS). Both surveys were conducted in a similar fashion (Sieber et al., 1991). For the NOES, NIOSH industrial hygienists visited nearly 4,500 workplaces across the United States and gathered information from non-agricultural workplaces with eight or more employees regulated by the Occupational Safety and Health Administration (OSHA). The surveyed workplaces included 522 industry types and 377 occupation categories. Surveyors collected data on agents in use at the facility, including trade name compounds, the occupations of the workers using the agents and the type of industry. Industries were classified using the Standard Industry Classification system (SIC) (Standard Industry Classification system) and the 1980 Bureau of Census occupation codes (1980 Census of Population and Housing, 1980). The survey resulted in the creation of a searchable database of nearly 13,000 different compounds with potential worker exposure based on industry and occupation.

1.2 Exposure Assessment

In the study of adverse effects, such as death or disease, a retrospective comparison is made between those with the adverse effect (cases) and those without (controls) the adverse effect in an effort to determine the differences between the two groups. Toxicology studies are typically prospective studies, conducted by exposing one group of animals to a particular agent and comparing disease (or other health endpoint) incidence in the exposed group to that in the unexposed group of animals and noting

any statistical differences related to the hypothesis being tested. Rarely (e.g., exposure chamber studies with volunteers under controlled exposure conditions) are humans used as the test population. Epidemiology studies are designed to look for differences in a specified population that may result in the adverse event of interest to the study. In epidemiology studies, exposure to a test agent may have occurred or might occur but human populations are not intentionally exposed to test agents.

Many types of studies are used by epidemiologists to evaluate the exposure-disease relationship. These include the cohort study where, typically, the study population is comprised of two groups; each disease free at the start of the study where one group is or has had the exposure of interest and the other is/has not. The study population is then followed for a period of time and the incidence of disease occurrence between the two groups is compared (Rothman and Greenland, 1998). An example of this type of study might include a particular factory or industry where those manufacturing the product are compared with the administrative or support staff of the same factory or industry. In a cohort study the measure of association is relative risk and is equal to the risk of disease in the exposed population divided by the risk of disease in the unexposed population. If the relative risk is greater than one, the exposed population is at an increased risk of disease (Gordis, 2008).

The second study design is the case-control study. In this study design, subjects are divided into cases (those with the disease state of interest) and controls (a non-diseased comparison group that is selected from the same population). The selection of controls is important and dependent on the exposure being studied. Controls might be siblings or friends of the controls; they might live in the same neighborhood or be chosen randomly, or they might be hospital-based controls selected from the same treatment facility as the cases but from a different clinic or department (Rothman and Greenland, 1998). In a case-control study the measure of association is the odds ratio. In a case-control study, the incidence of disease in the exposed versus non-exposed study subjects is not known, thus relative risk cannot be

calculated directly. The odds ratio approximates the relative risk in case-control studies assuming the disease of interest is rare and cases and controls are representative of the populations under observation. The odds ratio is defined as the probability that the case was exposed to the agent of interest divided by the probability that a control was exposed to the agent (Gordis, 2008).

Crombie (1981) discussed the limitations of the case-control study with respect to uncommon or rare exposures such as occupational exposures to carcinogens. The main limitation for the case-control study design is the large number of cases that would be needed to detect a significant odds ratio (e.g. OR=1.5). If a study has one control for each case and an incidence rate of 10 per 100,000, it is estimated that 900 cases and 900 controls would be needed to observe an odds ratio of 1.5 with a two-tailed test , $p \leq 0.05$ and 80% power. For a cohort study, 100,000 subjects would be needed to detect an incidence rate of 10.0 per 100,000. The case-control study is used to study rare diseases as the size of the study population required for a cohort study would be extremely large (Gordis, 2008). However, a large number of cases are needed to detect small increases in risk for rare exposures (Crombie, 1981).

Exposure assessment in occupational studies is often conducted by first identifying a disease state and then looking back, retrospectively, at the employment history of the study participant. In the case of occupational cancers, a long latency period between the time when the exposure occurred, and when the disease presents itself, makes the characterization of exposure challenging. An accurate work history is needed to assess the potential exposures an employee has experienced over his/her employment history. Payroll records or personnel records may be used to document the work history. These may have a general description of the job held (e.g., accountant, operator) or may be incomplete if the study participant has held several jobs during his career. Two commonly employed methods of evaluating work histories for exposure reconstruction, job exposure matrices and self-reported work histories, are discussed below.

1.2.1 Job Exposure Matrix

Knowing what chemicals are found in the workplace assists in the evaluation of worker exposure. Direct measurements, such as industrial hygiene monitoring of the workplace air, provide better measures of potential worker exposures. When exposure monitoring data are not available, a job exposure matrix (JEM) is often created to evaluate potential workplace chemical exposures. A job exposure matrix is a flexible tool that can be tailored to the type of exposure being evaluated. One axis may include the industry/occupation to be analyzed and the other the agent or chemical of interest. The cells of the matrix can represent different variables such as presence of the agent (yes or no), intensity of the exposure (high, medium or low), probability of exposure (high or low), etc. (Nieuwenhuijsen, 2003). The individual cells may be added, subtracted (i.e., credit for wearing personal protective equipment) or multiplied to generate a total score for each industry/occupation. Thus, a quantitative rating system for potential exposures can be generated.

The JEM can be designed to include a variety of variables. In a study of Polish rubber workers, a JEM was constructed to compare industrial hygiene monitoring data for several departments over a period of years. Industrial hygiene monitoring data for aromatic amines, inhalable aerosols and cyclohexane soluble matter were available for the departments for the years 1981-1996. Results showed that increased exposure to inhalable aerosols was observed in the early compounding stages of the manufacturing process while aromatic amine exposures were higher during the curing process when the rubber was heated. Some correlation was also noted between exposures and production volumes. The data from the matrix results continue to be used for an ongoing epidemiological study of the rubber workers (de Vocht et al., 2008).

JEMs may also be constructed for large population studies. Kauppinen et al. (2009) constructed job exposure matrices for the Nordic Occupational Cancer Study which analyzed over 300 occupations and more than 20 different compounds over four time periods from 1945-94. Census data for each country was used to determine the number of workers in a given occupation. From national databases of exposure monitoring data, a mean exposure level was generated for each occupation. Based on the study design, the number of occupation-exposure pairs was limited to 74. Exposure was estimated by multiplying the proportion of exposed to non-exposed workers times the mean concentration level of the agent in the given time periods. This matrix development allowed for comparison of occupations between the five Nordic Countries; Denmark, Finland, Iceland, Norway and Sweden (Kauppinen et al., 2009).

In a study conducted by Post et al. (1994) a JEM was created for a study of respiratory disease and occupation in Zutphen, Netherlands. The study followed 878 randomly sampled men for 25 years and evaluated ten specific exposures (e.g., welding fumes, pesticides, solvents, etc.) related to respiratory disease. Occupational histories were obtained from responses to self-reported questionnaires that were coded for occupation. The work histories were rated by experts for duration of exposure to one of the ten specific exposure groups and the time since the first exposure. The authors concluded that the JEM they had developed from self-reported data for their study cohort was consistent with generic external JEMs and that the use of self-reported data could be used to evaluate exposures in general population studies (Post et al., 1994).

Many different variables can be added to the industry/occupation JEM. Dosemeci et al. (1994) added three variables to a JEM study of methylene chloride exposure (with, at least, 21 years of exposure) and astrocytic brain cancer. These variables included a probability of exposure variable (high, medium or low), an overtime variable to reflect changes in the occupational exposure limits by decade, and a within

SIC code variable to reflect the possibly different exposures within the industry classification. In the highest exposure category, the relative risk was increased from 1.85 to 6.08 (95% CI: 0.64-5.42 and 95% CI: 1.11-43.8, respectively) with the addition of these three factors. However, the numbers of cases in this category was small. The authors concluded that the inclusion of additional variables increased the accuracy of the study (Dosemeci et al., 1994).

Sieber et al. (1991) and NIOSH have developed a JEM based on the NOHS. The NOHS-JEM used three exposure variables: industry, occupation in the industry, and potential exposure to the agent of interest. Potential exposure was defined as the agent being in use near employees, in a manner such that employees were likely to come in contact with it, and the exposure was likely to occur at least 30 minutes per week (on average) throughout the year. The intersection of the three axes provides an estimation of the workers potentially exposed to the agent of interest in a given industry and occupation. This JEM can similarly be used with the NOES database.

Ruder et al. (1990) used the NOHS and NOES data to examine occupational exposures to animal bladder tumorigens. The study was designed to estimate the number of workers with an increased risk of bladder cancer. A list of approximately 200 chemicals associated with bladder tumors was generated from the Registry of Toxic Effects of Chemical Substances (RTECS) and compared to the NOHS and NOES databases. Tables were generated showing the number of potentially exposed workers based on both industry and occupation. The results were compared with results reported in the literature. Case-control studies from the literature indicated that white men who were painters, machinists or truck drivers had an increased risk of bladder cancer. When compared with the results of the NOHS and NOES studies, no increased risk of bladder cancer was identified for these occupations. This study did reveal a potential increased risk of bladder cancer for nurses and electricians (Ruder et al., 1990).

1.2.2 Self-Reported Work Histories

By itself, a JEM may not adequately predict the exposure to chemicals in the workplace. In addition, workers may move from job to job or department to department within their careers, requiring worker input in addition to the JEM to evaluate their chemical exposures. Data may include a log of all jobs held, most recent job, longest held job, etc. (Teschke, 2002). Job histories may also be obtained by use of a questionnaire or by an interview. Regardless of how the data are obtained, the validity of the data is dependent on the memory of the individual providing the data and is subject to recall bias. In retrospective exposure assessments, self-reported data may be the only way to obtain information regarding work histories. Several studies have been conducted to test the reliability and validity of self-reported data (See Teschke, 2002 for a review).

Adegoke et al. (2004) compared self-reported work histories to a JEM in a case-control study of newly diagnosed leukemia patients in Shanghai, China. The study looked at exposure to benzene, other organic solvents, pesticides and electromagnetic frequencies (EMF). A JEM rated the potential exposures as none, low, moderate or high based on standardized industry and occupation codes. Self-reported work histories were collected through the use of a standardized questionnaire. Based on the questionnaire responses, participant exposure was rated as ever exposed or never exposed. Results showed good agreement between JEMs and self-reported work histories (kappa coefficients of agreement, K, ranged from 0.5 – 0.9). The sensitivity and specificity for the self-reported responses were also calculated. The sensitivity varied from 0.75-0.98 with the exception of the responses from the $\geq$ 50 year old group where the sensitivity was 0.44 for pesticide exposures. The specificity varied from 0.87-0.99 for all exposures. The authors concluded that the self-reported data was in agreement with the JEM. However, they did not find a significant association for leukemia status between cases and controls.

Schlaefer et al. (2009) conducted a study evaluating the validity of self-reported data compared with a JEM designed to evaluate occupational noise exposures. In this study, a review of the literature provided noise exposures and associated occupations that were used to create the JEM. Study participants were cases diagnosed with acoustic neuroma; controls were randomly selected from population registries. The intent of the study was to determine if an association existed between acoustic neuroma and loud occupational noise. Study subjects answered questions related to noise exposures through a computer assisted interview process. Using available noise monitoring data from government databases, exposures were compared to self-reported exposures based on the loudest job held and longest job held. Kappa statistics were calculated to assess the level of agreement between the JEM and the self-reported data (K = 1 complete agreement, K = -1 complete disagreement). Sensitivity and selectivity were also calculated (K range: 0.46-0.62; Sensitivity: 0.51-0.87 and Specificity: 0.70 – 0.93). The authors did not find a statistically significant association between acoustic neuroma and noise exposure. However, based on the kappa statistic results, sensitivity and specificity both the JEM and self-reported exposures reached similar conclusions (Schlaefer et al., 2009).

Information about a study participant's work history may also be obtained through the use of detailed questionnaires. Nieuwenhuijsen (2003) discussed several important factors that should be considered when designing a questionnaire. Questionnaires should be written in a language that the study populations understand. They should be clear and concise, should avoid asking for too much detail, and should use open-ended questions. Questionnaires should be designed with an idea as to how the responses will be analyzed. Open ended questions require interpretation before the analysis can be done and this may introduce misclassification bias. Interpretations should be made by well-trained professionals to avoid this type of bias (Nieuwenhuijsen, 2003).

Well-trained individuals or experts have been used to evaluate data relative to occupational exposures as they are thought to be more familiar with exposure variables, such as routes of exposure, manufacturing practices and ability to interpret results found in the literature. Swuste et al. (2008) described a study in which a team of five experts evaluated over 3,000 asbestos exposures to create a JEM. Using existing databases of industrial hygiene monitoring results collected from 1945-1994 and descriptions of industries and occupations, an estimate of historical asbestos exposure was generated for various occupations in the Netherlands.

Many studies (See Teschke et al., 2002 for review) compare results of self-reported data or JEM results with expert evaluations. In a retrospective exposure assessment conducted by Rybicki et al. (1997) three different measures of exposure assessment, self-reported, NIOSH-JEM and the professional judgment of an industrial hygienist were compared. The industrial hygienist provided an independent assessment of the exposures and this assessment was used as the reference category. This study examined the exposure to three metals; copper, lead and iron (N=115). Study participants had an average of 22.4 years in a single industry. Self reported data were collected from a comprehensive questionnaire. Percent agreement with expert evaluations, specificity and selectivity were quantified. All jobs were represented in the JEM. The self-reported data were in better agreement with the industrial hygienist's opinion than the JEM. When the self-reported data were combined with the JEM, results did not improve (Rybicki et al., 1997).

Rodvall et al. (1996) conducted a case-control study in Sweden to study the association between occupation and glioma. Cases and controls completed a questionnaire related to their work history and exposure to specific chemicals or group of chemicals (i.e., pesticides, solvents, insulating materials, etc.). An industrial hygienist reviewed the questionnaires to determine the possibility of exposure to the chemicals based on industry and occupation. Agreement between the self-reported data and expert

assessment was evaluated by calculation of the interclass coefficient, Kappa statistic. When cases and controls were compared to the expert assessment the Kappa statistic varied from K= 0.46-0.88, respectively.

Self-reported work histories are subject to recall bias and may contain errors with regard to agent and duration of exposure. Misclassification is also a problem as the details of the exposure to the agent in question may be unknown. In a review of exposure assessment methods used in case-control studies in the literature, Teschke (Teschke, 2002) concluded that the independent expert assessment was slightly better than self-reported information and that validity and reliability will vary greatly depending on study design and methods. The author further concluded that exposure assessment accuracy would be improved with the increased availability of quantitative data such as industrial hygiene monitoring or biomonitoring data.

1.3 Toxicology –General Considerations

1.3.1 Routes of Exposure

For an exposure to a chemical to occur, the chemical must not only be present in the environment, it must enter the body. The three major pathways by which a chemical enters the body are inhalation, ingestion, and absorption (through skin or eye). In the workplace, the primary routes of exposure are inhalation and skin absorption (Casserett and Doull, 2001). Adverse effects from the exposure are dependent on the frequency of exposure, the concentration of the chemical, and the length of time or duration of the exposure (Casserett and Doull, 2001). Workplace exposures may be a single acute exposure, subchronic exposure for weeks or months, or chronic exposure of months or years (Casserett and Doull, 2001). Acute effects include chemical burns from contact with a strong acid or base or irritation of the respiratory system from inhalation of noxious fumes. These exposures often cause

reversible damage to the body. Long-term exposures, i.e., subchronic or chronic, may result in irreparable and permanent damage to the body (Casserett and Doull, 2001).

1.3.2 Absorption, Distribution, Metabolism, and Excretion

When a chemical enters the body, several different responses may occur. The chemical may be eliminated unchanged through the lungs or gastrointestinal (GI) tract. The chemical may cause a direct toxic effect, the chemical may be metabolized to a less reactive stable metabolite and excreted, or it may form a more reactive metabolite that then reacts with target sites creating the toxic effect (Timbrell, 2001). Chemicals eliminated unchanged before reaching the circulatory system (first pass elimination) do not reach a target site and thus do not cause a toxic effect (Casserett and Doull, 2001). Once a chemical enters the circulatory system it may be transported to various organs and tissues where it is metabolized. This biotransformation may create stable metabolites that are excreted or eliminated from the body, or create a more reactive metabolite that will undergo further reactions and may result in a toxic effect. Metabolism may be a two phase process. Hydrolysis, reduction and oxidation reactions are Phase I reactions and may result in a small increase in hydrophilicity. Glucuronidation, sulfation, acetylation, methylation and conjugation with glutathione are examples of Phase II or biotransformation reactions (Casserett and Doull, 2001). These reactions may increase the hydrophilic properties of the compound and increase the excretion of the chemical.

Chemically reactive metabolites may be formed when stable compounds are metabolized by the cytochrome P-450 monooxygenase system or other enzymes. Epoxide metabolites may bond to cellular macromolecules resulting in necrosis or cancer. Free radicals may also be formed and may cause tissue damage (Lu, 1996).

1.3.3 Carcinogenesis

Response to a chemical exposure in the occupational setting is often undesirable. Toxic effects may be localized at the point of contact (such as a rash or skin irritation) or may affect a target organ. Toxic effects may be reversible or irreversible, and may have an immediate-onset or be delayed. It is possible that carcinogenic effects may first become apparent 10-20 years following exposure (Lu, 1996). Cancer, including lesions, neoplasms, or tissue growths, are defined by genetic variance at the germ cell level, and abnormal and autonomous cell growth that may be a response to environmental exposures. Neoplasms are either benign or malignant. A benign neoplasm is more commonly referred to as a tumor, while a malignant neoplasm is capable of metastatic secondary growths in cells away from the primary site. Malignant neoplasms are referred to as cancer. In toxicology studies, the exposure of a test population to a substance of interest that causes increases in neoplasm formation when compared to an unexposed population defines the term carcinogen (Casserett and Doull, 2001). Most evidence of the carcinogenicity of a chemical comes from animal studies. Carcinogenicity in humans is often observed following occupational exposure to the chemical agent (Lu, 1996).

Based on both human evidence and animal toxicity testing, the International Agency for Research on Cancer (IARC) has developed a classification system to rate the weight of evidence for carcinogenicity. The classifications are as follows: Group 1-Carcinogenic to humans; Group 2A- Probably carcinogenic to humans; Group 2B-Possibly carcinogenic to humans; Group 3-Not classifiable as to its carcinogenicity to humans, and Group 4-Probably not carcinogenic to human (IARC, 2006).

1.3.4 Carcinogen Mechanism

As stated above, upon entering the body a chemical may follow several pathways as follows: 1. the chemical enters the body and is eliminated unchanged; 2. the chemical is metabolized, then eliminated;

3. the chemical is eliminated following conjugation through covalent bonding; or 4.the chemical, as a reactive metabolite, may bind covalently with a macromolecule. These pathways are not mutually exclusive as a chemical may follow several different paths during its metabolism. If DNA is altered by the interaction with the reactive metabolite, cancer initiation may occur (Lu, 1996).

Many types of genes are involved in normal cell growth and development. Proto-oncogenes assist in cellular growth, replication and the maintenance of tissue. When mutations occur in proto-oncogenes the normal processes are interrupted resulting in uncontrollable cell growth and replication. These mutant alleles of proto-oncogenes are called oncogenes (Hans-Olov et al., 2002). Tumor-suppressor genes are also responsible for regulating cell growth and prevent cell growth when the growth-stimulatory signals are absent. Mutations in these genes result in a failure to inhibit cell growth (Hans-Olov et al., 2002). DNA repair genes, as their name implies, function by identifying altered DNA and repairing the mutation. If mutations in DNA repair genes occur, tumor-suppressor and proto-oncogene mutations may accumulate leading to cancer initiation and progression, and eventually leading to the formation of a neoplasm, i.e., cancer (Hans-Olov et al., 2002). Even with no mutation in DNA repair genes, mutations may still occur in tumor-suppressor or proto-oncogenes.

All cancers have some common features. These include uncontrolled cell growth, evasion of programmed cell death, changes in interaction with adjacent cells, avoidance of immune system functions and invasion into normal healthy tissues (Hans-Olov et al., 2002).

1.3.5 Neurocarcinogenesis

The brain and central nervous system (CNS) are protected from exposure to toxicants by an interface known as the "blood brain barrier" (Casserett and Doull, 2001). This barrier is made up of tightly joined endothelial cells that serve as a protective layer preventing the entry of toxicants to the CNS (Casserett

and Doull, 2001). Instead of passing between endothelial cells as other cells do, toxic molecules must pass through the endothelial cells of the brain (Casserett and Doull, 2001). Entry to the CNS is thus contingent on the lipid solubility of the toxicant and the ability of the toxicant to pass through the plasma membranes of the endothelial cells (Casserett and Doull, 2001). The causes of human brain cancer are largely unknown. High dose ionizing radiation and inherited genetic syndromes are two recognized causes of brain cancer (Davis, 2007).

1.4 Brain Cancer and Occupation

1.4.1 Brain Tumor Statistics

The Central Brain Tumor Registry of the United States (CBTRUS, 2009) is a non-profit corporation that compiles and disseminates information from 18 different participating state cancer registries on malignant and non-malignant brain and central nervous system tumors. CBTRUS recorded more than 85,000 tumors for the period 2002-2006 resulting in an incidence rate of 18.3 per 100,000 person-years age adjusted to the 2000 U.S. standard population. Ninety-three percent of these tumors were diagnosed in persons older than 20 years of age. By gender, males account for 43% of cases of primary brain tumors and females 57%. With regard to ethnicity, white have a higher incidence rate (18.5 per 100,000 person years) than blacks (15.8 per 100,000 person years). The difference is statistically significant. The difference in incident rates between Hispanics (16.1 per 100,000 person-years) and non-Hispanics (18.0 per 100,000 person-years) is also statistically significant. Tumors of the meninges are mostly non-malignant and represent 34% of all tumors. Another third of primary brain tumors are gliomas. Gliomas are tumors of the glial cells and include astrocytoma, glioblastoma, oligodendroglioma and others. Seventy-five percent of gliomas are astrocytomas and glioblastomas. (CBTRUS, 2009).

1.4.2 Industry, Occupation and Brain Cancer

To gain understanding of the etiology of brain cancer, the work environment has been investigated. Several studies have explored the association between occupation and the incidence of brain cancer. In a 2002 Swedish study, Navas-Acién et al. (2002) looked at a base population of men and women gainfully employed at the time of the 1970 census. This population was followed until the end of 1989. The 1970 census included information on occupation, industrial branch and residence in 1970. Using the Swedish Cancer Environment Register, specific rates for gliomas and meningiomas were computed. The overall person time (length of service) was calculated for each study participant and the expected number of cases was calculated for given occupations and occupational sectors. Thirteen chemical exposures were evaluated based on any combination of occupation and industrial branch compared to a job exposure matrix (JEM). The chemicals of interest included: "...arsenic, asbestos, chromium/nickel, lead, mercury, metallic compounds, oil mist, polycyclic aromatic hydrocarbons (PAH), pesticides or herbicides, pesticides or herbicides on peak-exposure (mainly applicators), petroleum products, solvents and textile dust " (Navas-Acién et al., 2002). The JEM classified exposures as no exposure, possible exposure, or probable exposure. Men exposed to arsenic or mercury were found to have an increased risk of glioma, with a possible correlation for those with exposure to petroleum products. None of the chemicals studied were consistently related to glioma or meningioma in women. This study demonstrates the application of the JEM and an exposure ranking system in brain cancer epidemiology (Navas-Acién et al., 2002).

In the U. S. San Francisco Bay area, a case-control study examined occupational risk factors for glioma using lifetime job histories (Carozza et al., 2000). Study participants provided information about each job held for at least six months, daily work activities, job duration and hours worked per week. Type of industry was coded using the 1987 Standard Industrial Classification (SIC) system. This study evaluated a

possible 10 year latency period for brain cancer by considering the work histories of study participants, excluding the most current ten years of the work history. Odds ratios for "ever employed", "employed less than 10 years", and "employed for 10 years or more" were estimated. No association was found with the occupational categories studied even when the 10 year latency period was considered. This study is noteworthy for its consideration of lifetime work histories and occupations. However, this study relied on the SIC coding and did not explore specific chemicals associated with each occupation (Carozza et al., 2000).

The San Francisco Bay area data were reanalyzed by Krishnan et al. (2003). Using the industry coding described above, the longest held job and ever held jobs were used as exposure variables. Longest held jobs as firefighters, physicians, material moving equipment operators and janitors were found to have elevated odds ratios. This study also linked exposure to job category, but did not attempt to identify potential chemical exposures (Krishnan et al., 2003).

Samkange-Zeeb et al. (2010) conducted a study of glioma, meningioma and acoustic neuroma in a German population to assess the association between these brain tumors and occupation. This case-control study included 844 cases and 1688 controls. Study participants provided information on their work histories during an interview and included all jobs held for more than one year. Occupations of interest included six broad categories; chemical, metal, agricultural, construction, electrical/electronic and transport. As an example of the broadness of the categories, the metal production category included foundry workers as well as motor vehicle mechanics and machine tool operators. The construction category included mine and quarry workers, trade workers and painters. The authors were interested in exposures to petrochemicals, solvents, asbestos, pesticides, metals and electromagnetic fields. The study did not find a significant association between occupation and glioma or meningioma in any of the categories examined (Samkange-Zeeb et al.).

In a study of occupational risk factors and brain cancer in Canada, Pan et al. (2005) used questionnaire data to examine potential associations between 18 chemicals and brain cancer. Cases were identified from cancer registries and controls were cancer-free participants from the National Enhanced Surveillance System. There were 1009 cases and 5039 controls included in the study. The chemicals of interest included asbestos, salts of arsenic, chromium, and cadmium, asphalt, benzidine, benzene, pesticides, vinyl chloride, radiation sources, welding and wood dust among others. Increased risk was associated with asphalt (OR=1.29; 95% CI = 1.02-1.62) and welding (OR=1.26; 95% CI = 0.98-1.45) exposures. Teaching, protective services, textile workers, construction trades and transportation workers were identified as having an increased risk of brain cancer.

Occupational risk factors for high and low grade glioma were evaluated in an international case-control study by Schlehofer et al. (2005). This study was carried out with data from eight countries and included 1178 cases and 1987 population based controls. Data from the centers were merged into pooled data sets. Information on occupation was ascertained from in-person interviews using a standardized questionnaire. Occupations were condensed into 16 different occupation groups and 20 substance exposures. In men, a slightly increased odds ratio was noted in metal occupations (OR=1.24; 95% CI = 0.96-1.2). For women who had worked in agricultural occupations a statistically significant decreased risk of glioma was reported (OR = 0.60; 95% CI = 0.36-0.99). Of the substance categories, none resulted in statistically significant results. The overall study conclusion was that occupational exposures were not a major source of increased risk for glioma (Schlehofer et al., 2005).

Zheng et al. (2001) conducted a population based case-control study in Iowa to look at occupational risk factors for brain cancer. Questionnaires were mailed to study participants with glioma. Approximately 74% of the questionnaires were completed by proxy. Controls were selected from driver's license records. There were 375 cases and 2434 controls who participated in the study. Industries and

occupations were coded using the SIC and SOC codes, respectively. Respondents reported all jobs held for five years or longer. Analyses were completed separately for men and women and statistically significant associations between glioma and occupation or industry were reported. For men, the highest statistically significant association was for the roofing, siding and sheet metal work industry (OR=22.3; 95% CI = 3.2-153). However, the number of cases and controls associated with the industry was small (4 cases and 2 controls). Some other industries with statistically significant increased risk of glioma included: Plumbing, heating, and air conditioning (3 cases, 11 controls; OR=2.8; 95% CI = 0.7-10.9); Newspaper (5 cases, 11 controls; OR=5.8; 95% CI = 1.9-17.9); rubber and plastic products (4 cases, 6 controls; OR=4.9; 95% CI = 1.2-19.2) (Zheng et al., 2001).

For women included in the Iowa study, several statistically significant associations between glioma and industry or occupation were observed. The textile and apparel industry was the industry with the highest statistically significant association (4 cases, 7 controls; OR=4.9; 95% CI = 1.3-18.3) and the highest statistically significant occupation was observed for general farm workers (4 cases, 1 control; OR= 13.4; 95% CI = 1.4-126). The authors reported that their findings were in agreement with previous studies and suggested further studies were warranted as they could not rule out chance association as a possible explanation for their results (Zheng et al., 2001).

1.4.3 Known or Suspected Animal Neurocarcinogens (ANCs)

The studies described above do not provide clear evidence that work in a particular industry or occupation increases the risk of developing brain cancer. However, looking at specific chemicals used in a particular industry or occupation may suggest an association with brain cancer.

No human neurocarcinogens have been identified to date. Thus, extrapolation from animal toxicity testing was used to develop a list of potential human neurocarcinogens. The premise of this hypothesis

was that chemicals neurotoxic to animals are potential human neurocarcinogens. If an animal neurocarcinogen is used in a particular industry or occupation it may be possible to detect an association between the ANC and human brain cancer.

There are several major sources of animal toxicity testing data. The National Toxicology Program (NTP) publishes a biannual report on carcinogens. This report is mandated by the United States Congress and provides information on the carcinogenicity, metabolism and potential to cause genetic damage in the body (NTP, 2009). For a chemical or mixture to be included in the NTP report, there must be evidence that the chemical or mixture is determined to be a human carcinogen or there is evidence to support a conclusion that the chemical is reasonably anticipated to be a carcinogen. These studies are scrutinized to determine if there is any evidence that the tested chemical causes brain cancer in animals.

The IARC is part of the World Health Organization and publishes monographs of chemical agents that may increase the risk of human cancer (IARC, 2009). IARC has been in existence since 1971 and has identified approximately 400 carcinogens or potential carcinogens.

The U.S. Environmental Protection Agency (EPA) maintains an electronic database called the Integrated Risk Information System (IRIS). IRIS contains data divided into non-cancer and cancer-causing effects. This database is primarily used for developing risk assessments based on several factors including toxicity benchmarks such as the cancer slope factor. The cancer slope factor is an estimate of increased cancer risk based on lifetime exposure to a chemical (EPA, 2009).

The National Institute for Occupational Safety and Health (NIOSH) puts forth recommendations related to occupational safety and health by publishing Recommended Exposure Limits (RELs) for many chemicals based on their research activities. NIOSH is part of the Centers for Disease Control and

Prevention (CDC) and was established at the same time that the Occupational Safety and Health Administration (OSHA) was established in 1970. OSHA has established Permissible Exposure Limits (PELs) for many chemicals found in industry. In addition to the chemical specific standards, there is a standard for non-specified carcinogens.

Finally, the Agency for Toxic Substances and Disease Registry (ATSDR) evaluates hazardous materials found at sites listed on the National Priorities List (NPL) and publishes information about the toxicity of the substance and an assessment of potential harm in the event of human contact (ATSDR, 2009).

Much of the long term animal carcinogen testing data has been compiled in the Carcinogenic Potency Database (CPD) created by the University of California, Berkeley and the Lawrence Berkeley Laboratory. This database strives to standardize data reported in the literature, and by the National Cancer Institute and the NTP. Berkeley researchers review the literature and develop a TD_{50} value for each chemical, which describes the carcinogenic potency as the lifetime daily dose (mg/kg/day that produces tumors in half of the test animals when compared to animals with no exposure (CPD, 2009). By reviewing the available toxicology literature and citations from these databases, over 40 known or suspected animal neurocarcinogens have been identified by the research group at the University of Illinois at Chicago (Davis, et al., 2006). These are listed in Table I. With this list of chemicals as a starting point it may be possible to advance the knowledge base of occupational chemical exposures as they relate to the etiology of brain cancer.

TABLE I - KNOWN OR SUSPECTED ANIMAL NEUROCARCINOGENS

1,3-Butadiene	Dimethylben(a)anthracene (DMBA)
1-H-Benzotriazole	Elaiomycin
1-Methyl-2-Benzylhydrazine	Ethyl methane sulphonate
1-Phenyl-3,3-Dimethyltriazene	Ethylene oxide
1-Pyridyl-3,3-Diethyltriazene	Ethylhydrazine
3,3'-Dimethoxybenzidine dihydrochloride	Ethylnitrosobiuret
3,3'-Dimethylbenzidine dihydrochloride	Ethylnitrosourea (ENU)
3-methylcholanthrene (MCA)	Furosemide
Acrylamide	Glycidol
Acrylonitrile	Isoprene
Azoethane	Lead Subacetate
Azoxyethane	Methyl methane sulfonate
Benzo(a)pyrene (BaP)	N,N',N'-Trimethyl-N-Nitrosourea (TMNU)
Bis(cloromethyl)ether	N,N-Dimethyl-N-Nitrosourea (DMNU)
Bromoethane	N-methyl-N-nitrosourea (MNU)
Chloroethane	p-Cresidine
CI Direct Blue 15	Procarbazine hydrochloride
Cyclophosphamide	Propane sultone
Diethylhydrazine	Propylene imine
Dimethyl sulfate	Vinyl Chloride

1.4.4 Biomarkers

The detection of chemicals or their metabolites in blood, urine or other body fluids is an indication that an exposure to the chemical has occurred. Biomonitoring describes what is absorbed by the body and how it is measured (Drablos et al., 2004). The detected chemicals or chemical/macromolecule compounds are designated as biomarkers of exposure, effect or susceptibility. Biomarkers of effect are measurable amounts of a chemical or its metabolites found in the body after exposure to the chemical has occurred. Biomarkers of effect represent a change in the body's responses following chemical exposure (i.e. biochemical, physiological, behavioral, etc.) that may be recognized as disease

manifestation or other sign of injury. Biomarkers of susceptibility are changes to the genetic makeup of a subject that may be inherited or acquired following exposure to a particular chemical, that alter the response of the subject to that chemical (National Research Council, 2006). Biomarkers of susceptibility may also be acquired, as the result of life choices such as poor diet or cigarette smoking (Drablos et al., 2004).

Biomarkers of exposure are usually short lived and, as such, may be of limited value in the investigation of carcinogens as the exposure typically occurs several years before the development of disease. Biomarkers of exposure do not necessarily indicate an adverse health outcome (Drablos et al., 2004). Even under similar exposure conditions, variations occur in the expression of biomarkers from individual to individual. Susceptibility to environmental mutagens also varies among individuals. Some individuals are unaffected by exposure to an environmental mutagen, others are susceptible.

One type of biomarker of effect is the hemoglobin adduct formed when the reactive metabolite of a chemical binds with the globin protein of the hemoglobin molecule. Hemoglobin adducts are found in the red blood cells. As these cells live for approximately 120 days, only recent exposures to the chemical under study can be measured. The resulting measurement of hemoglobin adducts is an indicator of exposure from all routes of entry. Hemoglobin adducts may be formed by alkylating agents such as 1,3-butadiene, acrylamide, acrylonitrile and ethylene oxide (Strachan, T. and Read, A., 2004).

Exposure to 1, 3-butadiene or acrylamide may form hemoglobin adducts during the metabolism of the chemicals and are the focus of the research presented in this dissertation. These chemicals are found in a number of occupational work environments, which increases the likelihood that study subjects will be available in sufficient numbers to enable quantitative analysis. 1, 3-butadiene is also present in cigarette smoke and acrylamide has recently been found in food (Besaratinia & Pfeifer, 2007). Evidence

for the inclusion of acrylamide and 1,3-butadiene in a list of known or suspected ANCs along with a discussion of their respective metabolic pathways is presented below. A discussion of polycyclic aromatic hydrocarbons (PAHs) and their role in the etiology of brain cancer is also included.

1.5 Study Chemicals

1.5.1 Acrylamide

Acrylamide (CAS: 79-06-1; Synonym: 2-Propenamide, Structural formula: $H_2C=CH-CO-NH_2$) has many industrial uses as both a polymer and copolymer and it is used in sewage treatment plants as a coagulant aid, as a grouting agent, in adhesives fibers and molded parts (ACGIH, 2005). Acrylamide is also used in research laboratories to make gels for electrophoresis. It is readily absorbed through the skin, in addition to exposure from dusts or vapors (ACGIH, 2005). Acrylamide exposure may also occur as the result of non-occupational exposures and is found in cigarette smoke and as polyacrylamide in cosmetics. Recently, acrylamide has been identified in various food sources including French fries, potato chips, coffee, breads, biscuits, crackers and breakfast cereals (Dybing, 2005). Acrylamide is formed during high temperature processing of these foods (Ghanayem et al., 2005).

The threshold limit value for acrylamide, established by the American Conference of Governmental Industrial Hygienists is 0.03 mg/m^3 with a skin notation. This exposure limit was set to minimize neurological effects such as muscle weakness, tremors and hallucinations and reduce the potential for cancer development, although cancer has been reported only in rodent studies (ACGIH, 2005). The OSHA-PEL is 0.30 mg/m^3 (29CFR 910.1000). Acrylamide is an IARC Group 2A carcinogen (probably carcinogenic to humans) (*Material safety data sheet - acrylamide*). Regulatory limits are tabulated in Table XLVIII, Appendix A. Acrylamide has an oral cancer slope factor (CSF) of 4.5 per mg/kg/day (IRIS, 1991). Acrylamide has not been linked to brain cancer in humans. The Carcinogen Potency Project

database lists two studies of acrylamide in two-year rodent studies where positive findings for brain cancer were reported. Johnson et al. (2006) conducted a two year drinking water study in male and female rats with doses of 0.01, 0.1, 0.5 or 2 mg/kg/day. Among other tumors, results showed increased brain and spinal cord tumors in the 2.0 mg/kg/day female group (7/61 female rats). The CSF was extrapolated from this drinking water study using results from the female Fischer 344 rat population. Tumors of the CNS, mammary and thyroid glands, uterus and oral cavity were reported (Johnson et al., 1986). Friedman et al. (1995) conducted a study of acrylamide in drinking water at doses of 0, 1 or 3mg/kg/day with male and female rats. In addition to other tumors, brain tumors were found in both male and female rats.

Studies of occupational exposure to acrylamide have reported symptoms such as contact dermatitis, non-specific neurological symptoms, excessive sweating and peripheral neuropathy. Sobel et al. (1986) conducted a mortality study with a cohort of monomer and polymer plant employees. This study included 371 workers at a chemical plant in Michigan. The authors were interested in cancers found in animal species including brain and central nervous system, thyroid, endocrine and mesotheliomas. Thirty-eight deaths were expected and 29 deaths were observed. No statistically significant excess deaths were reported and no connection between the reported deaths and the worker occupation were indicated by the study (Sobel et al., 1986).

Marsh et al. (2007) have updated a study of acrylamide workers in three United States and one Netherlands plant. The study period is 1925-2002 and includes a cohort of approximately 9000 workers. In this ongoing study company records were reviewed to determine work histories and industrial hygiene monitoring records were reviewed. Standard mortality ratios (SMRs) were computed using national and local rates. The SMR for brain and central nervous system was 0.67 (95% CI = 0.40-1.05). A

SMR of 1.38 (95% CI = 0.28-4.02) was reported for thyroid, etc. None of the mortality rates were statistically significant (Marsh, et al., 2007).

The metabolism of acrylamide is depicted in Figure 1 and follows two main metabolic pathways; glutathione conjugation which results in the formation of mercapturic acid products that are then excreted in the urine or catalyzed by Cytochrome P450 2E1 (CYP2E1) to form the epoxide, glycidamide (Fennell et al., 2005). Both acrylamide and glycidamide will react with hemoglobin forming stable adducts, N-(2-carbamoylethyl) valine (AA-Val – aka AA-Hb) and N-(2-carbamoyl-2-hydroxyethyl) valine (GA-Val –aka GA-Hb), which can be measured and are an indication that an exposure has occurred (Fennell et al., 2005). Glycidamide can also undergo further metabolism to form glyceramide or be detoxified by glutathione conjugation and eliminated in the urine (Dybing et al., 2005).

In a controlled human study, Fennell et al. (2005) measured AA-Val and GA-Val adducts following both dermal and oral dose administration. The oral dosing range varied from 0.5-3.0 mg/kg. Adduct formation increased linearly for both AA-Val and GA-Val, indicating that there was no saturation of the acrylamide to glycidamide metabolic pathway. Lower levels of hemoglobin adducts were formed following dermal exposure when compared to an equivalent oral dose of acrylamide. The authors concluded that acrylamide was eliminated from humans at a slower rate than from rats, and that the metabolism of acrylamide to glycidamide is slower in humans than rats (Fennell et al., 2005).

Glycidamide is mutagenic when tested in both the Salmonella assay and in bronchial epithelial cells while acrylamide is not (Fennell et al., 2005). As glycidamide is the metabolite that is responsible for the toxicological effects, measurements of both the acrylamide and glycidamide hemoglobin adducts permit the calculation of a ratio of glycidamide to acrylamide which is an indicator of CYP2E1 activity. Reports in the literature suggest that this ratio is highly variable between individuals suggesting that this

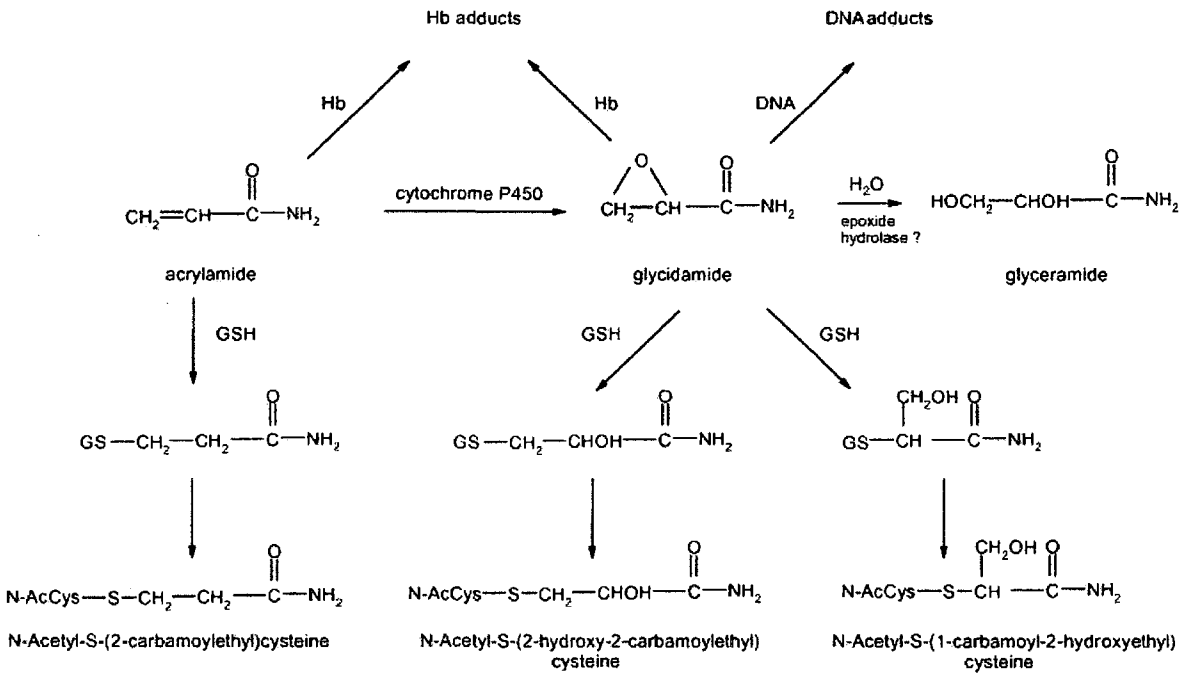

Figure 1. Acrylamide Metabolism. (Dybing et al., 2005-Used with permission)

inter-individual variability may account for the variation in response to exposures to toxic chemicals (Vesper, 2008). On average, acrylamide adducts are formed at a slower rate than glycidamide adducts; however, the levels of glycidamide are lower than acrylamide so at lower or moderate exposures to acrylamide, equal amounts or these adducts are formed resulting in a 1:1 GA-Hb to AA-Hb ratio (Dybing et al., 2005).

Metabolism of acrylamide to glycidamide is necessary for the expression of genotoxic effects. Levels of CYP2E1 drive the metabolism toward glycidamide formation. In a study conducted by Ghanayhem et al. (2005), CYP2E1-wild type (normal) and CYP2E1-null mice were dosed with 50 mg/kg acrylamide by intrarperitoneal injection and euthanized six hours later. Concentration of AA-Hb in the wild type mice

was 137 times lower than in the CYP2E1-null mice. The concentration of GA-Hb in wild type mice was 19 times higher than the concentration in the CYP2E1-null mice. These results demonstrate the importance of CYP2E1 in the metabolism of acrylamide to glycidamide and the influence of genotype on the direction of the metabolic reactions either toward detoxification and excretion in the urine or genotoxicity by glycidamide formation (Ghanayhem et al., 2005).

The direction of acrylamide metabolism can also be influenced by ethanol intake. In a cell culture study using human HepG2 hepatoma cells, Lamy et al. (2008) exposed the cells to acrylamide after they had been pretreated with ethanol for 24 hours. Results of the study suggested an increased expression of CYP2E1 in the cells and reduced levels of glutathione as compared to untreated cells. Thus, ethanol consumption in addition to acrylamide exposure would drive the metabolism of acrylamide toward the formation of glycidamide and potentially increased genotoxic effects (Lamy et al., 2008). As alcoholic beverages containing ethanol are often consumed with acrylamide containing foods (potato chips, French fries and crackers) higher glycidamide hemoglobin adduct levels may be observed in individuals making this lifestyle choice.

Differences in individual responses to acrylamide exposure may also be attributed to differences in gene coding for metabolizing enzymes. Duale et al. (2009) studied the ratio of GA-Hb to AA-Hb and found significant individual variations. The authors conducted a study to examine the association between the GA-Hb:AA-Hb ratio and gene coding for the following metabolizing genes; CPY2E1, EPHX1 (microsomal epoxide hydrolase, responsible for metabolism of glycidamide to glyceramide), GSTM1, GSTT1 and GSTP1 (glutathione-S-transferase producing genes responsible for glutathione conjugation). Blood samples were analyzed for hemoglobin adducts and genotyping for the genes referenced above was completed, using the wild type gene variant as the reference group. Statistically significant differences in the adduct ratio and glycidamide adducts were identified. Joint effects in the following combinations

were statistically significant for these variations of the genes: GSTM1-null + GSTT1-null; CYP2E1 (Val/Val) + GSTM1-null; CYP2E1 (Val/Val) + GSTM1-null +GSTT1-null and CYP2E1 (Val/Val) + GSTT1-null + EPHX1 (Tyr/Tyr) + EPHX1 (His/Arg) and resulted in higher ratios and glycidamide adducts when compared to the reference group. The difference in acrylamide adducts results for these gene variations were not statistically significant (Duale et al., 2009).

Several studies have been conducted to relate hemoglobin adduct formation to occupational exposures. Bergmark (1993) studied 41 workers in a chemical plant in China that was producing acrylamide. The factory workers were divided into four groups based on occupation: packaging; polymerization; ambulatory; and synthesis. These groups were compared with a control group. In addition, the authors compared adduct results to personal air monitoring data that were available at the time of the study. The adduct levels were found to be related to the job classification. Based on hemoglobin adduct formation, the authors further concluded that inhalation exposure was secondary to dermal exposure with respect to acrylamide absorption (Bergmark, 1993).

In a study of tunnel workers in Sweden (Hagmar et al., 2001), 210 workers who were exposed to acrylamide via a chemical grouting agent were evaluated for the exposure using hemoglobin adduct formation as a measure of internal dose. From a non-smoking, non-exposed population (N=18), it was determined that a background range of 0.02-0.07 nmol/g globin was normal. Using self-reported exposure data, the workers were divided into three groups; no exposure (exposure only from airborne or skin contact with contaminated water); some exposure (infrequent contact with the chemical grouting agent); and high exposure (frequent skin contact with the grouting agent). Statistically significant associations ($P>0.0001$) were found between the category of exposure and the hemoglobin adduct levels. The prevalence of peripheral nervous system symptoms such as headache, nausea,

numbness and tingling were also associated with the hemoglobin adduct levels in a dose-response relationship (Hagmar et al., 2001).

Paulsson et al. (2006) provided further evidence of the dose-response relationship between acrylamide exposure and hemoglobin adduct formation. Three case studies were discussed. The first case involved workers who transported acrylamide contaminated soil. The level of hemoglobin adduct was consistent with background levels and averaged 0.029 nmol/g globin. Acrylamide levels were slightly higher among smoking workers in this category. The second case involved workers in a glass window manufacturing facility who used the same chemical grouting agent described above to seal double paned windows. A 10 fold increase in adduct levels was found between the sealers and the non-smoking controls. Following receipt of these results, additional personal protective equipment was provided while this task was completed. The third case involved a male smoker who repaired concrete constructions using an acrylamide-based sealing agent for more than 15 years. His hemoglobin adduct level was very high (23 nmol/g globin) and he was showing symptoms of peripheral nervous system impairment. After replacement of the chemical sealing agent, the worker's hemoglobin adduct level dropped; however, his nervous system impairment did not resolve following removal from the exposure (Paulsson et al., 2006).

These studies show the usefulness of hemoglobin adduct analysis for the determination of internal dose and as a suitable biomarker of exposure for acrylamide-exposed workers.

1.5.2 1,3-Butadiene

1,3-butadiene (CAS: 106-99-0; Synonyms: Biethylene, Divinyl; Structural Formula: CH_2-CH-CH-CH_2) is a flammable, colorless gas at room temperature (ACGIH, 2001). It is an important monomer with uses in the manufacture of tires, and other synthetic rubber products (ACGIH). The TLV-TWA is 2 ppm based on the need to minimize the potential for cancer. The OSHA PEL is 1 ppm (ACGIH, 2005). Both IARC and

EPA classify 1,3-butadiene as a known human carcinogen, i.e., humans, IARC Group 1, EPA Group A chemical (IARC, IRIS). As this chemical is a gas at ambient temperature and pressure, the primary route of exposure is inhalation. Exposure of the chemical to air may result in the formation of potentially explosive peroxides. 1,3-butadiene is listed in the Carcinogen Potency Project database and its toxicity has been evaluated by ATSDR.

Many studies have explored the carcinogenicity of 1,3-butadiene. Melnick et al. (1993) reviewed two studies conducted by the NTP and one study conducted by the International Institute of Synthetic Rubber Producers (IISRP). The first NTP study was conducted in B6C3F1 mice (50 each, male and female) that were exposed to 0 (control, no exposure), 625 or 1250 ppm 1,3-butadiene for six hours/day, five days/week in an inhalation chamber. The study was stopped after 60-61 weeks due to reduced survival rates of both males and females. Malignant lymphomas originating in the thymus were observed following 20 weeks of exposure and were the suspected major cause of early death (Melnick et al., 1993).

In the second NTP study, mice were dosed at 0, 6.25, 20, 62.5, 200 or 625 ppm 1,3-butadiene in an inhalation chamber for six hours/day for five days/week for two years. Thymic lymphoma was again reported as the major cause of death for the 625 ppm group. Lymphoma was also observed in female mice exposed to 200 ppm butadiene. Many different tumor types were observed during the study including neoplasms in the heart, lung, forestomach, liver, ovary, mammary gland. Tumors were reported in the brain of male mice but their numbers were not statistically significant (Melnick et al., 1993).

The IISRP study was conducted in Sprague-Dawley rats. Male and female rats (100 each) were exposed to 0, 1000 or 8000 ppm in an inhalation exposure chamber for six hours/day, five days/week for two

years. Following exposure to 1,3-butadiene, increased incidences and/or dose-response trends were observed in several organs. Glial cell tumors of the brain were also observed in male rats (Melnick et al., 1993).

Between 1984 and 1987 NIOSH conducted a study involving thirty-nine 1,3-butadiene monomer, polymer and end user plants to determine the extent of exposure and to provide OSHA with assistance in the development of a new standard for 1,3-butadiene. At the time the OSHA PEL was 1000 ppm and the ACGIH TWA-TLV was 10 ppm (Fajen et al., 1990). The study included the completion of industrial hygiene surveys and the collection of both personal (full-shift, eight hours and short-term, 15-20 minutes) and area air samples. The effectiveness of engineering controls and leak prevention programs were also reviewed. In the monomer plants, employees in eight different jobs categories with exposure to 1,3-butadiene were monitored. Of the eight job categories, six (process technicians (4), laboratory technicians and maintenance personnel) had at least one full-shift personal monitoring result above 10 ppm (Fajen et al., 1990). Industrial hygiene monitoring results indicated that laboratory technicians voiding sample cylinders and workers loading or unloading rail cars experienced exposure concentrations above 35 ppm. In the polymer industry the highest full-shift sample concentration was 43.2 ppm for a maintenance technician. The highest short-term measurement was for a quality control technician sampling a barge containing 1,3-butadiene where an exposure concentration of 210 ppm was observed. A rubber tire plant and an industrial hose plant were selected to represent the end user facilities. Of the 124 samples analyzed for the end user facilities all sample results were less than the limit of detection (0.3 µg/sample). The arithmetic mean concentration for all job categories surveyed in this study was 2.7 ppm. The report concluded that engineering and administrative controls were adequate to maintain exposure limits below 10 ppm. By 2009 standards a number of overexposure results would be observed. This study is meaningful from the retrospective exposure assessment

perspective as work histories recorded today may include work periods during the time the study was completed (Fajen et al., 1990).

More recent studies have employed biomarker measurements to assess occupational exposures to 1,3-butadiene. Cytochrome P450 2E1 oxidizes 1,3-butadiene to three intermediate epoxides; 1,2-epoxy-3-butene (EB), 1,2,3,4-diepoxybutane (DEB) and 1,2-epoxy-3,4-butanediol (EB-diol) (Boysen et al., 2007). These three epoxides in turn form three hemoglobin adducts, N-(2-hydroxy-3-buten-1-yl)-valine (HB-Val), *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine (*pyr*-Val) and 2,3,4-trihydroxybutyl-valine (THB-Val). The metabolism of 1,3-butadiene is shown in Figure 2.

Cochrane and Skopek (1994) studied the three epoxide metabolites to determine their mutagenicity. Using TK6 human lymphoblastoid cells in culture, the authors were able to evaluate the mutagenic potential of ED, DEB and EB-diol. Mutagenic potential is defined as mutants per surviving cell per mole of compound added to solution (Cochrane and Skopek, 1994). Their results demonstrated that all three epoxide metabolites of 1,3-butadiene were mutagenic with mutagenic potency rated as follows: DEB >>>EB>EB-diol. The authors also identified a dose-response effect when the dose of epoxide metabolite was varied from zero to 900 μm with an exposure time of 24 hours (Cochrane and Skopek, 1994). The presence of the *pyr*-Val hemoglobin adduct suggests that an exposure to 1,3-butadiene has occurred (Boysen et al., 2007).

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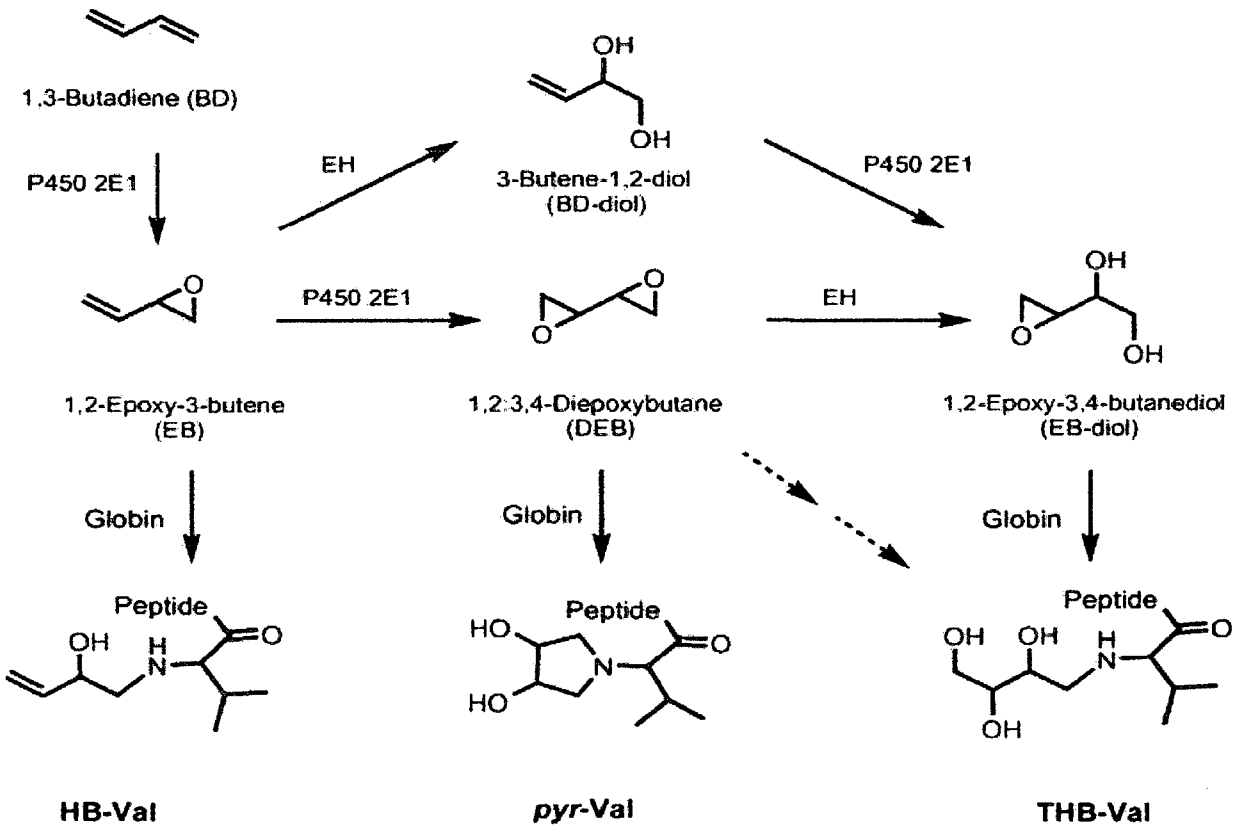

Figure 2. 1,3-Butadiene Metabolism. (Boysen et al. 2007 - used with permission)

>>>EB>EB-diol. The authors also identified a dose-response effect when the dose of epoxide metabolite was varied from zero to 900 µm with an exposure time of 24 hours (Cochrane and Skopek, 1994). The presence of the *pyr*-Val hemoglobin adduct suggests that an exposure to 1,3-butadiene has occurred (Boysen et al., 2007).

HB-Val was the first hemoglobin adduct studied with respect to 1,3-butadiene exposure. The *pyr*-Val adducts have been detected in mice and rats at concentrations comparable to 1,3-butadiene experimental concentration when analyzed by the same analytical methods (Albertini et al., 2001). The

authors concluded that if 1,3-butadiene is genotoxic at higher exposure levels, detection of the *pyr*-Val biomarker can be used to establish a lowest adverse effect level (LOAEL) to prevent irreversible genotoxicity (Albertini et al., 2001). Studies show that humans exposed to 1,3-butadiene produce relatively small amounts of HB-Val when compared to THB-Val (Boysen et al., 2007). Other studies have shown that an endogenous source may be responsible for the presence of THB-Val in several species when no known exposure has occurred. While THB-Val can be formed from DEB, it is primarily formed from the EB-diol epoxide (Boysen, et al., 2007). THB-Val adducts are formed in greater amounts than HB-Val or *pry*-Val adducts independent of exposure possibly due to the endogenous formation (Boysen et al., 2007). Because DEB is the most potent mutagen, HB-Val is formed in relatively small amounts and THB-Val may be formed from endogenous sources, *pyr*-Val adduct measurements may be the most representative hemoglobin adduct of human exposure to 1,3-butadiene.

Albertini et al. (2001) suggested that biomarkers might be useful in determining the internal dose of 1,3-butadiene in workers, but cautioned that the use of biomarkers for this purpose have not been validated. In these studies the biomarker is the dependent variable and the environmental exposure predicted by the biomarker is the independent variable (Albertini et al., 2001). This study aimed to validate the use of biomarkers for 1,3-butadiene. Workers completed an extensive questionnaire and personal air monitoring samples were collected. Of the 104 study participants, 51 were administrative controls, 23 worked in a laboratory and 30 worked in a polymerization production facility. Personal monitoring consisted of ten full shift samples for each study participant, collected over four months. Blood samples were collected on the last day of the four month exposure assessment period. Urine samples were collected before and after the shift on the last three days. Personal air monitoring results indicated that the female exposed group had statistically significant higher results than the female control group (female exposed group: mean=0.397 mg/m^3, SD=0.502 mg/m^3 versus female control

group: mean=0.008 mg/m^3, SD=0.005 mg/m^3). The male exposed group also had statistically significant higher results than the male control group (male exposed group: mean=0.808 mg/m^3, SD=1.646 versus male control group: mean=0.007 mg/m^3, SD=0.005 mg/m^3) (Albertini et al., 2001). The two urinary metabolites measured in this study were significantly different for the male groups; however, no statistically significant differences were seen between the female groups (Albertini et al., 2001).

Genotyping was also performed. The association of *GSTM1* with urinary metabolites of 1,3-butadiene was evaluated. A statistically significant difference for *GSTM1* positive individuals as compared to *GSTM1* null individuals was seen (N=53; difference between means = 0.0057; p=0.036). No statistically significant differences were noted for the *GSTT1* positive versus null individuals. Assays for sister chromatid exchanges and chromosomal aberrations showed no statistically significant differences between control and exposed groups (Albertini et al., 2001).

1.5.3 Polycyclic Aromatic Hydrocarbons

There are more than 100 chemicals classified as polycyclic aromatic hydrocarbons (PAHs). They are produced during the incomplete combustion of organic materials, such as coal, wood and oil. Phenanthrenes, naphthalene, and pyrene are PAHs (NHANES, 2009). Car exhaust, coal processing and tobacco smoke are some of the sources of PAHs found in the environment (NHANES, 2009).

The ATSDR included 17 PAHs in their toxicological profile of this class of compounds. Of these, three (acenaphthene, acenaphthylene, and anthracene) are produced commercially in the United States (ATSDR), and several others (including creosote, fluorine among others) are imported from other countries (ATSDR). The remaining sources of PAHs in the environment come from incomplete combustion as described above.

The greatest exposure to PAHs in the United States results from inhalation of tobacco smoke (active or passive), as well as from inhalation of wood smoke, and contaminated air, and ingestion of PAHs in foodstuffs and water(ATSDR, 2009). Skin absorption is also a potential route of exposure. Occupational exposures to PAHs may occur in industries such as coal gasification, coke production and iron and steel foundries (Bosetti et al., 2007).

The National Toxicology Program's 11th Report on Carcinogens lists 15 PAHs that are classified as reasonably anticipated human carcinogens. These include: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]acridine, dibenz[*a,j*]acridine, dibenz[*a,h*]anthracene, 7 H-ibenzo[*c,g*]carbazole, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a, l*]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene (NTP, 2005).

PAHs have been studied in a wide variety of species with varying types of exposures. These include: gavage, topical, inhalation, diet, drinking water (replaced by olive oil emulsion), subcutaneous injection, directly injected into the organ of interest (lung, kidney), intratracheal instillation, intraperitoneal, intravenous, bladder implantation, and intrabronchial implantation. These studies resulted in various neoplasms at the site of exposure (NTP, 2005).

Bosetti et al. (2007) completed a review of 57 occupational cohort studies of PAH exposures classified by industry. Generally, studies reported excess risks for lung/respiratory tract, larynx, bladder, kidney and skin. As an example, for aluminum production workers, 15 studies on nine cohorts were reviewed. One reanalyzed Norwegian study represented over 11,000 workers and more than 80 years of work histories dating back to 1920 with follow-up for cancer incidence from 1953-1996. No excess risk of lung cancer was reported, while an odds ratio of 1.3 (95% CI: 1.1-1.5) was found for bladder cancer (Bosetti et al., 2007).

For coal gasification plants five cohort studies were available for review. For all cancers of the respiratory system, 251 deaths were reported versus 104.7 expected. The pooled relative risk was 2.58

(95% CI: 2.28-2.92). For cancers of the urinary system, the pooled relative risk was 3.27 (95% CI: 2.06-5.19) (Bosetti et al., 2007).

Ten studies of coke production workers were reviewed. The pooled relative risk for lung cancer was 1.58 (95% CI: 1.47-1.69). While some studies reported excess risks for other cancers, the evidence was not consistent. No increased risk of bladder cancer was identified for this group of workers. For iron and steel foundry workers 10 cohort studies were reviewed. A pooled relative risk for all respiratory cancers of 1.4 (95% CI: 1.32-1.49), for bladder cancer of 1.29 (95% CI: 1.06-1.57) and kidney cancer of 1.30 (95% CI: 0.95-1.77) was found. Other studies included those evaluating bitumen fumes, coal tar and related products (three tar distillation, two each shale oil extraction, creosote, roofers and asphalt workers), carbon and graphite workers (seven cohort studies) and one study of chimney sweeps. Modest increases in the relative risks for lung and bladder cancer were reported (Bosetti, et al., 2007). According to the data presented in this article there were no cases of skin or brain cancer reported. The information contained in the pages above provides the foundation for the studies that were completed for this dissertation.

2.0 SPECIFIC AIMS

Prevention of disease is critical if the health of the population is to be achieved. To prevent disease, it is necessary to understand the etiology. Advancement in the technology necessary for detection of chemical exposures in the body and emerging theories about gene-environment interactions are now enabling us to examine the evidence of not only exposure, but also the cellular damage caused by these chemical exposures. The premise of this dissertation is that chemical exposures play a significant role in the etiology of glioma. With respect to cancer, the chemical exposure typically occurs several years before the disease state becomes apparent. The research presented in this dissertation investigates potential exposures to known or suspected animal neurocarcinogens found in the workplace in a brain cancer case-control study population. This dissertation builds on work that was included as part of the NCI's Specialized Programs of Research Excellence (SPORE) grant program. This study entitled "A Case-Control Study of Environmental Risk Factors and Polymorphisms in Metabolic Genes and the Risk and Outcomes of Primary Brain Tumors" consisted of two phases. Results from the pilot study were used to complete Specific Aim 1 and results from the main study were used to complete Specific Aim 2. Participants from both studies participated in the project described by Specific Aim 3. The specific aims of this dissertation are the following:

Specific Aim 1. To develop a relative cumulative exposure scoring method to inform exposure to known or suspected animal neurocarcinogens found in the workplace. To use this scoring method to quantify potential exposures to these chemicals in a glioma case-control study population. To use epidemiological methods to evaluate the exposure score results.

- Hypothesis: Through the use of self-reported work histories provided by study participants, an algorithm can be created to quantify cumulative occupational exposures to known or suspected animal neurocarcinogens.

Specific Aim 2. To analyze responses to a questionnaire completed by study participants to evaluate occupational exposures to acrylamide, 1,3-butadiene and polycyclic aromatic hydrocarbons. Occupational data were gathered from study participants using a questionnaire format that targeted specific known or suspected animal neurocarcinogens.

- Hypothesis: Analysis of the questionnaire responses from study participants will show a positive association between the chemicals named above and glioma in a glioma case-control study.

Specific Aim 3. To score the potential exposure to acrylamide and 1, 3-butadiene in a control population based on study participant's current occupation/industry and the potential exposures to acrylamide and 1,3-butadiene based on the SIC code of that occupation/industry and the findings of the NIOSH-NOES . To compare these scores to hemoglobin adduct levels of these chemicals in study participant blood specimens using analytical methods of detection. To correlate the results of the biological exposure results with exposure scores, demographic responses and other questionnaire responses provided by study participants.

- Hypothesis: Hemoglobin adducts of chemicals are valid biomarkers of exposure and can be used to validate the exposure scoring system described above.

3.0 METHODS

3.1 Scope of Work

This dissertation involves three interrelated projects. The first project uses occupational work histories collected from the pilot phase of a case-control study of glioma to investigate the association between the occupational environment and the etiology of glioma. The second project uses questionnaire data from a subsequent, main phase, case-control study to examine the association between occupational exposure to acrylamide, 1,3-butadiene and polycyclic aromatic hydrocarbons and glioma. The third project is a biomarker of exposure study that explores the relationship between hemoglobin adducts of acrylamide and 1,3-butadiene in study participant blood specimens and potential exposures to these chemicals in their workplaces.

3.2 Data Sources

3.2.1 Study Data

The UIC-SPH (Dr. Faith Davis – Principal Investigator) along with the Preston Robert Tisch Brain Tumor Center at Duke University Medical Center had a grant funded by the National Cancer Institute (NCI), which was part of the NCI's Specialized Programs of Research Excellence (SPORE) grant program. This study entitled "A Case-Control Study of Environmental Risk Factors and Polymorphisms in Metabolic Genes and the Risk and Outcomes of Primary Brain Tumors" consisted of two phases. In the pilot phase of the project (2004-2006), 229 cases and 348 (friend and sibling) controls were recruited by staff from NorthShore University HealthSystem in Illinois and the Preston Robert Tisch Brain Tumor Center at Duke University Medical Center in North Carolina. Cases enrolled in the study were defined as those patients presenting with a first primary brain tumor and a confirmed diagnosis of glioblastoma, astrocytoma or oligodendroglioma, English speaking and 18 – 80 years of age. The control population consisted of

friend or sibling, controls with hospital based controls added to the main study protocol. The self-reported work histories from the pilot study were used as the starting point for this dissertation. The work histories contained information about the industry where the individual worked, how long the individual worked there, the dates of employment and his/her primary job functions. Data were collected through use of a self-administered web-based questionnaire or an interviewer-administered telephone-based questionnaire (questions on both surveys were identical, but instructions differed based on how it was administered), which consisted of a number of modules (e.g., demographics, family history, smoking history, medical history, and environmental, occupational, and dietary exposure histories). In addition, study participants were asked to submit a complete paper-based self - administered occupational/work history. The main phase of the study began in 2006 and 412 cases and 776 hospital based controls were recruited through January 8, 2010 from NorthShore University HealthSystem in Illinois and the Preston Robert Tisch Brain Tumor Center at Duke University Medical Center in North Carolina. The questionnaire utilized in the pilot study was modified and expanded in the main study, although the modules were similar. Similar to the pilot study, data were collected through use of a self-administered web-based questionnaire or an interviewer-administered telephone-based questionnaire. With respect to the occupational section of the questionnaire used for the main study, participants were asked to respond to a series of questions related to their work history. These questions were designed to target specific industries and occupations associated with known or suspected ANC exposures and also gathered information concerning length of employment and use of personal protective equipment. In the main phase of the study, participants were not asked to complete a work history.

3.2.2 National Occupational Exposure Survey (NOES)

The National Institute for Occupational Safety and Health (NIOSH) conducted the National Occupational Exposure Survey (NOES) during 1981-1983. During the completion of this survey, trained industrial hygienists visited nearly 4,500 industrial facilities and collected data on the chemicals in use at the facility. Exposures to approximately 13,000 chemical and physical different agents in more than 500 industries and 377 occupations were recorded. Agents were cataloged by industry using the Standard Industrial Classification (SIC) codes and by occupation within the industry using the 1980 Bureau of Census codes. Data from the survey are maintained by NIOSH in a publically available web accessible database found at www.cdc.gov/NOES (*National occupational exposure survey.*1991).

3.2.3 The Standard Industry Classification System (SIC)

The SIC code system is used by the U. S. Department of Labor (DOL) to classify industries for use by the Bureau of Labor Statistics (BLS) and other purposes related to the activities of the federal government. The system divides industries into one of ten divisions with 100 major groups and further subdivisions within the major groups. Industries can then be assigned a four digit code representing their industry. The first two digits represent the major division and the second two digits represent the subdivisions. SIC codes are available in a web accessible format at www.osha.gov/oshstats/sicser.

3.2.4 The Standard Occupational Classification System (SOC)

Similar to the SIC system, the SOC classification system is used by the DOL and BLS to code occupations. There are 23 major groups and several subdivisions of the major groups used to classify specific occupations. The first two numbers of the SOC code represent the major division and four additional digits represent the subdivisions. SOC codes are available in a web accessible format found at

www.bls.gov/oes/2001/oes_stru.htm.

3.2.5 List of Known or Suspected Animal Neurocarcinogens

A list of more than 40 ANCs was prepared by the SPORE research group (See Table I). This list of chemicals was used as the basis for the studies described in this dissertation. The list of ANCs was compared to the NIOSH-NOES and 18 ANCs were common to both lists. This list of 18 chemicals, NOES survey codes and a chemical number used as an identifier throughout this document were used to generate the exposure scores is found in Table II. Available regulatory limits for these chemicals are found in Table XLI, Appendix A.

3.3 Researcher's Role

For this dissertation, the researcher's role was to analyze the study data generated by the pilot and main phases of the glioma case-control study. In addition, with input from the SPORE research team, the researcher executed a hemoglobin adduct study. The researcher had no input into the design of either the pilot or main studies or their execution.

3.4 Informed Consent and Institution Review Boards

All studies included in this dissertation were approved by the University of Illinois at Chicago Institution Review Board (IRB). These included the analyses of the pilot study (IRB # 2003-0075), the analyses of the main study (IRB #2005-0734) and the Hb-adduct study (IRB # 2007-0737). In addition, the institution review boards of Duke University Medical Center and NorthShore University HealthSystem also approved these studies. The University of North Carolina at Chapel Hill (UNC) Institution Review Board reviewed the study protocol for the hemoglobin adduct study and determined that their approval was not required as their laboratory was acting on a fee for service basis. Sample analysis was completed in

TABLE II - KNOWN OR SUSPECTED ANIMAL NEUROCARCINOGENS FOUND IN THE WORKPLACE AS SURVEYED BY NIOSH				
Chemical Number	Chemical	Synonym	CAS Number	NOES Number
	Acrylamide	Acrylamide	79-06-1	3540
1		Acrylamide, polymers	9003-05-8	X6599
	Acrylonitrile	Acrylonitrile	107-13-1	3800
		Acrylonitrile, polymer, processed		X6598
		Acrylonitrile, polymers	25014-41-9	X6615
2		Acrylonitrile, processed		X7427
3	Bis (chloromethyl) ether		542-88-1	X6315
4	Dimethyl sulfate		77-78-1	26880
5	Divinyl benzene		1321-74-0	83286
6	Ethylene oxide		75-21-8	32550
7	N-2-Fluorenylacetamide	2-Acetylaminofluorene	53-96-3	X6612
8	Glycidol	2,3-Epoxy-1-propanol	556-52-5	35120
9	1-H-Benzotriazole	1,2,3-Benzotriazole	95-14-7	82113
10	Bromoethane	Ethyl bromide	74-96-4	31900
	1,3-Butadiene	Butadiene, 1,3-	106-99-0	13410
		Butadiene, 1,3- polymers - processed		X8657
11		Butadiene, 1,3- polymers	9003-17-2	X6801
12	Chloroethane		75-00-3	31970
13	CI Direct Blue 15		2429-74-5	M4514
14	3,3'-Dimethylbenzidine dihydrochloride	O-Tolidine	612-82-8	X1372
15	3,3'-Dimethoxybenzidine dihydrochloride		20325-40-0	X7012
16	Ethyl methyl sulphonate	Ethyl methyl sulfonate	62-50-0	X6247
	Isoprene		78-79-5	40940
17		Isoprene, polymers	9003-31-0	X7314
	Vinyl chloride	Polyvinyl chloride, processed		X6596
18	Vinyl chloride	Chloroethylene	75-01-4	76445

part by the Centers for Disease Control and Prevention (CDC), also on a fee for service basis for UNC and CDC institution review board approval was not required.

3.5 Methods – Project One –Self-Reported Work Histories and Cumulative Exposures

3.5.1 Development of a Cumulative Exposure Score

To create the cumulative exposure score, three variables were assigned to each job reported by a study participant in the work history. These variables were 1) the number of ANCs found in the SIC code of the job (#ANCs), 2) the length of service in the job (Duration), and 3) an exposure intensity factor (EIF) based on the number of ANCs found in both the SIC and SOC codes for the job. The scores for each job were totaled for each study participant. The cumulative exposure score may be expressed as follows:

$$Cumulative\ Exposure\ Score = \sum_{job-1}^{n} Number\ of\ ANCs * Exposure\ Duration * Exposure\ Intensity\ Factor$$

The SIC codes where ANCs were found are shown Table XLI, Appendix B, and the ANC exposures by occupation within the SIC code is shown in Tables XLI-Table LXXXI, Appendix B. The exposure intensity factor ranges from 0 to 2 and was calculated as follows: If the industry in which a subject was employed was not surveyed by the NOES study the exposure intensity score of zero (0) is assigned. If the SIC code matched the industry reported by the study participant but the occupation did not, then an exposure intensity factor of 1 was used indicating a possible exposure. All ANCs found in a given industry may not have been associated with each occupation. Therefore, a percentage of ANCs found in the industry and the occupation was used to further define the exposure intensity factor. For example, if 10 ANCs were found in an industry based on the SIC code and five were also found in the occupation within that industry based on the SOC code, the exposure intensity factor would be 1.5. This would denote a

possible exposure to the ten ANCs identified for the industry and a probable exposure to five (50%) or half of the identified ANCs in that specific occupation. An exposure score for each job was estimated by multiplying the number of ANCs times the exposure duration times the exposure intensity factor. This process was repeated for each job reported by the study participant and the score for each job were totaled to generate the cumulative exposure score.

3.5.2 Statistical Analysis for Project One

Data were analyzed using SAS-version 9.1.3 (Cary, NC) statistical software. Demographics for the study population included age, gender and income and were obtained from the questionnaires. The differences between cumulative exposure scores for cases and controls were analyzed using the student t-test. The association between cumulative exposure scores and case-control status was estimated by calculation of odds ratios (OR) and 95% confidence intervals using the mean cumulative exposure of the control population as a cut point and logistic regression models. Cumulative exposure scores above the mean were designated as exposed and those below the mean were designated unexposed. Odds ratios for all cases and all controls were calculated and were adjusted for age, gender and income. A dichotomous income variable was created by dividing the income responses into two groups, those with an annual salary $\leq$ \$50,000 and those with a salary > \$50,000.

As not all cases had controls, odds ratios for matched pairs were calculated using conditional logistic methods for all cases with controls, all cases with friend controls and all cases with sibling controls individually and adjusted for age, gender and income. Additionally, all cases were frequency matched to controls based on age and gender and odds ratios generated. Frequency matching was completed by a computer program that randomly selecting a sibling or friend control for each case based on age and gender. In this manner, all cases were assigned a control and a 1:1 matched pair analysis was

performed. (Paired analyses were completed by Kristin Rankin, PhD for this study). Odds ratios were also generated for each ANC, SIC and SOC code reported by study participants and included in the NIOSH study. For these analyses the study population was divided into ever versus never exposed to calculate the odds ratios. If a study participant ever worked in the industry of the SIC code being analyzed, the participant was considered to be potentially exposed to that chemical.

To control for study participants who had a zero score because their industries were not included in the NIOSH study, these scores were dropped from the analysis and odds ratios and confidence limits were recalculated. To consider the possible bias from including sibling controls in the analysis, additional analyses excluding sibling controls were also conducted.

The reliability of the scoring method was examined by creating two additional variables. The first variable measured the number of jobs that were held in industries surveyed by NIOSH divided by the total number of jobs held by the study participant. The second variable was related to the length of employment and was based on the number of years of employment in NIOSH surveyed jobs divided by the total number of years of employment (statistical analysis of the reliability factors completed by Kristin Rankin, PhD).

3.6 Methods – Project Two – Use of Questionnaire Responses to Workplace Questions

3.6.1 Use of Questionnaire Data

In the main phase of the case-control study, participants answered questions that were designed to target specific ANCs. The six questions included in the occupational portion of the main study and the chemicals that were targeted for each question are shown in Tables LXXXII-LXXXIV, Appendix C. The first question asked if the participant ever worked outside the home. The second question asked questions related to the industries where the participant worked. The third question asked questions related to

the participant's occupation. The fourth question dealt with the use or production of certain materials in the workplace. The fifth question asked whether or not a participant worked in research or hospital clinical laboratories. The sixth question asked questions related to the presence of different types of exhaust fumes such as diesel, gasoline or wood burning stoves in the workplace. The questions pertaining to potential exposure to acrylamide, 1,3-butadiene and/or polycyclic aromatic hydrocarbons were identified and are the focus of this analysis. Questions one, two, three and six were used to complete this study.

3.6.2 Statistical Analysis – Project Two

Main study data were analyzed for potential exposure to acrylamide, 1,3-butadiene and PAHs using unconditional logistic regression techniques controlling for age, gender and income, and odds ratios and 95% confidence limits were calculated.

3.7 Methods – Project Three – Hemoglobin Adduct Study

3.7.1 Specimen Collection

Study participants were recruited from the existing control populations of one of two glioma case-control studies. In the first or pilot study, the control population consisted of siblings or friends of the cases (friend controls) and they completed a self-reported work history and study questionnaire. No blood samples were collected during the pilot study. In the second or main study, the control population was comprised of friend controls or hospital-based controls. In the main study, subjects completed a questionnaire which included a work history section. Additionally, some main study subjects also completed a self-reported work history. Main study participants also provided a blood sample which was stored for future studies. Pilot study subjects from NorthShore University HealthSystem were initially recruited for this study and asked to return to the clinic to provide a blood

sample and update their work histories with current employment status. As recruitment efforts resulted in minimal response from the pilot study participants, and subjects were currently being enrolled in the main study, main study subjects were also recruited to participate in this study. Blood samples from the main study subjects were used if sample collection was concurrent with recruitment for this study or if blood samples had been stored and a self-reported work history was available to determine current employment status. For new blood samples, the sample collection and preparation protocol from the University of North Carolina, Chapel Hill (UNC) were followed (See Appendix D). Samples were stored frozen until shipped to Dr. James Swenberg's laboratory at the University of North Carolina, Chapel Hill (UNC). Previously collected samples were processed according to main study protocols and stored frozen at Duke University Medical Center. Selected samples were shipped frozen to Dr. James Swenberg's laboratory.

3.7.2 1,3-Butadiene Hemoglobin Adduct Laboratory Analysis

Blood samples were prepared for analysis by harvesting the globin from the red blood cells and analyzed for the *pyr*-Val hemoglobin adduct according to the UNC protocol (See Appendix D). Briefly, the samples were hydrolyzed and filtered through an Immunoaffinity column and then analyzed by Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy. Results are reported as pmol/g hemoglobin.

3.7.3 Acrylamide Hemoglobin Adduct Laboratory Analysis

Globin samples were sent by UNC to the Centers for Disease Control and Prevention's (CDC's) National Center for Environmental Health for analysis. Acrylamide metabolism may produce two possible hemoglobin adducts, acrylamide-hemoglobin (AA-Hb) and glycidamide-hemoglobin (GA-Hb). These two adducts were analyzed simultaneously using high performance liquid chromatography/mass spectroscopy/mass spectroscopy (HPLC/MS/MS) methods (Vesper, 2008). The current laboratory

procedure manual is attached in Appendix D. The total hemoglobin in the sample was measured and results were reported in pmol adduct/g globin.

3.7.4 Data Analysis

An occupational variable was created for exposure to acrylamide or 1,3-butadiene based on the study participant's current employment status. Potential exposures were assigned based on the findings of the National Occupational Exposure Survey (NOES) conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1981-1983. Approximately 4500 industries participated in the survey and chemical inventories were completed. The survey was cataloged by SIC code and acrylamide and 1,3-butadiene were found in several industries. The NIOSH survey did not include all industries. Based on the Standard Industrial Classification (SIC) system and the NOES results, acrylamide was found in 19 industries and 1,3-butadiene was found in 20. If acrylamide or 1,3-butadiene was found to be present in the SIC code as identified by the NIOSH-NOES survey, the participant was considered to have been potentially exposed to that chemical. As the hemoglobin adduct results were not normally distributed, log-transformed data were used to complete the analyses. Currently exposed study subjects were compared to currently unexposed subjects using generalized linear models.

Demographic data were collected when study participants completed the questionnaire and included age, gender, education, income, state of residence and height and weight. Generalized linear models were used to determine if an association could be made between the study participant responses and hemoglobin adduct levels.

Both acrylamide and 1,3-butadiene are found in cigarette smoke. The questionnaire completed by study participants included several questions related to smoking history. The questions asked included whether or not the study participant currently smoked, ever smoked, ages they started and stopped

smoking, how many cigarettes they smoked in a day, if they smoked inside the home, if they lived with a smoker and how many, the number of years they lived with a smoker(s) and a rating of their exposure to second hand smoke. The association between the responses to these questions and the hemoglobin adduct results were completed using generalized linear models.

Acrylamide has been identified as a contaminant in various food sources. The questionnaire included a diet section which asked the study participant to rate the number of times they ate various foods during the week, month or year. From the questionnaire, a subset of 16 questions of a priori interest was analyzed. Not all questions were asked in both of the studies and some questions were combined in the main study but were asked individually in the pilot study. Some of the questions had four response levels, while others had up to nine different response levels. For questions with nine response levels, level one (never) was collapsed with level two (a few times per year) to correspond with level one (less than once per month) in the eight level response category. If questions used a four level response (never, rarely, sometimes or often) in one study and eight response levels in the other study, the eight levels were combined into four response levels. Thus, the response levels one and two (less than once per month and once per month) were collapsed into the never category, etc.

Not all study participants answered all diet history questions. This resulted in response levels with very small numbers of responses. For analysis purposes, questions with four response levels were collapsed into two response levels (never/rarely and sometimes/often). Questions with eight levels were collapsed into three levels as follow: 1) less than once per month, once per month and two to three times per month; 2) once a week or twice a week; 3) three to four times per week, five to six times per week, every day. Data were analyzed with these smaller categories.

The Fourth National Report on Human Exposure to Environmental Chemicals (NHANES) was published in December, 2009. Biomarkers of exposure to various chemicals, including hemoglobin adducts of acrylamide, were evaluated in the general population. Results of this study were compared to the NHANES data and included analysis of age, gender, AA-Hb and GA-Hb and the ratio of GA/AA results to determine the degree of agreement between the two datasets.

There were two extreme glycidamide results reported. Analysis of the glycidamide results was completed both with and without the outliers. The GA-Hb:AA-Hb ratios were calculated to permit comparison with the NHANES data after the acrylamide adduct levels for the two outliers were removed. The responses of the two outliers were examined to determine if any of the variables under study might contribute to their extreme glycidamide adduct results.

4.0 PAPER ONE

THE USE OF SELF-REPORTED WORK HISTORIES TO ASSESS WORKPLACE CHEMICAL EXPOSURES IN A BRAIN CANCER CASE-CONTROL STUDY

4.1 Introduction

The interaction of an individual with his/her work environment has been the focus of much attention. In the occupational setting, chemical exposures are rarely limited to one chemical. In addition, workers move from job to job and exposures change over time and with respect to each occupation of the worker. Identifying workplace chemicals that may lead to cancer is even more difficult to evaluate. The long latency period between exposure and disease leads to the questions of what, where, when and how the exposures occurred and to what relevant chemicals was the worker exposed at that time. All available data related to workplace exposures to the chemicals need to be assessed to determine if there is a link between workplace exposures and occupational cancers. Unfortunately, these records are generally not available and retrospective methods must be employed to quantify the exposure. The retrospective methods often include the reconstruction of the study participant's work history either through the use of questionnaires or completion of a self-reported work history.

The causes of brain cancer are largely unknown. High dose ionizing radiation and inherited genetic syndromes are two recognized causes (Davis, 2007). In humans, there are over 100 different brain tumor histologies, both malignant and non-malignant. Meningiomas are the most common brain tumor (33.4%), followed by glioblastomas (17.6%) and astrocytomas (7.4%) (CBTRUS, 2009). Thirty-three percent of all tumors and 80% of malignant tumors are gliomas. Seventy-seven percent of all gliomas are glioblastomas and astrocytomas (CBTRUS, 2009).

The aim of this study was to examine the association between potential exposures to specific groups of chemicals, known or suspected animal neurocarcinogens (ANCs), found in the work environment and glioma development in a case-control study population. Potential exposures to ANCs were indexed via cumulative exposure scores, which were derived from the completed work histories of participants in the study. The hypothesis was that through the use of self-reported work histories we could estimate an exposure score as an

indicator for potential cumulative occupational exposures to these ANCs and evaluate the association between these potential exposures and glioma in the study population.

4.2 Methods

Self reported work history data from a brain cancer case-control study were utilized. Study participants were recruited from the NorthShore University HealthSystem in Illinois and the Preston Robert Tisch Brain Tumor Center at Duke University Medical Center in North Carolina. Cases were enrolled in the study between 2003 and 2006 and were defined as those patients presenting with a first primary brain tumor (International Classification of Diseases for Oncology, 3rd edition, sites C70.0-C72.9) and a confirmed diagnosis of glioblastoma (International Classification of Diseases for Oncology, 3rd edition, histology codes 9440-9442), astrocytoma-grades 2 and 3 (9400-9411 and 9420) or oligodendroglioma (9382 and 9450-9451), English speaking, at least 18 years of age and a resident of the United States. The control population consisted of friends or siblings of the cases who were at least 18 years of age, English speaking, residents of the United States having no history of brain cancer. Following Institution Review Board approvals and completion of the informed consent process, 276 cases and 405 controls were recruited for the pilot study. Data were collected by administration of a questionnaire, which consisted of a number of modules (e.g., demographics, family history, smoking history, medical history, and environmental, occupational, and dietary exposure histories). In addition, study participants were asked to complete a separate occupational/work history. The self-reported work histories provided information about the industry in which the individual worked, how long the individual worked there, the dates of employment and his/her primary job functions. Work histories were completed by 149 cases (65% of total cases in the study) and 279 controls (80% of total controls in the study).

4.2.1 Development of a Cumulative Exposure Score

Following an extensive literature search, a list of more than 40 known or suspected animal neurocarcinogens was developed (Davis, et al., 2006). The chemicals on this list were compared to the chemical inventory created by NIOSH during the completion of the NOES, conducted during 1981-1983 (National Occupational Exposure

Survey, 1991). During the completion of this survey, trained industrial hygienists visited nearly 4,500 industrial facilities and collected data on the chemicals in use at the facility. Potential exposures to approximately 13,000 different chemical and physical agents in more than 500 industries and 377 occupations were reported (National Occupational Exposure Survey, 1991). In the NOES, chemicals were cataloged by industry using the SIC codes (Internet accessed on September 1, 2007 at www.osha.gov/pls/imis/sic_manual) and by occupation within the industry using the 1980 Bureau of Census codes (1980 Census of Population and Housing, 1980). The SIC code system is used by the federal government to classify industries into one of ninety-nine major categories. The Standard Occupation Classification (SOC) system is similarly designed for classification of worker occupations and replaces the 1980 Bureau of Census in this study (Internet accessed on September 1, 2007 at www.bls.gov/soc). There are 23 major occupation groups in this system. Each major group has many subgroups. The list of ANCs was compared to the NOES and 18 chemicals from this list were found in the workplaces surveyed by NIOSH (Table III).

To create the cumulative exposure score, three variables were assigned to each job reported by study participants; 1) number of ANCs found in the SIC code of the job (#ANCs), 2) the length of employment in the job in whole years (Duration), and 3) an exposure intensity factor (EIF) based on the number of ANCs found in both the SIC and SOC codes for the job. The exposure intensity factor had a range of 0 to 2 and was calculated as follows: If the industry in which a study participant was employed was not surveyed by the NOES study, the exposure intensity score of zero (0) was assigned. If the SIC code matched the industry reported by the study participant but the occupation did not, then an exposure intensity factor of 1 was used indicating a possible exposure. An exposure intensity factor of 2 was assigned if the study participant's job matched both industry and occupation as surveyed by NIOSH during the NOES. All ANCs found by NIOSH in a given industry may not have been associated with each occupation. Therefore, a percentage of animal neurocarcinogens found in the industry and the occupation was used to further define the exposure intensity factor. For example, if an industry had 10 ANCs based on the SIC code and five were also found for the occupation within that industry

TABLE III - KNOWN OR SUSPECTED ANIMAL NEUROCARCINOGENS FOUND IN THE WORKPLACE AS SURVEYED BY NIOSH

Known or Suspected Animal Neurocarcinogen	# SIC Codes where found	Known or Suspected Animal Neurocarcinogen	# SIC Codes where found
1,3-Butadiene	19	CI Direct Blue 15	6
1-H-Benzotriazole	32	Dimethyl sulfate	4
2-Acetylaminofluorene	1	Divinyl benzene	1
3,3'-Dimethoxybenzidine dihydrochloride	1	Ethyl bromide	11
3,3'-Dimethylbenzidine dihydrochloride	3	Ethyl methyl sulfonate	1
Acrylamide	20	Ethylene oxide	28
Acrylonitrile	15	Glycidol	2
Bis (chloromethyl) ether	1	Isoprene	20
Chloroethane	10	Vinyl chloride	25

based on the SOC code, the exposure intensity factor for that job was 1.5, denoting a possible exposure to the 10 ANCs identified for the industry and a probable exposure to five (50%) or half of the identified ANCs in that specific occupation. This process was repeated for each job reported by study participants and the scores for each job held by the study participant were totaled to generate the cumulative exposure score. Mathematically, the cumulative exposure score was calculated as follows:

$$Cumulative\ Exposure\ Score = \sum_{job-1}^{n} \#\ ANCs * Duration * Exposure\ Intensity\ Factor$$

4.2.2 Data Analysis

Data were analyzed using SAS-version 9.1.3 (Cary, NC) statistical software. Demographics for the study population included age, gender and income and were obtained from the questionnaires. The differences between cumulative exposure scores for cases and controls were analyzed using the student t-test. The association between cumulative exposure scores and case-control status was estimated by calculation of odds

ratios (OR) and 95% confidence intervals using the geometric mean cumulative exposure score of the control population as a cut point and logistic regression models. Cumulative exposure scores above the mean were designated as exposed and those below the mean were designated unexposed. To evaluate the reliability of the exposure scoring method, odds ratios were generated for the cumulative exposure scores based on the number of jobs an individual held in NIOSH surveyed facilities divided by the total number of jobs held by the individual and the number of years worked in NIOSH surveyed facilities divided by the total number of years worked. Crude odds ratios for all cases and all controls were calculated and were adjusted for age, gender and income. A dichotomous income variable was created by dividing the income responses into two groups, those with an annual salary $\leq$ \$50,000 and those with a salary > \$50,000. As not all cases had controls, odds ratios for matched pairs were calculated using conditional logistic methods for all cases with pair-matched controls, all cases with friend controls and all cases with sibling controls individually and adjusted for age, gender and income. Additionally, all cases were frequency matched to controls based on age and gender and odds ratios generated. Frequency matching was completed by a computer program that randomly selected a sibling or friend control for each case based on age and gender. In this manner, all cases were assigned a control and unconditional logistic regression was performed. Odds ratios were also generated for each ANC, SIC and SOC code reported by study participants and included in the NIOSH study. For these analyses the study population was divided into ever versus never exposed to calculate the odds ratios. If a study participant ever worked in the industry of the SIC code being analyzed, the participant was considered to be potentially exposed to that chemical.

4.3 Results

Work histories were obtained from 149 cases and 279 controls (129 sibling controls, 150 friend controls). Forty-six percent of the study population was male (81 cases and 116 controls) and 54% was female (68 cases and 163 controls). There were more male cases and more female controls in the study population and the gender differences were statistically significant (p=0.010). More than 95% of study participants were Caucasian. Selected characteristics of the study population were obtained from questionnaires completed at the time of

enrollment in the study and are shown in Table IV. Study participant ages were normally distributed with a mean age of 48 and a standard deviation of 12.9. The age differences in the study population were not statistically significant (p=0.75). Cases in the study population had confirmed diagnoses of one of three different types of brain tumors; glioblastoma, 50%, astrocytoma, 27% and oliglodendroma, 23%.

The cumulative exposure scores for cases and controls ranged from zero to 727. Cumulative exposure scores were higher in the control population than the case population. For cases, the cumulative exposure scores ranged from zero to 512 with an arithmetic mean score of 76.8 and a median score of 45.0. For controls, the cumulative exposure scores ranged from zero to 727 with an arithmetic mean score of 97.2 and a median score of 52.8. There were 91 study participants (27 cases and 64 controls) who held no jobs surveyed by NIOSH, resulting in a zero cumulative exposure score. No association between case/control status and potential exposure to ANCs could be assessed for these study participants. The full dataset was reduced by excluding these observations and the remaining 337 observations (122 cases and 215 controls) were further analyzed. Analyses of the full and reduced datasets were completed for comparison purposes. The difference in gender participation did change with the reduction in size of the study population; however, the differences remained statistically significant (p=0.04). There were four true zeros included in the reduced dataset as these study subjects worked in industries surveyed by NIOSH where no ANCs found. The characteristics of the revised study population are also shown in Table IV.

The distribution of the cumulative exposure scores was not normally distributed, driven to the right by high scores (N = 337; mean = 114.5; standard deviation = 119.5; kurtosis = 4.3; skewness = 1.82). A log-normal distribution was obtained by adding one unit to all cumulative exposure scores (to account for the zero scores) and transforming the data using natural logarithms (geometric mean = 60.5; geometric standard deviation = 2.72; kurtosis = 0.38; skewness = -0.78). The log normal distribution was used for all cumulative exposure score

TABLE IV - CHARACTERISTICS OF THE STUDY POPULATION

	Original Study Population										Study Population Adjusted by Total Score									
	All Cases	%	Sibling Controls	%	Friend Controls	%	All Controls	%	Total Cases and Controls	%	All Cases	%	Sibling Controls	%	Friend Controls	%	All Controls	%	Total Cases and Controls	%
Study Population	149	35	129	30	150	35	279	65	428	100	122	36	103	31	112	33	215	64	337	100
Gender																				
Male	81	55	45	35	71	47	116	42	197	46	69	57	37	36	59	53	96	45	165	49
Female	68	45	84	65	79	53	163	58	231	54	53	43	66	64	53	47	118	55	171	51
Total	149	100	129	100	150	100	279	100	428	100	122	100	103	100	112	100	215	100	337	100
Age (years)																				
18-29	10	7	11	9	12	8	23	8	33	8	8	7	6	6	4	4	10	5	18	5
30-39	32	22	23	18	26	17	49	18	81	19	27	22	16	16	24	22	40	19	67	20
40-49	34	22	37	29	28	19	65	24	99	23	26	21	33	32	21	19	54	25	80	24
50-59	43	29	33	25	45	30	78	28	121	29	38	31	26	25	34	30	60	28	98	29
60-69	23	16	20	15	35	23	55	19	78	18	18	15	17	17	27	24	44	21	62	18
>70	7	5	5	4	4	3	9	3	16	4	5	4	5	5	2	2	7	3	12	4
Total	149	100	129	100	150	100	279	100	428	100	122	100	103	100	112	100	215	100	337	100
Income*																				
< $ 25,000	12	8	7	5	10	7	17	6	29	7	10	8	7	7	6	6	13	6	23	7
$25,000 - $50,000	16	11	21	17	20	14	41	15	57	14	15	13	14	14	16	15	30	14	45	14
$51,000 - $75,000	25	17	29	23	23	15	52	19	77	18	20	17	25	25	16	15	41	20	61	19
$76,000 - $100,000	30	21	19	15	27	18	46	17	76	18	24	20	15	15	19	17	34	16	58	18
> $100,000	63	43	50	40	68	46	118	43	181	43	50	42	39	39	53	48	92	44	141	43
Total	146	100	126	100	148	100	274	100	420	100	119	100	100	100	110	100	210	100	329	100

*8 missing responses

analyses. Geometric mean results were adjusted by subtracting one unit to account for the one unit added prior to log transformation.

The control population included 103 study participants who were siblings of a case and 112 study participants who were friends of a case. The cumulative exposure scores of the sibling population had a geometric mean of 56.3 (SD = 3.10; range: 0-727) and the cumulative exposure scores of the friend population had a geometric mean of 75.0 (SD=2.74; Range 0-617). The geometric mean for the entire control population was 65.4 with a geometric standard deviation of 2.92. The difference between the cumulative exposure scores of cases and controls was not statistically significant (t= -1.42; p=0.16). The difference between the cumulative exposure scores of siblings and cases was not statistically significant (t = -0.36; p=0.71), while the difference between the cumulative exposure scores of friends and cases was statistically significant (t= -2.10; p=0.04). The difference between sibling and friend controls was not statistically significant (t= -1.52; p=0.13).

A total of 1983 individual jobs were reported by participants with an average number of six jobs reported per participant. Together, cases held 730 jobs and controls held 1253 jobs. The top five industries based on the number of jobs held in the SIC code were Educational Services, SIC 82, (222 jobs); Health Services, SIC 80, (205 jobs); Eating and Drinking Places, SIC 58, (127 jobs); Business Services, SIC 73 (99 jobs) and Miscellaneous Retail, SIC 59 (74 jobs). The top five occupations were Office and Administrative, SOC 43 (257 jobs); Retail Sales, SOC 41 (246 jobs); Management, SOC 11 (160 jobs) and Food Preparation and Services, SOC 35 (154 jobs) and Education, Training and Library, SOC 25 (129 jobs). Of the 1983 jobs scored, 937 (47%) were held in industries that were surveyed by NIOSH. The 18 ANCs included in this study were found in the workplace of 39 of the 43 SIC codes for industries that were surveyed by NIOSH for the NOES. There were no ANCs found in SIC codes 21, Tobacco Manufacturers; 46, Pipe Lines Except Natural Gas, and 51, Wholesale Trade-Non-durable Goods. There were no jobs reported for participants in SIC code 31, Leather and Leather Products.

The crude odds ratio comparing all unmatched cases to controls was 0.66 (95% CI = 0.42-1.03, p = 0.07). When adjusted for age, gender and income, the odds ratio was 0.65 (95% CI = 0.40-1.05, p = 0.08). These results suggest no statistically significant association between the cumulative exposure scores and the risk of glioma. Results of the matched pair analyses are shown in Table V.

TABLE V - ASSOCIATION BETWEEN HIGH (ABOVE THE GEOMETRIC MEAN) AND LOW (BELOW THE GOEMETRIC MEAN) CUMULATIVE EXPOSURE SCORES AND GLIOMA

	Total Cases and Controls	Total Cases	Total Controls	Odds Ratio	95% Confidence Interval	p-value
All unmatched cases and controls	337	122	215	0.66	0.42-1.03	0.07
All unmatched cases and controls*	329	119	210	0.65	0.40-1.05	0.08
Frequency matched cases and controls*	238	119	119	0.69	0.40-1.19	0.18
Matched cases and controls*	165	58	107	0.71	0.32-1.58	0.41
Cases matched to friend controls*	94	38	56	0.44	0.16-1.22	0.11
Cases matched to sibling controls*	91	40	51	0.43	0.14-1.27	0.51

*Adjusted for age, gender and income

Odds ratios were calculated based on two reliability factors. One reliability factor was based on the percentage of jobs held in NIOSH surveyed facilities and the other based on percentage of years worked in NIOSH surveyed facilities. Results are shown in Table VI. The distribution of these reliability factors is shown in Figures 3 and 4.

TABLE VI - RELIABILITY FACTORS - ASSOCIATION BETWEEN CUMULATIVE EXPOSURE SCORE AND CASE/CONTROL STATUS BASED ON YEARS OF SERVICE OR NUMBER OF JOBS HELD IN NIOSH SURVEYED INDUSTRIES ADJUSTED FOR AGE, GENDER AND INCOME

Percentage	Percentage of Years Worked in NIOSH Suveyed Industries						Percentage of Jobs Held in NIOSH Surveyed Industries					
	Total Cases and Controls	Cases	Controls	Odds Ratio	95% Confidence Interval	p-value	Total Cases and Controls	Cases	Controls	Odds Ratio	95% Confidence Interval	p-value
All unmatched cases and controls												
> 25%	240	87	153	0.41	0.22-0.08	0.008	275	100	175	0.61	0.36-1.03	0.07
≥ 50%	184	66	118	0.41	0.19-0.92	0.03	181	61	120	0.48	0.23-1.00	0.35
≥ 75%	126	41	85	0.21	0.07-0.63	0.005	88	32	56	0.18	0.05-0.71	0.01
100%	49	21	28	0.17	0.03-1.10	0.06	49	21	28	0.17	0.03-1.10	0.06
All matched cases and controls												
≥ 25%	140	47	93	0.36	0.09-1.48	0.4	166	54	112	0.83	0.34-2.03	0.68
≥ 50%	110	39	71	0.26	0.03-2.15	0.58	108	32	76	2.43	0.38-15.58	0.35
≥ 75%	70	22	48	Insufficient Data			46	15	31	Insufficient Data		
100%	Insufficient data											
Matched cases and sibling controls												
≥ 25%	81	36	45	0.31	0.06-1.62	0.5	91	41	50	0.35	0.09-1.39	0.13
≥ 50%	60	28	32	Insufficient Data			57	24	33	Insufficient Data		
≥ 75%	Insufficient data						Insufficient data					
100%	Insufficient data						Insufficient data					
Matched case and friend controls												
≥ 25%	82	32	48	Insufficient Data			98	36	62	0.44	0.15-1.30	0.14
≥ 50%	67	28	39	Insufficient Data			65	22	43	1.8	0.32-10.08	0.51
≥ 75%	Insufficient data						26	11	15	Insufficient Data		
100%	Insufficient data						Insufficient data					

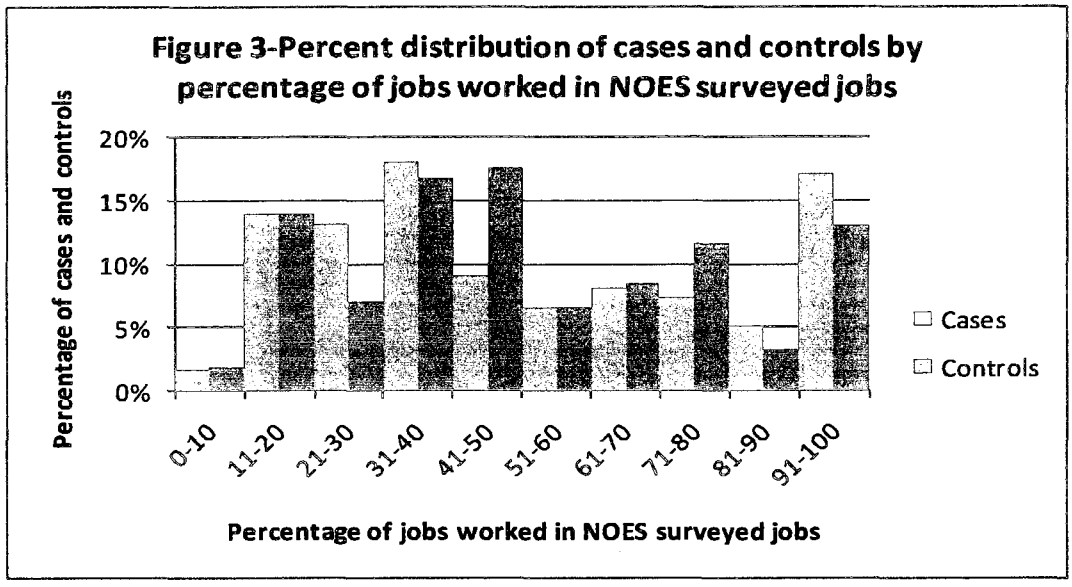
Figure 3-Percent distribution of cases and controls by percentage of jobs worked in NOES surveyed jobs
Percentage of cases and controls
20%
15%
10%
5%
0%
0-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
Cases
Controls
Percentage of jobs worked in NOES surveyed jobs

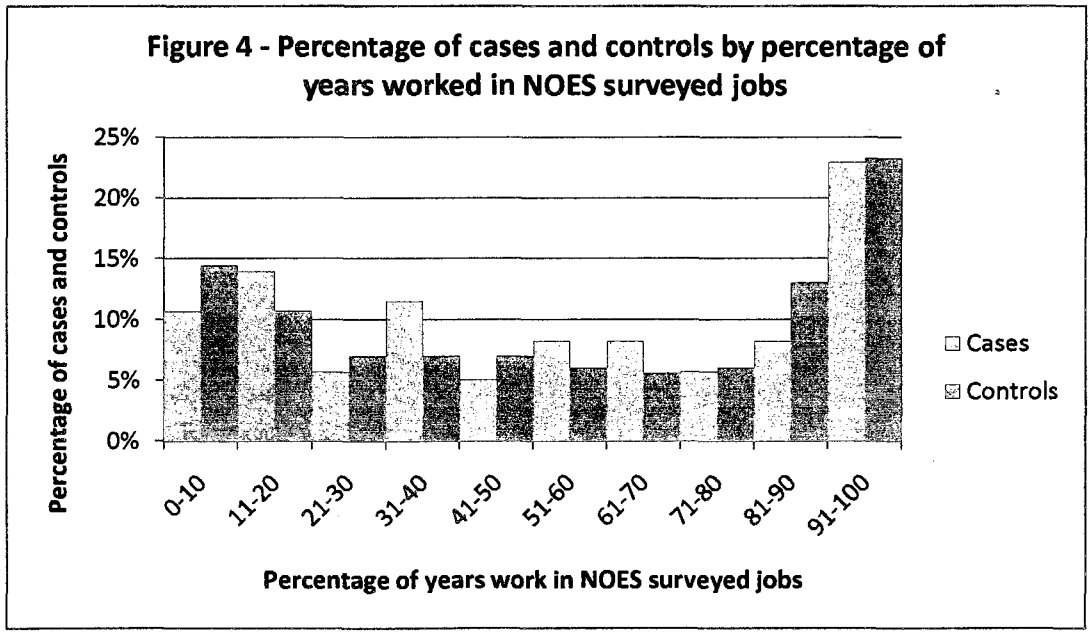
Figure 4 - Percentage of cases and controls by percentage of years worked in NOES surveyed jobs
Percentage of cases and controls
25%
20%
15%
10%
5%
0%
0-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
Cases
Controls
Percentage of years work in NOES surveyed jobs

Odds ratios were calculated for four categories: 1) the full dataset (N=428), 2) the full dataset, adjusted for age, gender and income, 3)the reduced dataset, adjusted for age, gender and income and including friend and sibling controls (N=337) and 4) the reduced dataset adjusted for age, gender and income, including only friend controls (N= 234). Results are shown in Tables VII-X. The majority of the odds ratios calculated by chemical were slightly elevated but not statistically significant. The highest odds ratio was for 3, 3'-Dimethylbenzidine dihydrochloride in all four categories. For this chemical, odds ratios varied from 1.92 (95% CI=0.94-3.92; p=0.07) for the full dataset (N=428), adjusted for age, gender and income to 1.87 (95% CI=0.79-4.41; p=0.15) for the reduced dataset, including only friend controls, adjusted for age, gender and income. 3, 3'-Dimethylbenzidine dihydrochloride was also the most statistically significant (p=0.07). This chemical is one of the benzidine dyes used in pigment production (National Toxicology Program, 1991) and was found in SIC code 20-Food and Kindred Products, SIC code 28-Chemical and Allied Products and SIC code 29-Petroleum and Coal Products SIC codes during the NIOSH study. A comparison spreadsheet of odds ratios and confidence intervals for all chemicals by study population category is shown in Table XI.

Similarly, odds ratios for study participants who ever worked in an industry compared to those who never worked in the industry based on SIC code were estimated (see Tables XII-XV). As the number of exposed cases and controls in each SIC code was small, the ability to detect an association was low. The highest odds ratio for industries by SIC code was calculated for SIC code 20, Food and Kindred Products in all four categories. Odds ratios ranged from the crude odds ratio of 2.88 (95% CI=0.80-10.38; p=0.09) to the odds ratio adjusted for age, gender and income for the reduced dataset using only friend controls of 5.77 (0.68-49.04; p=0.11). SIC code 20 includes food and beverage manufacturing and processing. The most statistically significant industry odds ratio based on p-values, was for SIC code 27, Printing and Publishing, in the reduced dataset using only friend controls (OR = 2.99; 95% CI=0.92-9.68; p=0.07). A

Table VII-CRUDE ODDS RATIOS FOR ANCS INCLUDED IN STUDY

Chemical	Cases N=149 Number Exposed	Controls N=279	Odds Ratio	95% CI		p-value
Acrylamide	94	171	1.08	0.72	1.63	0.72
Acrylonitrile	90	152	1.28	0.85	1.91	0.24
Bis (chloromethyl) ether	31	65	0.87	0.53	1.40	0.56
Dimethyl Sulfate	63	112	1.09	0.73	1.64	0.67
Ethylene Oxide	109	196	1.15	0.74	1.80	0.53
Glycidol	19	26	1.42	0.76	2.67	0.27
1-H-Benzotriazole	115	207	1.18	0.74	1.88	0.50
Bromoethane	84	149	1.13	0.76	1.68	0.56
1,3-Butadiene	98	177	1.11	0.73	1.68	0.63
Chloroethane	78	135	1.17	0.79	1.75	0.44
CI Direct Blue 15	29	52	1.06	0.64	1.75	0.83
3,3'-Dimethylbenzidine dihydrochloride	17	18	1.87	0.93	3.74	0.08
3,3'-Dimethoxybenzidine dihydrochloride	31	65	0.86	0.53	1.40	0.53
Ethyl methyl sulfonate	26	44	1.13	0.66	1.92	0.65
Isoprene	96	176	1.06	0.70	1.60	0.78
Vinyl Chloride	87	157	1.09	0.73	1.63	0.67
2-Acetylaminofluorene	26	44	1.13	0.66	1.92	0.65
Divinyl benzene	8	11	1.38	0.54	3.52	0.50

comparison spreadsheet of odds ratios and confidence intervals for all industries by study population category is shown in Table XVI.

Odds ratios were also calculated for study participants based on the SOC codes for occupation (See Tables XVII-XX). Unlike the odds ratios calculated for ANCs and Industries, the occupation with the highest odds ratio was different for each of the four categories. Of the crude odds ratios calculated for

TABLE VIII - CRUDE ODDS RATIOS FOR ANCS INCLUDED IN STUDY ADJUSTED FOR AGE, GENDER AND INCOME

Chemical	Cases N=146	Controls N=274	Odds Ratio	95% CI		p-value
	Number Exposed					
Acrylamide	91	166	1.03	0.68	1.57	0.88
Acrylonitrile	87	147	1.29	0.85	1.94	0.23
Bis (chloromethyl) ether	29	62	1.00	0.59	1.68	1.00
Dimethyl Sulfate	61	110	1.22	0.80	1.86	0.35
Ethylene Oxide	106	193	1.16	0.74	1.82	0.53
Glycidol	19	26	1.32	0.70	2.50	0.40
1-H-Benzotriazole	112	202	1.13	0.70	1.81	0.62
Bromoethane	81	145	1.16	0.77	1.74	0.49
1,3-Butadiene	95	172	1.09	0.71	1.67	0.68
Chloroethane	75	131	1.17	0.77	1.77	0.46
CI Direct Blue 15	28	52	0.89	0.52	1.50	0.65
3,3'-Dimethylbenzidine dihydrochloride	17	17	1.92	0.94	3.92	0.07
3,3'-Dimethoxybenzidine dihydrochloride	29	62	1.00	0.59	1.68	1.00
Ethyl methyl sulfonate	26	44	1.25	0.73	2.16	0.42
Isoprene	93	171	1.06	0.70	1.62	0.77
Vinyl Chloride	86	157	1.00	0.66	1.53	1.00
2-Acetylaminofluorene	26	42	1.25	0.73	2.16	0.42
Divinyl benzene	7	8	1.16	0.43	3.09	0.77

occupation, the highest odds ratio was calculated for SOC code 49, Installation, Maintenance and Repair (OR = 1.57; 95 %CI=0.73-3.36; P=0.24) while the most statistically significant odds ratio was calculated for SOC code 13; Business and Finance (OR=1.52; 95 %CI=0.93-2.48; p=0.10). When the crude odds ratio was adjusted for age, gender and income, the odds ratio for SIC code 49 was reduced to 1.16 (95 %CI=0.52-2.60; p=0.72). The highest and most statistically significant odds ratio for the crude odds ratio,

Table IX - ODDS RATIOS FOR ANCS IN REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME - FRIEND AND SIBLING CONTROLS

Chemical	Cases N=119	Controls N=210	Odds Ratio	95% CI		p-value
	Number Exposed					
Acrylamide	91	166	0.85	0.49	1.47	0.56
Acrylonitrile	87	147	1.24	0.75	2.06	0.41
Bis (chloromethyl) ether	29	62	0.92	0.53	1.60	0.77
Dimethyl Sulfate	61	110	1.14	0.71	1.83	0.59
Ethylene Oxide	106	193	0.95	0.44	2.04	0.89
Glycidol	19	26	1.29	0.67	2.46	0.45
1-H-Benzotriazole	112	202	0.66	0.23	1.89	0.44
Bromoethane	81	145	1.05	0.64	1.73	0.84
1,3-Butadiene	95	172	0.93	0.52	1.65	0.79
Chloroethane	75	131	1.11	0.68	1.80	0.68
CI Direct Blue 15	28	52	0.84	0.49	1.45	0.53
3,3'-Dimethylbenzidine dihydrochloride	17	17	1.88	0.91	3.87	0.09
3,3'-Dimethoxybenzidine dihydrochloride	29	62	0.92	0.53	1.60	0.77
Ethyl methyl sulfonate	26	44	1.15	0.66	2.02	0.63
Isoprene	93	171	0.88	0.50	1.56	0.67
Vinyl Chloride	86	157	0.83	0.49	1.40	0.48
2-Acetylaminofluorene	26	42	1.15	0.66	2.02	0.63
Divinyl benzene	7	8	1.12	0.42	2.99	0.83

adjusted for age, gender and income category was calculated for SOC code 39, Personal Care and Service occupations (OR = 1.64, 95% CI: 0.94-2.84, p = 0.08). This code includes occupations related to funeral homes, barber and beauty salons, flight attendants and recreation workers.

For the reduced dataset that included friend and sibling controls, the highest odds ratio was calculated for SOC code 21, Community and Social Services (OR=1.63; 95% CI=0.62-4.33, p=0.32). The odds ratio for SOC code 39, Personal Care and Service remained elevated but not statistically significant (OR= 1.54;

Table X - ODDS RATIOS FOR ANCS IN REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME - FRIEND CONTROLS ONLY

Chemical	Cases N=119	Controls N=110	Odds Ratio	95% CI		p-value
	Number Exposed					
Acrylamide	91	90	0.74	0.39	1.41	0.23
Acrylonitrile	87	79	1.08	0.60	1.94	0.36
Bis (chloromethyl) ether	29	31	0.84	0.45	1.59	0.60
Dimethyl Sulfate	61	58	1.00	0.58	1.72	0.32
Ethylene Oxide	106	102	0.67	0.26	1.74	0.41
Glycidol	19	16	1.12	0.54	2.31	0.77
1-H-Benzotriazole	112	107	0.46	0.12	1.84	0.27
Bromoethane	81	72	1.17	0.66	2.04	0.59
1,3-Butadiene	95	94	0.69	0.34	1.39	0.30
Chloromethane	75	65	1.24	0.72	2.14	0.43
CI Direct Blue 15	28	30	0.82	0.45	1.51	0.52
3,3'-Dimethylbenzidine dihydrochloride	17	9	1.87	0.79	4.41	0.15
3,3'-Dimethoxybenzidine dihydrochloride	29	31	0.84	0.45	1.59	0.60
Ethyl methyl sulfonate	26	23	1.05	0.55	1.99	0.88
Isoprene	93	90	0.82	0.43	1.60	0.57
Vinyl Chloride	86	82	0.87	0.47	1.59	0.64
2-Acetylaminofluorene	26	23	1.05	0.55	1.99	0.88
Divinyl benzene	7	6	1.13	0.36	3.51	0.84

95% CI=0.83-2.85, p=0.17) and the odds ratio for SOC code 49, Installation, Maintenance and Repair was reduced to 1.00 (95% CI=0.43-2.30; p=0.99). The odds ratio for SOC code 51 was significant at the 0.05 level with an inverse association between occupation and glioma (OR=0.55; 95% CI=0.30-1.01, p=0.05). For the reduced dataset that included only friend controls, the highest and most statistically significant odds ratio was for SOC code 27, Art, Design, Entertainment, Sports and Media occupations (OR=3.04; 95% CI=0.94-9.85, p=0.06). The odds ratios for SOC code 21; Community and Social Services (OR=1.55;

95% CI=0.48-4.97, p=0.46) and SOC code 39, Personal Care and Service (OR=1.37, 95% CI=0.67-2.80, p=0.39) remained elevated but not statistically significant. A non-significant inverse association was observed for SOC code 51, Production occupations (OR=0.55, 95% CI=0.28-1.09, p=0.09).

TABLE XI - COMPARISON OF ODDS RATIOS FOR ANCS INCLUDED IN STUDY

	Full Study -Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
Chemical	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=146	Controls N=274	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N=119	Controls N=110	Odds Ratio (95% CI)
	Number Exposed			Number Exposed			Number Exposed			Number Exposed		
Acrylamide	94	171	1.08 (0.72-1.63)	91	166	1.03 (0.68-1.57)	91	166	0.85 (0.49-1.47)	91	90	0.74 (0.39-1.41)
Acrylonitrile	90	152	1.28 (0.85-1.91)	87	147	1.29 (0.85-1.94)	87	147	1.24 (0.75-2.06)	87	79	1.08 (0.60-1.94)
Bis (chloromethyl) ether	31	65	0.87 (0.53-1.40)	29	62	1.00 (0.59-1.68)	29	62	0.92 (0.53-1.60)	29	31	0.84 (0.45-1.59)
Dimethyl Sulfate	63	112	1.09 (0.73-1.94)	61	110	1.22 (0.80-1.86)	61	110	1.14 (0.71-1.83)	61	58	1.00 (0.58-1.72)
Ethylene Oxide	109	196	1.15 (0.74-1.80)	106	193	1.16 (0.74-1.82)	106	193	0.95 (0.44-2.04)	106	102	0.67 (0.26-1.74)
Glycidol	19	26	1.42 (0.76-2.67)	19	26	1.32 (0.70-2.50)	19	26	1.29 (0.67-2.46)	19	16	1.12 (0.54-2.31)
1-H-Benzotriazole	115	207	1.18 (0.74-1.88)	112	202	1.13 (0.70-1.81)	112	202	0.66 (0.23-1.89)	112	107	0.46 (0.12-1.84)
Bromoethane	84	149	1.13 (0.76-1.68)	81	145	1.16 (0.77-1.74)	81	145	1.05 (0.64-1.73)	81	72	1.17 (0.66-2.04)
1,3-Butadiene	98	177	1.11 (0.73-1.68)	95	172	1.09 (0.71-1.67)	95	172	0.93 (0.52-1.65)	95	94	0.69 (0.34-1.39)
Chloroethane	78	135	1.17 (0.79-1.75)	75	131	1.17 (0.77-1.77)	75	131	1.11 (0.68-1.80)	75	65	1.24 (0.72-2.14)
CI Direct Blue 15	29	52	1.06 (0.64-1.75)	28	52	0.89 (0.52-1.50)	28	52	0.84 (0.49-1.45)	28	30	0.82 (0.45-1.51)
3,3'-Dimethylbenzidine dihydrochloride	17	18	1.87 (0.93-3.74)	17	17	1.92 (0.94-3.92)	17	17	1.88 (0.91-3.87)	17	9	1.87 (0.79-4.41)
3,3'-Dimethoxybenzidine dihydrochloride	31	65	0.86 (0.53-1.40)	29	62	1.00 (0.59-1.68)	29	62	0.92 (0.53-1.60)	29	31	0.84 (0.45-1.59)
Ethyl methyl sulfonate	26	44	1.13 (0.66-1.92)	26	44	1.25 (0.73-2.16)	26	44	1.15 (0.66-2.02)	26	23	1.05 (0.55-1.99)
Isoprene	96	176	1.06 (0.70-1.60)	93	171	1.06 (0.70-1.62)	93	171	0.88 (0.50-1.56)	93	90	0.82 (0.43-1.60)
Vinyl Chloride	87	157	1.09 (0.73-1.63)	86	157	1.00 (0.66-1.53)	86	157	0.83 (0.49-1.40)	86	82	0.87 (0.47-1.59)
2-Acetylaminofluorene	26	44	1.13 (0.66-1.92)	26	42	1.25 (0.73-2.16)	26	42	1.15 (0.66-2.02)	26	23	1.05 (0.55-1.99)
Divinyl benzene	8	11	1.38 (0.54-3.52)	7	8	1.16 (0.43-3.09)	7	8	1.12 (0.42-2.99)	7	6	1.13 (0.36-3.51)

TABLE XII - CRUDE ODDS RATIOS FOR FULL STUDY

SIC Code	SIC Description	Cases N=149	Controls N=279	Odds Ratio	95% CI		p-value
		Number Exposed					
7	AGRICULTURAL SERVICES	3	12	0.46	0.13	1.65	0.23
13	OIL AND GAS EXTRACTION	1	2	0.94	0.08	10.41	0.96
15	GENERAL BUILDING CONTRACTORS	7	7	1.92	0.66	5.57	0.23
16	HEAVY CONSTRUCTION CONTRACTORS	1	2	0.94	0.08	10.41	0.96
17	SPECIAL TRADE CONTRACTORS	14	12	2.31	1.04	5.13	0.04
20	FOOD AND KINDRED PRODUCTS	6	4	2.88	0.80	10.38	0.09
22	TEXTILE MILL PRODUCTS	2	4	0.94	0.17	5.17	0.94
23	APPAREL AND OTHER TEXTILE PRODUCTS	2	3	1.25	0.21	7.58	0.81
24	LUMBER AND WOOD PRODUCTS	2	2	1.88	0.26	13.51	0.53
25	FURNITURE AND FIXTURES	1	0	n/a	n/a	n/a	n/a
26	PAPER AND ALLIED PRODUCTS	1	4	0.47	0.05	4.19	0.49
27	PRINTING AND PUBLISHING	12	20	1.13	0.54	2.39	0.74
28	CHEMICALS AND ALLIED PRODUCTS	11	15	1.40	0.63	3.14	0.41
29	PETROLEUM AND COAL PRODUCTS	2	0	n/a	n/a	n/a	n/a
30	RUBBER AND MISC. PLASTICS PRODUCTS	3	5	1.13	0.27	4.78	0.87
32	STONE, CLAY, AND GLASS PRODUCTS	2	6	0.62	0.12	3.11	0.06
33	PRIMARY METAL INDUSTRIES	1	3	0.62	0.06	6.03	0.56
34	FABRICATED METAL PRODUCTS	10	13	1.47	0.63	3.44	0.37
35	MACHINERY, EXCEPT ELECTRICAL	8	16	0.93	0.39	2.23	0.88
36	ELECTRIC AND ELECTRONIC EQUIPMENT	11	13	1.63	0.71	3.74	0.25
37	TRANSPORTATION EQUIPMENT	8	11	1.38	0.54	3.52	0.50
38	INSTRUMENTS AND RELATED PRODUCTS	4	9	0.83	0.25	2.73	0.76
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	1	5	0.37	0.04	3.20	0.37
40	RAILROAD TRANSPORTATION	1	1	1.87	0.12	30.25	0.66
41	LOCAL AND INTERURBAN PASSENGER TRANSIT	2	4	0.94	0.17	5.17	0.94
42	TRUCKING AND WAREHOUSING	7	11	1.20	0.46	3.17	0.71
44	WATER TRANSPORTATION	1	1	1.88	0.12	30.25	0.66
45	TRANSPORTATION BY AIR	5	12	0.77	0.27	2.24	0.63
47	TRANSPORTATION SERVICES	6	6	1.91	0.61	6.03	0.27
48	COMMUNICATION	13	15	1.68	0.78	3.64	0.19
49	ELECTRIC, GAS, AND SANITARY SERVICES	0	7	n/a	n/a	n/a	n/a
50	WHOLESALE TRADE - DURABLE GOODS	9	15	1.13	0.48	2.65	0.78
55	AUTOMOTIVE DEALERS & SERVICE STATIONS	7	10	1.33	0.49	3.56	0.58
72	PERSONAL SERVICES	7	12	1.10	0.42	2.85	0.85
73	BUSINESS SERVICES	26	44	1.13	0.66	1.92	0.65
75	AUTO REPAIR, SERVICES, AND GARAGES	7	14	0.93	0.37	2.37	0.88
76	MISCELLANEOUS REPAIR SERVICES	0	4	n/a	n/a	n/a	n/a
80	HEALTH SERVICES	31	65	0.87	0.53	1.40	0.56
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS	3	3	1.89	0.38	9.48	0.44

TABLE XIII - CRUDE ODDS RATIOS FOR FULL STUDY ADJUSTED FOR AGE, GENDER AND INCOME

SIC Code	SIC Description	Cases N=146 Number Exposed	Controls N=274 Number Exposed	Odds Ratio	95% CI		p-value
7	AGRICULTURAL SERVICES	3	11	0.47	0.13	1.73	0.26
13	OIL AND GAS EXTRACTION	1	2	0.72	0.06	8.13	0.79
15	GENERAL BUILDING CONTRACTORS	7	7	1.72	0.58	5.07	0.32
16	HEAVY CONSTRUCTION CONTRACTORS	1	2	0.72	0.06	8.11	0.79
17	SPECIAL TRADE CONTRACTORS	14	12	2.02	0.89	4.58	0.09
20	FOOD AND KINDRED PRODUCTS	6	3	3.58	0.87	14.68	0.08
22	TEXTILE MILL PRODUCTS	2	4	0.85	0.15	4.77	0.86
23	APPAREL AND OTHER TEXTILE PRODUCTS	2	3	1.43	0.23	8.76	0.70
24	LUMBER AND WOOD PRODUCTS	2	2	1.65	0.23	12.04	0.62
25	FURNITURE AND FIXTURES	1	0	n/a	n/a	n/a	n/a
26	PAPER AND ALLIED PRODUCTS	1	4	0.53	0.06	4.83	0.57
27	PRINTING AND PUBLISHING	12	20	1.06	0.50	2.25	0.88
28	CHEMICALS AND ALLIED PRODUCTS	11	15	1.33	0.59	3.01	0.49
29	PETROLEUM AND COAL PRODUCTS	2	0	n/a	n/a	n/a	n/a
30	RUBBER AND MISC. PLASTICS PRODUCTS	3	5	1.05	0.24	4.54	0.95
32	STONE, CLAY, AND GLASS PRODUCTS	2	6	0.50	0.10	2.57	0.41
33	PRIMARY METAL INDUSTRIES	1	3	0.48	0.05	4.73	0.53
34	FABRICATED METAL PRODUCTS	10	13	1.32	0.56	3.12	0.53
35	MACHINERY, EXCEPT ELECTRICAL	8	16	0.82	0.34	1.99	0.66
36	ELECTRIC AND ELECTRONIC EQUIPMENT	11	13	1.49	0.63	3.49	0.36
37	TRANSPORTATION EQUIPMENT	7	11	1.16	0.43	3.09	0.77
38	INSTRUMENTS AND RELATED PRODUCTS	4	8	0.81	0.24	2.79	0.74
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	1	5	0.36	0.04	3.15	0.36
40	RAILROAD TRANSPORTATION	1	1	1.46	0.09	23.78	0.79
41	LOCAL AND INTERURBAN PASSENGER TRANSIT	2	4	0.93	0.17	5.24	0.94
42	TRUCKING AND WAREHOUSING	7	11	1.07	0.40	2.89	0.89
44	WATER TRANSPORTATION	1	1	1.89	0.11	31.31	0.66
45	TRANSPORTATION BY AIR	5	12	0.64	0.22	1.88	0.41
47	TRANSPORTATION SERVICES	6	6	1.98	0.62	6.33	0.25
48	COMMUNICATION	12	15	1.48	0.67	3.27	0.33
49	ELECTRIC, GAS, AND SANITARY SERVICES	0	7	n/a	n/a	n/a	n/a
50	WHOLESALE TRADE - DURABLE GOODS	8	15	0.95	0.39	2.32	0.91
55	AUTOMOTIVE DEALERS & SERVICE STATIONS	7	10	1.16	0.42	3.16	0.77
72	PERSONAL SERVICES	7	12	1.30	0.49	3.44	0.60
73	BUSINESS SERVICES	26	42	1.25	0.73	2.16	0.42
75	AUTO REPAIR, SERVICES, AND GARAGES	7	14	0.78	0.30	2.02	0.60
76	MISCELLANEOUS REPAIR SERVICES	0	4	n/a	n/a	n/a	n/a
80	HEALTH SERVICES	29	62	1.00	0.59	1.68	1.00
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS	3	3	2.23	0.44	11.39	0.34

TABLE XIV - ODDS RATIOS BY SIC CODE IN REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME-FRIEND AND SIBLING CONTROLS

SIC Code	SIC Description	Cases N=119	Controls N=210	Odds Ratio	95% CI		p-value
		Number Exposed					
7	AGRICULTURAL SERVICES	3	11	0.44	0.12	1.61	0.21
13	OIL AND GAS EXTRACTION	1	2	0.74	0.07	8.36	0.81
15	GENERAL BUILDING CONTRACTORS	7	7	1.68	0.57	4.98	0.35
16	HEAVY CONSTRUCTION CONTRACTORS	1	2	0.71	0.06	7.99	0.78
17	SPECIAL TRADE CONTRACTORS	14	12	1.96	0.86	4.47	0.11
20	FOOD AND KINDRED PRODUCTS	6	3	3.46	0.84	14.21	0.09
22	TEXTILE MILL PRODUCTS	2	4	0.79	0.14	4.43	0.79
23	APPAREL AND OTHER TEXTILE PRODUCTS	2	3	1.36	0.22	8.37	0.74
24	LUMBER AND WOOD PRODUCTS	2	2	1.57	0.21	11.52	0.66
25	FURNITURE AND FIXTURES	1	0	N/A			
26	PAPER AND ALLIED PRODUCTS	1	4	0.49	0.05	4.49	0.53
27	PRINTING AND PUBLISHING	12	20	1.02	0.48	2.18	0.96
28	CHEMICALS AND ALLIED PRODUCTS	11	15	1.31	0.58	2.99	0.52
29	PETROLEUM AND COAL PRODUCTS	2	0	N/A			
30	RUBBER AND MISC. PLASTICS PRODUCTS	3	5	1.00	0.23	4.34	1.00
32	STONE, CLAY, AND GLASS PRODUCTS	2	6	0.49	0.10	2.52	0.39
33	PRIMARY METAL INDUSTRIES	1	3	0.46	0.05	4.55	0.51
34	FABRICATED METAL PRODUCTS	10	13	1.27	0.54	3.03	0.59
35	MACHINERY, EXCEPT ELECTRICAL	8	16	0.81	0.33	2.00	0.65
36	ELECTRIC AND ELECTRONIC EQUIPMENT	11	13	1.48	0.62	3.53	0.37
37	TRANSPORTATION EQUIPMENT	7	11	1.12	0.42	3.00	0.83
38	INSTRUMENTS AND RELATED PRODUCTS	4	8	0.80	0.23	2.75	0.72
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	1	5	0.35	0.04	3.00	0.33
40	RAILROAD TRANSPORTATION	1	1	1.50	0.09	24.57	0.77
41	LOCAL AND INTERURBAN PASSENGER TRANSIT	2	4	0.82	0.14	4.65	0.82
42	TRUCKING AND WAREHOUSING	7	11	0.98	0.36	2.66	0.97
44	WATER TRANSPORTATION	1	1	1.68	0.10	27.39	0.72
45	TRANSPORTATION BY AIR	5	12	0.60	0.20	1.78	0.36
47	TRANSPORTATION SERVICES	6	6	1.84	0.57	5.93	0.31
48	COMMUNICATION	12	15	1.44	0.64	3.20	0.38
49	ELECTRIC, GAS, AND SANITARY SERVICES	0	7	N/A			
50	WHOLESALE TRADE - DURABLE GOODS	9	15	0.96	0.37	2.20	0.82
55	AUTOMOTIVE DEALERS & SERVICE STATIONS	7	10	1.11	0.41	3.07	0.83
72	PERSONAL SERVICES	7	12	1.18	0.44	3.14	0.74
73	BUSINESS SERVICES	26	42	1.15	0.66	2.02	0.63
75	AUTO REPAIR, SERVICES, AND GARAGES	7	14	0.71	0.27	1.87	0.49
76	MISCELLANEOUS REPAIR SERVICES	0	4	N/A			
80	HEALTH SERVICES	29	62	0.92	0.53	1.60	0.77
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS	3	3	2.00	0.39	10.28	0.41

TABLE XV - ODDS RATIOS BY SIC CODE IN REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME-FRIEND CONTROLS ONLY

SIC Code	SIC Description	Cases N= 122	Controls N= 112	Odds Ratio	95% CI		p-value
		Number Exposed					
7	AGRICULTURAL SERVICES	3	5	0.50	0.11	2.16	0.35
13	OIL AND GAS EXTRACTION	1	2	0.46	0.04	5.17	0.53
15	GENERAL BUILDING CONTRACTORS	7	3	2.28	0.57	9.12	0.24
16	HEAVY CONSTRUCTION CONTRACTORS	1	1	0.87	0.05	14.19	0.92
17	SPECIAL TRADE CONTRACTORS	14	7	1.98	0.75	5.18	0.17
20	FOOD AND KINDRED PRODUCTS	6	1	5.77	0.68	49.04	0.11
22	TEXTILE MILL PRODUCTS	2	2	0.84	0.11	6.16	0.86
23	APPAREL AND OTHER TEXTILE PRODUCTS	2	2	0.99	0.14	7.25	0.99
24	LUMBER AND WOOD PRODUCTS	2	1	1.69	0.15	19.35	0.67
25	FURNITURE AND FIXTURES	1	0	N/A			
26	PAPER AND ALLIED PRODUCTS	1	3	0.31	0.03	3.10	0.32
27	PRINTING AND PUBLISHING	12	4	2.99	0.92	9.68	0.07
28	CHEMICALS AND ALLIED PRODUCTS	11	8	1.31	0.50	3.40	0.58
29	PETROLEUM AND COAL PRODUCTS	2	0	N/A			
30	RUBBER AND MISC. PLASTICS PRODUCTS	3	3	0.93	0.18	4.77	0.93
32	STONE, CLAY, AND GLASS PRODUCTS	2	4	0.43	0.08	2.45	0.34
33	PRIMARY METAL INDUSTRIES	1	2	0.45	0.04	5.13	0.52
34	FABRICATED METAL PRODUCTS	10	10	0.91	0.36	2.28	0.84
35	MACHINERY, EXCEPT ELECTRICAL	8	11	0.66	0.25	1.75	0.41
36	ELECTRIC AND ELECTRONIC EQUIPMENT	11	8	1.36	0.52	3.60	0.53
37	TRANSPORTATION EQUIPMENT	7	6	1.13	0.36	3.51	0.84
38	INSTRUMENTS AND RELATED PRODUCTS	4	5	0.72	0.19	2.79	0.64
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	1	5	0.18	0.02	1.58	0.12
40	RAILROAD TRANSPORTATION	1	1	0.93	0.06	15.35	0.96
41	LOCAL AND INTERURBAN PASSENGER TRANSIT	2	1	1.88	0.16	21.60	0.61
42	TRUCKING AND WAREHOUSING	7	5	1.27	0.38	4.26	0.70
44	WATER TRANSPORTATION	1	1	0.87	0.05	14.13	0.92
45	TRANSPORTATION BY AIR	5	5	0.90	0.25	3.24	0.87
47	TRANSPORTATION SERVICES	6	3	1.82	0.44	7.54	0.41
48	COMMUNICATION	12	7	1.66	0.63	4.40	0.31
49	ELECTRIC, GAS, AND SANITARY SERVICES	0	5	N/A			
50	WHOLESALE TRADE - DURABLE GOODS	8	11	0.64	0.25	1.66	0.36
55	AUTOMOTIVE DEALERS & SERVICE STATIONS	7	6	1.06	0.34	3.33	0.91
72	PERSONAL SERVICES	7	3	2.34	0.58	9.55	0.24
73	BUSINESS SERVICES	26	23	1.05	0.55	1.99	0.88
75	AUTO REPAIR, SERVICES, AND GARAGES	7	9	0.62	0.22	1.78	0.37
76	MISCELLANEOUS REPAIR SERVICES	0	3	N/A			
80	HEALTH SERVICES	29	31	0.84	0.45	1.59	0.60
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS	3	0	N/A			

TABLE XVI - COMPARISON OF ODDS RATIOS FOR SIC CODES INCLUDED IN STUDY

		Full Study -Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
SIC Code	SIC Description	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=146	Controls N=274	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N= 119	Controls N= 110	Odds Ratio (95% CI)
		Number Exposed			Number Exposed			Number Exposed			Number Exposed		
7	AGRICULTURAL SERVICES	3	12	0.46 (0.13-1.65)	3	12	0.47 (0.13-1.73)	3	12	0.44 (0.12-1.61)	3	6	0.50 (0.11-2.16)
13	OIL AND GAS EXTRACTION	1	2	0.94 (0.08-10.41)	1	2	0.72 (0.06-8.13)	1	2	0.74 (0.07-8.36)	1	2	0.46 (.04-5.17)
15	GENERAL BUILDING CONTRACTORS	7	7	1.92 (0.66-5.57)	7	7	1.72 (0.58-5.07)	7	7	1.68 (0.57-4.98)	7	3	2.28 (0.57-9.12)
16	HEAVY CONSTRUCTION CONTRACTORS	1	2	0.94 (0.08-10.41)	1	2	0.72 (0.06-8.11)	1	2	0.71 (0.06-7.99)	1	1	0.87 (0.05-14.19)
17	SPECIAL TRADE CONTRACTORS	14	12	2.31 (1.04-5.13)	14	12	2.02 (0.89-4.58)	14	12	1.96 (0.86-4.47)	14	7	1.98 (0.75-5.18)
20	FOOD AND KINDRED PRODUCTS	6	4	2.88 (0.80-10.38)	6	4	3.58 (0.87-14.68)	6	4	3.46 (0.84-14.21)	6	2	5.77 (0.68-49.04)
22	TEXTILE MILL PRODUCTS	2	4	0.94 (0.17-5.17)	2	4	0.85 (0.15-4.77)	2	4	0.79 (0.14-4.43)	2	2	0.84 (0.11-6.16)
23	APPAREL AND OTHER TEXTILE PRODUCTS	2	3	1.25 (0.21-7.58)	2	3	1.43 (0.23-8.76)	2	3	1.36 (0.22-8.37)	2	2	0.99 (0.14-7.25)
24	LUMBER AND WOOD PRODUCTS	2	2	1.88 (0.26-13.51)	2	2	1.65 (0.23-12.04)	2	2	1.57 (0.21-11.52)	2	1	1.69 (0.15-19.35)
25	FURNITURE AND FIXTURES	1	0	n/a	1	0	n/a	1	0	n/a	1	0	n/a
26	PAPER AND ALLIED PRODUCTS	1	4	0.47 (0.05-4.19)	1	4	0.53 (0.06-4.83)	1	4	0.49 (0.05-4.49)	1	3	0.31 (0.03-3.10)
27	PRINTING AND PUBLISHING	12	20	1.13 (0.27-4.78)	12	20	1.06 (0.50-2.25)	12	20	1.02 (0.48-2.18)	12	4	2.99 (0.92-9.68)
28	CHEMICALS AND ALLIED PRODUCTS	11	15	1.40 (0.63-3.14)	11	15	1.33 (0.59-3.01)	11	15	1.31 (0.58-2.99)	11	8	1.31 (0.50-3.40)
29	PETROLEUM AND COAL PRODUCTS	2	0	n/a	2	0	n/a	2	0	n/a	2	0	n/a
30	RUBBER AND MISC. PLASTICS PRODUCTS	3	5	1.13 (0.54-2.39)	3	5	1.05 (0.24-4.54)	3	5	1.00 (0.23-4.34)	3	3	0.93 (0.18-4.77)
32	STONE, CLAY, AND GLASS PRODUCTS	2	6	0.62 (0.12-3.11)	2	6	0.50 (0.10-2.57)	2	6	0.49 (0.10-2.52)	2	4	0.43 (0.08-2.45)
33	PRIMARY METAL INDUSTRIES	1	3	0.62 (0.06-6.03)	1	3	0.48 (0.05-4.73)	1	3	0.46 (0.05-4.55)	1	2	0.45 (0.04-5.13)

TABLE XVI - COMPARISON OF ODDS RATIOS FOR SIC CODES INCLUDED IN STUDY (CONT)

		Full Study -Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
SIC Code	SIC Description	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=146	Controls N=274	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N= 119	Controls N= 110	Odds Ratio (95% CI)
		Number Exposed			Number Exposed			Number Exposed			Number Exposed		
34	FABRICATED METAL PRODUCTS	10	13	1.47 (0.63.-3.44)	10	13	1.32 (0.56-3.12)	10	13	1.27 (0.54-3.03)	10	10	0.91 (0.36-2.28)
35	MACHINERY, EXCEPT ELECTRICAL	8	16	0.93 (0.39-2.23)	8	16	0.82 (0.34-1.99)	8	16	0.81 (0.33-2.00)	8	11	0.66 (0.25-1.75)
36	ELECTRIC AND ELECTRONIC EQUIPMENT	11	13	1.63 (0.71-3.74)	11	13	1.49 (0.63-3.49)	11	13	1.48 (0.62-3.53)	11	8	1.36 (0.52-3.60)
37	TRANSPORTATION EQUIPMENT	8	11	1.38 (0.54-3.52)	8	11	1.16 (0.43-3.09)	8	11	1.12 (0.42-3.00)	8	6	1.13 (0.36-3.51)
38	INSTRUMENTS AND RELATED PRODUCTS	4	9	0.83 (0.25-2.73)	4	9	0.81 (0.24-2.79)	4	9	0.80 (0.23-2.75)	4	5	0.72 (0.19-2.79)
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	1	5	0.37 (0.04-3.20)	1	5	0.36 (0.04-3.15)	1	5	0.35 (0.04-3.00)	1	5	0.18 (0.02-1.58)
40	RAILROAD TRANSPORTATION	1	1	1.87 (0.12-30.25)	1	1	1.46 (0.09-23.78)	1	1	1.50 (0.09-24.57)	1	1	0.93 (0.06-15.35)
41	LOCAL AND INTERURBAN PASSENGER TRANSIT	2	4	0.94 (0.17-5.17)	2	4	0.93 (0.17-5.24)	2	4	0.82 (0.14-4.65)	2	1	1.88 (0.16-21.60)
42	TRUCKING AND WAREHOUSING	7	11	1.20 (0.46-3.17)	7	11	1.07 (0.40-2.89)	7	11	0.98 (0.36-2.66)	7	5	1.27 (0.38-4.26)
44	WATER TRANSPORTATION	1	1	1.88 (0.12-30.25	1	1	1.89 (0.11-31.31)	1	1	1.68 (0.10-27.39)	1	1	0.87 (0.05-14.13)
45	TRANSPORTATION BY AIR	5	12	0.77 (0.27-2.24)	5	12	0.64 (0.22-1.88)	5	12	0.60 (0.20-1.78)	5	5	0.90 (0.25-3.24)
47	TRANSPORTATION SERVICES	6	6	1.91 (0.61-6.03)	6	6	1.98 (0.62-6.33)	6	6	1.84 (0.57-5.93)	6	3	1.82 (0.44-7.54)
48	COMMUNICATION	13	15	1.68 (0.78-3.64)	13	15	1.48 (0.67-3.27)	13	15	1.44 (0.64-3.20)	13	7	1.66 (0.63-4.40)
49	ELECTRIC, GAS, AND SANITARY SERVICES	0	7	n/a	0	7	n/a	0	7	n/a	0	5	n/a
50	WHOLESALE TRADE - DURABLE GOODS	9	15	1.13 (0.48-2.65)	9	15	0.95 (0.39-2.32)	9	15	0.96 (0.37-2.20)	9	11	0.64 (0.25-1.66)
55	AUTOMOTIVE DEALERS & SERVICE STATIONS	7	10	1.33 (0.49-3.56)	7	10	1.16 (0.42-3.16)	7	10	1.11 (0.41-3.07)	7	6	1.06 (0.34-3.33)

TABLE XVI - COMPARISON OF ODDS RATIOS FOR SIC CODES INCLUDED IN STUDY (CONT)

		Full Study -Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
SIC Code	SIC Description	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=146	Controls N=274	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N= 119	Controls N= 110	Odds Ratio (95% CI)
		Number Exposed						Number Exposed			Number Exposed		
72	PERSONAL SERVICES	7	12	1.10 (0.42-2.85)	7	12	1.30 (0.49-3.44)	7	12	1.18 (0.44-3.14)	7	3	2.34 (0.58-9.55)
73	BUSINESS SERVICES	26	44	1.13 (0.66-1.92)	26	42	1.25 (0.73-2.16)	26	42	1.15 (0.66-2.02)	26	25	1.05 (0.55-1.99)
75	AUTO REPAIR, SERVICES, AND	7	14	0.93 (0.37-2.37)	7	14	0.78 (0.30-2.02)	7	14	0.71 (0.27-1.87)	7	9	0.62 (0.22-1.78)
76	MISCELLANEOUS REPAIR SERVICES	0	4	n/a	0	4	n/a	0	4	n/a	0	3	n/a
80	HEALTH SERVICES	31	65	0.87 (0.53-1.40)	29	62	1.00 (0.59-1.68)	29	62	0.92 (0.53-1.60)	31	32	0.84 (0.45-1.59)
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS	3	3	1.89 (0.38-9.48)	3	3	2.23 (0.44-11.39)	3	3	2.00 (0.39-10.28)	3	0	n/a

TABLE XVII - CRUDE ODDS RATIOS FOR OCCUPATIONS INCLUDED IN THE STUDY

SOC Code	Occupation	Cases N=149	Controls N=279	Odds Ratio	95% CI		p-value
		Number Exposed					
11	Management	37	62	1.16	0.73	1.84	0.54
13	Business and Finance	35	47	1.52	0.93	2.48	0.10
15	Computer and Mathematical	8	18	0.82	0.35	1.94	0.66
17	Architecture and Engineering	11	23	0.89	0.42	1.87	0.75
19	Life, Physical and Social Sciences	15	26	1.09	0.56	2.13	0.80
21	Community and Social Services	9	13	1.32	0.55	3.15	0.54
23	Legal	7	9	1.48	0.54	4.04	0.45
25	Education, Training and Library	34	71	0.87	0.54	1.38	0.55
27	Art, Design, Entertainment, Sports and Media	14	32	0.80	0.41	1.55	0.51
29	Healthcare Practitioners and Technical	11	32	0.62	0.30	1.26	0.18
31	Healthcare Support	12	23	0.98	0.47	2.02	0.95
33	Protective Services	9	14	1.22	0.51	2.88	0.66
35	Food Preparation and Serving	41	69	1.16	0.74	1.81	0.53
37	Building and Grounds Cleaning and Maintenance	8	18	0.82	0.35	1.94	0.66
39	Personal Care and Service	29	40	1.44	0.85	2.44	0.17
41	Sales	69	115	1.23	0.82	1.84	0.31
43	Office and Administrative Support	50	100	0.90	0.60	1.37	0.64
45	Farming, Fishing and Forestry	9	17	0.99	0.43	2.28	0.98
47	Construction and Extraction	15	25	1.14	0.58	2.23	0.71
49	Installation, Maintenance and Repair	13	16	1.57	0.73	3.36	0.24
51	Production	19	48	0.70	0.40	1.25	0.23
53	Transportation and Material Moving	21	38	1.04	0.59	1.85	0.89
55	Military	5	13	0.71	0.25	2.03	0.52

TABLE XVIII - ODDS RATIOS BY OCCUPATION ADJUSTED FOR AGE, GENDER AND INCOME

SOC Code	Occupation	Cases N=146	Controls N=274	Odds Ratio	95% CI		p-value
		Number Exposed					
11	Management	35	61	1.02	0.62	1.66	0.95
13	Business and Finance	35	47	1.48	0.90	2.42	0.12
15	Computer and Mathematical	8	18	0.75	0.31	1.78	0.51
17	Architecture and Engineering	11	22	0.77	0.35	1.66	0.50
19	Life, Physical and Social Sciences	15	24	1.09	0.55	2.17	0.81
21	Community and Social Services	9	13	1.54	0.63	3.75	0.34
23	Legal	6	9	1.25	0.43	3.63	0.68
25	Education, Training and Library	35	70	0.96	0.59	1.54	0.85
27	Art, Design, Entertainment, Sports and Media	13	31	0.83	0.42	1.66	0.60
29	Healthcare Practitioners and Technical	11	31	0.70	0.34	1.45	0.33
31	Healthcare Support	12	21	1.29	0.60	2.76	0.51
33	Protective Services	9	14	1.24	0.52	2.95	0.63
35	Food Preparation and Serving	40	69	1.16	0.73	1.86	0.53
37	Building and Grounds Cleaning and Maintenance	8	17	0.82	0.34	1.97	0.65
39	Personal Care and Service	29	40	1.64	0.94	2.84	0.08
41	Sales	67	113	1.29	0.85	1.95	0.23
43	Office and Administrative Support	48	98	1.06	0.67	1.69	0.80
45	Farming, Fishing and Forestry	9	17	0.90	0.39	2.09	0.80
47	Construction and Extraction	15	24	0.92	0.45	1.88	0.82
49	Installation, Maintenance and Repair	12	16	1.16	0.52	2.60	0.72
51	Production	18	48	0.58	0.32	1.06	0.07
53	Transportation and Material Moving	21	37	0.88	0.48	1.62	0.68
55	Military	5	13	0.52	0.18	1.56	0.25

TABLE XIX - ODDS RATIOS FOR OCCUPATIONS IN THE REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME-FRIEND AND SIBLING CONTROLS

SOC Code	Occupation	Cases N=119	Controls N=210	Odds Ratio	95% CI		p-value
		Number Exposed					
11	Management	31	50	1.11	0.65	1.89	0.71
13	Business and Finance	28	34	1.55	0.88	2.73	0.13
15	Computer and Mathematical	8	16	0.79	0.32	1.93	0.60
17	Architecture and Engineering	11	20	0.81	0.36	1.80	0.61
19	Life, Physical and Social Sciences	12	20	0.97	0.45	2.10	0.94
21	Community and Social Services	8	10	1.63	0.62	4.33	0.32
23	Legal	4	5	1.43	0.37	5.51	0.60
25	Education, Training and Library	24	46	0.97	0.55	1.71	0.90
27	Art, Design, Entertainment, Sports and Media	12	28	0.80	0.39	1.65	0.54
29	Healthcare Practitioners and Technical	10	31	0.59	0.27	1.27	0.18
31	Healthcare Support	11	21	1.05	0.47	2.32	0.91
33	Protective Services	6	13	0.81	0.30	2.19	0.67
35	Food Preparation and Serving	32	61	0.88	0.52	1.49	0.64
37	Building and Grounds Cleaning and Maintenance	7	13	0.86	0.33	2.25	0.75
39	Personal Care and Service	24	33	1.54	0.83	2.85	0.17
41	Sales	59	92	1.31	0.83	2.08	0.25
43	Office and Administrative Support	42	73	1.24	0.74	2.08	0.41
45	Farming, Fishing and Forestry	7	14	0.79	0.31	2.03	0.62
47	Construction and Extraction	15	25	0.89	0.43	1.85	0.76
49	Installation, Maintenance and Repair	11	16	1.00	0.43	2.30	0.99
51	Production	18	47	0.55	0.30	1.01	0.05
53	Transportation and Material Moving	20	36	0.80	0.42	1.50	0.48
55	Military	5	11	0.63	0.20	1.94	0.42

TABLE XX - ODDS RATIOS FOR OCCUPATIONS IN THE REDUCED STUDY POPULATION ADJUSTED FOR AGE, GENDER AND INCOME-FRIEND CONTROLS ONLY

SOC Code	Occupation	Cases N=119 Number Exposed	Controls N=110	Odds Ratio	95% CI		p-value
11	Management	31	29	1.01	0.56	1.84	0.97
13	Business and Finance	28	21	1.30	0.68	2.46	0.43
15	Computer and Mathematical	8	14	0.47	0.19	1.19	0.11
17	Architecture and Engineering	11	9	1.10	0.43	2.84	0.84
19	Life, Physical and Social Sciences	12	10	1.10	0.45	2.70	0.83
21	Community and Social Services	8	5	1.55	0.48	4.97	0.46
23	Legal	4	3	1.28	0.28	5.89	0.75
25	Education, Training and Library	24	20	1.12	0.57	2.20	0.74
27	Art, Design, Entertainment, Sports and Media	12	4	3.04	0.94	9.85	0.06
29	Healthcare Practitioners and Technical	10	13	0.71	0.29	1.72	0.45
31	Healthcare Support	11	9	1.11	0.43	2.89	0.82
33	Protective Services	6	6	0.93	0.29	2.98	0.90
35	Food Preparation and Serving	32	31	0.88	0.48	1.61	0.68
37	Building and Grounds Cleaning and Maintenance	7	8	0.73	0.25	2.12	0.56
39	Personal Care and Service	24	17	1.37	0.67	2.80	0.39
41	Sales	61	46	1.38	0.81	2.34	0.23
43	Office and Administrative Support	42	38	1.11	0.61	2.00	0.74
45	Farming, Fishing and Forestry	7	9	0.66	0.23	1.87	0.44
47	Construction and Extraction	15	13	1.03	0.44	2.39	0.95
49	Installation, Maintenance and Repair	11	11	0.83	0.33	2.09	0.68
51	Production	18	26	0.55	0.28	1.09	0.09
53	Transportation and Material Moving	20	21	0.82	0.41	1.64	0.57
55	Military	5	9	0.46	0.14	1.52	0.20

TABLE XXI - COMPARISON OF ODDS RATIOS FOR SOC CODES INCLUDED IN THE STUDY

		Full Study Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
SOC Code	SOC Code Description	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N= 119	Controls N= 110	Odds Ratio (95% CI)
		Number Exposed			Number Exposed			Number Exposed			Number Exposed		
11	Management	37	62	1.16 (0.73-1.84)	35	61	1.02 (0.62-1.66)	31	50	1.11 (0.65-1.89)	31	39	1.01 (0.56-1.84)
13	Business and Finance	35	47	1.52 (0.93-2.48)	35	47	1.48 (0.90-2.44)	28	34	1.55 (0.88-2.73)	28	21	1.30 (0.68-2.46)
15	Computer and Mathematical	8	18	0.82 (0.35-1.94)	8	18	0.75 (0.31-1.78)	8	16	0.79 (0.32-1.93)	8	14	0.47 (0.19-1.19)
17	Architecture and Engineering	11	23	0.89 (0.42-1.87)	11	22	0.77 (0.35-1.66)	11	20	0.81 (0.36-1.80)	11	9	1.10 (0.43-2.84)
19	Life, Physical and Social Sciences	15	26	1.09 (0.56-2.13)	15	24	1.09 (0.55-2.17)	12	20	0.97 (0.45-2.10)	12	10	1.10 (0.45-2.70)
21	Community and Social Services	9	13	1.32 (0.55-3.15)	9	13	1.54 (0.63-3.75)	8	10	1.63 (0.62-4.33)	8	5	1.55 (0.48-4.97)
23	Legal	7	9	1.48 (0.54-4.04)	6	9	1.25 (0.43-3.63)	4	5	1.43 (0.37-5.51)	4	3	1.28 (0.28-5.89)
25	Education, Training and Library	34	71	0.87 (0.54-1.38)	34	70	0.96 (0.59-1.54)	24	46	0.97 (0.55-1.71)	24	20	1.12 (0.57-2.20)
27	Art, Design, Entertainment, Sports and Media	14	32	0.80 (0.41-1.55)	13	31	0.83 (0.42-1.66)	12	28	0.80 (0.39-1.65)	12	4	3.04 (0.94-9.85)
29	Healthcare Practitioners and Technical	11	32	0.62 (0.30-1.26)	11	31	0.70 (0.34-1.45)	10	31	0.59 (0.27-1.27)	10	13	0.71 (0.29-1.72)
31	Healthcare Support	12	23	0.98 (0.47-2.02)	12	21	1.29 (0.60-2.76)	11	21	1.05 (0.47-2.32)	11	9	1.11 (0.43-2.89)
33	Protective Services	9	14	1.22 (0.51-2.88)	9	14	1.24 (0.52-2.95)	6	13	0.81 (0.30-2.19)	6	6	0.93 (0.29-2.98)
35	Food Preparation and Serving	41	69	1.16 (0.74-1.81)	40	69	1.16 (0.73-1.86)	32	61	0.88 (0.52-1.49)	32	31	0.88 (0.48-1.61)
37	Building and Grounds Cleaning and Maintenance	8	18	0.82 (0.35-1.94)	8	17	0.82 (0.34-1.97)	7	13	0.86 (0.33-2.25)	7	8	0.73 (0.25-2.12)

TABLE XXI - COMPARISON OF ODDS RATIOS FOR SOC CODES INCLUDED IN THE STUDY (CONT)

		Full Study Crude ORs			Full Study Adjusted ORs			Reduced Study-Adjusted-Friend and Sibling Controls			Reduced Study-Adjusted-Friend Controls Only		
SOC Code	SOC CodeDescription	Cases N=149	Controls N=279	Odds Ratio (95% CI)	Cases N=146	Controls N=274	Odds Ratio (95% CI)	Cases N=119	Controls N=210	Odds Ratio (95% CI)	Cases N= 119	Controls N= 110	Odds Ratio (95% CI)
		Number Exposed			Number Exposed			Number Exposed			Number Exposed		
39	Personal Care and Service	29	40	1.44 (0.85-2.44)	29	40	1.64 (0.94-2.84)	24	33	1.54 (0.83-2.85)	24	17	1.37 (0.67-2.80)
41	Sales	69	115	1.23 (0.82-1.84)	67	113	1.29 (0.85-1.95)	59	92	1.31 (0.83-2.08)	61	46	1.38 (0.81-2.34)
43	Office and Administrative Support	50	100	0.90 (0.60-1.37)	48	98	1.06 (0.67-1.69)	42	73	1.24 (0.74-2.08)	42	38	1.11 (0.61-2.00)
45	Farming, Fishing and Forestry	9	17	0.99 (0.43-2.28)	9	17	0.90 (0.39-2.09)	7	14	0.79 (0.31-2.03)	7	9	0.66 (0.23-1.87)
47	Construction and Extraction	15	25	1.14 (0.58-2.23)	15	24	0.92 (0.45-1.88)	15	25	0.89 (0.43-1.85)	15	13	1.03 (0.44-2.39)
49	Installation, Maintenance and Repair	13	16	1.57 (0.73-3.36)	12	16	1.16 (0.52-2.60)	11	16	1.00 (0.43-2.30)	11	11	0.83 (0.33-2.09)
51	Production	19	48	0.70 (0.40-1.25)	18	48	0.58 (0.32-1.06)	18	47	0.55 (0.30-1.01)	18	26	0.55 (0.28-1.09)
53	Transportation and Material Moving	21	38	1.04 (0.59-1.85)	21	37	0.88 (0.48-1.62)	20	36	0.80 (0.42-1.50)	20	21	0.82 (0.41-1.64)
55	Military	5	13	0.71 (0.25-2.03)	5	13	0.53 (0.18-1.56)	5	11	0.63 (0.20-1.94)	5	9	0.46 (0.14-1.52)

4.4 Discussion

This study investigates a specific group of chemicals that were potentially found in the workplaces of study participants. The chemicals were hypothesized to be homogeneously present in similar industries and, if they were found during the NOES survey of an industry, they would potentially be present in similar industries reported by study participants. From the NOES survey and the list of ANCs, the previous occupational brain cancer studies were expanded upon by creating a job exposure matrix (JEM) accounting for industry, occupation and chemical variables. The incorporation of duration and an exposure intensity factor permitted the development of cumulative exposure scores for each study participant. The statistical analysis of the dataset showed that a cumulative exposure score can be generated from self-reported work histories, and that the difference in cumulative exposure scores between cases and controls was statistically significant. However, the control population, on average, had higher scores than the cases. This inverse association with glioma was confirmed by the matched pair analysis, although the resulting odds ratios were not statistically significant.

Analysis of the two reliability factors based on the number of jobs worked and the number of years worked in NIOSH surveyed facilities provided mixed results. Odds ratios calculated for all unmatched cases and controls were statistically significant for each factor and for each percentage of jobs or years of service included in the analysis; however, the results indicated an inverse association with glioma. In addition, for the unmatched cases and controls a negative trend in the odds ratios was observed. Results of the matched pair analyses were not statistically significant and because of the small number of matches, odds ratio estimates were unstable or unable to be calculated. Calculation of the reliability factors did not add any additional insight into the association between occupation and glioma case/control status.

Since there were 91 study participants (18% cases and 23% controls) whose entire work history included jobs in industries that were not surveyed by NIOSH (zero scores) it seemed appropriate to reduce the

dataset by that number as conclusions about potential exposure to the ANCs of interest could not be made with confidence. The difference between friend and sibling controls was not statistically significant. However, since the difference in cumulative exposure scores between cases and sibling controls was not statistically significant and the difference in cumulative exposure scores between cases and friend controls was statistically significant it seemed appropriate to analyze the dataset with and without sibling controls. Regardless of the category analyzed, there were elevated odds ratios identified in the chemical, SIC code and SOC code analyses; however, as the number of potentially exposed cases and controls was small, most of the odds ratios that were calculated were not statistically significant. Several studies have attempted to evaluate a causal link between the work environment and the development of brain cancer (Navas-Acien et al., 2002; Carozza et al., 2000; Krishnan et al., 2003; Zheng et al., 2001; Schlehofer et al., 2005; Pan, et al., 2005; Samkange-Zeeb et al., 2010). In one study from Sweden, Navas-Acéin et al. (2002) looked at a base population of men and women gainfully employed at the time of the 1970 census and followed them until the end of 1989. Using the Swedish cancer environment register, specific rates for gliomas and meningiomas were computed. In this study, thirteen chemical exposures were evaluated based on any combination of occupation and industrial branch compared to a job exposure matrix (JEM). The industrial branch would be similar to the SIC code system used in this study. Men exposed to arsenic and mercury had an increased risk of gliomas, with a possible correlation for those exposed to petroleum products. None of the chemicals studied were consistently related to glioma or meningioma in women.

In the San Francisco Bay area, Carrozza et al. (2000) conducted a large case-control study to examine occupational risk factors associated with glioma using lifetime work histories. This study evaluated a possible 10 year latency period for brain cancer by considering the work histories of study participants while excluding the most current ten years of the work history. Odds ratios for "ever employed," "employed less than 10 years" and "employed for 10 years or more" were estimated. No association

was found between glioma and the occupational categories studied. The San Francisco Bay area data were reanalyzed by Krishnan et al. (2003). This study used the longest held job and ever held jobs as exposure variables. Longest held jobs as firefighters, physicians, material moving equipment operators and janitors were noted to have elevated odds ratios (Krishnan et al., 2003).

Samkange-Zeeb et al. (2010) conducted a study of glioma, meningioma and acoustic neuroma in a German population to assess the association between these brain tumors and occupation. This case-control study included 844 cases and 1688 controls. Study participants provided information on their work histories during an interview and included all jobs held for more than one year. Occupations of interest included six broad categories; chemical, metal, agricultural, construction, electrical/electronic and transport. As an example of the broadness of the categories, the metal production category included foundry workers as well as motor vehicle mechanics and machine tool operators. The construction category included mine and quarry workers, trade workers and painters. The authors were interested in exposures to petrochemicals, solvents, asbestos, pesticides, metals and electromagnetic fields. The study did not find a significant association between occupation and glioma or meningioma in any of the categories examined (Samkange-Zeeb et al.).

In a study of occupational risk factors and brain cancer in Canada, Pan et al. (2005) used questionnaire data to examine potential associations between 18 chemicals and brain cancer. Cases were identified from cancer registries and controls were cancer-free participants from the National Enhanced Surveillance System. There were 1009 cases and 5039 controls included in the study. The chemicals of interest included asbestos, salts of arsenic, chromium, and cadmium, asphalt, benzidine, benzene, pesticides, vinyl chloride, radiation sources, welding and wood dust among others. Increased risk was associated with asphalt (OR=1.29; 95% CI = 1.02-1.62) and welding (OR=1.26; 95% CI = 0.98-1.45) exposures. Teaching, protective services, textile workers, construction trades and transportation workers were identified as having an increased risk of brain cancer.

Occupational risk factors for high and low grade glioma were evaluated in an international case-control study by Schlehofer et al. (2005). This study was carried out with data from eight countries and included 1178 cases and 1987 population based controls. Data from the centers were merged into pooled data sets. Information on occupation was ascertained from in-person interviews using a standardized questionnaire. Occupations were condensed into 16 different occupation groups and 20 substance exposures. In men, a slightly increased odds ratio was noted in metal occupations (OR=1.24; 95% CI = 0.96-1.2). For women who had worked in agricultural occupations a statistically significant decreased risk of glioma was reported (OR = 0.60; 95% CI = 0.36-0.99). Of the substance categories, none resulted in statistically significant results. The overall study conclusion was that occupational exposures were not a major source of increased risk for glioma (Schlehofer et al., 2005).

Zheng et al. (2001) conducted a population based case-control study in Iowa to look at occupational risk factors for brain cancer. Questionnaires were mailed to study participants with glioma. Approximately 74% of the questionnaires were completed by proxy. Controls were selected from driver's license records. There were 375 cases and 2434 controls who participated in the study. Industries and occupations were coded using the SIC and SOC codes, respectively. Respondents reported all jobs held for five years or longer. Analyses were completed separately for men and women and statistically significant associations between glioma and occupation or industry were reported. For men, the highest statistically significant association was for the roofing, siding and sheet metal work industry (OR=22.3; 95% CI = 3.2-153). However, the number of cases and controls associated with the industry was small (4 cases and 2 controls). Some other industries with statistically significant increased risk of glioma included: Plumbing, heating, and air conditioning (3 cases, 11 controls; OR=2.8; 95% CI = 0.7-10.9); Newspaper (5 cases, 11 controls; OR=5.8; 95% CI = 1.9-17.9); rubber and plastic products (4 cases, 6 controls; OR=4.9; 95% CI = 1.2-19.2) (Zheng et al., 2001).

For women included in the Iowa study, several statistically significant associations between glioma and industry or occupation were observed. The textile and apparel industry was the industry with the highest statistically significant association (4 cases, 7 controls; OR=4.9; 95% CI = 1.3-18.3) and the highest statistically significant occupation was observed for general farm workers (4 cases, 1 control; OR= 13.4; 95% CI = 1.4-126). The authors reported that their findings were in agreement with previous studies and suggested further studies were warranted as they could not rule out chance association as a possible explanation for their results (Zheng et al., 2001).

The Occupational Safety and Health Administration was not in existence when many study participants reported employment on their work histories. In addition, regulatory chemical exposure limits have changed over time as the harmful effects of chemicals have been elucidated. As an example, the permissible exposure limit for vinyl chloride today is 1 ppm. In 1972, the exposure limit for vinyl chloride was 500 ppm (ACGIH, 2001). Even with air monitoring data from the industry in question, it is difficult to directly link a workplace exposure to a specific chemical with a specific disease. In this study the glioma cases are from different workplaces and there is no exposure monitoring data available.

In lieu of exposure monitoring data the use of self-reported work histories is used in epidemiological investigations to assess exposure to chemicals in the workplace. Teschke et al. (2001) summarized literature concerning the reliability and validity of self- reported work histories. Studies showed a general agreement between self-reported work histories and company, pension or union records (70-90%). The authors also referenced studies that found that reliability decreased for job title duration (67%), start date (62%) and work area assignments (50.6%). The authors suggested that expert assessment is usually the best approach for retrospective exposure evaluation and further stated that the reliability of self-reported data could be improved by using words familiar to the study subject and asking probing questions about the workplace. The authors concluded that a combination of self

reported data and/or company records along with exposure monitoring data would improve exposure assessment capabilities.

There were several limitations in this study. Glioma is a rare disease and the size of the study population (149 cases and 279 controls) is a significant limitation that reduces the power of the study. Crombie (1981) estimated that approximately 900 cases would be needed in a case-control study to detect a relative risk of 1.5 at 80% power and $p < 0.05$.

Study participants were asked to provide a complete work history including information about industry, occupation, job tasks and duration of each job. All coding of the work histories was completed by one individual blinded to case-control status. The two digit SIC and SOC codes used for the analyses provide broad categories to increase the number of individuals in each category. With a larger study population the four and six digit SIC and SOC codes, respectively, could provide more specific information. Use of the broad categories does reduce misclassification errors. For example, both carpenters and plumbers industries are in SIC code 17, Specialty Trade Contractors, with different four digit codes; however, neither code would, individually, have enough numbers to generate meaningful results. Use of the two digit codes puts both industries in the correct category. The SOC categories were used in similar fashion. However, recall bias on the part of the study participant and coding errors remain potential sources of bias in this study.

The NOES survey is 30 years old. While many processes may remain the same, it is likely that some changes have occurred in the facilities that were surveyed. The chemical usage may also have changed. This method does not take in to account any changes in regulatory limits for the ANCs that may have occurred since the NOES was completed. Therefore, the potential exposures identified then may no longer exist for some of the chemicals. However, 75% of study participants were employed during the time period when the NOES was conducted. Therefore, the use of NIOSH-NOES data appears appropriate for this study. It is important to recognize that while NIOSH surveyed nearly 4500 different

facilities there may be some bias in the survey data with respect to gender. Many industrial facilities have a larger male than female population. In this study there were more male cases and more female controls. This potential bias was somewhat reduced when the study population was reduced by removing participants from the dataset with zero scores.

4.5 Conclusions

While a cumulative exposure score for cases and controls was generated based on the potential for occupational exposure to 18 different ANCs the scores, an association with an increased risk of glioma was not observed. However, due to the small sample size and insufficient power, it is still possible that some chemicals, industries and occupations may be associated with an increased risk of glioma. Additional studies are needed to evaluate cumulative exposures to ANCs found in the workplace. Based on the results of this study, the ANCs with elevated odd ratios that may be of interest in future studies include acrylonitrile, glycidol, 3,3'-dimethylbenzidine dihydrochloride and divinyl benzene. Industries where further studies may be of interest include: SIC code 17-Speciality Trade Contractors, SIC code 20-Food and Kindred Products, SIC code 28-Chemical and Allied Products and SIC code 36-Electric and Electronic Equipment. Occupations where further study may be of interest include: SOC code 39-Personal Care and Service, and administrative occupations such as SOC code 13-Business and Finance. While the odds ratios generated in this study were not statistically significant, both elevated and protective effects were suggested and may provided evidence to suggest that further study of these industries, occupations and/or chemical exposures. By combining these methods with quantitative data such as personal air monitoring results and/or biomonitoring methods, the exposure assessment may have been more definitive.

5.0 PAPER TWO

USE OF QUESTIONNAIRE DATA TO EVALUATE THE ASSOCIATION BETWEEN OCCUPATIONAL CHEMICAL EXPOSURES AND BRAIN CANCER

5.1 Introduction

The causes of human brain cancer are largely unknown. High dose ionizing radiation and inherited genetic syndromes are two recognized causes of brain cancer (Davis, 2007). This case-control study was conducted to further the understanding of the etiology of glioma, as it relates to occupation.

The interaction of an individual with his/her work environment has been the focus of much attention as the workplace is a relatively constant source of exposure to chemicals. In the occupational setting, chemical exposures are rarely limited to one chemical. In addition, workers move from job to job and exposures change over time and with respect to each occupation of the worker. Identifying workplace chemicals that may lead to cancer is even more difficult to evaluate. The long latency period between exposure and disease leads to the questions of what, where, when and how the exposures occurred and what were the relevant chemicals to which the worker was exposed at that time. All available data related to workplace exposures to the chemicals need to be assessed to determine if there is a link between workplace exposures and occupational cancers.

Based on a review of the literature a group of more than 40 known or suspected animal neurocarcinogens (ANCs) were identified and are listed in Table I (Davis, 2003). This list was compared to the National Occupational Exposure Survey (NOES) completed by the National Institute for Occupational Safety and Health (NIOSH) during 1981-83. Eighteen ANCs (Table II) were found in the workplaces surveyed by NIOSH. Questionnaire responses were analyzed to determine the degree of association between potential exposure to known or suspected animal neurocarcinogens (ANCs) and cases of glioma in a case-control study. The focus of this analysis was on the questions that were specifically asked to evaluate the association between the potential exposure to acrylamide, 1,3-butadiene and polycyclic aromatic hydrocarbons (PAHs) and glioma. The hypothesis of this study was that there was an increased risk of glioma associated with exposure to these three ANCs in the

workplace and that by asking questions related to the study participant's work histories, particular industries or occupations with known exposure to these compounds may be identified.

5.2 Methods

Following Institution Review Board approvals and completion of informed consent procedures, study participants were enrolled in the study. Cases and controls were recruited by staff from NorthShore University HealthSystem in Illinois and the Preston Robert Tisch Brain Tumor Center at Duke University Medical Center in North Carolina. Cases were defined as those patients presenting with a first primary brain tumor (International Classification of Diseases for Oncology, 3rd edition, sites C70.0-C72.9) and a confirmed diagnosis of glioblastoma (International Classification of Diseases for Oncology, 3rd edition, histology codes 9440-9442), astrocytoma-grades 2 and 3 (9400-9411 and 9420) or oligodendroglioma (9382 and 9450-9451), English speaking, at least 18 years of age and a resident of the United States. The control population consisted of friends of the cases (friend controls) or hospital based controls (hospital controls) who were recruited from the orthopedic clinic at Duke University Medical Center (or by flyers posted at the medical center) or from the neurology clinic at NorthShore University HealthSystem and who were at least 18 years of age, English speaking, residents of the United States having no history of glioma or other relevant neurological condition.

5.2.1 Data Collection

Data were collected through the use of a self-administered web-based questionnaire or an interviewer-administered telephone-based questionnaire. The occupational history portion of the questionnaire was designed to identify potential exposures to specific ANCs expected to be found in the workplace, based on a literature review and was comprised of six questions. Question One identified those study participants who had ever worked outside the home for more than six months. If the study participant worked outside the home they were asked to complete the remaining questions. Question Two

pertained to specific industries of interest. Question Three pertained to specific occupations of interest. Question Four (not included in this study) pertained to manufactured products of interest. Question Five (not included in this study) related to research activities of interest. Question Six related to fumes and dusts. The four questions included in this study and the chemicals associated with each question are shown in Tables LXXXII-LXXXIV, Appendix C. Responses to questions related to potential exposure to acrylamide, 1,3-butadiene and/or polycyclic aromatic hydrocarbons (PAHs) were selected for analysis to evaluate the association between participant responses and glioma case-control status.

5.2.2 Statistical Analysis

All data were analyzed using SAS 9.1.3 statistical software (SAS Institute, Cary, NC). Study data were analyzed using unconditional logistic regression methods to determine the degree of association between potential exposure to the ANCs and study participant case/control status. Odds ratios and 95% confidence limits were calculated for acrylamide, 1,3-butadiene and PAHs for both industry and occupation questionnaire responses. All odds ratios were adjusted for age, gender and income.

5.3 Results

At the end of the study there were 1197 participants enrolled in this study. Of these, 412 cases and 776 controls had worked outside of the home for more than six months. The control population included both hospital-based controls and persons who were friends of the cases (friend controls). To avoid potential overmatching on occupation, the friend controls (N=174) were excluded from the analyses. The final population for this study included 412 cases and 602 hospital-based controls. The total study population was comprised of 52% males and 48% females. There was a statistically significant difference ($p=0.0001$) between males and females based on case-control status, with a larger percentage of male cases compared to controls. The age differences between cases and controls were

also statistically significant (p=0.0001), with controls being older than cases. Selected characteristics of the study population are shown in Table XXII.

Acrylamide was associated with 20 of the 39 industries included in Question Two of the occupational history questionnaire; 1,3-butadiene was associated with five of the industries and PAHs were associated with 12 of the industries (see Table LXXXII, Appendix C). Odds ratios, adjusted for age, gender and income, comparing the association between potential exposure to these chemicals and case/control status by industry were statistically significant at the 95% confidence level; however, the results indicate a decreased risk for those with glioma for individuals working in these industries. Results are shown in Table XXIII.

Question Three of the occupational history questionnaire included 51 occupation categories. Potential exposure to acrylamide was associated with 22 of the occupation categories; 1,3-butadiene was associated with 12 of the occupation categories and PAHs were associated with 13 of the occupation categories (see Table LXXXIII, Appendix C). Age, gender and income adjusted odds ratios comparing the association between the potential exposure to these chemicals and case/control status by occupation were statistically significant at the 95% confidence level for acrylamide and PAHs. The association between case/control status and 1,3-butadiene was not statistically significant. All odds ratios indicated a decreased risk for those with glioma for potentially exposed study participants working in the occupations included in the questionnaire. Results are shown in Table XXIII.

Question Six (see Table LXXXIV, Appendix C) of the occupational history questionnaire included a series of six questions related to potential workplace exposures to petroleum product exhaust, pesticides and dusts. These questions did not assess exposure to acrylamide. There were two queries related to 1,3-butadiene and four queries related to PAHs. Age, gender and income adjusted odds ratios indicated a

Table XXII - SELECTED CHARACTERISTICS OF THE STUDY POPULATION

		Cases	%	Controls	%	Totals	%
Study Population		412		602		1014	100
Gender	Male	247	60	277	46	524	52
	Female	165	40	325	54	490	48
	Total	412	100	602	100	1014	100
Age (years)	18-29	39	9	37	6	76	8
	30-39	48	12	55	9	103	10
	40-49	91	22	97	16	188	19
	50-59	116	28	159	27	275	27
	60-69	98	24	160	27	258	25
	>70	20	5	93	15	113	11
	Total*	412	100	601	100	1013	100
*1 missing value							
Income	<$50,000	107	26	221	38	328	33
	$51,000 - $75,000	137	34	217	37	354	36
	$76,000 - $100,000	87	22	97	16	184	19
	> $100,000	71	18	54	9	125	12
	Total*	402	100	589	100	991	100
*23missing values							

decreased risk for those with glioma for potentially exposed study participants; however, the results were not statistically significant at the 95% confidence level (see Table XXIII).

5.4 Discussion

Exposure assessment in occupational studies is often conducted by first identifying a disease state and then looking back, retrospectively, at the employment history of the study participant. In the case of occupational cancers, a long latency period between when the exposure occurred and when the disease presents itself makes the exposure assessment challenging. An accurate work history is needed to assess the potential exposures an employee has experienced. Often, payroll records or personnel

records are used to establish the work history. These may have a general description of the job held (i.e., accountant or production operator) or may be incomplete if the study participant has held several jobs during his career. Information about a study participant's work history may also be obtained through the use of detailed questionnaires. Nieuwenhuijsen (2003) discussed several important factors that should be considered when designing a questionnaire. Questionnaires should be written in a language that the study populations will understand, be clear and concise, and avoid asking for too much detail, and use open-ended questions. Questionnaires should also be designed with an idea as to how the responses will be analyzed. Open-ended questions will require interpretation before the analysis can be done and this interpretation may introduce misclassification bias which can be minimized by relying on well trained individuals (Nieuwenhuijsen, 2003).

As there are no known human neurocarcinogens, a search of the toxicology literature was conducted to prepare a list of ANCs that might also be found in the workplace. Using this list of more than 40 ANCs, (Table I) an occupational history questionnaire was generated that aimed to document potential ANC exposures in the various industries and occupations where the ANCs were found. Questions were intended to identify study participants that had a potential for exposure to these ANCs.

Potential exposures, specifically, to acrylamide, 1,3-butadiene and PAHs were evaluated to determine if an association existed between these chemicals, as assessed by responses to the occupational history portion of the questionnaire, and the case/control status of the study participant.

5.4.1 Acrylamide

Acrylamide has many industrial uses as both a polymer and copolymer and it is found in adhesives, grouting agents and molded parts, etc. (ACGIH, 2005). Acrylamide is also used in research laboratories to make gels for electrophoresis. In the workplace, exposure occurs through inhalation of dusts and

TABLE XXIII - ASSOCIATION BETWEEN THE POTENTIAL EXPOSURE TO THREE ANCS AND CASE/CONTROL STATUS BASED ON QUESTIONNAIRE RESPONSES

ANC	Odds Ratio Type	Cases N=412 Number Exposed	Controls N=602 Number Exposed	Odds Ratio	95% CI		p-value
Acrylamide							
Industry	Crude	120	213	0.75	0.57	0.98	0.04
	Adjusted*			0.73	0.56	0.97	0.03
Occupation	Crude	265	403	0.89	0.68	1.16	0.39
	Adjusted*			0.75	0.56	0.99	0.05
1,3-Butadiene							
Industry	Crude	25	66	0.53	0.33	0.85	0.008
	Adjusted*			0.47	0.29	0.78	0.003
Occupation	Crude	276	410	0.95	0.73	1.24	0.71
	Adjusted*			0.9	0.68	1.19	0.45
Dusts	Crude	78	111	1.03	0.75	1.42	0.84
	Adjusted*			0.85	0.60	1.12	0.37
PAHs							
Industry	Crude	39	82	0.65	0.43	0.99	0.04
	Adjusted*			0.57	0.37	0.88	0.01
Occupation	Crude	220	330	0.94	0.73	1.21	0.66
	Adjusted*			0.75	0.57	1	0.05
Dusts	Crude	131	192	1.00	0.76	1.30	0.97
	Adjusted*			0.80	0.59	1.07	0.13

*Adjusted for age, gender and income

vapors and it is also readily absorbed through the skin (ACGIH, 2005). The ACGIH TLV-TWA for acrylamide is 0.03 mg/m^3 with a skin notation. The limit is based on the effects of exposure on the central nervous system and for cancer, as reported only in animal studies (ACGIH, 2005). Acrylamide is an IARC Group 2A carcinogen, probably carcinogenic in humans (*Material safety data sheet - acrylamide.*).

The Carcinogen Potency Project database lists two studies of acrylamide in two year rodent studies where positive findings for brain cancer were reported. Johnson conducted a two year drinking water study in which male and female rats (no strain reported) received acrylamide doses of 0.01, 0.1. 0.5 or 2 mg/kg/day. Among other tumors, results showed increased brain and spinal cord tumors in the 2.0 mg/kg/day female group (7/61 female rats) (Johnson et al., 1986). Friedman conducted an oral study of acrylamide with doses of 0, 1 or 3mg/kg/day in drinking water with male and female rats. In addition to other tumors brain tumors were found in both male and female rats (Friedman et al., 1995).

Studies of occupational exposure to acrylamide have reported symptoms such as contact dermatitis, non-specific neurological symptoms, excessive sweating and peripheral neuropathy (ACGIH, 2001). Sobel et al. (1986) conducted a mortality study with a cohort of monomer and polymer plant employees. This study included 371 workers at a chemical plant in Michigan. The authors were interested in cancers found in animal species including brain and central nervous system, thyroid, endocrine and mesotheliomas. Thirty-eight deaths were expected and 29 deaths were observed. No statistically significant excess deaths were reported and no connection between the reported deaths and the worker occupation were indicated by the study (Sobel et al., 1986).

Marsh et al. (2007) have updated a study of acrylamide workers in three United States and one Netherlands plant. The study period was 1925-2002 and included a cohort of approximately 9000 workers. Company records were reviewed to determine work histories and industrial hygiene monitoring records were reviewed. Standard mortality ratios (SMRs) were computed using national and local rates. The SMR for brain and central nervous system was 0.67 (95% CI = 0.40-1.05). A SMR of 1.38 (95% CI = 0.28-4.02) was reported for thyroid, etc. None of the mortality rates were statistically significant (Marsh, et al., 2007).

In this study, potential exposures to acrylamide were evaluated using questions directed toward industries and occupations where exposure was anticipated, based on studies reported in the literature. The results of these analyses, while statistically significant, indicated an unexpected reduced risk of glioma with occupational exposure. Interestingly, these results suggest agreement with the findings of Marsh et al. (2007).

5.4.2 1,3-Butadiene

1,3-butadiene is a colorless and highly flammable gas at room temperature (ACGIH, 2001). It is an important monomer with uses in the manufacture of tires, and other synthetic rubber products (ACGIH, 2001). The TLV-TWA is 2 ppm based on the need to minimize the potential for cancer. The OSHA PEL is 1 ppm (ACGIH, 2001). Both IARC and EPA classify 1,3-butadiene as a known human carcinogen, Group 1 and Group A, respectively (IARC, IRIS). The primary route of exposure is inhalation. Exposure of the chemical to air may result in the formation of potentially explosive peroxides.

Many studies have explored the carcinogenicity of 1,3-butadiene. Melnick et al. (1993) reviewed two studies conducted by the NTP and one study conducted by the International Institute of Synthetic Rubber Producers (IISRP). The first NTP study was conducted in B6C3F1 mice (50 each, male and female) that were exposed to 0 (control, no exposure), 625 or 1250 ppm 1,3-butadiene for six hours/day, five days/week in an inhalation chamber. The study was stopped after 60-61 weeks due to reduced survival rates of both males and females. Malignant lymphomas originating in the thymus were observed following 20 weeks of exposure and were the suspected major cause of early death (Melnick et al., 1993).

In the second NTP study, mice were dosed at 0, 6.25, 20, 62.5, 200 or 625 ppm 1,3-butadiene in an inhalation chamber for six hours/day for five days/week for two years. Thymic lymphoma was again reported as the major cause of death for the 625 ppm group. Lymphoma was also observed in female

mice exposed to 200 ppm 1,3-butadiene. Many different tumor types were observed during the study including neoplasms in the heart, lung, liver, ovary, mammary gland among others. Tumors were reported in the brain of male mice but the results were not statistically significant (Melnick et al., 1993).

The IISRP study was conducted in Sprague-Dawley rats. Male and female rats (100 each) were exposed to 0, 1000 or 8000 ppm in an inhalation exposure chamber for six hours/day, five days/week for two years. Results indicated both increased incidences and/ or dose-response trends in several organs upon exposure to 1,3-butadiene with tumors reported at several sites including pancreas, testes in males and uterus in females. Glial cell tumors of the brain were also observed in male rats. Incidence rates were not provided (Melnick et al., 1993).

Between 1984 and 1987 NIOSH conducted a study involving thirty-nine 1,3-butadiene monomer, polymer and end user plants to determine the extent of exposure and to provide OSHA with assistance in the development of a new standard for 1,3-butadiene. At the time the OSHA PEL was 1000 ppm and the ACGIH TWA-TLV was 10 ppm (Fajen et al., 1990). The study included the completion of industrial hygiene surveys and the collection of both personal (full-shift, eight hours and short-term, 15-20 minutes) and area air samples. The effectiveness of engineering controls and leak prevention programs were also reviewed. In the monomer plants, employees in eight different jobs categories with exposure to 1,3-butadiene were monitored. Of the eight job categories, six had at least one full-shift personal monitoring result above 10 ppm. These job categories included tank farm and polymerization workers, laboratory technicians and maintenance personnel (Fajen et al., 1990). Industrial hygiene monitoring results indicated that laboratory technicians voiding sample cylinders and workers loading or unloading rail cars experienced exposure concentrations above 35 ppm. In the polymer industry the highest full-shift sample concentration was 43.2 ppm for a maintenance technician. The highest short-term measurement was for a quality control technician sampling a barge containing 1,3-butadiene where an

exposure concentration of 210 ppm was observed. A rubber tire plant and an industrial hose plant were selected to represent the end user facilities. Of the 124 samples analyzed for the end user facilities, all sample results were less than the limit of detection. The arithmetic mean concentration for all job categories surveyed in this study was 2.7 ppm. The report concluded that engineering and administrative controls were adequate to maintain exposure limits below 10 ppm. By 2009 standards a number of overexposure results would have been observed (Fajen et al., 1990).

In this study, potential exposures to 1,3-butadiene were also evaluated using questions directed toward industries and occupations where exposure was anticipated, based on studies reported in the literature. Based on responses to questions concerning industries where study participants had worked, the results were statistically significant; however, a reduced risk of glioma was indicated. Questions related to occupation also indicated a reduced risk of glioma with exposure to 1,3-butadiene and were also statistically significant.

5.4.3 PAH Results

There are more than 100 chemicals classified as polycyclic aromatic hydrocarbons (PAHs) that are products of incomplete combustion of organic matter. Car exhaust, coal processing and tobacco smoke are some of the sources of PAHs (NHANES).

The ATSDR included 17 PAHs in their toxicological profile. Of these only three, acenaphthene, acenaphthylene, and anthracene are produced commercially in the United States (ATSDR, 1995). Several other compounds including creosote, fluorine and others are imported from other countries (ATSDR, 2005). As PAHs are products of combustion, they have no known use. Occupational exposures to PAHs occur in industries such as coal and coke production, foundries, coal tar and related products, etc. (Bosetti, 2007). The greatest exposure to PAHs in the United States results from inhalation of

tobacco smoke (active or passive), inhalation of wood smoke, and contaminated air, and ingestion of PAHs in foodstuffs and water(ATSDR, 2009). Skin absorption is also a potential route of exposure.

The National Toxicology Program's 11th Report on Carcinogens lists 15 PAHs that are classified as reasonably anticipated human carcinogens. These include: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]acridine, dibenz[*a,j*]acridine, dibenz[*a,h*]anthracene, 7 H-ibenzo[*c,g*]carbazole, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a, l*]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene (NTP, 2005).

PAHs have been studied in a wide variety of species with varying types of exposures. These include: gavage, topical, inhalation, diet, drinking water (replaced by olive oil emulsion), subcutaneous injection directly injected into the organ of interest (lung, kidney), intratracheal instillation, intrarperitoneal, intravenous, bladder implantation, and intrabronchial implantation. These studies resulted in various neoplasms at the site of exposure (NTP, 2005).

Bosetti et al. (2007) completed a review of 57 occupational cohort studies of PAH exposures classified by industry. Generally, studies reported excess risks for lung/respiratory tract, larynx, bladder, kidney and skin. As an example, for aluminum production workers, 15 studies on nine cohorts were reviewed. One reanalyzed Norwegian study represented over 11,000 workers and more than 80 years of work histories dating back to 1920 with follow-up for cancer incidence from 1953-1996. No excess risk of lung cancer was reported (183.1 expected and 189 cases reported). In addition, the study found an odds ratio of 1.3 (95% CI: 1.1-1.5) for bladder cancer (Bosetti et al., 2007).

Five studies of coal gasification plants were reviewed. For all cancers of the respiratory system, 251 deaths were reported versus 104.7 expected. The pooled relative risk was 2.58 (95% CI: 2.28-2.92). For cancers of the urinary system, the pooled relative risk was 3.27 (95% CI: 2.06-5.19) (Bosetti et al., 2007).

Ten studies of coke production workers were reviewed. The pooled relative risk for lung cancer was 1.58 (95% CI: 1.47-1.69). While some studies reported excess risks for other cancers the evidence was not consistent. No increased risk of bladder cancer was identified for this group of workers.

For iron and steel foundry workers 10 cohort studies were reviewed. A pooled relative risk for all respiratory cancers of 1.4 (95% CI: 1.32-1.49), for bladder cancer of 1.29 (95% CI: 1.06-1.57) and kidney cancer of 1.30 (95% CI: 0.95-1.77) (Bosetti et al. 2007).

Remaining studies included bitumen fumes, coal tar and related products (three tar distillation, two each shale oil extraction, creosote, roofers and asphalt workers), carbon and graphite workers (seven cohort studies) and one study of chimney sweeps. Modest increases in the relative risks for lung and bladder cancer were reported (Bosetti et al., 2007). According to the data presented in this article there were no cases of skin or brain cancer reported.

In this study, odds ratios were calculated to evaluate the association between potential exposure to PAHs in the workplace and glioma. The adjusted odds ratios, based on industry and occupation questions were statistically significant at the 95% confidence level; however, the results indicate an inverse risk of glioma based on the association of potential workplace exposures with glioma status.

5.4.4 Study Limitations

The occupational exposure assessment study has two main limitations, the size of the study population and the lack of quantitative exposure measures such as personal air monitoring and/or area monitoring results. For occupational and environmental epidemiology studies, Crombie et al. (1983) estimates that approximately 900 cases would be needed to observe a relative risk of 1.5 with 80% power and p=0.05. While this study is relatively large compared to some studies, the number of cases would need to be increased by more than 50% to reach the suggested number of cases needed to see an increased risk.

The use of quantitative data such as air monitoring results would increase the reliability of predicted potential chemical exposures for the industries where the results were recorded. However, as study

participants represent a varied and diverse population any historical air monitoring results would be of limited use in evaluating personal exposures to ANCs.

The use of questionnaire data to assess potential occupational exposures to ANCs is limited by the number of questions to which a study participant can reasonably be expected to respond, in a given period of time. In this study only 49% of the study population indicated any work experience in the industries or occupations included in the occupational history portion of the questionnaire. Questions related to acrylamide, 1,3-butadiene and PAHs captured less than 30% of the study population with respect to occupational work experience.

Questions used in the occupational history questionnaire were directed toward manufacturing operations. In many instances, these industries and occupations were dominated by men. In the study population, 60% of the cases were male while only 47% of the controls were male. There may be a gender bias in the study based on study enrollment features and design.

5.5 Conclusions

This study suggests that current epidemiologic methods may not be sufficient to explain the etiology of occupational diseases, particularly in the case of glioma. The use of quantitative data such as personal and area air modeling results may improve the reliability of predictions of chemical exposure. The ultimate confirmation of exposure to chemicals may result from the development of methods to detect biomarkers of exposure in an individual. A study that supplements questionnaire data with air monitoring results and biomonitoring data may paint a clearer and more robust picture of actual workplace chemical exposures. Additionally, research in the area of occupational epidemiology needs to be shared and combined, through collaboration with others, to improve the ability to identify occupational disease etiologies.

6.0 PAPER THREE

EVALUATING CHEMICAL EXPOSURES USING HEMOGLOBIN ADDUCTS FOR ACRYLAMIDE AND 1,3-BUTADIENE

6.1 Introduction

The detection of chemicals or their metabolites in blood, urine or other body fluids is an indication that an exposure to a chemical has occurred. What is absorbed by the body and how we measure the absorbed dose describes the field of biomonitoring (Drablos et al., 2004). Biomarkers of exposure reflect the absorption of a chemical from an external exposure typically by inhalation, ingestion or absorption through the skin. Biomarkers of exposure represent exposures an individual received from all potential sources of exposure including occupational, environmental, and lifestyle choices such as diet and smoking behavior. Individual differences in genetic makeup may contribute to the biomarker variability between individuals. Absorption of a chemical in the workplace depends on several variables including, but not limited to, exposure concentration, work practices and the use/availability of personal protective equipment. Biomarkers of exposure may be a better measure of the actual dose an individual worker receives than airborne concentrations measured in the workplace environment. Demographics such as age, gender, place of residence and other variables are less controllable than diet and tobacco use but all of these variables may also influence biomarkers levels.

The purpose of this study was to examine the association between two chemicals found in the workplace, acrylamide and 1,3-butadiene, and hemoglobin adducts measurements to assess the influence of occupation, demographics, tobacco use and diet, on hemoglobin adduct formation. Available questionnaire data were compared to the hemoglobin adduct results. Because hemoglobin is found in erythrocytes and the lifespan of the erythrocyte is 120 days, any biomarker results reflect exposures that have occurred in the past 120 days.

6.2 Methods

6.2.1 Specimen Collection

Study participants were recruited from the existing control populations of one of two glioma case-control studies. In the first or pilot study, the control population consisted of siblings or friends of the cases (friend controls) and they completed a self-reported work history and study questionnaire. In the second or main study, the control population was comprised of friend controls or hospital-based controls. In the main study, subjects completed a questionnaire which included an occupational exposure assessment module. Additionally, some main study subjects also completed a self-reported work history. Main study participants provided a blood sample which was stored for future studies. As no red blood or whole blood samples were stored during the pilot study, pilot study subjects from NorthShore University HealthSystem were recruited for this study and asked to return to the clinic to provide an additional blood sample and update their work histories with current employment status. As recruitment efforts resulted in minimal response from the pilot study participants, and subjects were being enrolled in the main study, main study subjects were also identified for participation in this study. Blood samples from the main study subjects were used if sample collection was concurrent with recruitment for this study or if blood samples had been stored and a self-reported work history was available to determine current employment status. For new blood samples, the sample collection and preparation protocol from the University of North Carolina, Chapel Hill (UNC) were followed (See Appendix D). Samples were stored frozen until shipped to Dr. James Swenberg's Laboratory at the University of North Carolina, Chapel Hill (UNC). Previously collected samples were processed according to main study protocols and stored frozen at Duke University Medical Center. Stored samples, where a self-reported work history was available were shipped frozen to Dr. James Swenberg's Laboratory.

6.2.2 1,3-Butadiene Hemoglobin Adduct Laboratory Analysis

Blood samples were prepared for analysis by harvesting the globin from the red blood cells and analyzed for the *pyr*-Val hemoglobin adduct according to the UNC protocol (See Appendix D). Briefly, the samples were hydrolyzed and filtered through an Immunoaffinity column and then analyzed by Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy. Results are reported as pmol/g hemoglobin.

6.2.3 Acrylamide Hemoglobin Laboratory Analysis

Globin samples were sent by UNC to the Centers for Disease Control and Prevention's (CDC's) National Center for Environmental Health for analysis. Acrylamide metabolism may produce two possible hemoglobin adducts, acrylamide-hemoglobin (AA-Hb) and glycidamide-hemoglobin (GA-Hb). These two adducts were analyzed simultaneously using High Performance Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy (HPLC/MS/MS) methods (Vesper, 2008). The current laboratory procedure manual is attached in Appendix D. Hemoglobin adduct results were reported as pmol adduct/g globin.

6.2.4 Data Analysis

An occupational variable was created for exposure to acrylamide or 1,3-butadiene based on the study participant's current employment status. Potential exposures were assigned based on the findings of the National Occupational Exposure Survey (NOES) conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1981-1983. Approximately 4500 industries participated in the survey and chemical inventories were completed. The survey was cataloged by SIC code and acrylamide and 1,3-butadiene were found in several industries. The NIOSH survey did not include all industries. Based on the Standard Industrial Classification (SIC) system and the NOES results, acrylamide was found in 19 and 1,3-butadiene was found in 20 industries (See Table XLII for industries). If acrylamide or 1,3-

butadiene was found to be present in the SIC code as identified by the NIOSH-NOES survey, the participant was considered to have been potentially exposed to that chemical. As the hemoglobin adduct results were log normally distributed, log-transformed data were used to perform the statistical analyses. Currently exposed study subjects were compared to currently unexposed subjects using appropriate statistical tests.

Demographic data were collected when study participants completed the questionnaire and included age, gender, education, income, state of residence and height and weight. Generalized linear models were used to determine if an association could be made between the study participant responses and hemoglobin adduct levels.

Both acrylamide and 1,3-butadiene are found in cigarette smoke. The questionnaire completed by study participants included several questions related to smoking history. The questions asked included whether or not the study participant currently smoked, ever smoked, ages they started and stopped smoking, how many cigarettes they smoked in a day, if they smoked inside the home, if they lived with a smoker and how many, the number of years they lived with a smoker(s) and a rating of their exposure to second hand smoke. The association between the responses to these questions and the hemoglobin adduct results were completed using generalized linear models.

Acrylamide has been identified as a contaminant in various food sources. The questionnaire included a diet section which asked the study participant to rate the number of times they ate various foods during the week, month or year. From the questionnaire, a subset of 16 questions of a priori interest was analyzed. Not all questions were asked in both pilot and main phases of the study and some questions were combined in the main study but were asked individually in the pilot study. Some of the questions had four response levels, while others had up to nine different response levels. To merge responses to a

question with nine response levels with the same question with eight response levels, the first two response levels of the nine level questions were merged (i.e. never was merged with a few times per year to agree with the eight level response level one (less than once per month).

If questions used a four level response (never, rarely, sometimes or often) in one study and eight response levels in the other study, the eight levels were combined into four response levels. Thus, the response levels one and two (less than once per month and once per month) were collapsed into the never category, etc.

Not all study participants answered all diet history questions, resulting in response levels with very small numbers of responses. For analysis purposes, questions with four response levels were collapsed into two response levels (never/rarely and sometimes/often). Questions with eight levels were collapsed into three levels as follows: 1) less than once per month, once per month and two to three times per month; 2) once a week or twice a week; 3) three to four times per week, five to six times per week, every day. Data were analyzed with these smaller categories.

The Fourth National Report on Human Exposure to Environmental Chemicals (NHANES) was published in December, 2009. Biomarkers of exposure to various chemicals, including hemoglobin adducts of acrylamide, were evaluated in the general population. Results of this study were compared to the NHANES data and included analysis of age, gender, AA-Hb and GA-Hb and the ratio of GA/AA results to determine the degree of agreement between the two datasets.

There were two extreme glycidamide results reported. Analysis of the glycidamide results was completed both with and without these extreme values. The GA-Hb:AA-Hb ratios were calculated to permit comparison with the NHANES data after the acrylamide adduct levels for the two extreme values

were removed. The responses of the subjects with the two extreme values were examined to determine if any of the variables under study might contribute to their glycidamide adduct results.

6.3 Results

Participants completed a questionnaire with several modules including demographics, occupational work history, smoking history, diet and others. The questionnaires were similar but slightly different for the two studies. Fourteen of the study participants (eight sibling controls and six friend controls) were from the first study while 64 (three friend and 59 hospital-based controls) were from the second study. There were 78 blood samples sent to UNC for hemoglobin adduct analysis. Of these, two study participants did not complete the informed consent process and these sample results were discarded. Of the remaining 76 study participants, there were 12 who did not complete all of the modules of the questionnaire, in particular, the occupational history section. Responses varied for other questions, as well, so the number of study participant responsible for analysis is noted in tables. All 76 study participants did complete the informed consent process with institution review board (IRB) approvals from the University of Illinois at Chicago, NorthShore University HealthSystem or Duke University Medical Center. IRB approval was not required for UNC or the CDC as both organizations served as fee for service contractors in the study. Results are presented for the 76 samples analyzed for *pyr*-Val, acrylamide, and glycidamide hemoglobin adducts.

There were 52 females and 24 males with an age range of 23-85 years and a mean age of 55.7 years for males and 53.5 years for females. The study population was Caucasian with the exception of one black female, age 54. Study participants had completed various levels of education (21%-primary school, 37%-high school and 43%-college). Most study participants were married (68%). The income of study participants was evenly distributed between income levels. There were five current smokers, 31 (41%)

who had ever smoked and 49 (64%) who reported exposure to second hand smoke. Most of the study participants were living in Illinois (34%) or North Carolina (38%) with several other states included in the study population. Demographics of the study population are shown in Table XXIV.

The results for the pyr-Val adduct of 1,3-butadiene varied from 0.23-3.14 pmol/g globin, the acrylamide results varied from 14.7-380 pmol/g globin and the glycidamide results varied from 18.2-117,106 pmol/g globin. There were two extreme glycidamide results reported, thus, glycidamide results were analyzed with and without the extreme values. Log probability distribution plots are shown in Figures 5-7.

Figure 5 - Log probability distribution of *pyr*-Val adducts for *exposed and unexposed participants*

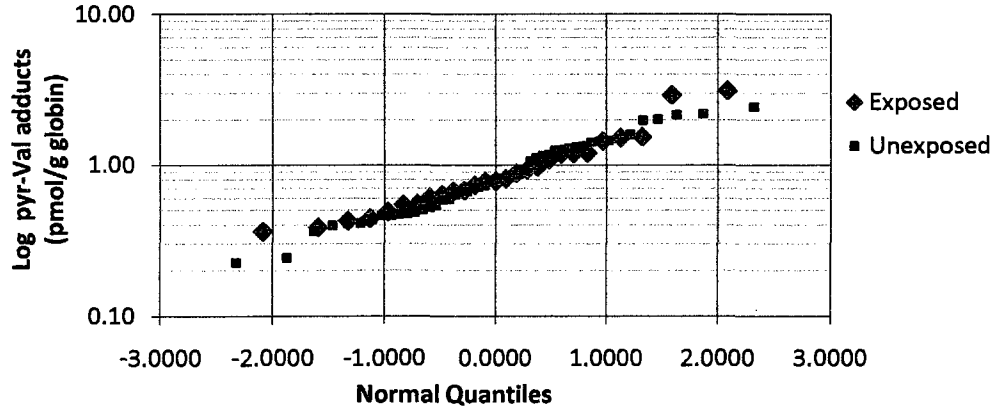

Figure 6 - Log probablility distribution of acrylamide adducts for exposed and unexposed participants

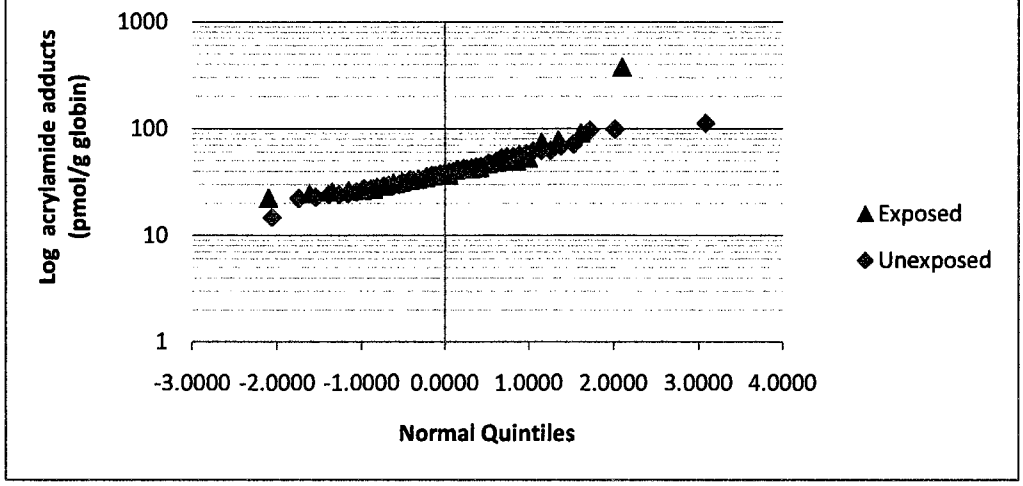

Figure 7 - Log probability distribution of glycidamide adducts for exposed and unexposed participants

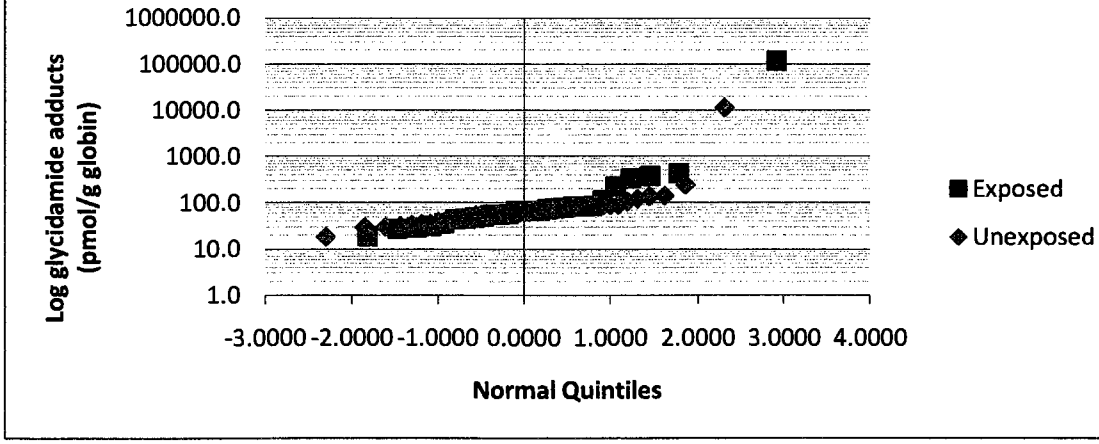

Table XXIV - SELECTED CHARACTERISTICS OF THE STUDY POPULATION

		Male	%	Female	%	Totals	%
Study Population		24	32	52	68	76	100
Age (years)	18-29	1	4	6	11	7	9
	30-39	2	8	5	10	7	9
	40-49	4	17	7	13	11	14
	50-59	8	33	13	25	21	28
	60-69	5	21	14	27	19	25
	70-79	3	13	3	6	6	8
	>80	1	4	4	8	5	7
	Total	24	100	52	100	76	100
Income	<$50,000	2	10	20	44	22	34
	$51,000 - $100,000	6	32	16	36	22	34
	>$100,000	11	58	9	20	20	32
	Total*	19	100	45	100	64	100
Education	0-8 years	4	20	12	26	16	24
	9-12 years	3	15	25	54	28	43
	>12 years	13	65	9	20	22	33
	Total**	20	100	46	100	66	100
Marital Status	Married	14	70	31	67	45	68
	Divorced	4	20	7	16	11	17
	Single	2	10	8	17	10	15
	Total**	20	100	46	100	66	100
Current Smoker**	No	18	90	43	93	61	92
	Yes	2	10	3	7	5	8
		20	100	46	100	66	100
State (Residence)**	Illinois	10	50	16	35	26	39
	North Carolina	7	35	22	48	29	44
	Ohio	1	5	1	2	2	3
	AR, OK, SC, TN, TX, VA	2	10	7	15	9	14
		20	100	46	100	66	100

*12 missing values; **10 missing values;

6.3.1 Occupational History Results

Current employment status was ascertained either from the work histories submitted when the study participant was enrolled in the study, or at the time of sample collection. Based on this information, the jobs were coded for industry using the Standard Industrial Classification (SIC) system. There were 22 (29%) study participants who were either retired or not working at the time of sample collection. There were 16 (21%) who were employed in SIC codes that were not surveyed as part of the NIOSH-NOES. Because the potential for exposure cannot be determined for those study participants who were working in non-NOES surveyed industries these 16 responses were removed from the study population and the analyses rerun. Removal of these observations did not change the statistical significance of the differences between exposed and unexposed groups. The majority of the remaining study participants held jobs in either SIC code 73, Business Services (11) or SIC code 80, Healthcare (13). From this information, exposure variables were created for acrylamide and 1,3-butadiene. (See Table LXXXV, Appendix D for raw data and code assignments). There were 12 study participants who did not complete the occupational history portion of the questionnaire. As the glycidamide results contained two extreme values analyses were completed with and without the extreme values. These analyses of occupational data did not show a statistically significant difference between the exposed and unexposed study populations. Results are shown in Table XXV.

6.3.2 Analysis of Demographic Data for Hemoglobin Adducts

Questionnaire data related to the demographics of the study population were analyzed to determine if there were significant differences by adduct levels. Demographics included gender, education, income, marital status and state of residence. Generalized linear models were used to interpret these differences.

6.3.2.1 Analysis of the *pyr*-Val Adduct Results by Demographics Responses

Female respondents had a higher geometric mean than males for the *pyr*-Val hemoglobin adduct. Study participants with more education than a college degree had lower *pyr*-Val adduct levels than those with a college degree or less education; however, the difference was not statistically significant. Single study participants had higher adduct levels than either married or divorced respondents; again, the difference was not significant. Differences in income were not significant. Also, differences in adduct levels were not significant for any of the 10-year age groups in the dataset.

The state of residence was examined. North Carolina respondents has the highest geometric mean *pyr*-Val adduct level (0.96 pmol/g globin), closely followed by other Southern states (0.93 pmol/g globin). The lowest adduct level reported was for the two respondents from Ohio (geometric mean = 0.46 pmol/g globin). The differences between states was not statistically significant ($F=2.10$; $p=0.11$). When comparing Northern States (Illinois and Ohio) to the remaining Southern states a statistically significant difference in the geometric means was found ($F=5.26$, $p=0.03$). North Carolina and Illinois had the most respondents. When either state was compared to all other states the differences were nearly statistically significant (IL versus all others – $F=3.11$; $p=0.08$, NC versus all others – $F=3.24$; $p=0.08$). When adduct level results for Illinois respondents were compared to North Carolina results, the differences were not statistically significant ($F=1.10$; $p=0.30$). All results are shown in Table XXVI.

6.3.2.2 Analysis of the Acrylamide/Glycidamide Adduct Results by Demographics Responses

Results of the demographic data were unremarkable for Acrylamide adduct results (See Table XXVII). Glycidamide results included two extreme values (discussed in detail below). For the demographic data

TABLE XXV - OCCUPATIONAL EXPOSURES AND HEMOBLOBIN ADDUCT LEVELS (PMOL/G GLOBIN)

Adduct	Exposed	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Standard Deviation	Minimum	Maximum	t-test	P-Value
1,3-Butadiene Adduct (*pyr*-Val)										
Currently Exposed										
	No	49	0.95	0.55	0.81	1.79	0.23	2.43	-0.31	0.75
	Yes	27	1.00	0.68	0.85	1.74	0.36	3.14		
Reduced Study Population										
	No	33	1.00	0.60	0.84	1.82	0.23	2.43	-0.05	0.96
	Yes	27	1.00	0.68	0.85	1.74	0.36	3.14		
Acrylamide Adduct (Acrylamide)										
Currently Exposed										
	No	48	43.71	20.42	39.96	1.52	14.70	113.00	-0.52	0.60
	Yes	28	53.81	66.07	42.36	1.74	22.50	380.00		
Reduced Study Population										
	No	32	46.04	23.13	41.39	1.57	14.70	113.00	-0.16	0.87
	Yes	28	53.81	66.07	42.36	1.74	22.50	380.00		
Acrylamide Adduct (Glycidamide)										
Currently Exposed										
	No	47	312.24	1664.67	69.37	2.44	18.20	11479.00	-0.88	0.38
	Yes	28	4300.96	22225.60	92.66	4.92	19.00	117706.00		
Reduced Study Population										
	No	31	75.38	43.21	66.08	1.68	18.20	244.00	-1.07	0.29
	Yes	28	4300.96	22225.60	92.66	4.92	19.00	117706.00		
Adjusted)										
Currently Exposed										
	No	46	69.49	37.67	62.07	1.60	18.20	244.00	-0.83	0.41
	Yes	27	100.78	107.88	71.09	2.17	19.00	421.00		
Reduced Study Population										
	No	31	75.38	43.21	66.08	1.68	18.2	244.00	-0.42	0.67
	Yes	27	100.78	107.88	92.66	2.17	19.00	421.00		

glycidamide results for the two outliers were in different groups, so that comparisons were offset by the two results (e.g. one male and one female, etc.). The glycidamide results were adjusted by removing the two outliers and the analyses were re-computed. Differences between the levels for the demographic variables were not statistically significant in any category. (See Tables XXVIII and XXIX).

TABLE XXVI - ANALYSIS OF VARIANCE FOR 1,3-BUTADIENE HEMOGLOBIN ADDUCTS BY DEMOGRAPHICS (PMOL/G GLOBIN)

Parameter	Level	N	Arithmetic Mean	Arithmetic Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Gender	Male	24	0.78	0.33	1.48	0.17	0.40	1.61	1.77	0.19
	Female	52	1.05	0.67	1.87	0.27	0.23	3.14		
Age	18-29	7	1.06	0.87	0.86	1.91	0.47	2.93	0.39	0.88
	30-39	7	1.00	0.68	0.79	2.18	0.24	2.16		
	40-49	11	0.87	0.37	0.80	1.54	0.41	1.44		
	50-59	21	1.07	0.63	0.94	1.68	0.41	3.14		
	60-69	19	0.83	0.46	0.71	1.77	0.23	2.02		
	70-79	6	1.00	0.65	0.87	1.74	0.55	2.19		
	>80	5	1.11	0.89	0.86	2.19	0.43	2.43		
Education	0-8 years	16	1.06	0.57	0.91	1.85	0.23	2.19	1.75	0.18
	8-12 years	28	1.10	0.69	0.92	1.84	0.36	3.14		
	>12 years	22	0.80	0.55	0.69	1.67	0.24	2.93		
Income	<$50,000	22	1.07	0.69	0.89	1.91	0.23	2.93	0.18	0.84
	>$50,000-$100,000	22	0.94	0.55	0.79	1.81	0.24	2.19		
	>$100,000	20	0.99	0.66	0.85	1.74	0.36	3.14		
Marital Status	Married	45	0.98	0.61	0.83	1.80	0.24	3.14	1.70	0.19
	Divorced	11	0.83	0.64	0.67	1.92	0.23	2.43		
	Single	10	1.18	0.66	1.07	1.55	0.61	2.93		
States	IL	26	0.79	0.35	0.71	1.63	0.24	1.42	2.10	0.11
	OH	2	0.46	0.23	0.46	1.05	0.44	0.47		
	NC	29	1.17	0.76	0.96	1.92	0.23	3.14		
	AR, OK, SC,TN, TX, VA	9	1.08	0.64	0.93	1.76	0.47	2.19		
North v.	IL & OH	28	0.77	0.34	0.69	1.62	0.24	1.42	5.26	0.03
South	All Others	38	1.15	0.73	0.95	1.87	0.23	3.14		
	IL vs.	26	0.79	0.35	0.71	1.63	0.24	1.42	3.11	0.08
	All Others	40	1.12	0.72	0.92	1.87	0.23	3.14		
	NC vs.	29	1.17	0.76	0.96	1.92	0.23	3.14	3.24	0.08
	All Others	37	0.84	0.45	0.74	1.67	0.24	2.19		
	IL vs.	26	0.79	0.35	0.71	1.63	0.24	1.42	1.10	0.30
	NC	29	1.17	0.76	0.96	1.92	0.23	3.14		

TABLE XXVII - ANALYSIS OF VARIANCE FOR ACRYLAMIDE HEMOGLOBIN ADDUCTS BY DEMOGRAPHICS (PMOL/G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Gender	Male	24	59.36	70.88	45.97	1.82	22.90	380.00	2.28	0.14
	Female	52	41.92	19.15	38.65	1.48	14.70	113.00		
Age	18-29	7	51.57	24.20	47.49	1.53	29.30	99.10	0.68	0.67
	30-39	7	34.74	7.52	34.08	1.23	26.80	46.50		
	40-49	11	42.30	14.32	40.40	1.37	24.80	78.60		
	50-59	21	61.60	76.77	45.46	1.94	22.30	380.00		
	60-69	19	41.78	19.14	38.08	1.56	14.70	91.90		
	70-79	6	45.80	17.02	43.12	1.47	27.30	68.70		
	>80	5	34.54	7.75	33.81	1.27	24.20	44.10		
Education	0-8 years	16	44.89	22.65	40.34	1.61	14.70	96.60	0.02	0.98
	8-12 years	28	43.84	20.87	40.49	1.46	22.30	113.00		
	>12 years	22	56.04	74.32	41.57	1.89	22.50	380.00		
Income	<$50,000	22	46.94	25.57	41.67	1.63	14.70	113.00	0.05	0.95
	>$50,000-$100,000	22	43.37	16.70	40.71	1.43	22.50	91.90		
	>$100,000	20	54.96	78.21	39.60	1.91	22.30	380.00		
Marital Status	Married	45	49.33	54.01	40.20	1.71	14.70	380.00	0.11	0.90
	Divorced	11	43.80	19.87	40.88	1.44	25.60	96.60		
	Single	10	47.69	23.35	43.60	1.54	24.30	99.10		
States	IL	26	42.66	16.28	39.79	1.47	14.70	78.60	1.24	0.30
	OH	2	58.05	6.29	57.88	1.11	53.60	62.50		
	NC	29	41.48	20.82	37.95	1.49	22.50	99.10		
	AR, OK, SC,TN, TX, VA	9	83.38	114.71	51.38	2.49	22.30	380.00		
North vs.	IL & OH	28	43.76	16.22	40.86	1.47	14.70	78.60	0.00	0.99
South	All Others	38	51.40	59.15	40.77	1.76	22.30	380.00		
	IL vs.	26	42.66	16.28	39.79	1.47	14.70	78.60	0.11	0.74
	All Others	40	51.74	57.64	41.49	1.74	22.30	380.00		
	NC vs.	29	41.48	20.82	37.95	1.49	22.50	99.10	1.13	0.29
	All Others	37	53.40	58.47	43.21	1.74	14.70	380.00		
	IL vs.	26	42.66	16.28	39.79	1.47	14.70	78.60	0.20	0.65
	NC	29	41.48	20.82	37.95	1.49	22.50	99.10		

TABLE XXVIII - ANALYSIS OF VARIANCE FOR GLYCIDAMIDE HEMOGLOBIN ADDUCTS BY DEMOGRAPHICS (PMOL/ G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Gender	Male	24	4989.90	24008.55	94.87	5.20	19.00	117706.00	1.03	0.31
	Female	51	300.87	1598.12	70.18	2.50	18.20	11479.00		
Age	18-29	7	67.57	33.81	61.74	1.56	33.20	138.00	0.21	0.97
	30-39	7	132.39	142.25	90.37	2.39	45.70	421.00		
	40-49	11	94.46	83.49	78.13	1.74	45.50	339.00		
	50-59	20	5962.68	26301.76	94.95	5.92	28.90	117706.00		
	60-69	19	659.00	2620.30	69.62	3.74	19.00	11479.00		
	70-79	6	90.25	78.47	70.40	2.09	29.40	244.00		
	>80	5	69.46	29.88	60.57	1.98	18.20	90.60		
Education	0-8 years	15	78.22	48.45	68.62	1.66	27.60	225.00	0.21	0.81
	8-12 years	28	506.03	2152.92	89.10	3.19	31.10	11479.00		
	>12 years	22	5412.64	25081.12	76.70	5.72	18.20	117706.00		
Income	<$50,000	21	613.71	2489.67	85.06	3.23	35.60	11479.00	0.27	0.76
	>$50,000-$100,000	22	96.44	104.03	67.76	2.20	18.20	421.00		
	>$100,000	20	5962.82	26301.74	89.49	6.18	19.00	117706.00		
Marital Status	Married	45	2696.15	17534.50	74.34	3.68	18.20	117706.00	0.30	0.74
	Divorced	10	1206.17	3609.54	105.22	5.32	35.00	11479.00		
	Single	10	103.19	88.57	82.85	1.90	35.60	339.00		
States	IL	26	506.24	2238.40	72.44	3.14	19.00	11479.00	1.24	0.30
	OH	2	72.55	13.79	71.89	1.21	62.80	82.30		
	NC	28	92.45	96.15	68.89	1.99	28.90	421.00		
	AR, OK, SC,TN, TX, VA	9	13169.36	39201.35	169.65	13.11	18.20	117706.00		
North v.	IL & OH	28	475.26	2156.90	72.40	3.01	19.00	11479.00	0.28	0.60
South	All Others	37	3273.32	19335.42	85.78	4.09	18.20	117706.00		
	IL vs.	26	506.24	2238.40	72.44	3.14	19.00	11479.00	0.24	0.62
	All Others	39	3109.10	18833.30	85.01	3.94	18.20	117706.00		
	NC vs.	28	92.46	96.15	68.89	1.99	28.90	421.00	0.64	0.43
	All Others	37	3563.01	19377.16	89.06	4.89	18.20	117706.00		
	IL vs.	26	506.24	2238.40	72.44	3.14	19.00	11479.00	0.04	0.84
	NC	28	92.45	96.15	68.89	1.99	28.90	421.00		

TABLE XXIX - ANALYSIS OF VARIANCE FOR GLYCIDAMIDE HEMOGLOBIN ADDUCTS BY DEMOGRAPHICS EXCLUDING TWO EXTREME VALUES (PMOL/G GLOBIN)

Parameter	Level	N	Arithmetic Mean	Arithmetic Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Gender	Male	23	89.20	77.08	69.60	1.98	19.00	339.00	0.38	0.54
	Female	50	77.31	71.45	63.37	1.76	18.20	421.00		
Age	18-29	7	67.56	33.81	61.74	1.56	33.20	138.00	0.94	0.47
	30-39	7	132.39	142.25	90.40	2.39	45.70	421.00		
	40-49	11	94.46	83.49	78.13	1.74	45.50	339.00		
	50-59	19	81.45	77.09	65.27	1.84	28.90	377.00		
	60-69	18	57.90	25.74	52.43	1.61	19.00	122.00		
	70-79	6	90.25	78.47	70.40	2.09	29.40	244.00		
	>80	5	69.46	29.88	60.57	1.98	18.20	90.60		
Education	0-8 years	15	78.22	48.45	68.61	1.66	27.60	225.00	1.62	0.21
	8-12 years	27	99.62	103.89	74.43	1.97	31.10	421.00		
	>12 years	21	65.34	48.27	54.08	1.85	18.20	244.00		
Income	<$50,000	20	70.45	24.81	66.56	1.41	35.60	138.00	0.14	0.87
	>$50,000-$100,000	22	96.44	104.03	67.76	2.20	18.20	421.00		
	>$100,000	19	81.59	84.36	61.32	2.01	19.00	377.00		
Marital Status	Married	44	82.29	82.73	62.88	1.95	18.20	421.00	0.82	0.44
	Divorced	9	64.74	17.46	62.47	1.34	35.00	90.60		
	Single	10	103.19	88.57	82.85	1.90	35.60	339.00		
States	IL	25	67.33	42.52	59.16	1.65	19.00	244.00	0.41	0.75
	OH	2	72.55	13.79	71.89		62.80	82.30		
	NC	28	92.45	96.15	68.89	2.00	28.90	421.00		
	AR, OK, SC,TN, TX, VA	8	102.28	99.61	74.88	2.30	18.20	339.00		
North v.	IL & OH	27	67.71	40.96	60.02	1.62	19.00	244.00	0.97	0.33
South	All Others	36	94.64	95.57	70.18	2.04	18.20	421.00		
	IL vs.	25	67.33	42.52	59.16	1.65	19.00	244.00	1.15	0.29
	All Others	38	93.47	93.11	70.27	2.00	18.20	421.00		
	NC vs.	28	92.45	96.15	68.89	2.00	28.90	421.00	0.30	0.59
	All Others	35	75.61	59.52	63.13	1.78	18.20	339.00		
	IL vs.	25	67.33	42.52	59.16	1.65	19.00	244.00	0.83	0.37
	NC	28	92.45	96.15	69.89	2.00	28.90	421.00		

6.3.3 Analysis of the Hemoglobin Adducts Results by Smoking Data Responses

Both acrylamide and 1,3-butadiene are found in cigarette smoke. The study questionnaire asked several questions about study participant's smoking history. These included current smoking status, if they ever smoked, age they started smoking, age they stopped smoking, whether or not the respondent smoked inside the home, the number of cigarettes smoked per day, if they lived with a smoker, for how long and

smoked, age they started smoking, age they stopped smoking, whether or not the respondent smoked inside the home, the number of cigarettes smoked per day, if they lived with a smoker, for how long and how many smokers did they live with and to how much second hand smoke they were exposed. There were five current smokers and 31 respondents who had ever smoked.

6.3.3.1 Analysis of the *pyr*-Val Adduct Results by Smoking Data Responses

Hemoglobin adduct results for 1,3-butadiene were not significantly different between current smokers and non-smokers (F=0.07; p=0.79); nor were most other variables. For the five current smokers there were two who reported smoking more than 20 cigarettes per day. The pyr-Val results were higher for this group (geometric mean = 1.14) when compared to smokers who smoked less than 20 cigarettes per day (geometric mean = 0.76); however, the difference was not statistically significant (F=0.37; p=0.59).

Respondents rated their exposure to second hand smoke as light, moderate or heavy. The pyr-Val results as they related to this question were statistically significant (F=3.46; p=0.04). Statistically significant differences were recorded for light versus heavy smokers (F=5.76; p=0.02); moderate versus heavy smokers (F=6.10; p=0.02) and light and moderate smokers versus heavy smokers (F=7.06, p=0.01). The difference between light and moderate smokers was not statistically significant (F=0.00; P=0.98). Results of all comparisons are shown in Table XXX.

6.3.3.2 Analysis of the Acrylamide/Glycidamide Adduct Results by Smoking Data Responses

There was a statistically significant difference in acrylamide adduct levels between current smokers and non-smokers (geometric mean for smokers = 80.46 pmol/g globin; geometric mean for non-smokers =38.80 pmol/g globin; F=11.85; p=0.001). There was also a statistically significant difference between those who had ever smoked when compared to those who had never smoked (F=5.07; p=0.03). Results were higher for smokers who smoked less than 20 cigarettes when compared to those smoking more than 20 cigarettes but the result was not statistically significant. The differences between all other smoking variables were not statistically significant. (See Table XXXI).

The glycidamide adduct results were appreciably higher when the two outlier results were included in the analyses; however none of the smoking variable results were statistically significant either with or without the inclusion of the extreme values. Smokers had higher glycidamide adduct levels than non-smokers. Those who had ever smoked had glycidamide adduct levels above those who had never smoked. Higher results were recorded if the respondent did not smoke inside the home, if the smoker smoked less than 20 cigarettes per day and if the respondent reported being exposed to a heavy amount of second hand smoke (See Tables XXXII and XXXIII).

TABLE XXX - ANALYSIS OF VARIANCE FOR *PYR*-VAL HEMOGLOBIN ADDUCTS BY SMOKING DATA (PMOL/G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Current smoker	No	61	0.98	0.62	0.83	1.79	0.23	3.14	0.07	0.79
	Yes	5	1.06	0.68	0.90	1.94	0.44	1.99		
Ever Smoked	No	35	1.00	0.67	0.84	1.77	0.24	3.14	0.01	0.91
	Yes	31	0.98	0.58	0.83	1.83	0.23	2.19		
Age start smoking	13-18	15	0.95	0.60	0.79	1.89	0.23	2.19	0.16	0.69
	>18	16	1.01	0.57	0.86	1.81	0.36	2.02		
Age stop smoking	18-32	12	0.92	0.55	0.79	1.79	0.36	2.16	0.07	0.80
	>32	14	1.01	0.60	0.84	1.92	0.23	2.19		
Smoke inside home	No	10	1.03	0.56	0.91	1.69	0.44	2.16	0.36	0.55
	Yes	21	0.96	0.60	0.79	1.91	0.23	2.19		
#Cigarettes/day ever smoked	<20	18	0.99	0.60	0.84	1.78	0.36	2.19	0.10	0.76
	>20	11	1.07	0.56	0.91	1.95	0.23	2.02		
#Cigarettes/day curent smokers	<20	3	0.98	0.88	0.76	2.30	0.44	1.99	0.37	0.59
	>20	2	1.19	0.50	1.14	1.54	0.84	1.54		
Ever live with smoker	No	49	1.00	0.62	0.84	1.81	0.23	3.14	0.03	0.85
	Yes	17	0.96	0.65	0.82	1.76	0.36	2.93		
# Smokers lived with	1	27	1.10	0.72	0.91	1.87	0.36	3.14	0.98	0.33
	>1	22	0.87	0.43	0.77	1.73	0.23	2.02		
# Years lived with smoker	<18	19	0.90	0.54	0.77	1.74	0.24	2.19	0.61	0.44
	>18	30	1.06	0.66	0.89	1.86	0.23	3.14		
Second hand smoke exposure	Light	18	0.93	0.62	0.77	1.89	0.23	2.19	3.46	0.04
	Moderate	23	0.87	0.43	0.77	1.69	0.24	2.01		
	Heavy	8	1.53	0.83	1.36	1.65	0.71	3.14		
Contrasts: Light v. Heavy (F=5.76; P=0.02); Moderate v. Heavy (F=6.10; P=0.02); Light v. Moderate (F=0.00; P=0.98); Light & Moderate v. Heavy (F=7.06; P=0.01)										

TABLE XXXI - ANALYSIS OF VARIANCE FOR ACRYLAMIDE HEMOGLOBIN ADDUCTS BY SMOKING DATA (PMOL/G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Current smoker	No	61	45.46	46.21	38.80	1.59	14.70	380.00	11.85	0.001
	Yes	5	83.56	24.69	80.46	1.37	53.60	113.00		
Ever Smoked	No	35	38.67	14.57	36.20	1.45	14.70	74.80	5.07	0.03
	Yes	31	59.27	64.06	47.19	1.78	22.90	380.00		
Age start smoking	13-18	15	45.43	17.79	42.75	1.42	24.80	96.60	0.85	0.36
	>18	16	72.24	86.83	51.77	2.07	22.90	380.00		
Age stop smoking	18-32	12	71.54	99.09	42.52	2.12	22.90	380.00	1.21	0.28
	>32	14	40.07	14.49	38.09	1.37	26.80	78.60		
Smoke inside home	No	10	56.20	24.95	51.62	1.54	26.80	99.10	0.35	0.56
	Yes	21	60.73	76.60	45.22	1.89	22.90	380.00		
#Cigarettes/day ever smoked	<20	18	52.42	26.60	47.25	1.57	26.80	113.00	0.01	0.91
	>20	11	73.77	103.50	48.47	2.22	22.90	380.00		
#Cigarettes/day curent smokers	<20	3	87.73	30.68	83.64	1.48	53.60	113.00	0.09	0.79
	>20	2	77.30	20.65	75.91	1.31	62.70	91.90		
Ever live with smoker	No	49	47.89	51.83	39.59	1.70	14.70	380.00	0.96	0.33
	Yes	17	49.65	22.75	45.35	1.54	24.20	99.10		
# Smokers lived with	1	27	53.54	68.09	40.76	1.84	14.70	380.00	0.19	0.66
	>1	22	40.96	17.61	38.21	1.44	22.30	96.60		
# Years lived with smoker	<18	19	43.28	18.28	40.35	1.45	22.50	96.60	0.04	0.84
	>18	30	50.82	64.94	39.13	1.80	14.70	380.00		
Second hand smoke exposure	Light	18	47.82	25.11	42.51	1.65	14.70	113.00	0.61	0.55
	Moderate	23	52.63	72.53	39.73	1.79	22.90	380.00		
	Heavy	8	34.44	8.80	33.41	1.31	22.30	44.20		

TABLE XXXII - ANALYSIS OF VARIANCE FOR GLYCIDAMIDE HEMOGLOBIN ADDUCTS BY SMOKING DATA (PMOL/G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Current smoker	No	60	2234.58	15231.35	81.24	3.76	18.20	117706.00	0.03	0.87
	Yes	5	76.58	22.25	73.70	1.38	44.20	106.00		
Ever Smoked	No	34	421.80	1955.74	75.37	3.08	18.20	11479.00	0.20	0.66
	Yes	31	3874.72	21126.28	86.84	4.21	27.60	117706.00		
Age start smoking	13-18	15	84.35	82.56	68.92	1.74	27.60	377.00	0.75	0.39
	>18	16	7428.19	29407.44	107.92	6.91	29.40	117706.00		
Age stop smoking	18-32	12	9873.40	33958.55	122.00	8.99	33.20	117706.00	0.86	0.36
	>32	14	89.70	88.74	68.80	1.99	27.60	377.00		
Smoke inside home	No	10	81.67	28.56	77.29	1.42	45.50	138.00	0.09	0.76
	Yes	21	5680.94	25668.27	91.79	5.70	27.60	117706.00		
#Cigarettes/day ever smoked	<20	18	89.48	77.07	73.75	1.78	29.40	377.00	0.84	0.37
	>20	11	10762.48	35469.16	124.02	10.09	27.60	117706.00		
#Cigarettes/day curent smokers	<20	3	86.57	17.69	85.40	1.22	71.40	106.00	1.96	0.26
	>20	2	61.60	24.61	59.09	1.51	44.20	79.00		
Ever live with smoker	No	48	2531.55	16977.85	75.99	3.45	19.00	117706.00	0.39	0.53
	Yes	17	761.36	2763.33	93.34	4.05	18.20	11479.00		
# Smokers lived with	1	26	4605.88	23068.11	85.77	4.97	27.60	117706.00	0.54	0.47
	>1	22	80.08	69.51	65.86	1.77	19.00	339.00		
# Years lived with smoker	<18	19	95.13	93.80	75.01	1.83	28.90	377.00	0.00	0.95
	>18	29	4127.83	21844.25	76.64	4.61	19.00	117706.00		
Second hand smoke exposure	Light	18	62.88	20.87	59.31	1.44	28.90	106.00	0.72	0.49
	Moderate	22	5439.77	25075.20	95.10	5.75	19.00	117706.00		
	Heavy	8	88.46	65.72	71.64	1.97	31.10	225.00		

TABLE XXXII - ANALYSIS OF VARIANCE FOR GLYCIDAMIDE HEMOGLOBIN ADDUCTS BY SMOKING DATA (PMOL/G GLOBIN)

Parameter	Level	N	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Minimum	Maximum	F-Test	P-Value
Current smoker	No	60	2234.58	15231.35	81.24	3.76	18.20	117706.00	0.03	0.87
	Yes	5	76.58	22.25	73.70	1.38	44.20	106.00		
Ever Smoked	No	34	421.80	1955.74	75.37	3.08	18.20	11479.00	0.20	0.66
	Yes	31	3874.72	21126.28	86.84	4.21	27.60	117706.00		
Age start smoking	13-18	15	84.35	82.56	68.92	1.74	27.60	377.00	0.75	0.39
	>18	16	7428.19	29407.44	107.92	6.91	29.40	117706.00		
Age stop smoking	18-32	12	9873.40	33958.55	122.00	8.99	33.20	117706.00	0.86	0.36
	>32	14	89.70	88.74	68.80	1.99	27.60	377.00		
Smoke inside home	No	10	81.67	28.56	77.29	1.42	45.50	138.00	0.09	0.76
	Yes	21	5680.94	25668.27	91.79	5.70	27.60	117706.00		
#Cigarettes/day ever smoked	<20	18	89.48	77.07	73.75	1.78	29.40	377.00	0.84	0.37
	>20	11	10762.48	35469.16	124.02	10.09	27.60	117706.00		
#Cigarettes/day curent smokers	<20	3	86.57	17.69	85.40	1.22	71.40	106.00	1.96	0.26
	>20	2	61.60	24.61	59.09	1.51	44.20	79.00		
Ever live with smoker	No	48	2531.55	16977.85	75.99	3.45	19.00	117706.00	0.39	0.53
	Yes	17	761.36	2763.33	93.34	4.05	18.20	11479.00		
# Smokers lived with	1	26	4605.88	23068.11	85.77	4.97	27.60	117706.00	0.54	0.47
	>1	22	80.08	69.51	65.86	1.77	19.00	339.00		
# Years lived with smoker	<18	19	95.13	93.80	75.01	1.83	28.90	377.00	0.00	0.95
	>18	29	4127.83	21844.25	76.64	4.61	19.00	117706.00		
Second hand smoke exposure	Light	18	62.88	20.87	59.31	1.44	28.90	106.00	0.72	0.49
	Moderate	22	5439.77	25075.20	95.10	5.75	19.00	117706.00		
	Heavy	8	88.46	65.72	71.64	1.97	31.10	225.00		

6.3.4 Analysis of Hemoglobin Adducts Results Compared to Diet Questionnaire Responses

Sixteen questions from the Food History Questionnaire were examined for an association with the hemoglobin adduct levels of acrylamide and 1,3-butadiene. The selected questions and the response distribution are shown in Table XXXIV. 1,3-butadiene is not normally associated with foodstuffs but the analysis was included for completeness. While the total number of possible responses is 76, the number of the responses varies as not all study participants answered all questions. For Questions 6-16 only responses from Main Study participants were included (N=50) as the Pilot Study Data Dictionary did not have corresponding variables for these questions. Response data was collapsed into two or three levels based on the number of initial responses and analyzed using generalized linear models.

For the *pyr*-Val adduct the result for Question 10, Cooked frankfurters/hotdogs was nearly significant ($F=2.65$, $p=0.08$); however, the majority of respondents (89%) reported infrequent consumption of these foods. For Question 13, Corn cakes, wheat cakes, the geometric mean of the *pyr*-Val adducts increased with the frequency of consumption; however, the results were not significant ($F=0.50$, $p=0.62$). As the frequency of consumption increased the geometric mean of the *pyr*-Val adducts decreased for Question 4, French fries, fried potatoes or potato chips, Question 5, Bakery products, etc., Question 6, Pretzels, corn snacks, salted crackers, Question 7, Toasted/burned or crispy foods in general, and Question 14, Margarine; however, these results were not significant. Results are shown in Table XXXV.

For the acrylamide adduct results, Question 3, Grilled, barbecued or blackened food ($F=2.22$, $p=0.14$); Question 5, Bakery products, etc. ($F=2.03$, $p=0.14$) and Question 15, Instant coffee ($F=1.81$, $p=0.20$) approached statistical significance. As consumption levels increased in Question 16, diet drinks with artificial sweeteners, the geometric means also increased. For Question 6, Pretzels, corn snacks, salted crackers, Question 9, Cooked bacon, Question 10, Cooked frankfurters/hotdogs and Question 15, Instant

TABLE XXXIV- SELECTED QUESTIONS FROM THE FOOD HISTORY QUESTIONNAIRE AND STUDY PARTICPANT RESPONSES

Question Number	Question Before two years ago how often did you eat:	Response Levels									
		1	2	3	4	5	6	7	8	Total	Missing
1	Broiled food?	8	4	30	16	x	x	x	x	58	18
2	Smoked food including fish, sausage?	10	6	19	3	x	x	x	x	38	38
3	Grilled or barbecued or blackened food?	16	8	15	7	x	x	x	x	46	30
4	French fries, fried potatoes or potato chips?	5	5	13	13	14	10	1	2	63	13
5	Bakery products, crisp bread, breakfast cereals, biscuits, toast?	1	2	6	5	11	14	12	11	62	14
6	Pretzels, corn snacks, salted crackers?	7	5	3	8	7	9	4	0	43	33
7	Toasted/burned or crispy food in general?	6	3	4	7	6	5	2	2	35	41
8	Ham?	23	8	3	4	1	1	0	1	41	35
9	Cooked Bacon?	23	8	3	4	1	1	0	1	41	35
10	Cooked frankfurters/hotdogs?	19	8	12	3	1	1	0	0	44	32
11	Sandwich meats such as bologna or salami?	9	4	12	9	9	1	0	1	45	37
12	Wheat, corn, millet?	6	4	3	5	2	6	2	12	40	36
13	Corn cakes, wheat cakes?	5	3	4	2	1	1	1	0	17	59
14	Margarine?	1	0	3	3	6	7	6	7	33	43
15	Instant coffee?	4	0	2	0	2	1	1	7	17	59
16	Diet drinks with artificial sweetener?	2	1	1	2	1	5	2	11	25	51

Response Levels for Questions 1-3: 1=Never; 2=Rarely; 3=Sometimes; 4=Often
Response Levels for Questions 4-16: 1=Less than once/month; 2=Once/month; 3=Two to three times/month; 4=Once/week; 5=Twice/week; 6=Three to four times/week; 7=Five to six times/week; 8=Every Day; Missing= Study participant did not answer the question

coffee, the geometric means decreased as consumption levels increased. These results were not statistically significant (See Table XXXVI).

For the glycidamide adduct results the results were highly influenced by the two extreme values. The responses to Question 12, Wheat, corn, millet were statistically significant (F=6.58, p=0.004) and

TABLE XXXV ANALYSIS OF VARIANCE FOR *PYR*-VAL ADDUCTS BY DIET RESPONSES (PMOL/G GLOBIN)

Question	Level	Responses	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Min.	Max.	F-test	P-Value
1	1	12	1.14	0.58	0.99	1.77	0.36	2.19	1.45	0.23
	2	46	0.95	0.67	0.78	1.86	0.23	3.14		
2	1	16	0.81	0.45	0.71	1.72	0.24	2.02	0.14	0.71
	2	22	0.92	0.64	0.76	1.85	0.23	2.93		
3	1	24	0.96	0.44	0.86	1.64	0.36	2.02	0.04	0.85
	2	22	1.15	0.87	0.89	2.10	0.23	3.14		
4	1	23	1.14	0.71	0.95	1.85	3.14	0.71	1.54	0.22
	2	27	1.00	0.65	0.84	1.84	0.23	2.93		
	3	13	0.74	0.33	0.67	2.15	0.24	1.30		
5	1	9	1.15	0.66	0.97	1.86	0.43	2.19	0.79	0.46
	2	16	1.07	0.68	0.89	1.90	0.24	2.93		
	3	37	0.90	0.61	0.76	1.77	0.23	3.14		
6	1	15	1.19	0.70	0.99	1.90	0.36	2.43	0.97	0.39
	2	15	1.09	0.88	0.84	2.08	0.23	3.14		
	3	13	0.81	0.49	0.71	1.68	0.36	2.16		
7	1	13	1.39	0.79	1.17	1.92	0.41	2.93	2.37	0.11
	2	13	1.04	0.72	0.89	1.72	0.47	3.14		
	3	9	0.79	0.57	0.65	1.93	0.23	2.16		
8	1	34	1.02	0.69	0.23	1.86	0.23	3.14	0.02	0.98
	2	5	0.96	0.70	0.80	1.90	0.23	2.16		
	3	2	1.01	0.85	0.81	2.63	0.41	1.61		
9	1	33	1.01	0.70	0.83	1.86	0.23	3.14	0.55	0.58
	2	9	1.22	0.67	1.06	1.79	0.43	2.43		
	3	2	1.01	0.85	0.81	2.63	0.41	1.61		
10	1	39	1.11	0.71	0.93	1.82	0.36	3.14	2.65	0.08
	2	4	0.55	0.28	0.49	1.78	0.23	0.92		
	3	1	1.61	n/a	1.61	n/a	1.61	1.61		
11	1	25	1.12	0.66	0.96	1.75	0.41	2.93	1.18	0.32
	2	12	0.91	0.78	0.72	1.99	0.23	3.14		
	3	2	1.24	0.51	1.19	1.53	0.88	1.61		
12	1	13	1.12	0.69	0.96	1.75	0.41	2.93	0.94	0.40
	2	7	0.68	0.22	0.65	1.40	0.40	0.98		
	3	20	1.17	0.82	0.92	2.10	0.23	3.14		
13	1	12	0.89	0.80	0.70	1.92	0.40	3.14	0.50	0.62
	2	3	1.34	1.01	0.93	3.43	0.23	2.19		
	3	2	1.52	1.29	1.22	2.67	0.61	2.43		
14	1	4	1.25	0.56	1.10	1.93	0.41	1.61	0.56	0.58
	2	9	1.13	0.69	0.97	1.81	0.49	2.43		
	3	20	1.02	0.84	0.79	2.05	0.23	3.14		
15	1	6	1.13	0.54	1.01	1.69	0.47	1.99	2.00	0.17
	2	2	0.54	0.19	0.53	1.43	0.41	0.68		
	3	9	0.72	0.36	0.65	1.63	0.36	1.33		
16	1	4	0.75	0.42	0.67	1.71	0.40	1.33	0.68	0.52
	2	3	0.91	0.54	0.82	1.75	0.53	1.53		
	3	18	1.16	0.74	0.97	1.75	0.36	3.14		

TABLE XXXVI ANALYSIS OF VARIANCE FOR ACRYLAMIDE ADDUCTS BY DIET RESPONSES (PMOL/G GLOBIN)

Question	Level	Responses	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Min.	Max.	F-test	P-Value
1	1	12	47.71	21.77	43.67	1.54	22.30	96.60	0.17	0.68
	2	46	49.94	53.77	40.76	1.71	14.70	380.00		
2	1	16	37.57	12.61	35.41	1.45	14.70	62.50	1.39	0.25
	2	22	56.88	73.77	43.22	1.82	22.90	380.00		
3	1	24	41.34	18.97	38.14	1.48	22.30	99.10	2.22	0.14
	2	22	63.71	74.90	48.11	1.91	22.90	380.00		
4	1	23	47.17	22.96	42.98	1.53	22.30	113.00	0.57	0.57
	2	27	50.57	67.94	38.11	1.84	14.70	380.00		
	3	13	48.12	20.66	44.71	1.47	25.60	99.10		
5	1	9	84.49	114.09	53.47	2.41	22.30	380.00	2.03	0.14
	2	16	39.59	20.73	35.59	1.59	14.70	96.60		
	3	37	42.81	17.00	40.07	1.43	24.30	99.10		
6	1	15	49.81	24.95	45.03	1.57	24.80	113.00	0.07	0.93
	2	15	61.69	88.96	43.13	1.99	22.30	380.00		
	3	13	46.68	25.49	41.61	1.61	22.90	99.10		
7	1	13	45.52	24.96	40.83	1.59	22.30	113.00	0.34	0.71
	2	13	68.22	95.75	46.20	2.11	22.90	380.00		
	3	9	39.54	11.25	38.22	1.32	26.80	62.70		
8	1	34	50.28	61.26	39.73	1.73	22.30	380.00	0.87	0.43
	2	5	58.08	23.64	54.71	1.46	35.30	96.60		
	3	2	36.15	9.26	35.55	1.30	29.60	42.70		
9	1	33	53.77	62.55	42.22	1.79	22.30	380.00	0.10	0.91
	2	9	44.94	22.23	41.29	1.51	27.30	96.60		
	3	2	36.15	9.26	35.55	1.30	29.60	42.70		
10	1	39	52.47	57.08	42.85	1.70	22.30	380.00	0.27	0.76
	2	4	46.60	34.31	39.11	1.92	22.90	96.60		
	3	1	29.60	n/a	29.60	n/a	29.60	29.60		
11	1	25	56.88	69.93	43.63	1.83	22.30	380.00	0.32	0.73
	2	12	50.82	25.74	45.54	1.62	25.80	99.10		
	3	2	32.45	4.03	32.32	1.13	29.60	35.30		
12	1	13	49.43	25.78	44.35	1.60	22.90	113.00	1.48	0.24
	2	7	49.66	24.44	45.24	1.58	27.40	96.60		
	3	20	37.85	12.99	36.00	1.37	22.30	72.20		
13	1	12	35.17	9.91	33.99	1.31	24.20	54.80	0.60	0.56
	2	3	43.03	14.38	41.44	1.40	29.60	58.20		
	3	2	36.70	10.47	35.95	1.34	29.30	44.10		
14	1	4	48.90	30.56	42.70	1.80	24.80	91.90	0.45	0.64
	2	9	54.97	25.75	50.43	1.53	30.70	99.10		
	3	20	55.04	77.42	40.74	1.83	22.30	380.00		
15	1	6	57.85	38.15	48.63	1.89	24.20	113.00	1.81	0.20
	2	2	34.75	11.24	33.87	1.39	26.80	42.70		
	3	9	32.73	7.16	32.03	1.25	22.90	42.90		
16	1	4	33.48	6.78	32.97	1.22	27.30	41.20	0.88	0.43
	2	3	40.10	15.49	38.19	1.46	26.80	57.10		
	3	18	49.48	24.13	44.61	1.59	22.30	113.00		

approached statistical significance for Question 3, Grilled, barbecued or blackened food; Question 5, Bakery products, etc.; Question 6, Pretzels, corn snacks, salted crackers, and Question 7, Toasted/burned or crispy food in general. When the two extreme values were removed and the data re-analyzed, Question 12, Wheat, corn and millet remained statistically significant (F=3.70, p=0.03). Those questions that initially approached statistical significance when the two extreme values were included were no longer of interest. Results are shown in Tables XXXVII and XXXVIII.

6.3.5 Comparison to NHANES Results or Acrylamide and Glycidamide Analyses

The latest NHANES report was released in December, 2009 and presented data collected during 2003-2004. This report provided biomonitoring information related to acrylamide exposures in the form of hemoglobin adducts for acrylamide and glycidamide. In each category for both current smokers and non-smokers the acrylamide results of this study were lower than the NHANES results. For the glycidamide results, the results of this study were lower for non-smokers and higher for current smokers than the NHANES results. The resulting ratios of GA-Hb to AA-Hb were also higher in all categories on this study except for smokers 60 years of age or older where they were nearly the same (ratio, this study= 0.78 vs. NHANES study=0.81). The results of the NHANES study as compared to the findings of this study are shown in Table XXXIX.

TABLE XXXVII ANALYSIS OF VARIANCE FOR GLYCIDAMIDE ADDUCTS BY DIET RESPONSES (PMOL/G GLOBIN)

Question	Level	Responses	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Min.	Max.	F-test	P-Value
1	1	12	69.60	25.87	66.26	1.37	40.50	143.00	0.30	0.58
	2	46	2947.41	17578.42	84.18	4.42	18.20	117706.00		
2	1	16	72.76	45.09	63.93	1.66	27.60	225.00	0.49	0.49
	2	22	5427.05	25077.96	87.62	5.71	18.20	117706.00		
3	1	23	83.83	86.29	63.62	1.98	18.20	421.00	2.00	0.16
	2	22	5448.31	25073.28	109.20	5.46	31.10	117706.00		
4	1	23	80.20	77.82	65.63	1.75	29.40	421.00	0.68	0.51
	2	27	4443.56	22635.89	85.90	4.98	18.20	117706.00		
	3	12	1037.00	3288.78	111.43	4.83	27.60	11479.00		
5	1	9	13142.63	39211.27	159.02	12.10	40.50	117706.00	1.87	0.16
	2	16	67.51	50.60	55.97	1.83	18.20	225.00		
	3	36	411.87	1899.62	81.99	2.99	19.00	11479.00		
6	1	15	66.71	23.18	63.06	1.42	29.40	115.00	2.25	0.12
	2	14	9319.65	31342.74	176.55	11.54	18.20	117706.00		
	3	13	84.18	92.00	64.03	1.92	31.30	377.00		
7	1	13	90.73	89.29	70.46	1.98	18.20	377.00	1.71	0.20
	2	13	10030.22	32504.97	192.70	12.23	31.10	117706.00		
	3	9	84.84	95.80	64.06	1.90	43.80	339.00		
8	1	34	3554.23	20170.35	85.00	4.37	18.20	117706.00	0.10	0.90
	2	5	65.44	15.21	63.94	1.28	44.20	85.20		
	3	2	73.60	24.04	71.61	1.39	56.60	90.60		
9	1	32	3749.18	20794.81	77.28	4.28	18.20	17706.00	0.25	0.78
	2	9	167.48	160.56	110.16	2.60	38.60	421.00		
	3	2	73.60	24.04	71.61	1.39	56.60	90.60		
10	1	39	3403.98	18873.03	96.65	4.93	18.20	117706.00	0.12	0.87
	2	3	61.53	8.57	61.15	1.14	55.90	71.40		
	3	1	90.60	n/a	90.60	n/a	90.60	90.60		
11	1	24	5018.87	24002.53	112.03	5.43	18.20	117706.00	0.10	0.91
	2	12	1014.68	3295.53	90.51	4.84	31.10	11479.00		
	3	2	79.10	16.26	78.26	1.23	67.60	90.60		
12	1	13	72.52	32.25	66.33	1.56	29.40	143.00	6.58	0.004
	2	7	1801.36	4269.67	245.82	6.93	31.30	11479.00		
	3	20	74.09	84.14	57.57	1.85	18.20	421.00		
13	1	12	109.72	123.50	68.22	2.65	18.20	421.00	0.02	0.98
	2	3	73.90	16.65	72.63	1.26	57.30	90.60		
	3	2	64.50	29.27	61.09	1.60	43.80	85.20		
14	1	4	77.18	16.22	75.72	1.26	53.90	90.60	0.23	0.79
	2	9	125.80	100.59	99.30	2.02	35.60	339.00		
	3	19	6894.04	26960.74	135.82	8.70	29.40	117760.00		
15	1	6	84.77	52.56	67.18	2.28	18.20	143.00	0.12	0.89
	2	2	51.80	6.79	51.58	1.14	47.00	56.60		
	3	8	78.34	61.87	65.04	1.82	31.30	225.00		
16	1	4	55.40	25.05	51.14	1.59	31.30	85.40	0.50	0.61
	2	3	184.83	205.48	119.62	3.10	47.00	421.00		
	3	18	727.18	2684.72	95.74	3.93	31.10	11479.00		

TABLE XXXVIII ANALYSIS OF VARIANCE FOR GLYCIDAMIDE ADDUCTS BY DIET RESPONSES EXCLUDING TWO EXTREME VALUES(PMOL/G GLOBIN)

Question	Level	Responses	Arimethetic Mean	Standard Deviation	Geometric Mean	Geometric Strandard Deviation	Min.	Max.	F-test	P-Value
1	0	12	69.60	25.87	66.26	1.37	40.50	143.00	0.05	0.82
	1	43	80.20	72.85	63.45	1.90	18.20	377.00		
2	0	16	72.76	45.09	63.93	1.66	27.60	225.00	0.02	0.89
	1	21	80.43	75.37	62.18	1.98	18.20	339.00		
3	0	23	83.83	86.29	63.62	1.98	18.20	421.00	1.01	0.32
	1	21	102.70	96.17	78.31	1.99	31.10	377.00		
4	1	23	80.20	77.82	65.63	1.75	29.40	421.00	0.14	0.87
	2	26	87.31	88.60	65.06	2.04	18.20	377.00		
	3	11	88.24	63.25	73.12	1.86	27.60	244.00		
5	1	8	72.21	20.07	69.62	1.34	40.50	106.00	0.80	0.45
	2	16	67.50	50.60	55.97	1.83	18.20	225.00		
	3	35	95.66	96.83	71.19	2.02	19.00	421.00		
6	1	15	66.71	23.18	63.06	1.42	29.40	115.00	0.18	0.84
	2	12	107.51	116.32	72.52	2.42	18.20	421.00		
	3	13	84.17	92.00	64.03	1.92	31.30	377.00		
7	1	13	90.73	89.29	70.46	1.98	18.20	377.00	0.10	0.91
	2	11	109.81	121.09	74.17	2.35	31.10	421.00		
	3	9	84.44	95.80	64.06	1.90	43.80	339.00		
8	1	33	95.08	100.23	68.27	2.11	18.20	421.00	0.03	0.98
	2	5	65.44	15.21	63.94	1.28	44.20	85.20		
	3	2	73.60	24.04	71.61	1.39	56.60	90.60		
9	1	31	73.15	52.49	61.01	1.79	18.20	244.00	2.70	0.08
	2	9	167.48	160.56	110.16	2.60	38.60	421.00		
	3	2	73.60	24.04	71.61	1.39	56.60	90.60		
10	1	37	96.49	98.17	70.11	2.09	18.20	421.00	0.12	0.89
	2	3	61.53	8.57	61.15	1.14	55.90	71.40		
	3	1	90.60	n/a	90.60	n/a	90.60	90.60		
11	1	23	119.43	117.47	82.79	2.31	18.20	421.00	0.89	0.42
	2	11	63.37	29.71	58.27	1.52	31.10	138.00		
	3	2	79.10	16.26	78.26	1.23	67.60	90.60		
12	1	13	72.52	32.25	66.33	1.56	29.40	143.00	3.70	0.03
	2	6	188.42	151.17	129.54	2.79	31.30	377.00		
	3	20	74.09	84.14	57.57	1.85	18.20	421.00		
13	1	12	109.72	123.50	68.22	2.65	18.20	421.00	0.02	0.98
	2	3	73.90	16.65	72.63	1.26	57.30	90.60		
	3	2	64.50	29.27	61.09	1.60	43.80	85.20		
14	1	4	77.18	16.22	75.72	1.26	53.90	90.60	0.62	0.55
	2	9	125.80	100.59	99.30	2.02	35.60	339.00		
	3	17	105.98	120.80	70.28	2.32	29.40	421.00		
15	1	6	84.77	52.56	67.18	2.28	18.20	143.00	0.12	0.89
	2	2	51.80	6.79	51.58	1.14	47.00	56.60		
	3	8	78.34	61.87	65.04	1.82	31.30	225.00		
16	1	4	55.40	25.05	51.14	1.59	31.30	85.40	1.20	0.32
	2	3	184.83	205.48	119.62	3.10	47.00	421.00		
	3	17	94.72	89.88	72.24	1.99	31.10	377.00		

TABLE XXXIX - GEOMETRIC MEANS (PMOL/G GLOBIN) AND 95% CONFIDENCE INTERVALS FOR ACRYLAMIDE, GLYCIDAMIDE AND ADDUCT RATIOS FOR SELECT GROUPS OF SMOKERS AND NON-SMOKERS COMPARING STUDY RESULTS TO NHANES RESULTS

		Study Results				NHANES Results				
		Non-smokers				Non-smokers				
Group		N	GM-AA-Hb (95% CI)	GM-GA-Hb (95% CI)	GM-GA-Hb:AA-Hb (95% CI)	N	GM-AA-Hb (95% CI)	N	GM-GA-Hb (95% CI)	N	GM-GA-Hb:AA-Hb (95% CI)
All		58	37.27 (33.95-40.91	65.80 (55.60-77.86)	1.77 (1.50-2.08)	5686	50.00 (48.50-51.7)	5809	51.10 (49.00-53.20)	5464	1.01 (0.97-1.06)
Gender	Female	41	36.45 (32.59-40.71)	62.76 (52.10-75.60)	1.72 (1.43-2.08)	3050	49.50 (48.20-50.80)	3111	51.80 (49.10-54.70)	2931	1.04 (0.99-1.09)
	Male	17	39.32 (32.49-47.59)	71.80 (50.06-230.87)	1.88 (1.32-2.67)	2636	50.70 (48.40-53.10)	2698	50.20 (48.40-55.30)	2533	0.98 (0.94-1.02)
Age Group	20-39 years	14	40.23 (32.57-49.69)	74.70 (50.08-111.41)	1.86 (1.24-2.79)	951	51.00 (48.50-53.80)	972	51.70 (48.40-55.30)	919	1.00 (0.95-1.06)
	40-59 years	22	38.17 (32.83-44.38)	71.61 (53.50-95.85)	1.88 (1.42-2.48)	784	48.60 (46.10-51.20)	792	49.10 (47.00-51.20)	753	1.00 (0.95-1.06)
	> 60 years	22	34.66 (31.49-40.76)	55.77 (43.13-72.18)	1.61 (1.25-2.07)	1252	44.40 (42.60-46.20)	1249	41.00 (38.10-44.10)	1199	0.92 (0.86-0.98)
		Smokers				Smokers					
Group		N	GM-AA-Hb (95% CI)	GM-GA-Hb (95% CI)	GM-GA-Hb:AA-Hb (95% CI)	N	GM-AA-Hb (95% CI)		GM-GA-Hb (95% CI)		GM-GA-Hb:AA-Hb (95% CI)
All		5	80.46 (54.5-118.78)	73.70 (49.49-109.76)	0.92 (0.62-1.35)	1316	113.00 (103.00-123.00)	1357	93.50 (86.50-101.00)	1282	0.82 (0.79-0.85)
Gender	Female	3	86.67 (41.26-182.10)	71.80 (23.76-216.94)	0.83 (0.58-1.19)	483	120.00 (108.00-133.00)	496	104.00 (94.90-115.00)	466	0.86 (0.82-0.90)
	Male	2	71.96 (1.71-3035.80)	76.65 (31.09-1890.4)	1.07 (0.01-110.83)	833	108.00 (97.20-121.00)	861	87.20 (79.40-95.70)	816	0.80 (0.76-0.83)
Age Group	20-39 years	0	N/A			444	118.00 (106.00-131.00)	464	98.20 (88.50-109.00)	435	0.84 (0.80-0.88)
	40-59 years	3	83.64 (15.78-222.15)	85.40 (51.94-140.41)	1.02 (0.40-2.58)	371	116.00 (106.00-127.00)	376	94.20 (87.10-102.00)	362	0.79 ((0.75-0.83)
	> 60 years	2	75.91 (6.69-861.41)	59.09 (1.48-2364.87)	0.78 (0.22-2.75)	252	93.50 (84.20-104.00)	258	77.20 (70.10-85.10)	243	0.81 (0.76-0.86)

6.4 Discussion

6.4.1 1,3-Butadiene Hemoglobin Adducts

1,3-butadiene is used in the manufacturing of tires and other synthetic rubber products. It is also present in automobile exhaust; cigarette smoke and wood fires (ATSDR). The detection of the *pyr*-Val adducts in this study is indicative of the improvement in laboratory detection methods, as to date there have been no human studies where *pyr*-Val adducts have been reported. The presence of the *pyr*-Val adduct indicates that all study participants had been exposed to 1,3-butadiene within the past four months. The concentrations of *pyr*-Val presented here may be small, but may also be an indicator of the ubiquitous presence of 1,3-butadiene in the environment.

Evaluations of occupational exposures to 1,3-butadiene in this study did not suggest an association between potential exposure and hemoglobin adduct results as the reported levels were not statistically significantly different between exposed and unexposed subjects. Use of the questionnaire data to assign exposure potentials, while tailored to a specific exposure scenario may be too broad to identify potential associations between occupations and adduct levels. Since there are no human data reported in the literature, it is not feasible to determine if the results presented in this study are high, low or normal.

As 1,3-butadiene is found in cigarette smoke the smoking history of study participants was of interest. None of the five study participants who were current smokers had the highest *pyr*-Val adduct levels. As rated by the study participants, exposure to heavy levels of second hand smoke is suggestive of a statistically significant source of exposure. Differences between residences in northern states versus southern states were statistically significant and may also represent cultural differences, in that, exposure to second hand smoke may be more common in the southern states.

Responses to questions related to diet were not expected to influence the *pyr*-Val levels and none of the 16 questions analyzed in this study identified statistically significant differences between response groups. Results were nearly significant (p=0.08) for the responses to the frequency of frankfurter consumption; however, only one study subject consumed these with any regularity. Also, nearly significant (p=0.11) were responses to the question concerning toasted/burned or crispy food in general. Interestingly, the geometric mean decreased as the frequency of consumption increased. Although not significant, the geometric means increased as the frequency of consumption increased for corn or wheat cakes and artificially sweetened drinks.

The metabolism of 1,3-butadiene results in the formation of three epoxides; 1,2-epoxy-3-butene (EB), 1,2,3,4-diepoxybutane (DEB) and 1,2-epoxy-3,4-butanediol (EB-diol). These three epoxides in turn form three hemoglobin adducts, N-(2-hydroxy-3-buten-1-yl)-valine (HB-Val), *N,N*-(2,3-dihydroxy-1,4-butadiyl)-valine (*pyr*-Val) and 2,3,4-trihydroxybutyl-valine (THB-Val). Formation of the epoxides is mediated by the Cytochrome P-450 2E1 (CYP2E1) system as shown in Figure 1 in the Background Section (Boysen et al., 2007). Cochrane and Skopek (Cochrane and Skopek, 1994) studied the three epoxide metabolites to determine their mutagenicity. Using TK6 human lymphoblastoid cells in culture medium, the authors were able to evaluate the mutagenic potential of ED, DEB and EB-diol. All three epoxides are mutagenic with the DEB epoxide being the most mutagenic (Cochrane and Skopek, 1994). DEB will react with hemoglobin to form the *pyr*-Val hemoglobin adduct. Two of the hemoglobin adducts, HB-Val and THB-Val, have been detected (Boysen et al., 2007); however, to date, detection of the *pyr*-Val hemoglobin adduct in humans has not been reported in the literature.

HB-Val was the first hemoglobin adduct studied with respect to 1,3-butadiene exposure. Studies showed that humans exposed to 1,3-butadiene produced relatively small amounts of HB-Val when compared to THB-Val (Boysen et al., 2007). Other studies have shown that an endogenous source may

be responsible for the presence of THB-Val in several species when no known exposure has occurred. While THB-Val can be formed from DEB, it is primarily formed from the EB-diol epoxide (Boysen et al., 2007). THB-Val adducts are formed in greater amounts than HB-Val or *pry*-Val adducts independent of exposure, possibly due to the endogenous formation (Boysen et al., 2007). Because DEB is the most potent mutagen, HB-Val is formed in relatively small amounts, and THB-Val may be formed from endogenous sources, the *pry*-Val adduct measurements may be the most meaningful hemoglobin adduct of human exposure to 1,3-butadiene (Boysen et al., 2007).

Albertini et al. (2001) conducted a study to validate the biomarkers of 1,3-butadiene in workers using an extensive questionnaire and personal air monitoring samples. Of the 104 study participants, 51 were administrative controls, 23 worked in a laboratory and 30 worked in a polymerization production facility. Personal monitoring consisted of ten separate full shift samples for each study participant, collected over four months. Blood samples were collected on the last day of the four-month exposure assessment period. Air monitoring results up to 9.8 mg/m^3 were reported for female workers and up to 12.6 mg/m^3 for male workers. In this study, the *pyr*-Val adduct was not detected or was present at levels below the limit of detection. This result is an indication of the difference in species, as the *pyr*-Val adducts have been detected in mice and rats exposed to 2.21mg/m^3 in inhalation studies (Albertini et al., 2007).

6.4.2 Acrylamide Hemoglobin Adducts

Unlike the pyr-Val hemoglobin adduct of 1,3-butadiene, acrylamide hemoglobin adducts have been the subject of many studies. The metabolism of acrylamide and the formation of the two acrylamide hemoglobin adducts, AA-Hb and GA-Hb have been studied at length in the workplace, although glycidamide adducts are less frequently reported in the literature. A sample of acrylamide adduct results reported in occupational studies is shown in Table XL. Glycidamide is also capable of forming

DNA adducts and is considered responsible for acrylamide's genotoxic capabilities (Vesper et al., 2010). Acrylamide metabolism follows two paths (See Figure 2-Background Section) and may be detoxified and eliminated by glutathione conjugation or oxygenated to glycidamide by CYP2E1. Glycidamide undergoes further metabolism to glyceramide, forms DNA adducts or is eliminated by glutathione conjugation (Dybing et al., 2005).

Acrylamide adducts have been measured in the workplace. Bergmark (1993) studied 41 workers in a chemical plant in China that was producing acrylamide. The factory workers were divided into four groups based on occupation: packaging, polymerization, ambulatory, and synthesis. These groups were compared to a control group. In addition, the authors compared adduct results to personal air monitoring results that were available at the time of the study. For polymerization workers the geometric mean air concentration was 1.52 mg/m^3 and in the synthesis area the geometric mean air concentration was 0.73 mg/m^3. Based on the rate of reactions an expected acrylamide level was calculated as 930 pmol/g/globin for the polymerization workers while the actual level was 7300 pmol/g globin. While the adduct levels were found to be related to the job classifications, the authors concluded that inhalation exposure was secondary to dermal exposure, (indicated by the observation of skin peeling of the hands of workers) with respect to acrylamide absorption based on hemoglobin adduct formation (Bergmark, 1993).

In a study of tunnel workers in Sweden by Hagmar et al. (2001), 210 workers who were exposed to acrylamide as a chemical grouting agent were evaluated, using hemoglobin adduct formation as a measure of internal dose. The background level of AA-Hb ranged from 20-70 pmol/g globin in a non-exposed population (N=18). Using self-reported exposure data, the workers were divided into three groups: 1) no exposure (exposure only from airborne or skin contact with contaminated water), 2) some exposure (infrequent contact with the chemical grouting agent), and 3) high exposure (frequent skin

TABLE XL - OCCUPATIONAL STUDIES OF ACRYLAMIDE EXPOSURE USING HEMOGLOBIN ADDUCTS AS AN INDICATOR OF EXPOSURE

Study	N	Acrylamide Adduct Levels (pmol/g globin)
Bergmark et al., 1993 Workers in a chemical synthesis plant in China		
Controls	10	0
Packaging	5	3900 ± 2500
Polymerization	12	7300 ± 3400
Ambulatory	8	9600 ± 6800
Synthesis	14	14700 ± 10600
Hagmar et al., 2001 N= 210 tunnel workers using grout containing acrylamide for two months		
Reference population		
Non-smokers/no exposure	18	20-70
47 workers	47	<80
89 workers	89	80-290
36 workers	36	300-1000
38 workers	38	>1000
Paulsson, et al., 2001 2 case studies		
Case Study 1 - Soil transport		
Non-smoking workers - 11	11	29
Smoking workers - 6	6	110
Non-smoking controls - 2	2	23
Case Study 2 - Glass work, non-smokers		
Worker A (first year)	1	310
Worker B (first year)	1	94
Worker A (second year)	1	200
Worker C (second year)	1	190
Control	1	35

contact with the grouting agent). Statistically significant associations (P>0.0001) were found between the category of exposure and the hemoglobin adduct levels. The prevalence of peripheral nervous system symptoms such as headache, nausea, numbness and tingling were also associated with the hemoglobin adduct levels in a dose-response relationship (Hagmar et al., 2001).

The analysis of AA-Hb results for this study did not show a statistically significant association between adduct formation and potential exposure based on occupation/industry coding. The highest result for the AA-Hb adduct was 380 pmol/g globin for a software engineer. Based on industry/occupation the study participant was coded for SIC code 73-Business Services with a potential exposure to acrylamide. The next four highest results were recorded for an airline customer service representative, accountant, home repair person and administrator for a cancer information center. Only the administrator, SIC code-80 had a potential exposure to acrylamide in the workplace. The five lowest reported results, 14.7-24.3 pmol/g globin were reported for two retired study participants, a teacher, a pottery glazer and a plasma products research scientist. Only the research scientist (SIC code 80-Health Services) had a potential for exposure based on the NOES. Both the pottery glazer and teacher were coded to SIC codes not surveyed by NIOSH.

A small increased risk was identified for the GA-Hb adduct formation but the increased odds ratio was not statistically significant. The highest GA-Hb adduct result, 117,706 pmol/g globin was reported for the same software engineer with the highest AA-Hb result, referenced above. Based on the SIC code 73-Business Services, there is a potential for exposure; however, this result is much higher when compared to the other reported results for the study population. The second highest reported result for the study population, 11,479 pmol/g globin was for a teacher, SIC code 82-Education. SIC code 82 was not included in the NIOSH survey. The three remaining of the highest five results, 339-421 pmol/g globin, were reported for a research coordinator, nurse and another software engineer. Of these five, based on

SIC codes, all of these study participants would have had a potential exposure to acrylamide except the teacher. However, as the education industry was not surveyed, it cannot be definitively concluded that there is no potential for exposure, only that we don't know if a potential exposure exists.

Analysis of the demographic data did not aid in the detection of the potential sources of acrylamide exposure, although males had a nearly statistically significant difference in acrylamide adduct levels as compared to females (males-45.97 pmol/g globin versus females 38.65 pmol/g globin; p=0.14). Alternatively, the analysis of the smoking data did provide some insight into the potential source of exposure. Both current and ever smokers had statistically significant differences in acrylamide adduct levels. Current smokers had acrylamide adduct levels more than twice the level for non-smokers (Smokers-80.46 pmol/g globin, non-smokers-38.80 pmol/g globin, p=0.001). The difference in means between current smokers and ever smokers was smaller; however, statistically significant (Ever smokers – 47.19 pmol/g globin, never smokers-36.20 pmol/g globin; p=0.03). Smoking variable analyses did not result in any statistically significant result for glycidamide adduct levels. Under the premise that hemoglobin adducts represent exposures that have occurred in the past four months the difference between those study participants who never smoked should not be different from those who were ever smokers. The statistical significance of the result cannot be explained.

Responses to the diet history questions did not suggest an association with acrylamide adduct levels. Responses were nearly significant for the question related to grilled, barbecued or blackened foods (more frequent consumption-48.11 pmol/g globin; less frequent consumption 38.14 pmol/g globin; p=0.14); bakery product consumption (p=0.14; less frequently consumed had highest geometric mean) and instant coffee drinkers (p=0.20, highest levels in lowest consumption group). For the glycidamide results, the response to the wheat, corn and millet question was statistically significant (p=0.004, moderate consumption level, highest geometric mean) questions for grilled, barbequed or blackened

foods (p=0.16, highest geometric mean in most frequent consumption group); pretzels, corn snacks and salt crackers (p=0.12, moderate consumption level-highest geometric mean) and toasted, burned or crispy food in general (p=0.20, moderate consumption level-highest geometric mean) were nearly statistically significant. When the two extreme glycidamide adduct level responses were removed and the data reanalyzed the wheat, corn and millet responses were still statistically significantly different (p=0.03; highest geometric mean in moderate consumption level). A nearly statistically significant result was reported for those who consumed cooked bacon (p=0.08, highest geometric mean in moderate group). There were only two instances where a positive trend was seen between increased consumption and increased adduct levels. These were: For the pyr-Val adduct levels for Question 13, Corn cakes or wheat cakes and for acrylamide adducts and Question 16, Diet drinks with artificial sweeteners.

6.4.3 Comparison to NHANES Data

The source of the exposure for these study participants is unclear. Acrylamide is found in food, cigarette smoke, and drinking water and can also be absorbed through the skin by direct contact with acrylamide containing compounds (NHANES, 2009). The Fourth National Report on Human Exposure to Environmental Chemicals (NHANES) has recently been published by CDC (NHANES, 2009). During the years 2003-2004, 7101 blood samples from study participants across the U.S. were analyzed for the presence of hemoglobin adducts.

The acrylamide adduct results in this study are all lower than the NHANES results, while the glycidamide results are higher (See Table XXXIX). The AA-Hb adduct results from our study were statistically significantly different from those reported in the NHANES study ($t = -7.43$. $p = < 0.0001$), while the GA-

Hb adduct results were not statistically significantly different from those reported by the NHANES study ($t = 1.40$, $p = 0.16$).

Glycidamide is mutagenic when tested in both the Salmonella assay and in bronchial epithelial cells while acrylamide is not (Fennell et al., 2005). As glycidamide is the metabolite that is responsible for the toxicological effects, measurements of both the acrylamide and glycidamide hemoglobin adducts permit the calculation of a ratio of glycidamide to acrylamide which is an indicator of CYP2E1 activity. Reports in the literature suggest that this ratio is highly variable between individuals suggesting that this inter-individual variability may be responsible for the variation in response to exposures to toxic chemicals (Vesper, 2008). On average, acrylamide adducts are formed at a slower rate than glycidamide adducts; however, at lower or moderate exposures of acrylamide, the level of glycidamide in the body is lower than acrylamide therefore, equal amounts of these adducts are formed resulting in a 1:1 GA-Hb to AA-Hb ratio (Dybing et al., 2005). The glycidamide to acrylamide ratios are much higher than the expected 1:1 ratio, with the exception of the smokers who as a group were fairly close to the NHANES data although the geometric means for both adducts were lower than the NHANES results.

In a controlled human study, Fennell et al. (2005) measured AA-Val and GA-Val adducts following both dermal and oral dose administration. The oral dosing range varied from 0.5-3.0 mg/kg. Adduct formation increased linearly for both AA-Val and GA-Val, indicating that there was no saturation of the acrylamide to glycidamide metabolic pathway. This may explain why there were two very high glycidamide results reported in this study.

6.4.4 Analysis of Extreme Results

There were two study participants with extreme glycidamide adduct results. As glycidamide is the genotoxic adduct, the possible sources of acrylamide are of interest.

The highest glycidamide level was reported for a white, 56 year old male software engineer (AA-Hb = 380 pmol/g globin; GA-Hb = 117,706 pmol/g globin; *pyr*-Val = 0.54 pmol/g globin). As a software engineer, the study participant was placed in SIC code 73, Business services with a current potential exposure to both acrylamide and 1,3-butadiene. The study participant is a former smoker but not smoking now and reports moderate exposure to second hand smoke. The study participant's dietary profile indicates consumption of the following food items and frequency of each as follows: broiled food and smoked food - sometimes; grilled, blackened or barbecued food - often, French fries - twice a week, bakery products, etc. - once a month, pretzels and crispy/burned food - once a week, ham and bacon - less than once a month, hotdogs - once a month, sandwich meats - two to three times a week and margarine - three to four times a week. Body surface area (BSA) may play a role in the glycidamide levels as a higher BSA may indicate a higher blood volume. Based on the equation for BSA (BSA = √height (in.)*weight (lbs.)/3131) the BSA for this study participant was 2.20 m^2 (Mosteller, 1987). The median for the NHANES study was 1.75 m^2.

The second highest glycidamide level belongs to a white, 67 year old female teacher, whose adduct levels were AA-Hb-62.2 pmol/g globin, GA=Hb-11,479 pmol/g globin; *pyr*-Val-0.79 pmol/g globin. As a teacher, she was working in SIC code 82, Education. This industry was not surveyed by NIOSH. She has no current or historical exposure to either acrylamide or 1,3-butadiene. The study participant has never smoked and did not respond to the second hand smoke question. The study participant's dietary profile indicates consumption of the following food items and frequency of each as follows: grilled or barbecued food - twice a week, crispy food in general, etc. - once a week, hotdogs - two to three times a month, cooked bacon - once a week, wheat, corn or millet - once a week, bakery products - five to six times a week, French fries - five or six times a week, margarine -five or six times a week and drinks diet drinks every day. BSA = 1.43 m^2.

There is not enough information about these extreme values to draw any definitive conclusions about their unusually high levels of glycidamide hemoglobin adducts. In addition to diet, occupation and other lifestyle choices such as smoking behavior, there may be other explanations for the glycidamide adduct formation. Metabolism of acrylamide to glycidamide is necessary for the expression of genotoxic effects. Levels of CYP2E1 drive the metabolism toward glycidamide formation. In a study conducted by Ghanayhem et al. (2005), CYP2E1-wild type (normal) and CYP2E1-null mice were dosed with 50 mg/kg acrylamide by intrarperitoneal injection and euthanized six hours later. Concentration of AA-Hb in the wild type mice was 137 times lower than in the CYP2E1-null mice. The concentration of GA-Hb in wild type mice was 19 times higher than the concentration in the CYP2E1-null mice. These results demonstrate the importance of CYP2E1 in the metabolism of acrylamide to glycidamide and the influence of genotype on the direction of the metabolic reactions (Ghanayhem et al., 2005).

The direction of acrylamide metabolism either toward detoxification and excretion in the urine or genotoxicity by glycidamide formation can also be influenced by ethanol intake. In a cell culture study using human HepG2 hepatoma cells, Lamy et al. (2008) exposed the cells to acrylamide after they had been pretreated with ethanol for 24 hours. Results of the study suggested an increased expression of CYP2E1 in the cells and reduced levels of glutathione as compared to untreated cells. Thus, ethanol consumption in addition to acrylamide exposure would drive the metabolism of acrylamide toward the formation of glycidamide and potentially increased genotoxic effects (Lamy et al., 2008). As ethanol containing beverages are often consumed with acrylamide containing foods, potato chips, French fries and crackers, higher glycidamide hemoglobin adduct levels may be observed in individuals making this lifestyle choice.

6.4.5 Limitations

Acrylamide analyses were completed in the same laboratory that conducted the NHANES studies and presumably under the same working conditions. Samples were handled up to three different times by different laboratories and even though the extreme value samples were analyzed twice a possible lab error cannot be discounted. There is a statistically significant difference between the fresh samples and the stored samples for the *pyr*-Val with higher mean adduct levels observed in the stored samples (geometric mean stored samples = 0.98 pmol/g globin, geometric mean fresh samples = 0.71 pmol/g globin; p=0.01). There were no statistically significant difference in the acrylamide or glycidamide adduct results between fresh and stored samples.

The study size is small and a larger study population may normalize the results of the laboratory analysis. The study serves as a starting point and results should be interpreted with great care. Respondents were asked to think about their eating habits two years before the questionnaire was completed and the assumption is that these habits have not changed in that two year period. The biomarker results cannot be paired with any workplace air sampling results for the chemicals and exposure is rated as potential. The opportunity for misclassification bias to influence the results exists both from the coding of industries and occupations and from the respondent's possible misunderstanding of the questions. With a large percentage of the population retired the number of respondents with current exposure to acrylamide and/or 1,3-butadiene is limited. Finally, not all study participants completed the full questionnaire minimizing the usefulness of the data.

6.5 Conclusions

This study demonstrates that exposures to 1,3-butadiene have occurred in the past four months, most likely due to the ubiquitous presence of this chemical in the environment. Epoxides are formed during

1,3-butadiene metabolism and are mutagenic. The DEB epoxide is the most mutagenic chemical intermediate and *pyr*-Val is the hemoglobin adduct formed when DEB reacts with hemoglobin. With nearly one third of the study population unemployed, retired or otherwise not potentially exposed to 1,3-butadiene no conclusion can be made as to the contribution of workplace exposure to hemoglobin adduct formation. An association between *pyr*-Val adduct levels and second hand smoke exposure is suggested, and provides some confirmation that exposure to cigarette smoke can increase adduct formation.

The associations between acrylamide adduct formation and cigarette smoking was confirmed by this study. Again, the association between workplace exposures and acrylamide adduct formation is unclear due to the composition of the study population.

The detection of hemoglobin adducts for 1,3-butadiene and acrylamide in this small study is hypothesis generating in nature, and demonstrates the need for further studies. Studies of CYP2E1 and glutathione-S-transferase genotypes along with more informative diet information including alcohol consumption, refined occupational information, environmental risk factors, medical history, prescription drug use and cosmetic use are all risk factors which may influence hemoglobin adduct formation and exposure assessment considerations and deserve further study.

7.0 CONCLUDING REMARKS

This dissertation demonstrates the need for collaboration between toxicology, epidemiology and exposure assessment fields of study. The hypothesis that a cumulative exposure score can be generated from work histories is sound; however, professional judgment might be useful to provide an additional variable that reflects the potential exposure.

Detailed questionnaires, too, have their place in the exposure assessment process. By themselves, questions about industry and occupation experiences are subject to interpretation by the study participant and need to be clear and to the point of the study.

Perhaps the most promising development in exposure assessment methodology is the use of biomarker technology. The detection of the *pry*-Val hemoglobin adduct of 1,3-butadiene is exciting as this adduct has not been detected in human subjects prior to this study. That a statistically significant difference in adduct levels between perceived exposure to second hand smoke as reported by study subjects and this adduct provides evidence that exposure to second hand smoke can influence the formation of mutagenic metabolites.

The influence of smoking history was evident with the results of the acrylamide adduct analyses. The differences between adduct levels for current smokers as compared to those who never smoked were statistically significant.

Less clear are the influences of diet on hemoglobin adduct formation. Diet history question responses were limited and an assumption had to be made that dietary pattern had not changed in the two years before the study participants were enrolled in the study. Further study of diet is suggested by this study.

The influence of occupation on adduct formation is not evident as a result of this study. A larger study with more focused survey questions may be beneficial. Also of note is the announcement by OSHA that they are providing access to their industrial hygiene monitoring database. Use of this database may permit a better understanding of the workplace exposures to hazardous chemicals and may also permit the detailed analysis of historical data for specific industries and occupations. The suggestion of an increased risk of glioma associated with 3,3'-dimethylbenzidine dihydrochloride suggests that further studies of this chemical are needed to better characterize the exposure.

These studies were limited by the small study populations. The possibility of misclassification bias based on self-reported work histories and usage of broad classification categories such as the two digit SIC and SOC codes should be considered when interpreting these results. The lack of exposure monitoring data is another limitation that prevents a better understanding of the results. This limitation may be minimized in the future as the understanding of biomarker results improves.

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APPENDICIES

APPENDIX A

TABLE XLI - REGULATORY LIMITS FOR ANCS

Chemical Number	Chemical	CAS Number	OSHA Limit	ACGIH Limit	IARC Group	NIOSH REL	Listed by Carcinogen Potency Project
1	Acrylamide	79-06-1	0.3 mg/m3	0.03 mg/m3	A3	0.03 mg/m3	Y
2	Acrylonitrile	107-13-1	40 ppm	20 ppm	A4	20 ppm	Y
3	Bis (chloromethyl) ether	542-88-1	0.001 ppm	0.001 ppm	1		Y
4	Dimethyl sulfate	77-78-1	1 ppm	0.1 ppm	2A	0.1 ppm	N
5	Divinyl benzene	1321-74-0	10 ppm	10 ppm	N/A	10 ppm	N
6	Ethylene oxide	75-21-8	1 ppm	1 ppm	1	< 0.1 ppm	Y
7	N-2-Fluorenylacetamide	53-96-3			1		Y
8	Glycidol	556-52-5	50 ppm	2 ppm	2A	25 ppm	Y
9	1-H-Benzotriazole	95-14-7	n/a	n/a	N/A	n/a	Y
10	Bromoethane	74-96-4	200 ppm	5 ppm	3	200 ppm	Y
11	1,3-Butadiene	106-99-0			1		Y
12	Chloroethane	75-00-3	1000 ppm	100 ppm	3	n/a	Y
13	CI Direct Blue 15	2429-74-5	n/a	n/a	2B	n/a	Y
14	3,3'-Dimethylbenzidine dihydrochloride	612-82-8	n/a	n/a	2B	n/a	Y
14	3,3'-Dimethoxybenzidine dihydrochloride	20325-40-0	n/a	n/a	N/A	n/a	Y
16	Ethyl methyl sulphonate	62-50-0	n/a	n/a	2B	n/a	N
17	Isoprene	78-79-5	n/a	n/a	2B	n/a	Y
18	Vinyl chloride	75-01-4	1 ppm	1 ppm	1	n/a	Y

APPENDIX B

TABLE XLII - KNOWN OR SUSPECTED ANCS BY SIC CODE AS IDENTIFIED BY NIOSH

SIC Code	SIC Code Description	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
7	AGRICULTURAL SERVICES						X												
13	OIL AND GAS EXTRACTION	X					X			X									
15	GENERAL BUILDING CONTRACTORS		X										X						
16	HEAVY CONSTRUCTION CONTRACTORS									X		X							X
17	SPECIAL TRADE CONTRACTORS		X							X		X							X
20	FOOD AND KINDRED PRODUCTS	X	X				X			X					X				X
21	TOBACCO MANUFACTURERS																		
22	TEXTILE MILL PRODUCTS	X	X		X		X			X		X		X				X	X
23	APPAREL AND OTHER TEXTILE PRODUCTS		X				X			X	X							X	X
24	LUMBER AND WOOD PRODUCTS	X								X				X				X	
25	FURNITURE AND FIXTURES	X					X												X
26	PAPER AND ALLIED PRODUCTS	X	X				X			X		X						X	X
27	PRINTING AND PUBLISHING						X			X	X		X					X	X
28	CHEMICALS AND ALLIED PRODUCTS	X	X		X		X		X	X	X	X	X		X			X	X
29	PETROLEUM AND COAL PRODUCTS	X					X			X	X	X	X		X			X	X
30	RUBBER AND MISC. PLASTICS PRODUCTS	X	X				X			X		X						X	X
31	LEATHER AND LEATHER PRODUCTS						X				X	X						X	
32	STONE, CLAY, AND GLASS PRODUCTS	X					X			X								X	X
33	PRIMARY METAL INDUSTRIES	X					X			X								X	X
34	FABRICATED METAL PRODUCTS	X					X		X	X		X	X	X				X	X
35	MACHINERY, EXCEPT ELECTRICAL	X					X			X	X	X	X	X				X	X
36	ELECTRIC AND ELECTRONIC EQUIPMENT	X					X			X		X	X					X	X
37	TRANSPORTATION EQUIPMENT	X	X			X	X			X	X	X	X	X				X	X

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE XLII - KNOWN OR SUSPECTED ANCS BY SIC CODE AS IDENTIFIED BY NIOSH (CONTINUED)

SIC Code	SIC Code Description	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
38	INSTRUMENTS AND RELATED PRODUCTS		X				X			X		X	X					X	X
39	MISCELLANEOUS MANUFACTURING INDUSTRIES		X				X			X								X	X
40	RAILROAD TRANSPORTATION						X												
41	LOCAL AND INTERURBAN PASSENGER TRANSIT						X			X									
42	TRUCKING AND WAREHOUSING	X								X	X							X	X
44	WATER TRANSPORTATION									X									X
45	TRANSPORTATION BY AIR	X								X				X					X
46	PIPE LINES, EXCEPT NATURAL GAS																		
47	TRANSPORTATION SERVICES																		
48	COMMUNICATION						X												
49	ELECTRIC, GAS, AND SANITARY SERVICES	X	X				X			X		X							
50	WHOLESALE TRADE - DURABLE GOODS						X			X		X							X
51	WHOLESALE TRADE - NONDURABLE GOODS																		
55	AUTOMOTIVE DEALERS & SERVICE STATIONS									X		X							
72	PERSONAL SERVICES						X			X									
73	BUSINESS SERVICES	X	X		X		X	X		X	X	X					X	X	X
75	AUTO REPAIR, SERVICES, AND GARAGES		X							X		X							X
76	MISCELLANEOUS REPAIR SERVICES									X									
80	HEALTH SERVICES	X	X	X	X		X			X	X	X	X			X		X	
84	MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS										X								

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

Table XLIII SIC CODE 7 - AGRICULTURAL SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
487	Animal caretaker, except farm						x												

Table XLIV SIC CODE 13 - OIL AND GAS EXTRACTION - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
518	Industrial machinery repairers	x								x									
617	Mining occupations	x					x												

Table XLV SIC CODE 15 - GENERAL BUILDING CONTRACTORS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
567	Carpenters		x										x						
585	Plumbers, pipefitters, steamfitters		x																
643	Boilermakers		x																
757	Separating, filtering & clarifying		x																
869	Construction laborers		x										x						

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE XLVI SIC CODE 16 - HEAVY CONSTRUCTION CONTRACTORS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
516	Heavy equipment mechanics									x		x							
869	Construction laborers																		x

TABLE XLVII SIC CODE 17 - SPECIAL TRADE CONTRACTORS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
453	Janitors and cleaners									x									
534	HVAC Mechanics											x							x
558	Supervisors		x																
575	Electricians											x							x
576	Electrician apprentices											x							x
585	Plumbers, pipefitters, steamfitters									x									
589	Glazers		x																
595	Roofers		x																
596	Sheet metal duct installers		x																
599	Construction trades		x																
653	Sheet metal workers		x							x									
844	Operating engineers									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE XLVIII SIC CODE 20 - FOOD AND KINDRED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
56	Industrial engineers	x																	
453	Janitors and cleaners	x	x				x			x									
507	Bus, truck & stationary engine mechanics									x									
516	Heavy equipment mechanics									x									
534	HVAC mechanics									x									
547	Specified mechanics and repairers									x									
633	Production supervisors														x				
696	Stationary engineers	x																	
754	Packaging and filling machine operators						x												
777	Miscellaneous machine operators																		x
887	Vehicle washers and equipment cleaners						x												

TABLE XLIX SIC CODE 22 - TEXTILE MILL PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
95	RNs						x												
213	Electrical and electronic technicians						x												
216	Engineering technicians						x												x
235	Technicians									x									
364	Traffic, shipping and receiving clerks		x																
453	Janitors and cleaners		x				x			x									x
518	Industrial machinery repairers									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE XLIX SIC CODE 22 - TEXTILE MILL PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
534	HVAC mechanics									x									
547	Specified mechanics and repairers									x									
549	Mechanics and repairers, ns		x																
734	Printing machine operators		x									x							x
738	Winding and twisting machine operators		x																
744	Textile sewing machine operators		x				x					x							
749	Miscellaneous textile machine operators	x	x				x											x	x
756	Mixing & blending operators						x			x		x		x					
759	Painting and paint spraying operators		x									x							
766	Furnace, kiln, & oven operators, not food	x																	
777	Miscellaneous machine operators	x	x		x		x					x							
783	Welders and cutters						x												
785	Assemblers						x												
796	Product inspectors, checkers, and examiners						x												
856	Industrial truck and tractor equipment operators						x												
859	Misc material moving equipment operators									x									
873	Production helpers						x												
878	Machine feeders and offbearers						x												
883	Freight, stock, & material movers, hand									x									
889	Laborers except construction		x																

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-Cl Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE L SIC CODE 23 - APPAREL AND OTHER TEXTILE PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
364	Traffic, shipping and receiving clerks									x									
365	Stock and inventory clerks						x												
453	Janitors and cleaners		x				x			x									
518	Industrial machinery repairers		x																
667	Tailors		x				x			x									
734	Printing machine operators										x								
738	Winding and twisting operators		x																
744	Textile sewing machine operators		x				x			x									
747	Pressing machine operators									x									
749	Miscellaneous textile machine operators		x																
753	Cementing and gluing machine operators																	x	
765	Folding machine operators		x																
777	Miscellaneousmachine operators		x				x												x
878	Machine feeders and offbearers		x																
888	Hand packers and packagers		x																

TABLE LI SIC CODE 24 - LUMBER AND WOOD PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
453	Janitors and cleaners																	x	
547	Specified mechanics and repairers									x									
549	Mechanics and repairers, ns									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LI SIC CODE 24 - LUMBER AND WOOD PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
637	Machinists																	x	
777	Miscellaneous machine operarors	x												x					
785	Assemblers																	x	

TABLE LII SIC CODE 25 - FURNITURE AND FIXTURES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
453	Janitors and cleaners	x					x												
518	Industrial machine repairers																		x
633	Production supervisors																		x
668	Upholsterers																		x
727	Sawing machine operators																		x
729	Nailing and tacking machine operators																		x
744	Textile sewing machine operators																		x
749	Miscellaneous textile machine operators																		x
759	Painting and paint spraying operators																		x
769	Slicing and cutting machine operators																		x
785	Assemblers																		x
888	Hand packers and packagers																		x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LIII SIC CODE 26 - PAPER AND ALLIED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
364	Traffic, shipping and receiving clerks																	x	
453	Janitors and cleaners	x								x								x	x
518	Industrial machine repairers	x								x									
633	Production supervisors																		x
655	Miscellaneous precision metal workers		x																x
696	Stationary engineers	x																	
734	Printing machine operators																	x	x
749	Miscellaneous textile machine operators																		x
756	Mixing and blending machine operators																	x	x
759	Painting and paint spraying						x					x							
764	Washing, cleaning, & pickling machine operators	x																	
766	Furnace, kiln and oven operators, except food						x												
768	Crushing and grinding machine operators						x												x
769	Slicing and cutting machine operators						x												
777	Miscellaneous machine operators	x					x			x									x
873	Production operators																	x	
878	Machine feeders and offbearers	x																	

TABLE LIV SIC CODE 27 - PRINTING AND PUBLISHING - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
189	Photographers						x											x	

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LIV SIC CODE 27 - PRINTING AND PUBLISHING - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
195	Editors and reporters						x												
213	Electrical and electronic technicians						x			x									
216	Engineering technicians						x												
364	Traffic, shipping and receiving clerks										x								
379	General office clerks						x			x									
385	Data-entry keyers										x								
453	Janitors and cleaners						x			x	x								x
516	Heavy equipment operators									x									
549	Mechanics and repairers, not specified																	x	
723	Metal plate machine operators						x												
734	Printing machine operators						x			x	x							x	x
735	Photoengravers and litographers						x			x	x								
736	Typesetters and compositors						x			x								x	
737	Miscellaneous printing machine operators						x			x	x								
749	Miscellaneous textile machine operators						x												x
753	Cement and gluing machine operators						x												
756	Mixing and blending machine operators																		x
774	Photographic process machineoperators						x			x								x	
777	Miscellaneous machine operators						x			x			x						
783	Welders and cutters									x									
789	Hand painting, coating, and decorating occupations																		x
859	Misc. material moving equipment operators												x						
878	Machine feeders and offbearers																		x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LIV SIC CODE 27 - PRINTING AND PUBLISHING - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
888	Hand packers and packagers																	x	x

TABLE LV SIC CODE 28 - CHEMICALS AND ALLIED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19	Managers and administrators																		x
48	Chemical engineers						x		x			x	x					x	x
73	Chemists, except biochemists	x	x									x						x	
213	Electrical and electronic technicians											x							
216	Engineering technicians		x																x
223	Biological technicians	x			x		x												
224	Chemical technicians	x	x		x		x		x			x	x					x	x
225	Science technicians		x				x					x							x
235	Technicians									x		x							x
364	Traffic, shipping & receiving clerks																		x
389	Administrative support occupations																		x
453	Janitors and cleaners	x	x		x		x			x	x	x	x					x	x
519	Machinery maintenance occupations				x		x						x						
535	Camera, watch, and musical instrument repairers						x		x			x	x					x	
549	Mechanics and repairers not specified				x		x			x			x						x
567	Carpenters				x		x						x						
575	Electricians						x		x			x	x						x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LV SIC CODE 28 - CHEMICALS AND ALLIED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
585	Plumbers, pipefitters, steamfitters				x		x					x	x						x
593	Insulation workers				x		x						x						
633	Production supervisors						x		x			x	x					x	x
637	Machinists				x		x					x	x						x
653	Sheet metal workers				x								x						
694	Water & sewage treatment plant operators				x					x			x						
695	Power plant operators									x									
696	Stationary engineers											x						x	
757	Separating, filtering, & clarifying machine operators	x	x		x		x		x				x						x
759	Painting and paint spraying operators				x		x						x						x
766	Furnace, kiln, & oven operators, except food				x								x						
768	Crushing and grinding machine operators	x																	
769	Slicing and cutting machine operators								x										
777	Miscellaneous machine operators	x	x		x		x						x		x				x
783	Welder and cutters				x		x						x						x
785	Assemblers						x												
796	Production inspectors, checkers, & examiners		x		x		x						x						
797	Production testers		x																
856	Industrial truck and tractor equipment operators											x							
859	Misc. material moving equipment operators	x	x				x					x						x	x
877	Stock handlers and baggers																	x	
878	Machine feeders and offbearers												x						
883	Freight, stock, & material movers, hand	x																	

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LV SIC CODE 28 - CHEMICALS AND ALLIED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
887	Vehicle washers and equipment cleaners		x																
888	Hand packers and packagers						x												x
889	Laborers, except construction		x				x											x	x

TABLE LVI SIC CODE 29 - PETROLEUM REFINING AND RELATED PRODUCTS - OCCUPATIONS ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19	Managers and administrators									x									
73	Chemists, except biochemists									x									
213	Electrical and electronic technicians											x			x				x
224	Chemical technicians									x	x	x	x						
225	Science technicians											x			x				x
356	Mail clerks, except postal clerks											x							
357	Messengers						x												
453	Janitors and cleaners						x			x									
505	Automobile mechanics									x									
519	Machinery maintenance						x												
575	Electricians						x												
585	Plumbers						x					x			x				x
633	Supervisors, production operations	x																	
694	Waste and sewage treatment	x																	
699	Miscellaneous plant and system operators									x	x	x							

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LVI SIC CODE 29 - PETROLEUM REFINING AND RELATED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	anc Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
719	Molding and casting machine operators						x												
753	Cementing and gluing machine operators						x												
754	Packaging and filling machine operators									x									
756	Mixing and blending machine operators						x			x			x						
757	Separating, filtering, and clarifying machine oper											x						x	
759	Painting & paint spraying machine operators						x					x			x				x
766	Furnace, kiln, and oven operators, ex food											x							
769	Slicing and cutting machine operators						x												
777	Misc machine operators	x					x			x		x			x			x	x
783	Welders and cutters						x												
856	Industrial truck and tractor equipment operators									x									
859	Miscellaneous material moving equipment oper																		x
889	Laborers, except construction									x									

TABLE LVII SIC CODE 30 - RUBBER AND MISC. PLASTIC PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
73	Chemists, except biochemists																		x
216	Engineering techicians		x									x						x	x
224	Chemical technicianss		x									x							
335	File clerks											x							
345	Duplicating machine operators									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LVII SIC CODE 30 - RUBBER AND MISC. PLASTIC PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
357	Messengers						x					x						x	
364	Traffic, shipping, and receiveing clerks											x							
365	Strock and inventory clerks									x									
453	Janitors and cleaners	x					x			x		x						x	
519	Machinery maintenance occupations						x					x						x	
549	Mechanics and repairers, ns											x							
575	Electricians						x					x						x	
585	Plumbers, pipefitters, and steamfitters						x					x						x	
633	Production supervisors											x						x	
634	Tool and die makers									x									
653	Sheet metal workers						x					x						x	
684	Miscellaneous precision workers											x							
696	Stationary engineers											x							
705	Milling and planing machine operators		x																
709	Grinding, abrading, buffing & polishing mach oper																	x	
715	Misc metal, plastic, stone & glass workng mach oper											x							
717	Fabricating machine operators											x							
719	Molding and casting machine operators		x				x					x						x	
749	Miscellaneous textile machine operators											x						x	
753	Cementing and gluing machine operators						x					x						x	
755	Extruding and forming machine operators						x												
756	Mixing and blending machine operators		x				x					x						x	x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LVII SIC CODE 30 - RUBBER AND MISC. PLASTIC PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
757	Separating, filtering, & clarifying machine operators											x						x	
759	Painting and paint spraying machine operators		x				x					x						x	x
766	Furnace, kiln, & oven operators, except food	x																	
769	Slicing and cutting machine operators						x					x						x	
777	Miscellaneous machine operators		x				x					x						x	x
783	Welders and cutters						x											x	
785	Assemblers											x						x	
796	Production inspectors, checkers, & examiners		x																
856	Industrial truck and tractor equipment operators											x						x	
859	Misc material moving equipment operators		x									x							
873	Production helpers											x						x	
878	Machine feeders and offbearers											x						x	
883	Freight, stock, and material movers, hand																	x	
888	Hand packers and packagers																		x

TABLE LVIII SIC CODE 31 - LEATHER AND LEATHER PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
368	Weighers, measures, and checkers						x												
453	Janitors and cleaners																	x	
734	Printing machine operators										x								
745	Shoe machine operators											x						x	

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LVIII SIC CODE 31 - LEATHER AND LEATHER PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
753	Cementing and gluing machine operators											x						x	
756	Mixing and blending						x												
759	Paint and paint spraying						x												
769	Slicing and cutting machine operators											x							
777	Miscellaneous machine opererators						x												
785	Assemblers																	x	
795	Miscellaneous hand working occupations						x												
889	Laborers, except construction						x												

TABLE LIX SIC CODE 32 - STONE, CLAY, AND GLASS PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
208	Health technologists and technicians																		x
453	Janitors and cleaners	x					x			x									
516	Heavy equipment mechanics									x									
549	Mechanics and repairers, ns									x									
789	Hand painting, coating, & decorating occupations																	x	

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LX SIC CODE 33 - PRIMARY METAL INDUSTRIES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
55	Electrical and electronic engineers									x									
453	Janitors and clearners	x								x									
505	Automobile mechanics									x									
507	Bus, truck, & stationary engine mechanics									x									
516	Heavy equipment mechanics									x									
518	Industrial machinery repairers	x								x									
519	Machinery maintenance occupations									x									
523	Electronic repairers, communication, & industrial eq									x									
539	Mechanical controls and valve repairers									x									
544	Millwrights									x									
547	Specified mechanics and repairers									x									
549	Mechanics and repairers, ns									x									
563	Brickmasons and stonemasons									x									
575	Electicians	x								x									
585	Plumbers, pipefitters, and steamfitters	x								x								x	
643	Boilermakers									x									
675	Hand molders and shapers	x																	
696	Stationary engineers	x								x									
699	Miscellaneous plant and system operators	x																	
709	Grinding, abrading, buffing, & polishing mach oper						x			x								x	
713	Forging machine operators									x									
719	Molding and casting machine operators									x									
724	Heat treating equipment operators									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LX SIC CODE 33 - PRIMARY METAL INDUSTRIES - OCCUPATIONS WHERE ANC FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
757	Seperating, filtering, & clarifying machine operators	x								x									
759	Paint and paint spraying machine operators									x									x
766	Furnace, kiln, and oven operators, except food	x					x												
769	Slicing and cutting machine operators																	x	
777	Miscellaneous machine operators	x								x									
789	Hand painting, coating, and decorating occupations									x									
849	Crane and tower operators									x									
865	Helpers, construction																	x	
873	Production helpers	x								x									
889	Laborers, except construction									x									

TABLE LXI SIC CODE 34 - FABRICATED METAL PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
56	Industrial engineers																		x
59	Engineers									x				x					
73	Chemists, except biochemists									x		x							
216	Engineering technicians						x			x		x							
225	Science technicians	x																	
364	Traffic, shipping, & receiving clerks									x									
365	Stock and inventory clerks									x									x
453	Janitors and cleaners	x								x									x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXI SIC CODE 34 - FABRICATED METAL PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
507	Bus, truck, and stationary engine mechanics						x			x									
518	Industrial machinery repairers						x			x									
633	Supervisors, production occupations	x					x			x									
634	Tool and die makers									x									
637	Machinists									x									
675	Hand molders and shapers, except jewelers											x							
684	Miscellaneous precision workers									x									
696	Stationary engineers	x																	
703	Lathe and turning machine set-up operators																		x
706	Punching and stamping press machine operators	x							x	x									x
709	Grinding, abrading, buffing, & polishing mach oper											x						x	
713	Forging machine operators								x										
717	Fabricating machine operators									x									
719	Molding and casting machine operators											x							
723	Metal plating machine operators						x			x									x
724	Heat treating equipment operators									x									
725	Miscellaneous metal & plastic processing mach oper									x									
734	Printing machine operators	x																	
749	Miscellaneous textile machine operators																		x
753	Cementing & gluing machine operators												x						
755	Extruding and forming machine operators											x							x
756	Mixing and blending machine operators						x			x									
759	Painting and paint spraying machine operators											x							x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-Cl Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXI SIC CODE 34 - FABRICATED METAL PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
777	Miscellaneous machine operators	x					x			x		x		x					x
783	Welders and cutters									x									x
785	Assemblers								x	x			x						x
796	Production inspectors, checkers, and examiners											x							
799	Graders and sorters, except agricultural									x									
856	Industrial truck and tractor equipment operators																		x
859	Miscellaneous material moving equipment oper									x		x							
873	Production helpers	x																	x
888	Hand packers and packagers																	x	

TABLE LXII SIC CODE 35 - INDUSTRIAL MACHINERY, EXCEPT ELECTRICAL - OCCUPATIONS ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
59	Engineers									x									x
185	Designers									x									
189	Photographers									x								x	
216	Engineering technicians									x									x
235	Technicians ns																	x	
364	Traffic, shipping, and receiving clerks	x																	
453	Janitors and cleaners						x			x	x	x	x						
518	Industrial machine repairers									x									
519	Machinery maintenance occupations									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXII SIC CODE 35 - INDUSTRIAL MACHINERY, EXCEPT ELECTRICAL - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
534	HVAC mechanics									x									
535	Camera, watch, and musical instrument repairers						x												
547	Specified mechanics and repairers									x									
549	Mechanics and repairers, ns												x						
579	Painters, construction and maintenance																		x
585	Plumbers, pipefitters, steamfitters																		x
633	Production supervisors									x									
634	Tool and die makers						x			x									
636	Precision assemblers, metal									x									
637	Machinists						x			x				x				x	
644	Precision grinders, fitters, & tool sharpeners									x									
645	Patternmakers & model makers, metal									x									
683	Electrical and electronic equipment assemblers									x									x
684	Miscellaneous precision workers									x									
704	Lathe and turning machines									x									
705	Milling and planing machine operators									x									
706	Punching and stamp press machine operators									x									
708	Drilling and boring machine operators									x								x	
709	Grinding, abrading, buffing, & polishing mach oper									x								x	
713	Forging machine operators									x									
715	Misc metal, plastic, stone & glass working oper									x									
717	Fabricating machine operators									x									
723	Metal plating machine operators									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXII SIC CODE 35 - INDUSTRIAL MACHINERY, EXCEPT ELECTRICAL - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
724	Heat treating equipment operators									x									
725	Misc metal & plastic processing machine operators									x									
734	Printing machine operators									x									
756	Mixing and blending machine operators						x												
759	Painting and spray painting machine operators						x						x						
766	Furnace, kiln & oven operators, except food									x									
769	Slicing and cutting machine operators									x									
774	Photographic process machine operators									x									
777	Miscellaneous machine operators						x			x								x	
783	Welders and cutters									x									
785	Assemblers	x								x			x					x	x
787	Hand molding, casting, and forming occupations									x									
793	Hand engraving and printing occupations									x									
796	Production inspectors, checkers, & examiners									x				x					
804	Truck drivers, heavy									x									
856	Industrial truck and tractor equipment operators						x												
859	Miscellaneous material moving equipment oper									x									
888	Hand packers and packagers																		x
889	Laborers, except construction									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-Cl Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIII SIC CODE 36 - ELECTRONIC AND OTHER ELECTRICAL EQUIPMENT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
5	Administrators and officials, public administration												x						
189	Photographers						x			x									
213	Electrical and electronic technicians						x			x								x	x
216	Engineering technicians									x									
224	Chemical technicians											x							x
335	File clerks											x							
364	Traffic, shipping and receiving clerks																		x
365	Stock and inventory clerks																	x	
453	Janitors and cleaners						x			x		x							x
507	Bus, truck and stationary engine mechanics									x									
519	Machinery maintenance occupations									x									x
534	HVAC mechanics									x									
535	Camera, watch, & musical instrument repairers									x									
549	Mechanics and repairers, ns									x									
585	Plumbers, pipefitters, and steamfitters									x									
633	Production supervisors						x												
634	Tool and die makers									x									
637	Machinists						x			x									
675	Hand molders and shapers									x									x
683	Electrical and electronic equipment assemblers									x									x
689	Inspectors, testers, and graders						x												x
696	Stationary engineers	x								x									
703	Lathe and turning machine operators											x							x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIII SIC CODE 36 - ELECTRONIC AND OTHER ELECTRICAL EQUIPMENT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONT)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
706	Punching and stamping machine operators											x							
709	Grinding, abrading, buffing, & polishing mach oper									x									x
719	Molding and casting machine operators									x		x							
723	Metal plating machine operators						x			x									
734	Printing machine operators						x			x									
755	Extruding and forming machine operators																		x
756	Mixing and blending machine operators						x			x									x
759	Painting and paint spraying operators																		x
766	Furnace, kiln, and oven operators, except food									x		x							
768	Crushing and grinding machine operators																		x
774	Photographic process machine operators						x			x									
777	Miscellaneous machine operators	x					x			x		x							
779	Machine operators, ns						x												
783	Welders and cutters									x									
784	Solderers and brazers																		x
785	Assemblers						x			x			x					x	x
796	Production inspectors, checkers, and examiners						x			x									
797	Production testers																		x
859	Misc material moving equipment operators											x							

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIV SIC CODE 37 - TRANSPORTATION EQUIPMENT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
44	Aerospace engineers					x				x									
57	Mechanical engineers						x												
215	Mechanical enginering technicians						x												
216	Engineering technicians									x		x							x
224	Chemical technicians									x									
225	Science technicians						x			x									
363	Production coordinators									x									
364	Traffic, shipping, and receiving clerks						x												
365	Stock and inventory clerks						x			x									
453	Janitors and cleaners						x			x								x	x
503	Supervisors, mechanics and repairers																	x	
508	Aircraft engine mechanics						x												
514	Auto body and related repairers																	x	
518	Industrial machinery repairers						x			x									
519	Machinery maintenance occupations									x									
575	Electricians									x								x	
579	Painters, construction and maintenance									x									x
633	Production supervisors																	x	
634	Tool and die makers									x									x
637	Machinists						x			x									
649	Engravers		x																
653	Sheet metal workers		x																
668	Upholsterers																		x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-Cl Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIV SIC CODE 37 - TRANSPORTATION EQUIPMENT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONT)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
684	Miscellaneous precision workers									x									
689	Inspectors, testers, graders									x									
695	Power plant operators						x			x									
703	Lathe and turning machine set-up operators									x			x						
704	Lathe and turning machine operators									x									
705	Milling and planing machine operators									x									
706	Punching and stamping press machine operators		x				x					x							
708	Drilling and boring machine operators									x									
709	Grinding, abrading, buffing, & polishing mach oper						x			x								x	
715	Misc metal, plastic, stone & glass working oper									x									
717	Fabricating machine operators									x									
719	Molding and casting machine operators	x																	
723	Metal plating machine operators						x												
724	Heat treating equipment operators						x			x									
744	Textile sewing machine operators												x						
753	Cementing and gluing machine operators																	x	
755	Extruding and forming machine operators																		x
756	Mixing and blending machine operators									x									
759	Painting and paint spraying machine operators									x									
769	Slicing and cutting machine operators									x									
774	Photographic process machine operators						x			x	x								
777	Miscellaneous machine operators		x				x			x		x	x	x					
785	Assemblers						x			x								x	x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIV SIC CODE 37 - TRANSPORTATION EQUIPMENT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONT)

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
789	Hand painting, coating, and decorating occupations									x									
796	Production inspectors, checkers and examiners	x								x								x	
856	Industrial truck and tractor equipment operators						x												
873	Production helpers												x						x
888	Hand packers and packagers						x												
889	Laborers, except construction						x											x	

TABLE LXV SIC CODE 38 - INSTRUMENTS AND RELATED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
73	Chemists, except biochemists									x									
208	Health technologists and technicians												x						
235	Technicians		x				x			x		x	x					x	
345	Duplicating machine operators						x												
453	Janitors and cleaners						x					x							
634	Tool and die makers									x									
637	Machinists									x									
644	Precision grinders, fitters, & tool sharpeners									x									
719	Molding and casting machine operators									x		x							
723	Metal plating machine operators									x									
755	Extruding and forming machine operators											x							
756	Mixing and blending machine operators						x			x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXV SIC CODE 38 - INSTRUMENTS AND RELATED PRODUCTS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
759	Painting and paint spraying machine operators																		x
777	Miscellaneous machine operators						x			x									
785	Assemblers						x			x									
796	Production inspectors, checkers, and examiners									x									
859	Misc material moving equipment operators											x							
888	Hand packers and packagers		x																
889	Laborers, except construction									x									

TABLE LXVI SIC CODE 39 - MISCELLANEOUS MANUFACTURING INDUSTRIES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19	Managers and administrators																		x
365	Stock and inventory clerks									x									
453	Janitors and cleaners						x			x								x	x
518	Industrial machine repairers									x									
633	Production supervisors									x								x	x
634	Tool and die makers									x									
637	Machinists									x									
696	Stationary engineers																		x
719	Molding and casting									x									x
723	Metal plating machine operators						x			x									
753	Cementing and gluing machine operators																		x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXVI SIC CODE 39 - MISCELLANEOUS MANUFACTURING INDUSTRIES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONT)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
756	Mixing and blending machine operators																		x
758	Compressing & compacting machine operators																	x	x
759	Painting and spay painting machine operators									x									x
766	Furnace, kiln, & oven operators, except food									x									
777	Miscellaneous machine operators																	x	x
785	Assemblers		x							x									x

TABLE LXVII SIC CODE 40 - RAILROAD TRANSPORTATION - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
637	Machinists						x												

TABLE LXVIII SIC CODE 41 - LOCAL AND SUBURBAN PASSENGER TRANSIT - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
365	Stock and inventory clerks						x												
453	Janitors and cleaners									x									
505	Automobile mechanics									x									
507	Bus, truck, and stationary engine mechanics						x												

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXIX SIC CODE 42 - MOTOR FREIGHT TRANSPORTATION AND WAREHOUSING - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
364	Traffic, shipping, and receiving clerks																	x	
453	Janitors and cleaners	x								x									
507	Bus, truck & stationary engine mechanics	x								x									
547	Specified mechanics and repairers									x									
723	Metal plating machine operators																		x
734	Printing machine operators										x								

TABLE LXX SIC CODE 44 - WATER TRANSPORTATION - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
453	Janitors and cleaners									x									
549	Mechanics and repairers, ns																		x
759	Painting and paint spraying machine operators																		x

TABLE LXXI SIC CODE 45 - TRANSPORTATION BY AIR - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
235	Technicians									x									
453	Janitors and cleaners									x									
505	Automobile mechanics	x								x									
508	Aircraft engine mechanics									x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXI SIC CODE 45 - TRANSPORTATION BY AIR - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
515	Aircraft mechanics, except engine									x									
518	Industrial machine repairers									x									
547	Specified mechanics and repairers									x									x
637	Machinists									x									
653	Sheet metal workers									x									
689	Inspectors, testers, and graders									x									
709	Grinding, abrading, buffing, & polishing mach oper									x									
759	Painting and spray painting machine operators									x									
785	Assemblers									x									
797	Production testers									x									
889	Laborers, except construction									x				x					

TABLE LXXII SIC CODE 48 - COMMUNICATIONS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
529	Telephone installers and repairers						x												

TABLE LXXIII SIC CODE 49 - ELECTRIC, GAS AND SANITARY SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
453	Janitors and cleaners		x				x			x									

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXIII SIC CODE 49 - ELECTRIC, GAS AND SANITARY SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
549	Mechanics and repairers, ns									x									
575	Electricians						x												
577	Electrical power installers and repairers											x							
694	Water and sewage treatment plant operators	x																	
696	Stationary engineers									x									
777	Miscellaneous machine operators									x		x							
869	Construction laborers											x							

TABLE LXXIV SIC CODE 50 - WHOLESALE TRADE - DURABLE GOODS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19	Managers and administrators																		x
313	Secretaries																		x
505	Automobile mechanics									x									
549	Mechanics and repairers, ns						x												
704	Lathe and turning machine operators						x												
756	Mixing and blending machine operators											x							
769	Slicing and cutting machine operators						x												
783	Welders and cutters						x												

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXV SIC CODE 55 - AUTOMOTIVE DEALERS AND SERVICE STATIONS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
243	Supervisors & proprietors, sales occupations									x									
505	Auto mechanics									x		x							
804	Truck drivers, heavy									x									

TABLE LXXVI SIC CODE 72 - PERSONAL SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
19	Managers and administrartors						x												
274	Sales workers, other commodities						x												
747	Pressing machine operators						x												
748	Laundering and dry cleaning machine operators						x			x									
774	Photographic process machine operators						x												
796	Production inspectors, checkers, and examiners						x												
889	Laborers, except construction						x												

TABLE LXXVII SIC CODE 73 - BUSINESS SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
73	Chemists, except biochemists	x	x		x		x				x	x						x	x
75	Geologists & geodesists	x					x				x	x							
78	Biological and life scientists				x			x									x		x

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXVII SIC CODE 73 - BUSINESS SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
83	Medical scientists																x		
213	Electrical and electronic technicians									x									
216	Engineering technicians	x					x					x							x
224	Chemical technicians		x		x														
225	Science technicians	x																	
319	Receptionists									x									
364	Traffic, shipping, and receiving clerks									x									
453	Janitors and cleaners	x																x	x
633	Production supervisors									x									
677	Optical goods workers									x									
736	Typesetters and compositors						x												
774	Photographic process machine operators									x								x	
777	Miscellaneous machine operators									x	x								

TABLE LXXVIII SIC CODE 75 - AUTOMOTIVE REPAIR, SERVICES, AND PARKING - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
505	Automobile mechanics									x									x
549	Mechanics and repairers, ns									x									
637	Machinists		x									x							

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-Cl Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXIX SIC CODE 76 - MISCELLANEOUS REPAIR SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
505	Automobile mechanics									x									

TABLE LXXX SIC CODE 80 - HEALTH SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
15	Managers, medicine and health						x												
19	Managers and administrators	x																	
27	Personnel, training, and labor relations specialists						x												
33	Purchasing agents and buyers						x												
73	Chemists, except biochemists	x																	
78	Biological and life scientists				x		x												
83	Medical scientists						x												
84	Physicians						x			x			x						
58	Dentists												x						
95	RNs		x				x					x	x					x	
96	Pharmacists						x						x						
98	Inhalation therapists						x												
103	Physical therapists						x						x						
106	Physician's assistants												x						
189	Photographers									x									
203	Clinical laboratory technologists & technicians	x		x	x		x			x			x			x			
204	Dental hygienists									x			x						

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXX SIC CODE 80 - HEALTH SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
206	Radiologic technicians						x			x									
207	LPNs						x					x	x						
208	Health technologists and technicians						x					x	x						
224	Chemical technicians	x																	
235	Technicians	x					x												
303	Supervisors, general office												x						
319	Receptionists						x												
365	Stock and inventory clerks						x												
379	General office clerks						x												
389	Administrative support occupations						x												
426	Guards and police, except public service									x									
445	Dental assistants						x			x									
446	Health aides, except nursing						x						x						
447	Nursing aides, orderlies, and attendants		x				x						x						
449	Maids and housemen						x												
453	Janitors and cleaners	x	x				x			x	x	x							
549	Mechanics and repairers, ns									x									
575	Electricians											x							
579	Painters, construction and maintenance																	x	
633	Production supervisors						x												
643	Boilermakers									x									
678	Dental laboratory & medical appliance technicians						x						x						

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

TABLE LXXX SIC CODE 80 - HEALTH SERVICES - OCCUPATIONS WHERE ANCS FOUND BY NIOSH (CONTINUED)

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
696	Stationary engineers									x									
734	Printing machine operators										x								
748	Laundering and dry cleaning machine operators						x												
766	Furnace, kiln, & oven operators, except food	x					x			x	x								
774	Photographic process machine operators									x									
777	Miscellaneous machine operators						x			x									
877	Stock handlers and baggers						x												

TABLE LXXXI SIC CODE 84 - MUSEUMS, BOTANICAL, ZOOLOGICAL GARDENS - OCCUPATIONS WHERE ANCS FOUND BY NIOSH

Census Code	Occupation	ANC Number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
734	Printing machine operators										x								

Legend: 1-Acrylamide; 2-Acrylontrile; 3-Bis (chloromethyl) ether; 4-Dimethyl sulfate; 5-Divinyl benzene; 6-Ethylene oxide; 7-N-2-Fluorenylacetamide; 8-Glycidol; 9-1-H-Benzotriazole; 10-Bromoethane; 11-1,3-butadiene; 12-Chloroethane; 13-CI Direct Blue; 14-3,3'-Dimethylbenzidine dihydrochloride; 15-3,3'Dimethoxybenzidine dihydrochloride; 16-Ethyl methyl sulfonate; 17-Isoprene; 18-Vinyl chloride

APPENDIX C

TABLE LXXXII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTIONS ONE AND TWO

Question 1. Since the age of 16, did you ever work for 6 months or more? We are interested in every part-time (between 16 and 34 hours per week) or full-time (35 or more hours per week) job, whether paid or unpaid, that you have held for 6 months or longer.

Question 2. Please check the industries at which you have been employed throughout your life. Please check all that apply and indicate for each the age you started that job, how many years worked at that job, and briefly describe your job title and task(s) you performed.

SIC	Acryl	Diene	PAH	Variable	*Question #*	*Chemical(s)*
1	1	1	0	O2_5ck4	Agriculture	Acrylamide, Ethylene Oxicide, Bromoethane, Chloroethane, Dimethyl sulfide, Nitroso compounds, Propane sultone, 1,3-Butadiene, 1-H-Benzotriazole, Glycidol, p-Cresidine, Acrylonitrile, Methyl methane sulfonate, Cyclophosphamide, Isoprene
2	0	0	0	O2_4ck2	Fishing	Nitroso compounds, Ethylene oxide
8	1	1	1	O2_4ck3	Forestry	Ethylene oxide, Acrylamide, Benzo(a)pyrene, Isoprene, 1,3-Butadiene
10	1	0	0	O2_3ck6	Ore processing	Acrylamide
13	1	0	1	O2_2ck6	Oil shale exploration	Acrylamide, Benzo(a)pyrene, Ethylene oxide, 1-H-Benzotriazole
14	1	0	1	O2_3ck1	Mineral processing / mining	Acrylamide, Polycyclic Aromatic Hydrocarbons
17	0	0	0	O2_7ck4	Air conditioning/ Cooling	1-H-Benzotriazole
20	0	0	1	O2_5ck6	Food Smoking	Benzo(a)pyrene
20	1	1	0	O2_5ck5	Food packaging	Acrylonitrile, 1,3-Butadiene, Acrylamide, Vinyl chloride
20	1	0	0	O2_5ck8	Food processing	Nitroso compounds, Acrylamide, Acrylonitrile, Ethylene oxide, Diphenhydramine hydrochloride, 1-H-Benzotrialzole, C I Direct Blue 15
22	1	0	0	O2_5ck1	Weaving or fabric mill	Nitroso compounds, Acrylamide
22	0	0	0	O2_2ck3	Acrylic Fiber	Acrylonitrile

TABLE LXXXII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTIONS ONE AND TWO (CONTINUED)

SIC	Acryl	Diene	PAH	Variable	*Question #*	*Chemical(s)*
26	1	0	0	O2_4ck4	Pulp and paper	Propalene imine, Acrylamide, Ethylene oxide, 3, 3'-Dimethylbenzidiene dihydrochloride, 1-H-Benzotriazole, Isoprene
26	0	0	0	O4_4ck5	Paper dying	C I Direct Blue 15, 3,3'-dimethoxybenzidine dihydrochloride, 3,3'-dimthylbenzidine dihydrochloride, Nitroso compounds, Dimethyl sulfide
27	1	0	0	O2_5ck3	Printing / Publishing	Nitroso compounds, Bromoethane, Chloroethane, 1-H-Benzotriazole, Ethylene oxide, Acrylamide
28	1	0	0	O2ack2	Inorganic Chemical	Acrylonitrile, Acrylamide
28	0	0	0	O2_2ck4	Resins	Propylene imine, Acrylonitrile, 3,3'-dimethybenzidine dihydrochloride, p-Cresidine
28	0	0	0	O2_1ck4	Cleaning product manufacture	Nitroso compounds, Dimethyl sulfide, Propane sultone
28	1	0	0	O2_5ck2	Dye packaging and processing	Nitroso compounds, 3,3'Dimethylbenzidine dihydrochloride, C I Direct Blue 15, Acrylamide, Propane sultone, Dimethyl sulfide, Acrylonitrile, 3,3'-Dimethoxybenzidine dihydrochloride, p-Cresidine, Glycidol, Chloroethane
28	1	0	0	O2ck8	Chemical industry (general)	Nitroso compounds, Ethylene oxide, Vinyl chloride, N-acetyl-2-acetylaminofluorene, Bromoethane, Chloroethane, Bis(chloromethyl)ether, Propylene iminel, Acrylamide, Acrylonitrile, Propane sultone, Ethylene oxide, Diphenyhdroamine hydrochloride, Isoprene, 1-H-Benzotriazole, 3,3'Diemthylbenzidine dihydrochloride, Methyl methane sulfonate, 1,3-Butadiene, Ethyl methyl sulfonate, Azoethane, 1-Phenyl 3,3 dimethyltriazene, Lead subacetate
28	0	0	0	O2_7ck2	Pharmaceuticals	Nitroso compounds, Propylene imine, Bromoethane, Chloroethane, Glycidol, Propane sultone, Dimethyl sulfide, Ethylene oxide, C I Direct Blue 15, Elaiomycin, Acrylonitrile, Cyclophosphamide, Procarbazine hydrochloride, Diphenhydromine hydrochloride, 1-Phenyl-3,3-dimethyltriazene

TABLE LXXXII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTIONS ONE AND TWO (CONTINUED)

SIC	Acryl	Diene	PAH	Variable	*Question #*	*Chemical(s)*
28	1	0	0	O2ack1	Organic Chemical Synthesis	1-H-Benzotriazole, Propane sultone, Acrylonitrile, Vinyl chloride, Dimethyl sulfide, Chloroethane, Acrylamide, Bromoethane, Azoethane
29	1	1	1	O2ck4	Petrochemical	1,3-Butadiene, Vinyl chloride, Benzo(a)pyrene, 3 and 20-Methylcholanthrene, Chloroethane, Acrylamide, Ethylene oxide, Bromoethane, Isoprene, 1-H-Benzotriazole, 3,3'-Dimethylbenzidine dihydrochloride, 1-Phenyl-33 dimethyltriazene
29	0	0	1	O2ck5	Asphalt	Benzo(a)pyrene, Polycyclic aromatic hydrocarbons
30	0	1	1	O2_2ck1	Rubber	Propylene imine, Nitroso compounds, Ethylene oxide, Acrylonitrile, 1-H-Benzotriazole, 3,3'-Dimethoxybenzidine dihydrochloride, Chloroethane, 1,3-Butadiene, Benzo(a)pyrene, Bis(chloromethyl)ether
30	1	1	0	O2_1ck5	PVC or VC processing	Vinyl chloride, Ethylene oxide, Acrylamide, Acrylonitrile, 1,3-Butadiene, 1-H-Benzotriazole, Chlorethane
31	0	0	0	O2_2ck5	Leather Tanning	Nitroso compounds
31	0	0	0	O2_2ck2	Leather	Nitroso compounds, Ethylene oxide, Bromoethane
33	1	0	1	O2_3ck2	Steel making	Acrylamide, Propane sultone, Polycyclic aromatic hydrocarbons
33	0	0	1	O2_3ck3	Coking	Polycylic aromatic hydrocarbons
33	0	0	1	O2_3ck4	Aluminum production / wood preservation	Chloroethane, Polycyclic aromatic hydrocarbons
34	1	0	0	O2_3ck5	Fabricated metal	Nitroso compounds, Ethylene oxide, Acrylamide, Glycidol, Chloroethane, C I Direct Blue 15, 1-H-Benzotriazole
35	1	0	1	O2ck1	Engine manufacturing	Bromoethane, Chloroethane, Polycylic aromatic hydrocarbons, Nitroso compounds, Acrylonitrile, Vinyl chloride, Acrylamide
38	0	0	0	O2ck6	Aerospace	Acrylonitrile
49	0	0	1	O2ck2	Coal fired utility	Benzo(a)pyrene, 3 and 20 Methylcholanthrene
49	0	0	0	O2ck3	Energy production	Dimethyl sulfide
49	1	0	0	O2_6ck2	Sewage treatment	Nitroso compounds, Chloroethane, Acrylamide, 3,3'-Dimethylbenzidine dihydrochloride

TABLE LXXXII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTIONS ONE AND TWO (CONTINUED)

SIC	Acryl	Diene	PAH	Variable	*Question #*	*Chemical(s)*
49	0	0	0	O2_6ck3	Water and wastewater treatment	Methyl methane sulfate, Bis(chloromethyl)ether, Dimethyl sulfide
49	1	0	0	O2_6ck4	Waste management/ incineration OR hazardous waste management	Nitroso compounds, Chloroethane, 1,3-Butadiene, Acrylonitrile, Bromoethane, Polycylic aromatic hydrocarbons, Vinyl chloride, Acrylamide, Lead subacetate, Ethylene oxide, Propane sultone, Propylene imine, Dimethyl sulfide, C I Direct Blue 15, 3,3'-Dimethylbenzidine dihydrochloride, 3,3'-domethoxybenzidine dihydrochloride, 2-Acetylaminofluorene, Chloroethane

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE

Question: O3. Please check all occupations that apply to you from the list given below, including the jobs that you performed as a teenager. Please check all that apply and indicate for each the age you started that job, how many years worked at that job, and describe how often you wear or wore personal protective equipment (respirators, masks, coveralls).

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
11	1	0	0	O3_8ck4	Executive	Ethylene oxide, Acrylamide, Chloroethane, Furosemide, 1-H-Benzotriazole
19	1	1	0	O3_3ck3	Chemist/Biochemist / Chemical laboratory technician	Nitroso Compounds, Etylene oxide, Vinyl chloride, N-acetyl-2-acetylaminofluorene, Bromoethane, Chloroethane, Bis(chloromethyl)ether, Propylene imine, Acrylamide, Acrylonitrile, Propane Sultone, Ethylene oxide, Glycidol, Diphenhydramine hydrochloride, Isoprene, 1-H Benzotriazole, 3,3'-Dimethylbenzidine dihydrochloride, 1,3-Butadiene, Azoethane, Lead subacetate, Methyl methane sulfonate, Ethyl methyl sufonate, 1-Phenyl 3,3 dimethyltriazene
27	1	1	0	O3_7ack4	Artist Painting	Lead subacetate, Chloroethane, Acrylamide, Propylene imine, 1,3-Butadiene
27	1	0	0	O3_7ack1	Artist using Fabric/Textile	CI Direct Blue 15 15, 3,3'-Dimethylbenzidine dihydrochloride, 3,3'-Dimethoxybenzidine dihydrochloride, Nitroso compounds, Vinyl chloride, Glycidol, Acrylonitrile, Acrylamide, Dimethyl sulfate, Ethylene oxide, Chloroethane, Propane sultone, p-Cresidine
27	1	0	0	O3_7ack3	Artist - ceramics	Acrylamide
27	0	0	0	O3_6ck5	Photographer involved with developing his/her films or any other film developer	1-H-Benzotriazole, Glycidol, 3,3'-Dimethylbenzidine dihydrochloride, C I Direct Blue 15
27	0	0	0	O3_6ck6	Cinematographer	C I Direct Blue 15
27	0	0	0	O3_7ack2	Artist using Metal	Chloroethane, 1-H-Benzotriazole, Propane sultone

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE (CONTINUED)

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
29	0	0	0	O3_4ck1	Physician/Surgeon/Dentist /Nurse/Hospital Aid/Hospice Worker/Dental Hygienist and Assistants	Ethylene oxide, Chloroethane, Diphenhydramine hydrochloride
33	0	0	1	O3ck2	Soldier in a war or combat operation	Dimethyl sulfate, Polycyclic aromatic hydrocarbons
33	1	0	0	O3ck4	Police/ agent working with explosives	Nitroso compounds, Acrylamide
35	1	1	1	O3_8ck2	Cook / Restaurant server/ hostess/ busperson	PAHs, Isoprene, Acrylamide, Nitroso compounds, 1,3-Butadiene
35	1	0	0	O3_4ck6	Worker who chemically washes/peels fruits and vegetables	Acrylamide
37	1	1	0	O3_5ck3	Lawn maintenance worker	Nitroso compounds, Propane sultone, Ethylene oxide, 1-H-Benzotriazole, Methyl methane sulfonate, Cyclophosphamide, 1,3-Butaidene, Acrylonitrile, Glycidol, Acrylamide, Bromoethane, Chloroethane, p-Cresidine, Dimethyl sulfate, Isoprene
37	1	1	0	O3_5ck4	Exterminator	Nitroso compounds, Propane sulfone, Ehylene oxide, Bromoethane, Chloroethane, Dimethyl sulfate, Isoprene, Glycidol, p-Cresidine, Acrylonitrile, 1,3-Butadiene, Methyl methane sulfonate, Cyclophosphamide, Acrylamide
37	0	0	1	O3_4ck3	Cleaning personnel in a hospital/clinic/cancer research center	Cyclophosphamide, Procarbazine Hydrochloride, Bischloroethylnitrosourea, Nitroso compounds, Methyl methane sulfonate, Glycidol, Ethylene oxide, Ethyl methyl sulfonate, Elaiomycin, Polyclic aromatic hdrocarbons, Propylene imine

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE (CONTINUED)

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
37	0	0	0	O3_3ck4	Personnel involved with sterilization of equipment/devices/instruments/materials OR Fumigator of products (e.g., spices, bedding, clothing, animal feed, foods, fur) OR Beekeper	Ethylene oxide
37	0	0	0	O3_5ck7	Cleaning service provider including maid, janitor	Propane sultone, Chloroethane, Nitroso compounds, Dimethyl sulfate
39	1	0	0	O3_7ck2	Makeup Artist	Nitroso compounds, Acrylamide, Vinyl chloride, Acrylonitrile, Etylene oxide, C I Direct Blue 15
39	1	0	0	O3_7ck3	Barber / Hair dresser / hair colorist/stylist	Nitroso compounds, Acrylamide
43	0	0	0	O3_8ck3	Administrative Support	Bromoethane, Chloroethane, Diphenhydramine hydrochloride, 1-H-Benzotriazole, 3,3'-Dimethylbenzidine hydrochloride, Ethylene oxide
45	1	0	0	O3_4ck5	Farmer / Farm worker	Nitroso compounds, Ethylene oxide, Isoprene, Bromoethane, Chloroethane, Dimethyl sulfide, Glycidol, p-Cresidine, Acrylonitrile, Acrylamide
45	0	0	0	O3_4ck7	Sheep shearer (chemical)	Cyclophosphamide
45	0	0	0	O3_5ck1	Fisherman	Ethylene oxide, Bromoethane
47	1	1	1	O3_1ck2	Construction worker / any job spent on construction site	Polycyclic aromatic hydrocarbons, Nitroso compounds, 1,3-Butadiene, Isoprene, Acrylonitrile, Acrylamide, Chloroethane, Ethylene oxide, Vinyl chloride

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE (CONTINUED)

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
47	0	1	1	O3_1ck1	Toll-booth attendant/ C.S. at drive through window/ Taxi/Bus driver/ Mail carrier/ courier/ Transportation and Material moving/ Truck driver	Polycyclic aromatic hydrocarbons, Nitroso compounds, 1,3-Butadiene, Isoprene, Ethylene oxide
47	1	1	0	O3_1ck4	Plumber/Pipe fitter	Chloroethane, Acrylamide, Vinyl chloride, 1,3-Butadiene
47	1	1	0	O3_6ck7	Painter (houses, cars, etc.)	Acrylamide, Propylene imine, Chloroethane, 1,3-Butadiene, Chloroethane, Lead subacetate
47	0	0	1	O3_6ck3	Roofer	Benzo(a)pyrene, Polycyclic aromatic hydrocarbons, Chloroethane
47	1	0	0	O3_1ck3	Worker involved with pipe grouting and sealing OR grouter	Acrylamide
47	0	0	0	O3_1ck5	Floor installer/ window installer	Vinyl chloride
49	1	1	1	O3ck5	Auto mechanic	Polycyclic aromatic hydrocarbons, Nitroso compounds, Chloroethane, 1,3-Butadiene, Isoprene, Acrylonitrile, Vinyl chloride, Acrylamide, Ethylene oxide, Dimethyl sulfate, 1-H-Benzotriazole, Isoprene
49	0	0	1	O3_6ck2	Chimney sweeper	Benzo(a)pyrene
49	1	0	0	O3_2ck4	Precision Production and Repair	Acrylamide, Acrylonitrile, Ethylene oxide, Glycidol
49	0	0	0	O3_1ck7	Cable installer	Vinyl chloride, Furosidimide
49	0	0	0	O3_2ack2	Airplane Maintenance Personnel/ Airplane mechanic	1-H-Benzotriazole, Glycidol

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE (CONTINUED)

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
49	0	0	0	O3_2ck5	Air conditioning maintenance personnel	1-H-Benzotriazole
49	0	0	0	O3_3ck7	Swimming pool service worker	3,3'-Dimethylbenzidine dihydrochloride
51	1	1	0	O3_2ck2	Fabricator/Welder of PVC products	Acrylamide, Acrylonitrile, Ethylene Oxide, Vinyl chloride, 1,3-Butadiene
51	0	0	1	O3_1ck8	Blacksmith	Benzo(a)pyrene
51	0	0	1	O3_5ck5	Worker involved with maturation of tobacco leaves	Ethylene oxide, Polycyclic aromatic hydrocarbons
51	0	0	1	O3_5ck6	Wood treatment worker (Ex: thermal-dip treatment, pressure-treatment)	Polycyclic aromatic hydrocarbons
51	1	0	0	O3_4ck4	Laundry or dry cleaning machine operator	Chloroethane, Nitroso compounds, Acrylamide, Glycidol, Vinyl chloride, Propane sultone, Bis(chloromethyl)ether
51	1	0	0	O3_6ck8	Textile treatment worker	Vinyl chloride, Bis(chloromethyl)ether, Acrylamide, Glycidol, C I Direct Blue 15, Propane sultone, 3,3'-Dimethylbenzidine, 3,3'-Dimethoxybenzidine, Nitroso compounds, p-Cresidine, Acrylonitrile
51	0	0	0	O3_2ck3	Machinist	Ethylene oxide, Bromoethane, Chloroethane, C I Direct Blue 15, Diphenhydramine hydrochloride, Isoprene, 1-H-Benzotriazole,3,3'-Dimethylbenzidine dihydrochloride
51	0	0	0	O3_4ck2	Launderer at a hospital or clinic	Cyclophosphamide, Procarbazine hydrochloride, Bischloroethylnitrosourea, Nitroso compounds, Propane sultone, Ethylene oxide

TABLE LXXXIII - OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION THREE (CONTINUED)

SOC	Acry	1,3-But	PAH	VarName	*Question #*	*Chemical(s)*
51	0	0	0	O3_7ck1	Paper/ Textile dyer / Textile sewing machine operator	C I Direct Blue 15, 3,3'Diomethylbenzidine dihydrochloride, Nitroso compounds, 3,3'-Dimethoxybenzidine dihydrochloride, Propane sultone
53	0	1	1	O3ck6	Gas station attendant	Polycyclic aromatic hydrocarbons, Nitroso compounds, Chloroethane, 1,3-Butadiene, Isoprene, Ethylene oxide, Bromoethane
53	0	0	1	O3_2ack3	Outdoor airport gate personnel directing planes to gate	1-H-Benzotriazole, Polyclic aromatic hydrocarbons
53	0	0	0	O3_2ack1	Airplane pilot/Flight attendent	1-H-Benzotriazole
53	0	0	0	O3_5ck2	Sailor/Cruise ship crew/ or any other personnel working out on the sea in a ship	Bromoethane

TABLE LXXXIV OCCUPATIONAL HISTORY QUESTIONNAIRE - QUESTION SIX

O6. Were/have you been exposed to any of the following during your work day? Please check all that apply and indicate for each the age you started that job, how many years worked at that job, and how often you were exposed. Please briefly describe your job title and task(s) you performed for each.)

Acry	Diene	PAH		*Question #*	*Chemical(s)*
0	1	1	O6ck1	Gasoline exhaust	1,3-Butadiene, Polycyclic aromatic hydrocarbons, Isoprene, Ethylene oxide, Nitroso Compounds
0	0	1	O6ck2	Diesel exhaust	Polycyclic aromatic hydrocarbons, Nitroso compounds
0	0	0	O6ck3	Turbine engine exhaust (airplane/jet engine)	Isoprene
0	1	1	O6ck4	Emissions from coal/oil/wood burning	Polycyclic aeromatic hydrocarbons, Isoprene, Chloroethane, 1,3-Butadiene
0	0	0	O6ck5	Chemicals used as pesticides, herbicides, insecticides, fungicides to control fleas, roaches, ants, or other insects or for lawncare and weed control	Dimethyl sulfide, Bromoethane, Methyl methane sulfonate, Cyclophosphamide, Ethylene oxide, Acrylonitrile, Chloroethane, p-Cresidine, Nitroso compounds
0	0	1	O6ck6	Dust	Polycyclic aromatic hydrocarbons, C I Direct Blue 15, 3,3'-Dimethylbenzidine dihydrochloride, 3,3'-Dimethoxybenzidine dihydrochloride, N-acetyl-2-acetylaminofluorene,

APPENDIX D

TABLE LXXXV – DATA TABLE FOR HEMOGLOBIN ADDUCT STUDY

Gender	Age	Smoke	Industry/Occupation	UIC #	CDC#	AA	GA	pyr-val	SIC Code	Acryl	Diene
M	71	No	Retired	21050	75	30.2	29.4	0.59	0	0	0
F	73	No	Retired	21051	64	35.6	67.8	0.64	0	0	0
M	56	No	Software Engineer	22146	14	380	117706.0	0.54	73	1	1
F	58	Yes	Customer Service Airlines	22152	19	113	106.0	1.99	47	0	0
F	50	No	Clinical Research Asst	22184	61	41.2	85.4	0.49	80	1	1
F	71	No	Retired	22273	25	58.2	73.8	2.19	0	0	0
F	50	No	Retired	22301	60	44.2	143.0	1.16	0	0	0
F	66	No	Retired	22332	22	62.7	44.2	0.84	0	0	0
F	53	No	Pottery Glazer	22386	65	22.3	40.5	1.45	89	0	0
F	51	No	Retired	22396	74	32.7	42.9	1.06	0	0	0
F	81	No	Retired	22406	29	36.6	68.1	0.43	0	0	0
M	43	No	Fire Fighter	22417	21	42.7	56.6	0.41	92	0	0
F	26	No	Biomedical Engineer	22438	78	32.9	33.2	0.47	87	0	0
M	38	No	Computer Technical Support - Phones	22462	18	26.8	47.0	0.68	73	1	1
F	24	No	RA-Breast CA Study	22501	67	47.4	56.7	2.93	80	1	1
M	45	No	Software Engineer	22503	62	42.7	339.0	0.98	73	1	1
F	32	No	Research Cord	22505	26	36.4	421.0	1.53	80	1	1
F	85	No	Retired	22521	16	44.1	85.2	2.43	0	0	0
F	43	No	Clinical Research Coord-Acryl Gels	22526	15	33.3	45.5	1.44	80	1	1
M	36	No	Software Engineer	22534	63	32.3	225.0	0.81	73	1	1
M	55	Yes	Home Repair	22550	23	96.6	71.4	0.50	15	0	0
F	50	No	Diagnostic Clinic Mgr	22557	72	25.8	31.1	3.14	80	1	1
F	79	No	Retired	22571	24	27.3	38.6	1.33	0	0	0
F	62	No	Retired	22581	66	41.3	57.3	0.23	0	0	0
M	66	No	Teacher	22587	13	22.9	55.9	0.92	82	0	0
F	63	No	Retired	22610	71	30.7	62.8	2.02	0	0	0
F	33	No	Retired	22611	73	46.5	58.8	2.16	0	0	0
F	34	No	Physical Therapist	22614	27	42.9	72.3	1.20	80	1	1
F	83	No	Retired	22617	76	24.2	18.2	0.47	0	0	0
F	64	Yes	Cancer Info Ctr-Admin	22622	70	91.9	79.0	1.55	80	1	1
F	52	No	Nurse	22635	59	27.4	377.0	0.93	80	1	1
F	23	No	Acct-Publishing	22640	69	99.1	138.0	1.30	27	0	0
F	56	No	Retired	22651	77	25.6	*	0.54	0	0	0
F	27	No	Grants Assoc-Med Ctr	22652	68	29.3	43.8	0.61	80	1	1
M	83	No	Retired	22654	28	29.6	90.6	1.61	0	0	0
M	56	No	Sales - Trucking	30024	2	34.2	67.0	1.11	42	1	0
F	45	No	Maintenance	30025	56	42.2	79.9	1.26	86	0	0

*Insufficient Sample

TABLE LXXXV – DATA TABLE FOR HEMOGLOBIN ADDUCT STUDY (CONTINUED)

Gender	Age	Smoke	Industry/Occupation	UIC #	CDC#	AA	GA	pyr-val	SIC Code	Acryl	Diene
F	43	No	Healthcare Consulting	30089	38	50.1	83.0	0.67	73	1	1
F	31	No	Architect	30097	55	29.7	56.9	0.24	87	0	0
F	66	No	Retired	30116	54	14.7	62.3	1.28	0	0	0
F	65	No	Airline Pilot	30217	35	25	19.0	1.24	45	1	0
F	60	No	Interior Design-Sales	30226	36	49.2	27.6	0.39	73	1	1
F	42	No	Insurance	30276	8	37.8	54.8	0.66	64	0	0
F	64	No	Retired	30289	57	39.4	61.7	0.82	0	0	0
F	46	No	School Nurse	30354	41	40.9	82.7	0.87	82	0	0
F	62	No	Retired	30379	40	24.3	89.9	0.81	0	0	0
F	55	No	Research Scientist - Plasma Products	30409	9	22.5	28.9	0.63	80	1	1
M	53	Yes	Sales-Chem Eq	30427	42	53.6	82.3	0.44	35	1	1
F	28	No	Not working	30555	37	62.5	62.8	0.47	0	0	0
M	72	No	Retired	40299	3	68.7	87.9	0.70	0	0	0
M	60	No	Retired	40300	1	36.6	72.0	0.71	0	0	0
M	48	No	Mfg Elec Equip	40173	58	78.6	115.0	0.74	36	1	1
F	54	No	Hosp Housekeeping	40281	30	31	35.6	1.19	80	1	1
F	57	No	Administrative Asst	40303	11	43.5	76.2	1.09	73	1	1
M	41	No	Real Estate Sales	40308	39	45.7	72.5	0.66	65	0	0
M	68	No	Desktop Publisher	40309	10	33.4	35.0	0.48	27	0	0
F	46	No	Office Worker	40311	49	26.5	56.2	0.43	73	1	1
F	66	No	Literary Consultant	40312	5	39.6	30.2	0.86	27	0	0
F	57	Yes	Education	40314	33	53.5	49.4	1.44	82	0	0
F	33	No	Athletic Trainer	40316	52	28.6	45.7	0.36	72	0	0
M	30	No	Higher Ed Admin	40319	43	54.5	70.8	0.73	82	0	0
M	52	No	Police Officer	40320	34	27.3	34.7	1.49	92	0	0
M	71	No	Auto Parts Sales	40321	7	54.8	244.0	0.55	55	0	1
M	55	No	HS Teacher	40322	45	57.1	86.5	0.53	82	0	0
F	41	No	Paralegal	40325	12	24.8	53.9	1.42	81	0	0
F	67	No	Teacher	40326	6	62.2	11479.0	0.79	82	0	0
M	50	No	Software Sales	40327	44	50.3	67.8	0.78	73	1	1
F	60	No	Administrative Asst	40328	31	74.8	55.6	1.20	73	1	1
F	81	No	Retired	40329	48	38.2	85.2	0.59	0	0	0
M	64	No	Agricultural Construction	40330	32	72.2	56.7	1.15	15	0	0
F	62	No	Not working	40331	51	49.3	85.2	0.41	0	0	0
F	66	No	Retired	40332	50	32.7	122.0	0.46	0	0	0
M	67	No	Teacher	40333	4	28.3	31.3	0.40	82	0	0
F	66	No	Admin-Dr's office	40335	53	37.5	50.1	0.36	80	1	1
F	29	No	Secretary-Hospital	40336	47	35.3	67.6	0.88	80	1	1
M	56	No	Systems Analyst	40339	46	37.1	66.3	0.78	73	1	1

The Laboratory of Molecular Carcinogenesis and Mutagenesis

The University of North Carolina at Chapel Hill

SOP No.: LMC013 **Edition No.: 4**

Title: Collection of blood and isolation of red blood cells (RBC) from humans

1. Scope

This SOP applies to all lymphocyte samples to be sent to the Laboratory of Molecular Carcinogenesis, University of North Carolina at Chapel Hill for analysis.

2. Responsibility for Administration of the Procedures

The responsibility for the performance of this procedure lies with the technical staff participating in the study.

3. Introduction and background information

The objective of this research is to provide RBC for the measurement of N-terminal valine adducts at the University of North Carolina Laboratories of Molecular Carcinogenesis and Mutagenesis. The sample preparation is described here as a Standard Operating Procedure (SOP **LMC0013**).

Acceptance

Author: _____ Date: _____
(Patricia B. Upton, Research Analyst)

Principal Investigator: _____ Date: _____
(James A. Swenberg, P.I.)

4. Procedures
   1. Safety Recommendations

Laboratory coats, appropriate gloves and safety glasses will be worn when handling blood, processing blood samples, and when using hazardous and/or toxic chemicals.

2. Summary of the method
   1 Blood will be drawn into a Vacutainer tube with sodium heparin or EDTA as anticoagulant.

3. Apparatus
   1 Vacutainer tubes with heparin or EDTA (green cap or lavender cap), preferable 10 mL capacity
   2 Syringes (10-20 mL) and 18 ga needles (or whatever ga needle is preferred)
   3 Indelible marking pen
   4 15 mL centrifuge tubes (Falcon, product # 353097)
   5 Centrifuge (with a swinging bucket rotor) **IMPORTANT**
   Must be capable of 1500×g and hold 13×100 mm test tubes.

4. Chemicals (of sufficient purity for LC-MS analysis)
   1 Sodium Chloride (ACS reagent)
   2 Distilled water (ddw)

5. Reagent Solutions
   1 Isotonic saline solution (0.9% NaCl in ddw), sterile
   0.9 g NaCl in 100 mL ddw

6. Sample Identification and Storage
   1 Each sample should have a unique identifying number. These numbers will be documented in a laboratory notebook.
   2 For each sample, 1-2 vacuatainer tube(s) (for collection of blood) should be labeled with sample number and date.

7. Sample preparation
   1 Collect 10-20 mL whole blood into heparinized 10-20 mL syringe with an 18 ga needle (or use the vacutainer system). Transfer blood to labeled vacutainer tube. Mix by gentle inversion 8 times.
   2 Blood samples can be kept up to 2 hours before centrifugation (so that all blood is centrifuged at the same time). Centrifuge at room temperature for 15 minutes at 1500 ×g.
   3 From this centrifugation, remove plasma and buffy coat and discard.
   4 Add an equal volume of isotonic saline. Mix by gentle inversion. Centrifuge at room temperature for 15 minutes at 1500 ×g.
   5 Discard supernatant. Repeat 2 more times.
   6 Store washed red cells at -70°C until shipped to UNC.

8. Data and Reporting
   1 All data will be recorded and stored in laboratory notebooks and laboratory binders (appendices). Deviations from standard procedures will be documented in the laboratory notebook.

9. Samples Storage and Shipment
   1 All samples will be stored at -70°C until shipped to UNC.
   2 At the time of shipment, samples will be packed on dry ice and sent priority overnight to UNC:

Pat Upton 919-966-6141
Dept of Envr Sci & Eng
348 Rosenau Hall
Chapel Hill, NC 27599

   3 Notify UNC when package is to be delivered.

The Laboratory of Molecular Carcinogenesis and Mutagenesis

The University of North Carolina at Chapel Hill

SOP No.: LMC015 **Edition No.: 1**

Title: **Analysis of the *pyr*-V adduct**

1. Aim

This SOP applies to all red blood cell (RBC) samples to be sent to the Laboratory of Molecular Carcinogenesis, University of North Carolina at Chapel Hill for analysis of the cyclic pyr-V adduct

2. Responsibility for Administration of the Procedures

The responsibility for the performance of this procedure lies with the research and technical staff participating in the study.

3. Introduction and background information

The objective of this research is to provide the procedure for analysis of the cyclic N-terminal valine adduct at the University of North Carolina Laboratories of Molecular Carcinogenesis and Mutagenesis. The sample preparation is described here as a Standard Operating Procedure (SOP LMC015).

Acceptance

Author: _____ Date: _____
(Nadia I. Georgieva & Gunnar Boysen)

Principal Investigator: _____ Date: _____
(James A. Swenberg, P.I.)

4. Procedures

1. Safety Recommendations

Laboratory coats, appropriate gloves and safety glasses will be worn when handling blood, processing blood samples, and when using hazardous and/or toxic chemicals.

2. Summary of the method

Extracted globin from RBC (mouse, rat or human) will be hydrolysed by trypsin-biotin-agarose together with the proper internal standard (IST), samples will be filtered on Centricon-3 columns and applied on Immunoaffinity (IA) columns for clean-up. Samples will be then eluted in 5% formic acid, filtered on Microspin filters, dried again, and dissolved in 15mM ammonium formate for analysis by LC-MS/MS.

3. Instruments and Supplies

1 Centricon-3 columns are from Millipore (Bedford, MA)
2 Microspin filter tubes (regenerated cellulose, 0.2 um) are from Alltech Associates, Inc. (Dearfield, IL).
3 Ultracentrifuge (Sorval), microcentrifuge, speed-vac, environmental shaker, uncubator (substitutions are acceptable)
4 1100 Capillary HPLC (Agilent, Wilmington, DE)
5 TSQ-Quantum (ThermoFinnigan, San Jose, CA)

4. Chemicals

1 Trypsin-biotin-agarose can be ordered through Sigma- Aldrich (T4019, 25U, St. Louis, MO)
2 Protein-A Sepharose CL4B will be purchased from Pharmacia (Upsala, Sweden)
3 The polyclonal antibody (AB) was produced by AnaSpec, Inc. (San Jose, CA) and are a proprietary reagent of our lab.
4 All other reagents and solvents should be ACS grade or higher

5. Sample Identification and Storage

1 Each sample should have a unique identifying number. These numbers will be documented in a laboratory notebook.

2 Samples will be analyzed in set of 16 consisting of 2 blanks (control globin), 2 positive controls (control globin spiked with standard peptide (1-11)), and 12 unknown samples.

3 With each set of samples, 4 calibration solution containing 50, 150 250 and 350 fmol/µl analyte and 500 fmol/µl IST will be analyzed.

6. Sample preparation and analysis

1 Globin (10 mg for mice, 20 mg for rats, 50 mg for humans) will be dissolved in 1-5 ml of 0.1M ammonium bicarbonate, pH 8.0 and 10 – 100 µl of SDS added

2 Samples will be incubated for 1 h at 37°C (shaking).

3 To each sample 2 pmol from the corresponding IST will be added.

4 Globin will be digested with 50-500 µl of trypsin-biotin-agarose suspension at 37°C for 20 h.

5 Samples will be filtered through Centricon-3 filters and dried by centrifugal lyophilization.

6 IA columns will be prepared as described by Ham et al (Chem.Res. Toxicol., *12*, 1240-1246 (1999)). Before use they will be conditioned two times with PBS buffer. Samples will be dissolved in 300-600 µl of HPLC water and loaded on the IA columns. Extensive washing with water, and elution in 5% formic acid will follow and the solvents will be removed.

7 Samples will be re-dissolved in 200 µl HPLC-water and filtered through Microspin columns. Dried again and store at -20°C

7. LC-MS/MS analysis

1 A 0.3 × 150 mm Vydac C18 MS (238MS5.315), 5 µm column will be operated with a linear gradient of 100% 15 mM ammonium formate, pH 3.4, to 70% methanol-15 mM ammonium formate, pH 3.4, in 10 min, at a flow rate of 7 µl/min.

2 The peptides will be detected in single reaction monitoring (SRM) mode, monitoring the transition of the single charged analyte (mouse and rat peptides m/z 833, human m/z 815) and internal standard ions (mouse and rat peptides m/z 836, human m/z 818) to the a_1-fragment (pyr-V, m/z 158).

3 The MS conditions will be as follows; spray voltage 2800 V, heated capillary temperature 350°C.

4 For analysis by LC-MS/MS samples will be dissolved in 10-20 µl 15mM ammonium formate pH 3.4.

5 Depending on expected adduct levels 1 to 8 µl will be injected with an 1100 capillary autosampler (Agilent, Wilmington, DE).

8. Data Analysis

1 All data will be recorded and stored in laboratory notebooks and laboratory binders (appendices). Deviations from standard procedures will be documented in the laboratory notebook.

2 Adduct level will be calculated as follows:

$$amount\,(analyte) = \frac{area\,(analyte)}{area\,(IST)} \times amount\,(IST) \times RF$$

The response factor (RF) will correct for the differences in MS response of analyte and IST and will be calculated from the 4 calibration standards that will be run with each set of samples.

9. Samples Storage and Shipment

1 All samples will be stored at -70°C until shipped to UNC.

2 At the time of shipment, samples will be packed on dry ice and sent priority overnight to UNC:

Pat Upton 919-966-6141
Dept of Envr Sci & Eng
348 Rosenau Hall
Chapel Hill, NC 27599

3 Notify UNC when package is to be delivered.

Figure 1: *pyr*-V (1-11) Peptide Sequences (mouse, rat and human)

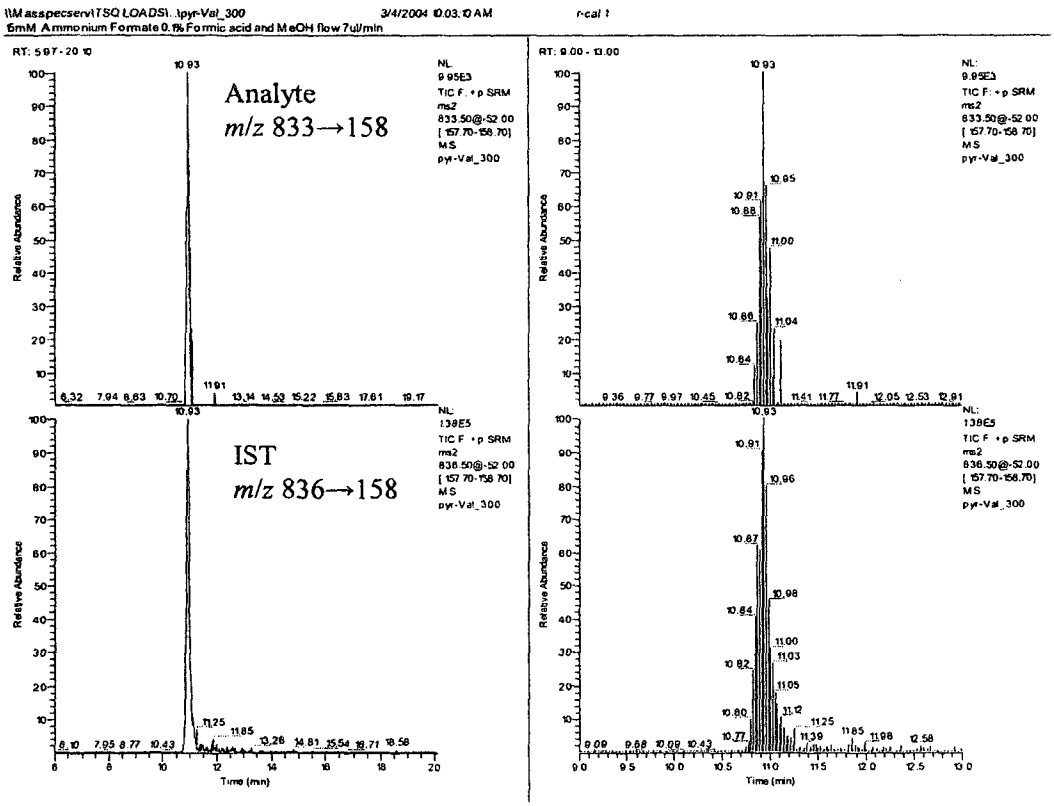
Mouse: *pyr*-Val-Leu*-Ser-Gly-Glu-Asp-Lys/-Ser-Asn-Ile-Lys
(***pyr*****-mV)** **V - L - S - G - E - D - K /- S - N - I - K**

Rat: *pyr*-Val-Leu*-Ser-Ala-Asp-Asp-Lys/-Thr-Asn-Ile-Lys
(***pyr*****-rV)** **V - L - S - A - D - D - K / -T - N - I - K**

Human: *pyr*-Val-Leu*-Ser- Pro-Ala-Asp-Lys/-Thr-Asn-Val-Lys
(***pyr*****-hV)** **V - L - S - P - A - D - K / -T - N - V - K**

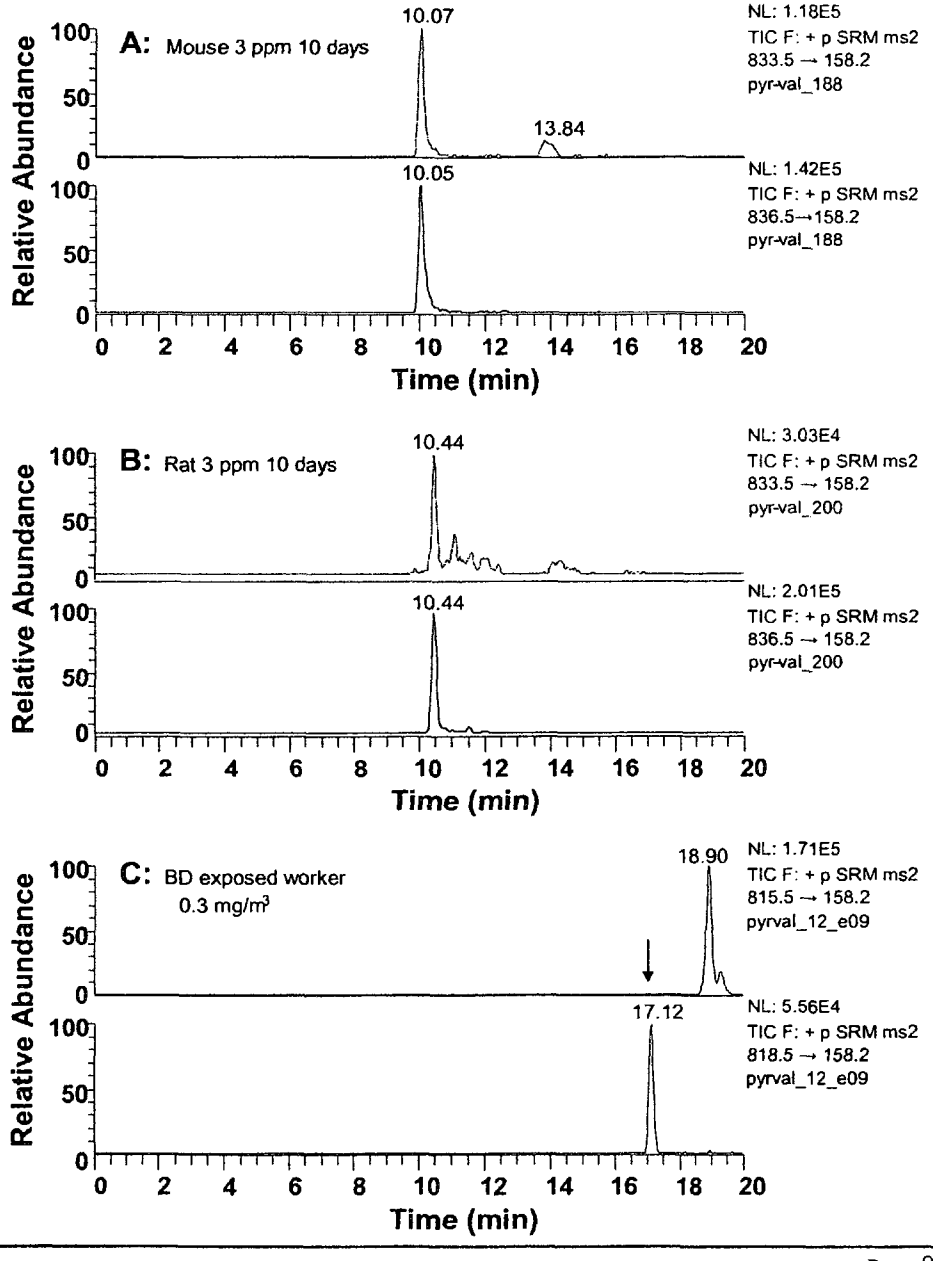
* d_3 in Internal standards
/ hydrolyzed by trypsin

Figure 2: Calibration standard (50 fmol analyte on column)



On the left; ion-chromatograms.
On the right; ion chromatograms in stick mode to demonstrate the number of points (scans) over the peak.

Figure 3: Ion-Chromatograms of *pyr*-V Samples



Laboratory Procedure Manual

Analyte: N-terminal hemoglobin adducts of acrylamide and glycidamide

Matrix: Red Blood Cells/Whole Blood

Method: Liquid Chromatography Tandem Mass Spectrometry

Method No:

Revised:

as performed by:

Clinical Chemistry Branch
Division of Laboratory Sciences
National Center for Environmental Health

contact:

Dr. Hubert W. Vesper
Phone: 770-488-4191
Fax: 404-638-5393
Email: HVesper@cdc.gov

Dr. Eric J. Sampson, Director
Division of Laboratory Sciences

November 2008

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

A tabular list of the released variables follows:

Data File Name	Variable Name	SAS Label
l06AGE_C	LBXACR	Acrylamide (pmoL/G Hb)
	LBXGLY	Glycidamide(pmoL/G Hb)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A. Clinical relevance

Acrylamide was identified as being neurotoxic to animals and humans (i,ii), mutagenic to male germ cells (iii) and probably genotoxic (iv). It is classified as probably carcinogenic to humans by the International Agency for Research on Cancer, and as a potential occupational carcinogen by the Occupational Safety and Health Agency. Research on non-occupational human exposure to acrylamide, were initially driven to elucidate unexplained background biomarker concentrations in control subjects of occupational studies. These studies identified tobacco smoke (v,vi,vii,viii) and food (ix,x,xi,xii) as sources of acrylamide exposure in the general population. The finding that the general population is being exposed to acrylamide created the need for biomonitoring of acrylamide exposure. The assessment of human exposure in the general population will define the extend of exposure and the evaluation of possible associations between exposure to acrylamide and health effects.

Glycidamide, the primary metabolite of acrylamide, has a higher reactivity towards nucleophilic reagents than acrylamide and, in contrast to acrylamide, forms DNA-adducts. Further, results from animal studies suggest that genetic damage in somatic and germ cells is dependent upon the metabolism of acrylamide to glycidamide by CYP2E1 (xiii,xiv,xv). The metabolism of acrylamide to glycidamide seems to depend on exposure dose and exposure path. Because of the concentration and exposure path dependent metabolism of acrylamide and the differences in toxicity of acrylamide and glycidamide, measurement of acrylamide as well as glycidamide adducts are needed to obtain comprehensive information about acrylamide exposure and possible risks associated with this exposure.

B. Test principle

This procedure describes a method to measure hemoglobin adducts of acrylamide and its primary metabolite glycidamide in human whole blood or erythrocytes. Specifically, the reaction products with the N-terminal valine of the hemoglobin protein chains (N-[2-carbamoylethyl]valine and N-[2-hydroxycarbamoyl-ethyl]valine for acrylamide and glycidamide adducts, respectively) are measured. This method is based on modified Edman reaction, which uses the effect of N-alkylated amino acids being able to form Edman products in neutral or alkaline conditions without changing the pH to acidic conditions required in conventional Edman reaction procedures (xvi).

It was first described for N-terminal hemoglobin adducts of ethylene oxide, propylene oxide and styrene oxide (xvii) and later optimized to increase yield of Edman products of these adducts (xviii). This optimized method was then successfully applied to adducts produced by other chemicals such as acrylamide, glycidamide and acrylonitrile (xix,xx,xxi,xxii). This optimized method was further refined and modified in-house to increase sensitivity and enable automation (xxiii) The procedure described here consists of 4 parts:

1) Preparation of the specimen for measurement of hemoglobin adducts of acrylamide and glycidamide; 2) Total hemoglobin measurement in the sample solution used for hemoglobin adduct measurements; 3)Modified Edman reaction in the sample solution and isolation of Edman products; 4)Analysis of Edman products by HPLC/MS/MS and results processing.

Because results are reported in pmol adduct per g hemoglobin, the amount of hemoglobin used for the modified Edman reaction needs to be known. Therefore, this procedure includes a measurement procedure for total hemoglobin. It is a commercial assay kit based on a well-established procedure commonly used in clinical chemistry. Quantitation of the acrylamide and glycidamide hemoglobin adduct is performed using octapeptides with the same amino acid sequence as the N-terminal of the beta-chain of hemoglobin and with acrylamide and glycidamide attached at the valine (AA-VHLTPEEK, GA-VHLTPEEK) and the corresponding stable isotope labeled AA-Val(13C5 15N)-HLTPEEK and GA-Val(13C5 15N)-HLTPEEK as internal standards. Total hemoglobin measurement is performed using calibrators provided with the manufacture's assay kit.

2. SAFETY PRECAUTIONS

A. General Safety

Consider all specimens as potentially positive for infectious agents including HIV, hepatitis B and hepatitis C. We recommend the hepatitis B vaccination series for all analysts working with whole blood and /or serum.

Observe all safety regulations as detailed in the Division (DLS) Safety Manual. Additional information can be found in your lab's chemical hygiene plan.

Observe universal precautions; wear protective gloves, lab coat, and safety glasses during all steps of this method. Discard any residual sample material into auto cleavable bags.

Place all disposable plastic, glassware, and paper (pipette tips, autosampler vials, gloves etc.) that are in contact with blood or blood products in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Use disposable bench diapers during sample preparation and serum/ blood handling and discard after use. Also wipe all contaminated work surface with 10% bleach solution when work is finished.

Acetic acid: Handle with extreme care. It is caustic and toxic; avoid contact with skin and eyes. Do not store with inorganic acids.
Organic solvents: Handle only in well-ventilated areas or as required under a fume hood.
Isopropyl Ether: Forms explosive peroxides on prolonged storage. Keep container in well ventilated place.
Pentafluoropenyl isothiocyanate: Do not breathe vapor. Do not get in eyes, on skin, or clothing. Handle only in well-ventilated areas.
Formaldehide: Toxic to reproduction, wear appropriate personal protective equipment, clothing, safety goggles, and gloves. Avoid contact with skin or eyes. Use adequate ventilation. Store away from acids.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for all chemicals are readily available in the MSDS section as hard copies in the laboratory. MSDSs for other chemicals can be viewed at http://www.ilpi.com/msds/index.html or at http://intranet.cdc.gov/ohs.

B. Waste Disposal

Operators of this method should take the CDC-OHS Hazardous Chemical Waste Management Course (initial and yearly refreshers).

1. Waste to be placed into Biohazard Autoclave Bags & Pans
   All biological samples, pipette tips, all disposable plastic well plates used during sample preparation, papers which contact blood or red blood cells, gloves, etc.).
2. Waste to be placed into Sharps Containers
   Glass pipette tips, broken glass, cuvettes for HemoCue measurement, etc. Large broken glass which will not fit in the sharps container should be placed in a separate autoclave pan from other waste and labeled as "broken glass" (see the "Autoclaving" section of the CDC safety policies and practices manual located in the laboratory).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT RESULT ENTRY

A. For NHANES, data is transmitted electronically Westat's ISIS computer system and transferred from there to NCHS. Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file to the Westat ISIS computer, and are eventually forwarded to NCHS. Westat also prepares the abnormal report notifications for the NCHS Survey Physician.

B. Files stored on the network or CDC mainframe are automatically backed up nightly by DLS LAN support staff and CDC Data Center staff, respectively. Backup of the daily data containing all raw data files and result files for each run are the responsibility of the analyst. Typically these files are backed up once a week onto a floppy disk or a CD-ROM using a CD writer.

C. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. General specimen requirements

No fasting or special time of day for specimen collection is required. Specimens for acrylamide- and glycidamide hemoglobin adducts analysis may be fresh or frozen erythrocytes or EDTA-whole blood. This procedure was evaluated with EDTA-whole blood and erythrocytes obtained from EDTA-whole blood. The applicability of this method for other coagulants was not tested.

A 1.0 mL sample of whole blood or 0.5 mL packed red blood cells is preferable to allow for repeat analyses; a sample volume of 550 µL of whole blood or lyzed red blood cells is required for analysis. More sample is needed if blood clots are present in the tube.
The appropriate amount of whole blood or red blood cells is dispensed into a 2.0-mL cryogenic vials with the external screw cap. These cryovials should be labeled in accordance to CDC and NCEH/DLS policies and regulations.

If there are analytes other than acrylamide and glycidamide to be measured, the sample needs to be divided; the appropriate amount of blood or lyzed red blood cells should be transferred into a sterile cryovial labeled in accordance to CDC and NCEH/DLS policies and regulations.

Other specimen handling conditions are outlined in the Policies and Procedures Manual of DLS (copies are available in the Protein Biomarker Laboratory and the electronic copy of this file is located at Q:\SHARE\DLS CLIA Folder).

B. Specimen Storage

Specimens collected in the field can be shipped refrigerated at 5°C or frozen on dry ice. Specimens can be kept refrigerated for 3 days. For long term storage samples are stored at -70°C. Samples are stable for at least 5 years if stored at -70°C. Two to three freeze-thaw cycles did not show any changes in values. However, multiple freeze-thaw cycles of diluted whole blood samples possibly increases the formation of blood clots, which complicates the analysis of the samples. Therefore, diluted whole blood samples should not undergo more than 3 freeze-thaw cycles.

C. Specimen Storage and Handling During Testing

Specimens are allowed to reach room temperature during preparation. Once the samples are ready to be analyzed by HPLC/MS/MS, they are placed into the autosampler tray. The unused portion of the patient specimen is returned to the freezer. Diluted samples (see section 2 can be stored refrigerated over night.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Sources for equipment and instrumentation

1. Reagent preparation

   a. Accumet AB 15 pH meter (Fisher scientific, Suwanee, GA) with Orion Micro-Combination pH electrode, pH range 0-14, temperature range 0-100 °C. (Thermo Electron Corp., Bellefonte, PA).
   b. Sartorius Analytical Balance (Accurate "0.00 mg" Digital Scales and Electronic Balances, Edgewood, NY.)

c. Sartorius Chemical Balance (Electronic "0.000 g" Balances, Edgewood, NY.)
d. Fisherbrand Desiccator Cabinets, Tall (Fisher scientific, Suwanee, GA). the Dessicant, 6-8 Mesh 100% (EMD Chemicals, Gibbstown, NJ).

2. Sample processing

a. Tecan (Tecan US., Research Triangle Park, NC). Model Genesis Freedom 200 with Model Pos ID V2200 barcode reader
b. Matrix (Matrix Technologies Corporation, Hudson, NH). Plate Mate 2X2
c. Lab rotator Barnstead with flat surface rotor (Lab-Line, Melrose Park, IL)
d. Vortex- Genie 2 with well plate adapter (Scientific Industries Inc, Bohemia, NY)
e. Precision Oven (Thelco Laboratories, Torrance, CA).
f. SPE 215 system with 8 needle arm (Gilson Inc., Middleton, WI)
g. Eppendorf Centrifuge 5810 R V4.2 with A-4-62 rotor (GMI, Ramsey, MN)
h. GeneVac EZ-2 Evaporation System with Side Bridge holders and universal rotor (GeneVac Inc., Valley Cottage, NY)
i. Hamilton Dispenser Microlab 500 Series with 2- 25.0 ml syringes (Hamilton Co., Reno, NV)
j. Eppendorf 8-channel pipette 50-1200 µl, (Eppendorf, Westbury, NY)
k. Gilson 1000 µl pipette (Gilson Inc., Middleton, WI)
l. Gilson 200 µl pipette, fixed (Gilson Inc., Middleton, WI)
m. Gilson 10 µl pipette (Gilson Inc., Middleton, WI)
n. Eppendorf repeater pipette (Eppendorf, Ramsey, MN)
o. Hand held scanner (Symbol, Technologies. Inc, Bohemia, NY.)
p. Transfer pipettes (Samco scientific, San Fernando, CA).
q. Fisherbrand 20" Flexible-Arm lamp (Fisher scientific, Suwanee, GA) with halogen bulb.
r. Boekel Orbitron Rotator, model II (Fisher scientific, Suwanee, GA).
s. Microman, 10µL positive-displacement pipette (Gilson, Inc, Middleton, WI).
t. Fisherbrand Octagonal stirring bars, 1"L x 5/16" D (Fisher scientific, Suwanee, GA).
u. Amber Glass Bottles 60 mL(Fisher scientific, Suwanee, GA).

3. Sample measurement

a. Power Wave 200 Spectrophotometer (Bio-Tek Instruments, Winooski, VT).
b. Finnigan TSQ Quantum Ultra™ with atmospheric pressure chemical ionization unit (Thermo Electron, San Jose, CA).
c. Surveyor HPLC with autosampler and photodiode array detector (Thermo Electron, San Jose, CA).
d. HemoCue (HemoCue Inc., Lake Forest, CA.)

B. Sources for General Laboratory Equipment and Consumables

1. Reagent preparation

a. 1 mL, 2mL, 3mL and 4mL volumetric glass pipettes
b. Various glass beakers, 2L volumetric flask, four 500mL and two 100mL graduated cylinders, and Teflon seal 1L and 2L glass bottles, silanized volumetric flask, silanized volumetric pipettes, class A glassware
c. Pyrex Culture Tubes, (Corning 16x100 mm, 13X100mm)
d. BD Vacutainer K3 EDTA (Fisher scientific, Suwanee, GA)
e. 15.0 ml plastic falcon tube (KSE, Durham, NC).
f. Plastic Tube (Fisher scientific, Suwanee, GA)

2. Sample processing

a. 48 well, 5 mL, rectangular well, pyramid bottom, natural polypropylene plate (Innovative Microplate, Chicopee, MA).
b. 96 well, microtiter plate, Nonbinding Surface (NBS*) (Fisher Scientific, Suwanee, GA).
c. 48 well, 7.5 ml fritted plate, with 25 µM PE frit, Long drip (Innovative Microplate, Chicopee, MA).
d. 48 well, 7.5 mL, rectangular well, pyramid bottom, natural polypropylene (Innovative Microplate, Chicopee, MA).
e. 96 well, 2 ml, deep well plate (VWR International, West Chester, PA).
f. Bulk Isolute Sorbent HM-N (Biotage, Charlottesville, VA).
g. 1000 µl, clear pipetting tips for Tecan (Molecular Bio Products, San Diago, CA).
h. 1000 µl, conductive pipetting tips for Tecan (Molecular Bio Products, San Diego, CA).
i. 200µl, conductive pipetting tips for Tecan (Molecular Bio Products, San Diego, CA).
j. 300 µl, clear extended length with filter tips for Matrix (Fisher scientific, Suwanee, GA)
k. 2 mil PE 96-well pattern sealing film (Bio Tech Solutions, Mt. Laurel, NJ).

l. Arctic sealer silicon PTFE film (Arctic White LLC, Bethlehem, PA).
m. 96 well, 1mL filter plate, with 0.2μM GHP (Pall Corporation, Ann Arbor, MI).
n. Nalgene 2mL cryovials with ext-tread (fisher scientific, Suwanee, GA).
o. Microcuvettes for Hemocue (Aura Tech, Greensboro, NC).
p. CP10ST (0.5μL-10μL) M10 pipette tips for Microman (Gilson, Inc, Middleton, WI).
q. Aluminum Foil (Fisher scientific, Suwanee, GA)

3. Sample measurement

a. HPLC column: Termo Hypersil GOLD, 50 x 2.1 mm, 1.9 μ, (Termo Electron, Holbrook, NY)
b. Guard column: Krudkatcher filter (Phenomenex, Torrance, CA)

C. Sources for Chemicals and Standards

1. Reagent preparation

a. Cyanmethemoglobin Diluent Powder (CMH Solution), (Fisher Scientific, Suwanee, GA).
b. Cyanmethemoglobin Standard (CMH Standard),(Fisher Scientific, Suwanee, GA).
c. Mili-Q Water (Aqua Solutions, Jasper, GA)
d. Acrylamide Octapeptide (Bachem Bioscience Inc., King of Prussia, PA).
e. Glycidamide Octapeptide (Bachem Bioscience Inc., King of Prussia, PA).
f. Labeled Acrylamide Octapeptide (Bachem Bioscience Inc., King of Prussia, PA).
g. Labeled Glycidamide Octapeptide (Bachem Bioscience Inc., King of Prussia, PA).
h. Acetic Acid ACS grade (J. T.Baker, Phillipsburg, NJ)
i. Ammonium acetate (Fisher scientific, Suwanee, GA)
j. Hemo Point H2, Hemoglobin Control, 6 vials (Stanbio Labortory, Boerne, TX).

2. Sample processing
a. Formamide ACS grade (USB, Cleveland, OH).
b. Pentafluorophenyl Isothiocyanate 96 %(Alfa Aesar, Ward Hill, MA).
c. Isopropyl Ether ACS grade (EMD, Gibbston, NJ).

d. Toluene ACS/HPLC grade (Burdick and Jackson, Muskegon, MI).
e. Ethylacetate HPLC grade (EMD, Gibbston, NJ).

3. Sample measurement

   a. Methanol HPLC grade 99.9 % (Caledon Laboratories, Ontario, Canada).
   b. Deionized water with resistance to at least 15 megaOhm-cm and filter before use, using 0.45 µm nylon filters

4. Calibrators and internal Standards

   The following calibrators and internal standards were obtained from Bachem and used as custom synthesis products:

   a. Acrylamide Octapeptide (AA-VHLTPEEK):
      Labeled as (-)-AA-VHLTPEEK,
      FW=1022.5 g/mol,
      Chemical formula: C45H74N12O15
      Chemical purity: 71 %
   b. Glycidamide Octapeptide (GA-VHLTPEEK):
      Labeled as (-)-GA-VHLTPEEK,
      FW=1038.5 g/mol,
      Chemical formula: C45H74N12O16
      Chemical purity: 71 %
   c. Labeled Acrylamide Octapeptide [AA-Val(13C5,15N)-HLTPEEK]:
      Labeled as (-)-AA-V*HLTPEEK,
      FW=1028.2 g/mol,
      Chemical purity: 80 %
      Isotopic purity: >99%
   d. Labeled Glycidamide Octapeptide [GA-Val(13C5,15N)-HLTPEEK]
      Labeled as (-)-GA-V*HLTPEEK,
      FW=1045.2 g/mol,
      Chemical purity: >99%
      Isotopic Purity: >99%

D. Preparation of Reagents and Materials

Prepare all reagents using deionized water with resistance to at least 18 megaOhm-cm.and filter before use, using 0.45 µm nylon filters.

1. Reagents used for total hemoglobin measurement

Preparation Cyanmethemoglogin (CMH Solution)
According to the manufacturer's procedure, this solution can either be purchased already diluted in liquid form or can be prepared from a premixed reagent powder.

Prepare CMH Solution from reagent powder by reconstituting 4 packages of CMH Diluent Powder in a 2.0-L volumetric flask using Mili-Q water according to the manufacturer's recommendation. The CMH Diluent Powder consists of (0.05 g KCN, 0.20 g potassium ferricyanide, 1.00 g sodium bicarbonate).

2.0-L CMH solution is sufficient for approximately 768 samples including controls. CMH Solution is also used as the calibrator dilution solvent and used as blank in the calibration curve.

Store CMH solution in a dark amber bottle (or bottle wrapped in aluminum foil) at room temperature (18-26 °C) for no longer than 2 years.

Note: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Minimize dust generation and accumulation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. Acids should not be used around sodium cyanide or potassium cyanide unless absolutely necessary and then only after careful planning. Use only with adequate ventilation or respiratory protection.

2. Reagents used for Edman reaction and purification of Edman products

   a. 1.58 M Acetic Acid Solution for adjusting the pH of the blood samples.
      This solution is used for adjusting the pH of the blood samples to be between 5.5 and 6.5.
      Pipette 2 mL of concentrated acetic acid (use a borosilicate disposable 1.0 mL glass pipette) in 20 mL of deionized water in a Pyrex culture tube and mix thoroughly. Solution needs to be prepared fresh at the day of use. 12 mL of 1.58 M acetic acid solution is sufficient for (4) 48-wellplates.
   b. 0.52 M Acetic Acid Solution
      This solution is used for adjusting the pH of the 400 mM ammonium acetate solution.
      Pipette 290 µL of concentrated acetic acid in 10 mL of deionized water (use a borosilicate disposable 1.0 mL glass pipette) in a Pyrex culture tube and mix thoroughly. Prepare solution fresh as needed.
   c. 400 mM Ammonium acetate buffer solution

This solution is used for adjusting the pH of the calibrator solutions.
Dissolve 1.52 g ammonium acetate (use Sartorius chemical balance) in 40 mL of water using a 100 mL tube. Adjust the pH of the solution by titrating with 0.52 M acetic acid solution to pH 5.0 (± 0.2 pH). Adjust the solution to a final volume of 50 mL.
Prepare solution every four months or as needed.

d. 6 mM K3EDTA saline solution
This solution is used to dilute the samples 1:0.33 (v/v) to facilitate automated pipetting.
Dissolve 0.0175 g K3EDTA in 7 mL using saline solution using a Pyrex culture tube. Prepare solution as needed.

e. Liquid-liquid extraction solution
Add 500 mL isopropylether, 400 mL of ethylacetate and 100 mL toluene in a in a 1-L glass bottle, use separate graduated cylinders for each solvent. Close the bottle and mix thoroughly (Isopropylether/Ethylacetate/Toluene - 50/40/10 v/v/v).
This solution needs to be prepared fresh at the day of use. 2L of this solution is sufficient for four 48-well plates.

CAUTION! Isopropylether, Etheylacetate, Toluene are volatile organic compounds. Wear gloves, safety glasses, lab coat and/or apron, and work only inside a properly operating chemical fume hood.

f. Preparation of extraction plate
Fill the extraction filter plate (48-well 7.5-mL filter plate with 25 µm filter and long drip) with Isolute Sorbent HM-N (diatomaceous earth). The plate should be filled up to 0.5 cm or ¼ inch to the top. Tap all sides of the plate to get a uniform packing. Plates can be prepared the day before and stored in a desiccator.

Note: Perform packing of the plate in the hood to avoid inhaling of sorbent dust.

3. Reagents used for analysis of Edman products by HPLC/MS/MS

Solvent C methanol
Solvent D (methanol/water 50:50 v/v): Add 500 mL methanol and 500 mL water in 1-L glass bottle. Use separate graduated cylinders for each solvent. The HPLC mobile phase is made up daily or as needed.

4. Standards and Calibrators

a. Preparation of Calibrators for acrylamide and glycidamide adduct measurement

All glassware that is in contact with the octapeptide calibrators must be silanized otherwise losses due to adsorption of the peptides to the glass are observed resulting in inaccurate calibrators.

The calibrators for acrylamide and glycidamide adduct measurements are prepared using acrylamide and glycidamide octapeptides (AA-VHLTPEEK and GA-VHLTPEEK). Seven different concentrations are prepared with concentrations of 1.25, 2.5, 5.0, 10, 20, 40 and 80 nM octapeptides of acrylamide and glycidamide. This concentration range covers acrylamide and glycidamide adduct values observed in the general population, non-smoking, smoking and non-occupationally exposed people.

(1) Acrylamide Octapeptide (AO) stock solution of 72.9 μM
Dissolve 10.5 mg of acrylamide octapeptide (use Sartorius Analytical Balance) in 100 mL deionized-water in a silanized, volumetric 100-mL flask.

(2) Glycidamide Octapeptide (GO) stock solution of 75.6 μM
Dissolve 2.77 mg of glycidamide octapeptide (use Sartorius Analytical Balance) in 25 mL deionized-water in a silanized, volumetric 25-mL flask.

Note: if new batches of calibrators with different purities are used, adjust the amount of octapeptide appropriately.

(3) Calibrator stock solution 1000 nM
Pipette 1370 μL of the 72.9 μM acrylamide octapeptide stock solution and 1320 μL of the 75.6 μM glycidamide octapeptide stock solution in a 100-mL volumetric flask and add water to the 100 mL. Mix the solution thoroughly.

(4) Calibrator solutions
Prepare 80, 40, 20, 10, 5.0, 2.5 and 1.25 nM calibrator solution from the 1000 nM solution by serial dilution. All pipettes used need to be silanized volumetric pipettes. From this solution, prepare the calibrator solutions as described in Table 1.
Take 50 mL of the 1000 nM solution into a 500 mL volumetric flask and dilute to the mark to obtain a 100 nM standard.

Concentration of calibrator solution	Volume to take from calibrator solution	Dilute to final volume	Obtained concentration of calibrator solution
100 nM	160 mL	200 mL	80 nM
80 nM	100 mL	200 mL	40 nM

40 nM	100 mL	200 mL	20 nM
20 nM	100 mL	200 mL	10 nM
10 nM	100 mL	200 mL	5.0 nM
5.0 nM	100 mL	200 mL	2.5 nM
2.5 nM	100 mL	200 mL	1.25 nM

Table 1: Dilution scheme for acrylamide and glycidamide calibrator solutions

Aliquot 0.9 mL of each calibrator level into a 2.0 mL cryovials and store calibrators at -70 °C. One cryovial 0.9 mL is sufficient for 4 analytical runs (4 x 48-wellplates), volumes described are sufficient for a total of 500 analytical runs.

5. Preparation of Internal standard solutions for acrylamide and glycidamide adducts measurement

   a. Labeled acrylamide octapeptide stock solution (LAO) 66 µM
   Dissolve 0.84 mg of labeled acrylamide octapeptide (use Sartorius Analytical Balance) in 10 mL deionized-water bottle using a 10-mL silanized, volumetric flask.

   b. Labeled glycidamide octapeptide stock solution (LGO) 164 µM
   Dissolve 1.69 mg of labeled glycidamide octapeptide (use Sartorius Analytical Balance) in 10 mL deionized-water using a 10 mL silanized, volumetric flask.

   c. Internal Standard Solution
   This solution contains 33 nM labeled acrylamide octapeptide and 82 nM labeled glycidamide octapeptide.

   Pipette 500 mL of the LAO and 500 mL LGO stock solution into a 1000 mL volumetric flask (use silanized volumetric glassware only). Store the solution in 5 mL aliquots in a 10 mL falcon tubes at -70 °C. 1000 mL is sufficient for 10,000 analytical samples.

Note: Diluted standards are stable for at least 4 years when stored at -70 °C

6. Preparation of Calibrators for Hemoglobin Measurement
   Calibrators are prepared according to the Stanbio manufacturer's procedure recommendation that is provided as package insert with the reagents and chemicals.
   Dilute the CMH standard A (20 g/dL) provided by the manufacturer with CMH solution prepared in section 0 using the dilution scheme shown in Table 2. The following concentrations of calibration solutions are prepared: 20, 15, 10, 5, and 0 g/dL.

Concentration of CMH standards solution A	Volume to take from calibrator solution	Dilute with CMH solution to final volume	Obtained concentration of calibrator solution
20 g/dL	4 mL	0 mL	20 g/dL
20 g/dL	3 mL	4 mL	15 g/dL
20 g/dL	2 mL	4 mL	10 g/dL
20 g/dL	1 mL	4 mL	5 g/dL
20 g/dL	0 mL	4 mL	0 g/dL

Table 2: Dilution scheme for total hemoglobin calibrator solutions

A new calibration curve must be prepared with each sample set.
See manufacturer's recommendation for stability of the hemoglobin standards

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Higher order reference materials are currently not available for these analytes or measurements. Exchange of calibrators with other laboratories measuring the same analyte with different methodologies has been performed and the concentration of the calibrators has been confirmed.

Results of in-house recovery studies showed a mean (±SD) recovery of 96.0%±1.9 for acrylamide- and glycidamide hemoglobin adducts added externally to whole blood and red blood cells at different levels (0.5-20 pmol /200µL)

In-house studies showed that aqueous calibration gave same results as calibration in serum. Daily calibrations will thus be performed in aqueous solutions.

All necessary calculations are performed in an Excel spreadsheet after copying the result tables containing the integrated areas, calculated using the quantitation settings on the Finnigan Xcalbur into Excel. After calculating area ratios between the analytes and their internal standards, a linear calibration standard curve, not forced through zero calculated on the basis of single analysis is generated. The zero point calibrator available has only internal standard. The concentrations are calculated from the regression equation based on area ratios between analyte and internal standard.

At the end of each run, the calibration curve is reanalyzed as unknowns. The measured concentrations of these calibrators must agree within the assigned values of their set values for acrylamide and glycidamide respectively.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Daily Analysis of Samples

1. Preparation of the MS/MS system
   All instrument tuning and mass calibration is performed according to manufacturer's instructions and recommendations. The tuning and mass calibration of the Finnigan Quantum is performed by infusion either running the instrument in Auto-Tune or using Manual Tune using a solution of polytyrosin. The instrument settings defined in this tuning and calibration step are saved on the local hard drive (Usually tuning and calibration is performed after regular preventive maintenance twice a year or after cleaning the Q0 unit).

   All other method related instruction materials and manuals (such as daily required maintenance files) are kept in the laboratory next to the instrument.

   The corona discharge needle is checked every day for cleanliness. If discharge needle is not clean (indicated by dark coloration of the needle), it is cleaned by wiping the needle with a polishing cloth.

   Sample tube is flushed with methanol daily. Q 0 is cleaned approximately every two months.

   The pump oil in the rough pump is changed approximately every six months. The oil level is checked once a week.

2. Preparation of the HPLC system

   A Krudkatcher that acts as a pre-column is replaced approximately every 300 injections or when pressure increases profoundly.

   The injection port is periodically checked for clogging by doing a syringe and a needle wash before every sequence. The liquid should flow freely through the waste line.

   Assure that solvents are full and that the waste is emptied every day and that there are no air bubbles in the line. LC column is replaced approximately every 1500 injections, if separation performance is insufficient or pressure gets too high (> 290 bars).

3. Preparation of the Tecan system
   Before using the TECAN instrument flush lines with 25 ml of 5 % methanol and tighten all eight cones on the tips, also tighten all diluter syringe connections.

   Assure that no air bubbles are in the Teflon lining. Assure that the rinse solvent bottle has sufficient solutions and empty waste bottle as needed.

4. Preparation of the Gilson system
   Before using the Gilson instrument, flush lines with 5 ml of elution solvent two times. 8.0 ml of mix solvent will not overflow in the plate since the packed plate absorbs at least 1.0 ml of solvent.

   Assure that no air bubbles are in the Teflon lining. Empty the waste bottle as needed.

5. Preparation of the Matrix system
   Insert 96 tip magazines into the 96 channel pipetting head. Assure pipettor or tips are 96 5µL-300µL long tips (5536+5537).

B. Preparation of Samples for Analysis

The descriptions described here are setup to process (4) 48-wellplates, with each plate having its own calibrators, QCs and run ID. Less than four plates can be processed by making appropriate adjustments for reagents and samples.

1. Preliminaries
   This section contains a description on how samples are diluted, homogenized, assessed for blood clots and registered for tracking of sample ID throughout the analysis process.

   The dilution of the samples will reduce the viscosity of the sample and enable accurate and precise pipetting of the sample using automated pipetting systems. Labels are printed using a Sato label printer for all the plates and necessary tubes for proper identification.

   Blood clots can occur especially in frozen samples. They prevent the accurate and precise pipetting of the sample. Therefore, samples need to be tested for blood clots visually and either removed manually or settled on the bottom of the

sample vial by centrifugation to minimize interfere with the pipetting process.

Because samples are transferred from the vial to the well plate, it is important to know the sample ID of each cell in the well plate. Using the Tecan instrument with a defined program for sample transfer enables the reliable tracking of samples in the well plate. Certain vial positions relate to certain cells in the well plate

2. Sample preparation procedure
Allow frozen samples, quality controls, buffer, internal standard and calibrators to reach ambient temperature. Homogenize all samples, quality controls and standards by gently mixing the vials for about 15 minutes using a rotator.

Dilute blood samples using Tecan program entitled: Dilution 0.3 task from cryovial to cryovial. 1:0.33 (v/v) with 6 mM K3EDTA saline solution and homogenize gently using laboratory rotator.

Centrifuge blood samples and quality control materials at 15 °C and 4000 rpm for 10 min using the centrifuge. This step is to move blood clots at the bottom of the cryovials. After centrifugation, visually inspect each sample tube for air bubbles; remove them if present to facilitate automated pipetting.

Place all sample vials, quality control vials, calibrators and reagents on the Tecan instrument according to the scheme depending on the tasks being performed. All bar-coded vials need to be aligned in a manner that the barcode reader installed in the Tecan instrument can read it properly. Therefore, face barcodes to the right-hand side of the 16 position racks and confirm that they can be scanned.

Scan all vials and reagents using the barcode reader installed on the Tecan (execute program: Task 1: HB-measure 4_Plates.gem). Manually scan vials that could not be read with the Tecan instrument using the hand held scanner attached to the instrument computer. If the barcode cannot be read by the scanner installed on the Tecan instrument, the program will prompt to an option to manually enter IDs using the keypad or to scan the tube and rack number using a hand held scanner that can be read with the Tecan barcode reader. After the scanning is successfully completed, a file is created ("barcode

ID file") that contains the sample ID from the barcode, the position of the vial and the position of the reagents on the Tecan instrument.

Assure that the scan results are saved as ".gem"-file on the instrument computer at [C drive:// Program files/Gemini/ Output/Archive]. A special file management program ("Lab Rat") automatically copies this file to the network drive (Q:\CCB 8), while renaming it using the current date, time and seconds format.

After scanning the vials, the program "Task 1: HB-measure 4_Plates.gem " executes pipetting of 550 µL of diluted blood samples using 1000 µL conductive tips to 48-well 5 mL plates. This plate in named "Dilution Plate" The program will pause with a wait prompt to allow assessing samples in the 48-well 5 mL plate for blood clots using a lamp. If a blood clot is observed, it is removed by the operator using a pipette tip. If blood clot is large, sample is removed from the cell and the same sample is pipetted by hand to the plate without blood clots. After plate was assessed and corrective actions have been taken as needed place Dilution plate on the Matrix Plate Mate 2X2 instrument one dilution plate at a time.

Refer to section 8C of this document for the hemoglobin measurement procedure, followed by the Edman procedure in section d.E. Note the hemoglobin measurement procedure has to be performed first before continuing with the Edman procedure.

If samples are manually pipetted, scan the barcode of each vial individually and note the position of each individual sample in the well plate and transfer samples manually..

Note: Barcodes from NHANES cover the entire cryovial disabling the Pos ID to detect whether a tube is present. Therefore, cryovials need to be placed on the Tecan rack and raised by 1 centimeter by placing a small cap below the tube. This will allow the barcode reader to sense that there is a tube present to be scanned.

C. Hemoglobin Measurement

1. Preliminaries

The hemoglobin measurement provides information about the hemoglobin concentration in the diluted sample solution used for modified Edman reaction. With the concentration of the sample solution and the specific volume used for modified Edman reaction, the amount of hemoglobin used for modified Edman reaction is calculated. This result is later used to calculate the reported results for acrylamide and glycidamide hemoglobin adducts in pmol adduct per g hemoglobin. This step is necessary, because the dilution of the specimens with EDTA saline solution 1:0.33 v/v cannot be performed accurately and precisely with the Tecan instrument because of the high viscosity of whole blood and erythrocytes.

The measurement procedure performed in this protocol is based on a commercial clinical assay. In principle, the hemoglobin and its derivatives, except sulfohemoglobin, are oxidized to methemoglobin by ferricyanide in the presence of alkali. Cyanmethemoglobin is formed from methemoglobin by reaction with cyanide (CMH solution). The resulting red colored complex has peak absorption at 540 nm, the value of which is proportional to total hemoglobin concentration.

2. Hemoglobin measurement procedure

   a. Pipette 2.5 mL of the CMH Solution using the Hamilton pipettor into a 5 mL 48-well pyramid bottom well plate. Place plate with CMH solution and dilution plate with blood samples on the Matrix worktable.

   b. Manually add 10 µL of the hemoglobin QC pools using positive displacement pipette in the 2.5 mL CMH solution to the appropriate cells in each 48 well plate to the following positions:

	Plate 1	Plate 2	Plate 3	Plate 4
low QC to cell:	1f	2f	3f	4f
medium QC to cell:	1g	2g	3g	4g
high QC to cell:	1h	2h	3h	4h

Note: the batch ID and expiration date of the QC materials.

   c. Transfer 20 µL of diluted blood sample (transfer is performed using two tips using 10 µL volume in each tip) prepared in section 2. to the plate containing the CMH solution and mix thoroughly by running Matrix program "Task1. HB-measurement". Each Dilution Plate containing 550µL diluted

blood for the CMH measurement is placed on the Matrix separately, after the first plate CMH measurement is performed then the second Dilution Plate is placed on the Matrix Plate Mate 2X2. 96 channel pipettes are used. Each sample is being measured in duplicates, but later the two cells are averaged from the duplicate measurements. See Table **Error! Reference source not found.** for sample transfer to the 96 well microtitor plate (MTP)

d. Read the absorption of the empty 96-well MTP
Create a new folder one time on the C drive to store the raw data from the UV/VIS plate reader. (i.e., HB_reading_AA folder)

Open the Powerwave software (KC_4) on the computer and load program "HB_reading_AA.

e. Place the 96-well MTP on Matrix instrument and transfer 200 µL sample solution in duplicate measurements prepared in step 2) to the 96-well MTP uses the Matrix program "Task 1. HB_Measurement".

One 96-well microtiter plate will have duplicate measurements of one sample. Using a 200 µL fixed-volume Gilson pipette, pipette the hemoglobin calibrators to the 96 well MTP manually to the following positions:

HB calibrators	1st 96-well MTP	2nd 96-well MTP	3rd 96-well MTP	4th 96-well MTP
Blank	1a and 2a	3a and	5a and	7a and
5 g/dl	1b and 2b	4a	6a	8a
calibrator	1c and 2c	3b and	5b and	7b and
10g/dl	1d and 2d	4b	6b	8b
calibrator	1e and 2e	3c and	5c and	7c and
15g/dl		4c	6c	8c
calibrator		3d and	5d and	7d and
20g/dl		4d	6d	8d
calibrator		3e and	5e and	7e and
		4e	6e	8e

f. Insert the 96-well microtiter plate with the sample solutions back in Powerwave 200 plate reader and select "OK" to measure the absorbance at 540 nm of the sample solution. Each MTP is measured individually once the final absorbance is

measured for the first MTP, the second, third and fourth MTP can be measured thereafter one at a time.

g. After reading the absorption in step 5, the instrument software automatically subtracted the absorbance of the empty plate (obtained in step 3) from the absorbance (obtained in step 5) and stores the results as text-file on the local C drive (HB_reading_AA folder). The software automatically prompts to save raw absorbance data as a text file. The file is imported in Excel and saved as file using the date and the run IDs of the plates being measured (HB_raw_[Date][ID].xls) in the appropriate folder in q-drive.

3. Hemoglobin Measurement Data Processing
The absorbance of the calibrators and the known concentration of the calibrators are used to calculate a calibration curve using linear regression. The x-axis represents the concentration while the y-axis represents the absorbance. The total hemoglobin content in the samples is calculated using the calibration curve.

Note: All calculations can be performed using an MSExcel template named "HB_Calc_2006_Plate 1 and 2.xls and HB_Calc_2006_Plate 3 and 4.xls". Use the appropriate template according to the plate 1 and 2 or plate 3 and 4 to assure proper sample assignment. The template assumes the samples to be arranged in the plate as described in Table **Error! Reference source not found.**

a. Open MSExcel and import the data created in 0
b. Open file "Hb_Calculations_2006.xls"
c. Copy all absorption data from HB_raw_[Date][ID].xls to file "Hb_Calc_2006_Plate_X and X.xls depending which plate you are working with", sheet "Plate R. Raw 1" for the first plate 48-well plates and to "Plate R. Raw 2" for the second 48-well plates to cells A4 through M11 using the "Past Special Link" function.

Note: All absorption data can be copied and pasted in one step for each 96 well plate. The designated area in the Hb_Calc_2006_Plate_X and X.xls file is setup for the data as they were produced by the Powerwave Software.

d. Save the modified "Hb_Calc_2006_Plate_X and X.xls"-file under the appropriate name using the current date and corresponding run ID in the q-drive.
Once the instrument readings are inserted in the template file, the following calculations are performed automatically:

- From the duplicate samples and calibrator absorbance measurements the average absorbance is calculated.
- The average absorbance of the calibrators is plotted against the total hemoglobin concentration.
- A linear regression is performed using the "linreg" function in MSExcel.
- The total hemoglobin content for each samples and QCs is calculated by using the obtained calibration curve.

e. Open the appropriate file containing Barcode Reading Data generated by the Tecan Pos ID mark the first column to sort all values A to Z and copy the information in the renamed template file HB_calc_2006_Plate X and X.xls into worksheet "Barcode R. Raw". The total hemoglobin measurement results are then automatically assigned to the correct sample ID.

4. Hemoglobin Measurement Data Evaluation

a. Assess the calibration curve data for any apparent unusual data.
b. Compare the obtained QC values against the manufacturers established values.
c. Assure that the total hemoglobin concentration in the sample solution is not less than 7.6 g/dL and no more than 14.2 g/dL to assure that there is any pipetting error. Note this concentration is for a diluted blood samples 1:0.3 v/v if it would be undiluted it would be out side of the normal hemoglobin physiological range.
d. If values for QCs are within the limits and the total hemoglobin concentration is within the desired range, continue with modified Edman reaction. Otherwise repeat measurements using the HemoCue device.
e. If concentration values deviate from CMH to HC values use the HC value and manually type in concentration into the worksheet "Barcode & abs. to AA calc."
f. Barcode & abs. to AA calc worksheet automatically calculates weather additional volume is required to be added to the samples to have at least 0.025 g of hemoglobin.
g. Make appropriate adjustments such as using more sample volume for Edman reaction if necessary.

Note: The template is setup to highlight total hemoglobin values that are not within the desired range using the conditional formatting function. If hemoglobin values are too low, the template automatically calculates the additional volume that needs to be added on top of the 350 µL volume neccessary for the Edman reaction in section d.D.

D. Modified Edman reaction and analyte purification

In this step the N-terminal valine containing the acrylamide and glycidamide is cleaved from the hemoglobin protein chains with the Edman reagent. The resulting pentafluorophenyl thiohydantoin derivatives are isolated from the reaction mixture by liquid-liquid extraction on diatomaceous earth and prepared further for HPLC/MS/MS analysis.

1. Modified Edman reaction and analyte purification procedure
   Add calibrators, internal standards, reagents and supplies on Tecan according to Tecan layout for "Task 2. Calibrators 4 Paltes.gem" for the addition of reagent and amounts.

   To prepare the calibrators in each plate load program "Task 2. Calibrators 4 Plate.gem" and run program. The program performs the following tasks:

   a. Pipette 200 µL calibrators using a 1000 µL conductive tips in new 48-well 5-mL plate called "Edman Plate" see Article XV.
   b. Pipette 200 µL deionized water as blank in the assigned well.
   c. Add 100 µL internal standard solution using 200 µL conductive pipette tip in wells containing the calibrators and the blank.
   d. Pipette 80 µL of 400 mM Ammonium Acetate Buffer solution to the calibrators to adjust pH to 7.0+/-0.5.

2. To transfer exact volumes of the blood samples from each plate to a new plate load Matrix program "Task2. Blood transfer 350µL" and run program. The program performs the following task.

   a. Pipette 350 µL samples and QCs using 300 µL extended length clear tips with filter using the Matrix Plate mate 2x2 from the Dilution Plate 1 described in section 2 to the Edman Plate 1. Perform each plate individually by loading / starting the program as many times as plates are available.

3. To add formaldehide solution to the Edman plate use 300 µL extended length clear tips with filter using the Matrix Plate mate 2x2, perform the formaldehide addition one plate at a time. This requires loading / starting the program as many times as plates there are to add formaldehide too. Load program "Task 3. Adding FA" and run program. The program performs the following tasks.

   a. Pipette 1.5 ml of formaldehide using 300 µL clear pipettes in all wells.

b. The 96 channel tips use two tips to transfer 600 µL of volume in one cell at a time and repeat the process and changes volume addition up till 1.5 ml total volume is reached.

4. To prepare the samples for each plate, load Tecan program "Task 3. Edman 4 Plate.gem" and run program. The program performs the following tasks:

   a. Pipette 100 µL internal standard solution using 200 µL conductive pipette tip in all wells containing sample.
   b. Pipette 55 µL 0.22 M Acetic Acid using 200 µL conductive tips in all wells containing samples to adjust pH to 7.0.
   c. Pipette 10 µL Edman reagent using a 200 µL conductive tips in all wells of the plate.
   d. Seal plates and transfer them into the oven.
   e. Incubate samples at 55 °C for 2 hours. Assure samples are shaken during that time using a laboratory shaker (Lab Rotator in the oven is set to dial 9).
   f. Let sample cool to room temperature and remove seal.

5. To transfer samples to the extraction plate for liquid-liquid extraction, arrange plates and supplies on Matrix and load and run program "Task 4. Blood transfer 2.5mls". This program transfers all sample solutions to the filter plates using 300 µL extended length, clear pipette tips with filter.

   a. Transfer the derivatized blood samples to the 7.5 ml 48 well filter plates filled with Isolute material.
   b. Wait 5 minutes for samples to properly penetrate in sorbent material.
   c. Transfers filter plates containing the samples to the Gilson instrument. Arrange extraction plates / collection plate in a fashion that the extraction plate is on the bottom in respect to the filter plate (7.5-mL 48-well plates).
   d. Load and run program "Task 1. CE 8.0 mL elution.Elute". The program dispenses 8.0 mL of a solution of Isopropylether/ Ethylacetate/Toluene in portions of 1 mL (2 times 0.5 mL) per well.
   e. Remove elution solvents by placing the collection plates containing the extraction solutions in a GeneVac EZ-2 concentrator. Instrument setting is set on "low boiling and no heat".
   f. After solvent has been removed. Wash the walls with ethylacetate solution to recover the analytes on the side of the walls. Use the repeater pipette with a 10 mL tip and set the dial on 1 (corresponds to 200 µL dispense volume). Add 200 µL of

ethylacetate to each wall of collection plate. Then, add an additional 200 µL to the wider walls of the wells (total wash volume: 1,200 µL).

g. Vortex plate on level 4 for 30 seconds.

6. Arrange collection plates containing ethylacetate and supplies on Tecan instrument. Use Tecan instrument by loading and running program "Task 5. Wall wash transfer 4 Plates.gem".

   a. Transfer samples recovered from the wall wash step to a new 96-well 2-mL plate called "Reconstitution Plate"
   b. Remove solvents by placing the Reconstitution Pates in a GeneVac EZ-2 concentrator. The instrument is set to "low boiling point" and no lamp setting.
   c. Dry Reconstitution Plate for 4 hours, during this time the solvent evaporated completely.

7. To reconstitute the samples load Matrix program "Task5. Reconstitution Step" and run program. The program performs the following tasks.

   a. Arrange plates according to Figure 19
   b. Reconstitute samples with methanol and water by loading and running program "Task 5. Reconstitute Step". This program adds 80 µL of methanol and 120 µL of water to each well.
   c. Mixes plate using 96 channel Matrix tips.
   d. Transfer sample to filter plate (GHP 0.2 µm filters) containing a new 96 well 2 ml collection plate called " Final Plate" below the filter plate. This program transfers the samples in the filter plate. The filter plate and Final Plate then is transferred to the centrifuge manually. Two rubber bands are place on the plates before centrifuging. The samples are filtered by centrifuging for 10 min at 3700 rpm and 15 °C.
   e. After filtration is finished the Final Plate is sealed and transfer for analysis by HPLC/MS/MS.

E. Analysis of Edman products by HPLC/MS/MS

In this section, a run sequence is created using the information obtained from scanning the barcodes of the sample vials (section 2). The runs sequence is then used by the HPLC/MS/MS software to analyze the samples in the wellplates.

1. Creating a run sequence

   A run sequence consists of (2) 48-well plates combined in on 96-well plate and additional samples such as water and instrument

controls that are added to assess carry-over and instrument function. The sequence is created by combining the Barcode ID file with a sequence template file.

a. Load template file "Sequence_1 and 2_2006.XLS or Sequence_3 and 4_2006.XLS" depending on which plates you are working with.
b. Load Barcode ID file, sort all IDs using the A to Z short function
c. Copy information from Barcode ID file to "Sequence_X and X_2006.XLS" into worksheet "Barcode ID" file using the "Paste Special Link" function. After copying the information, the sequence information is automatically compiled.
d. Save sheet "Export Sequence" as "CSV" file using the date and RunIDs as name (i.e., 061206_P_255_256). Use the date when sample preparation was started.
e. Open Finnigan Xcalibur software and activate "Sequence Setup".
f. Import the CSV file and add additional information such as injection volume, path or instrument method as needed.
g. Save sequence using the same name as used for the CSV file.
h. Insert Shutdown method to shut down the instrument at the end of the run if no more plates will be run.
i. Preliminary instrument setup and performance check
   (1) Make sure that all the solvent reservoirs are full or have enough solvent to run the sequence. Check the Argon gas pressure. It should be about 20 psi. Make sure the tank is not empty.
   (2) Empty the waste solvent reservoir of the LC system into the LC waste 4L bottle labeled for acrylamide project located under the sink.
   (3) Turn the flow on the LC on and wait until pressure is stable and record the HPLC pressure before each sequence run in the appropriate log book.

F. Chromatographic and mass spectrometric conditions (for additional information see page 52)

HPLC system: ThermoFinnigan Surveyor HPLC system (San Jose, CA)
HPLC column: Thermo Hypersil GOLD, (50 x 2.1 mm, 1.9 µ, Termo Electron, Holbrook, NY)
Column temperature: 50 °C
Eluent: Methanol and water (63/37 v/v) C methanol, D methanol water 1:1
Flow rate: 400 µL/min

Injection conditions:	50 µL (no waste option) with needle wash and syringe flush after each injection.
MS system:	ThermoFinnigan TSQ Quantum Ultra (San Jose, CA)
Ionization conditions:	Atmospheric pressure chemical ionization (APCI), positive ion mode at 4.5 µA, and 375°C vaporizer temperature

Detection conditions: single reaction monitoring (SRM) at 10 eV collision energy of transitions

m/z 396 → m/z 379 for AA-Val-PFPTH,

m/z 402 → m/z 385 for AA-Val(13C5,15N)-PFPTH,

m/z 412 → m/z 395 for GA-Val-PFPTH and

m/z 418 → m/z 401 for GA-Val(13C5,15N)-PFPTH

For confirmatory purposes the transitions

m/z 396 → m/z 337 for AA-Val-PFPTH and

m/z 412 → m/z 357 GA-Val-PFPTH are monitored.

G. Final instrument setup and sample sequence run

1. Load the sequence created in section 8.d.1.
2. Place the sample plates that correspond to the sequence loaded in the auto sampler of the LC system. Make sure cell A1 of the plate is at the left back position and the plates are positioned on the rack corresponding to the sequence.
3. Run the sequence to analyze samples.

H. Processing of HPLC/MS/MS data

1. Transfer raw data files obtained from the HPLC/MS/MS measurements to the corresponding folder on the Network (Q-drive).
2. Open Finnigan Xcalibur and activate Sequence set up, make sure the files are in the right directory.
3. Perform automated data integration using the XCalibur processing program "021006 AAGA conf ions short column". Save file reprocessed.

4. Assess automated integration for correctness and adjust integration as needed manually.
5. Save integration results as xqn-file using the same file name as used for the corresponding sequence file.
6. Create a hardcopy of each integrated peak using the report template named "AA report template.doc".
7. Export integration results as MSExcel file (use option "Short Excel Report" in Xcalibur Quan Browser). For saving the file, use the same file name as used for the corresponding sequence file.
8. Open MSExcel and load template file "AA_calculation_2006_CC Data for plate 1&2.xls or AA_calculation_2006_CC Data for plate 3&4.xls" and load the MSExcel export file created with Finnigan Xcalibur.
9. Copy sheets of "AA_calculation_2006_CC Data for plate 1&2.xls or AA_calculation_2006_CC Data for plate 3&4.xls" to export file. Close excel sheet and save current file using the same file name as used for the corresponding sequence file with plate number the addition of the word "data" at the end of the file name.
10. In the "Summary" sheet of the file, update all links to refer to the sheets in the current file.
11. Open file containing the corresponding hemoglobin measurement data (see section: 0) and insert results from worksheet "Barcode & abs. to AA calc" from columns A,B and C to the Summery sheet to AA calculation_2006_CC Data for plate X&X.XLS depending which plate you are working on to columns P,Q and R.
12. Assess if sample IDs from hemoglobin measurement match sample IDs of adduct measurements. If IDs match, the correct reportable result is calculated automatically. If sample IDs do not match, assess the reasons and make appropriate adjustments.
13. After links have been updated, calibration curves, acrylamide and glycidamide adduct values are calculated automatically.

Note: The template automatically performs an ID match test and highlights sample IDs that do not match. Further, the template automatically highlights data that are outside the calibration curve.

9. REPORTABLE RANGE OF RESULTS

This method is linear for acrylamide and glycidamide adducts in the range 1.25 – 80 nmol/L. Assuming 25 mg of hemoglobin were used for adduct measurements, the linear range would translate to 40 pmol/g hemoglobin and 20 pmol/g hemoglobin and 125 pmol/g hemoglobin and 350 pmol/g hemoglobin for glycidamide- and acrylamide adducts respectively. Samples outside this range are re-analyzed for confirmation before results are released. Sample above this range will be diluted further with water. Samples below this range will be processed by

diluting them less or by using higher sample volumes for the Edman reaction.

10. QUALITY CONTROL (QC) PROCEDURES

A. Bench QC Materials

The bench QC pools used in this method comprise three levels of concentration spanning the "low-normal" to "high-normal" ranges of the analyte of interest. The intent of bench QC is for the analyst to evaluate the performance of the analytical system. The analyst inserts the bench QC specimens in each sample set (set of samples that are processed together in one batch).

Pending on the sample specimen being used for analysis three levels of either erythrocyte controls, or whole blood controls are analyzed in duplicate in each run as bench quality control materials. Hemoglobin adduct ranges of 25 - 50 pmol/g hemoglobin (low QC pool), 50 – 150 pmol/g hemoglobin (medium QC pool) and 150 –400 pmol/g hemoglobin (high QC pool) are targeted. Erythrocytes or whole blood (1000 µL) is aliquot into 2.0 mL cryovials, capped, and frozen. The QC pools are stored at -70 °C and are stable for at least 4 years.

Means plus range limit for all pools are established by analyzing duplicates for at least 20 consecutive runs. The low QC pool is prepared by selecting and pooling blood from non-smoking individuals. Smoking status can be determined by using cotinine measurements using test such as TobacAlert from Nymox Corp (www.tobacalert.com). The medium QC pool is prepared by mixing a high QC pool with a low QC pool 1:1 (v/v). The high QC pools are obtained from people actively smoking tobacco products.

The quality control materials for total hemoglobin measurements provided by the assay manufacturer are used and assessed according to the manufacturer's recommendations.

B. Blind QC Materials

Blind QC samples are QC materials placed in vials, labeled, and processed so that they are indistinguishable from the subject samples handled by the analyst. The supervisor decodes and reviews the results of the blind specimens without the analyst knowing of their presence in the runs. At least one low-normal concentration and one high-normal concentration QC material should be run in the laboratory for this purpose.

C. Calibration and Calibration Verification Procedures

Assessment of runs using QC materials

The results from the QC pools are checked after each run. The system is declared "in control" if all three QC results are within 2s limits and the run is accepted. If one of the three QC results is outside the 2s limits then apply rules below and reject if any condition is met - the run is then declared "out of control":

* 13s: Any of the three QC results are outside the 3s limit
* 2x2s: Two of the three QC results in the run are outside the 2s limit (same side of mean)
* R4s: Sequential QC results (either within the run or across runs) are outside the 2s limit on the opposite sides of the mean
* 10x: Ten sequential QC results (across pools and across runs) are on the same side of the mean

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC.

The initial limits are established by analyzing pool material in 20 consecutive runs and then are re-evaluated quarterly. When necessary, limits are updated to include more runs.

While a study is in progress, electronic copies of the QC results from each run are stored in the analyte-specific folder. Electronic copies of the tracking of the QC results over time are stored in the analyte-specific folder. A hardcopy of the QC results from each run is also kept by the analyst.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Assess whether the hardware is functioning properly. Make sure the MS calibrations are proper. Check the instrument calibration.

Check the autosampler and UV signal for proper sample injections.

Look for sample preparation errors, specimen, right volume of buffer etc.

Check the calibrations of the pipettes.

If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.
Do not report analytical results for runs not in statistical control.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

No known interferences exist with this method for the detection and quantitation of acrylamide and glycidamide adducts. However, other unknown compounds are detected with this method. Poor chromatographic separation may cause interferences with these compounds.

Acrylamide and glycidamide adducts are formed due to exposure coming from food, smoking and certain occupational activities related to handling of acrylamide. The adduct concentrations measured with this method do not provide any information about the exposure source(s).
Though adduct values of people exposed to tobacco smoke are higher than in groups not exposed to tobacco smoke, data from individuals cannot be used to assign it to one or the other group, because of the high inter-individual variability and the overlap of adduct values in both groups.

13. REFERENCE RANGES (NORMAL VALUES)

Reference ranges have not been established yet. An in-house assessment using a convenience sample from 96 individuals found the following adduct values: the median globin adduct values are 129 pmol/g globin (range: 27 - 453 pmol/g globin) and 97 pmol/g globin (range: 27 – 240 pmol/g globin) for AA-Hb and GA-Hb, respectively. Higher values have been reported in the literature and were related to occupational exposures.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Currently, no information is available linking hemoglobin adduct data to certain health outcomes.

15. Test Result Reporting System; Protocol for Reporting Critical Calls

Not applicable

16. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

The Microsoft Access database is used to keep records and track specimen's results. If analyses are used for smaller, non-NHANES studies, records are kept on file on the CDC network and on CDs or DVDs.

Records including related QA/QC data are maintained for 10 years after completion of the study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual specimen from these analyses for non-NHANES studies may be discarded at the request of the principal investigator, or may be transferred to the CDC CASPIR facility for use by other investigators. Very little residual material will be available after NHANES analyses are completed, and these vials may be routinely autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored in a freezer at -70°C. The specimen ID is read off of the vial by a barcode reader attached to the computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the DIF file containing the electronic copy of the results is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping a notebook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies.

17. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

Because the analysis of hemoglobin adducts of acrylamide and glycidamide is inherently complex and challenging, there are no acceptable alternative methods of analysis in the laboratory. If the analytical system fails, we recommend that the specimens be stored at -70 °C until the analytical system is restored to functionality.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

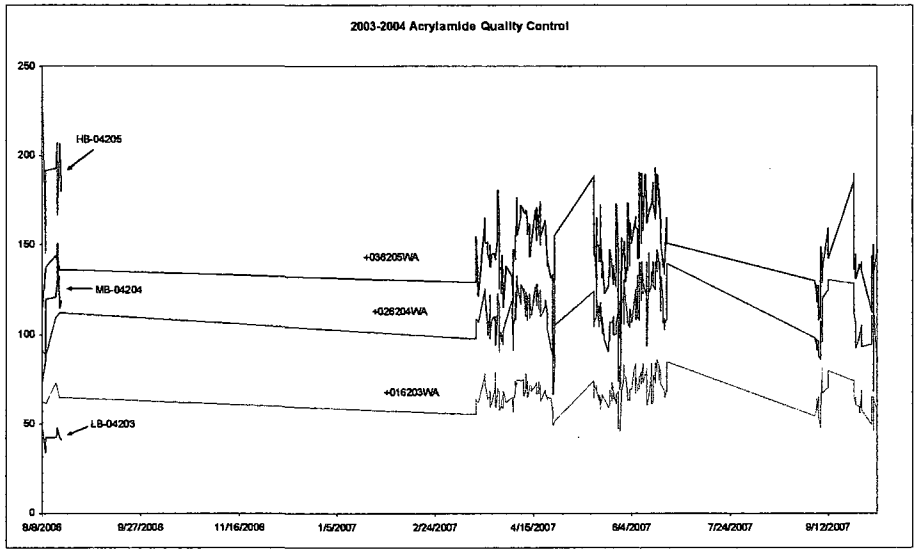
All shipments are recorded on the NHANES Shipping Log upon receipt. Actions taken during the course of analysis, result reporting, and specimen retention are also recorded on the log.

19. Summary Statistics and QC Graphs

A. Acrylamide

Summary Statistics for Acrylamide by Lot

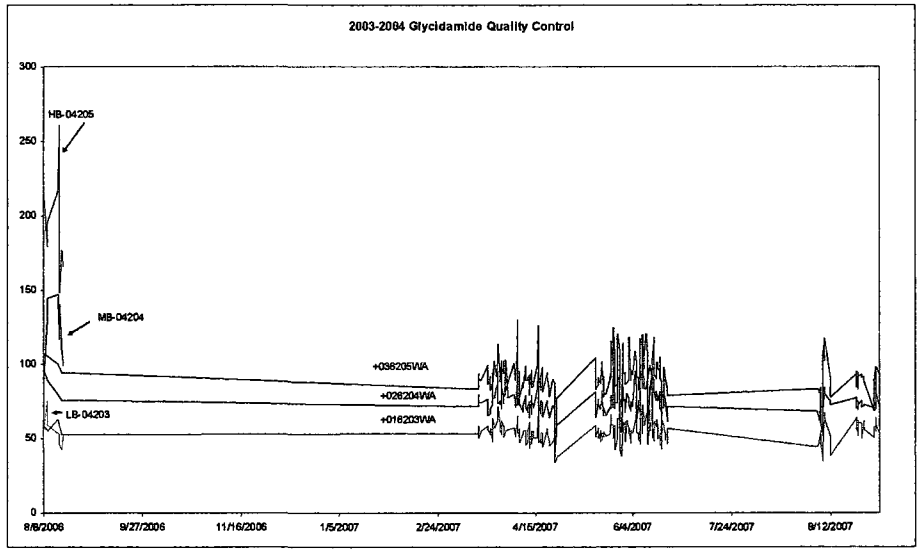
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
LB-04203	11	8/8/2006	8/18/2006	41.04	4.87	11.9
+016203WA	210	8/8/2006	10/7/2007	67.31	7.91	11.8
+026204WA	211	8/8/2006	10/7/2007	111.45	15.06	13.5
MB-04204	12	8/8/2006	8/18/2006	114.63	21.41	18.7
+036205WA	209	8/8/2006	10/7/2007	148.27	19.57	13.2
HB-04205	12	8/8/2006	8/18/2006	182.19	32.66	17.9



B. Glycidamide

Summary Statistics for Glycidamide by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
LB-04203	11	8/8/2006	8/18/2006	53.34	9.84	18.4
+016203WA	212	8/8/2006	10/7/2007	54.63	7.02	12.8
+026204WA	216	8/8/2006	10/7/2007	74.7	9.06	12.1
+036205WA	214	8/8/2006	10/7/2007	92.28	11.48	12.4
MB-04204	12	8/8/2006	8/18/2006	124.82	21.08	16.9
HB-04205	12	8/8/2006	8/18/2006	193.12	38.02	19.7



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2007	Teaching Assistant, EOHS 400 – Principles of Environmental Health, University of Illinois at Chicago
2006-2009	Teaching Assistant, PH 303 –Environmental Health Sciences, Northwestern University, Feinberg School of Medicine, MD/MPH program

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AWARDS, HONORS, DISTINCTIONS

2009 American Industrial Hygiene Association, Chicago Section – Second Place, Student Night Poster Session

2007 James P. Keogh Memorial Scholarship – American Public Health Association Occupational Health and Safety Section

2006 – 2009 NIOSH Traineeship in Industrial Hygiene

1999 Abbott Laboratories - Excellence in Safety, Health, Environment and Energy – STAR Safety Awareness Program

1998 Abbott Laboratories Chairman's Award – Alcohol Emissions Reduction Program

1998 Abbott Laboratories Hospital Products Division President's Award – Most Improved Safety Program

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Shimek, J., Lewis, J.F.M., Rankin, K., Erdal, S. and Davis, F. (2009) Occupational Chemical Exposure Assessment in a Brain Cancer Case Control Study, Student Poster Session American Industrial Hygiene Conference and Exhibition, Toronto, Ont. Canada.

Shimek, J., Lewis, J.F.M., Erdal, S. and Davis, F. (2009) Occupational Chemical Exposure Assessment in a Brain Cancer Case Control Study, Poster, Student Night, American Industrial Association, Chicago Section, Chicago, IL, Second Place.

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2004- 2006 Senior Environmental, Health and Safety Specialist
Hospira, Inc., Global EHS Office, Lake Forest, IL

Major tasks: Drafted first Sustainability Report for new company, planned three day corporate wide EHS conference, provided EHS support for off-site facilities, prepared EHS policy manual for new company, designed EHS Awards program, corporate EHS auditor.

1994-2004 Manager, Environmental Health and Safety
Abbott Laboratories Hospital Products Division, North Chicago, IL

Major tasks: Responsible for EHS program for a pharmaceutical solutions manufacturing facility, obtained Title V, water and hazardous waste permits and maintained environmental compliance programs, reduced OSHA reportable incidents, developed OSHA compliance mechanisms.

1993-1994 Senior Industrial Hygienist
Abbott Laboratories, Pharmaceutical Products Division

Major tasks: Provided EHS support for pharmaceutical research and development including Product Development, Pilot Plant, Potent Drug and Toxicology labs including laboratory safety inspections and development of safe handling procedures for experimental materials.

1992-1993 Senior Occupational Safety and Health Specialist
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Major tasks: Provided EHS program management by contract for a large medical center.

1987-1992 Manager, Environmental Health and Safety
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Major tasks: Established the first Environmental Health and Safety office for a large medical center and directed all safety related activities including oversight of new construction activities and development of hazard communication, bloodborne pathogen standard and infectious waste management compliance programs.

1980-1985 Representative
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Major tasks: Provided property/fire protection engineering services for large insurance carrier.

1978-1980 Representative
Factory Mutual Engineering Association

Major tasks: Provided fire protection engineering services for insured properties.