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A) FINAL PROGRESS REPORT

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List of Terms and Abbreviations

AA-Hb	Acrylamide hemoglobin adduct of Acrylamide
ANC	Animal Neurocarcinogen
CI	Confidence Interval
DEB	1,2,3,4-diepoxybutane
EIF	Exposure Intensity Factor
F	F test
GA-Hb	Glycidamide hemoglobin adduct of Acrylamide
HPLC/MS/MS	High performance liquid chromatography/mass spectroscopy/mass spectroscopy
IRB	Institution Review Board
JEM	Job Exposure Matrix
NHANES	National
NIOSH	National Institute for Occupational Safety and Health
NOES	National Occupational Exposure Survey
OR	Odds Ratio
P	Probability
PAH	Polycyclic aromatic hydrocarbon
pmol/g globin	Pico-mole/gram of globin
pyr-Val	N,N-(2,3-dihydroxy-1,4-butadiyl)-valine
SIC	Standard Industry Classification System
SOC	Standard Occupation Classification System

Abstract

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. The association between chemical exposures in the workplace and diseases such as cancer are difficult to observe for several reasons, including the nature and duration of the exposure, latency period between exposure and disease and individual parameters such as occupation and use of personal protective equipment. Recognition of the association between chemical exposure and diseases such as cancer are important if disease prevention is to be achieved. A group of 18 known or suspected animal neurocarcinogens (ANCs) found in the workplace were considered in this project. The association between potential workplace chemical exposures and the risk of glioma was examined.

First, self-reported work histories were used to develop a cumulative exposure score for each study participant. The entire work history was considered due to potentially long latency period between exposure and glioma diagnosis. The cumulative exposure score was based on the 18 ANCs found in the workplace, the length of time the study participant spent in that workplace and the occupation of the study participant while in that workplace. Scores were compared to glioma case/control status to identify possible associations. Associations between glioma case/control status and industry, occupation and individual ANCs were also evaluated.

While a cumulative exposure score was generated based on the potential for occupational exposure to 18 different ANCs, these scores were not associated with an increased risk of glioma. The number of study participants assigned to the individual industry, occupation and ANC exposure groups were small. In some instances the odds ratios that were calculated for the exposure groups indicated an increased risk of glioma based on exposure but were not statistically significant. An increased risk of glioma based on potential exposure to one chemical, 3,3'-dimethylbenzidine dihydrochloride was detected and nearly statistically significant (p=0.07). SIC code 17-Special Trade Contractors and SIC code-20-Food and Kindred Products were two industries where an increased risk of glioma was detected but the resulting odds ratio was not statistically significant. These industries may warrant further study with a larger study population.

Second, questionnaire data was used to examine the association between three compounds, acrylamide, 1,3-butadiene and polycyclic aromatic hydrocarbons (PAHs) and glioma. Potential exposures were identified by study participant responses to questions related to industries and occupations where these chemicals were known to be present. Ever exposed versus never exposed variables were created to evaluate potential associations. Using questionnaire data, no statistically significant results were obtained for study chemicals.

Finally, a biomarker of exposure study was conducted to examine the relationship between exposure to acrylamide and 1,3-butadiene, as determined by hemoglobin adduct analysis, and potential workplace exposures. Blood samples were analyzed for the presence of hemoglobin adducts and

compared to potential occupational exposure to the chemicals based on current work status. Available data including demographics, smoking history and food frequency questionnaires were also examined.

Analysis of the blood samples did detect the first reported observation of the *pyr*-Val hemoglobin adduct for 1,3-butadiene which is significant in that this adduct represents the formation of the most mutagenic epoxide formed during the metabolism of 1,3-butadiene and had not previously been observed in human blood samples. The biomarker study was small and did not provide strong evidence for occupational exposure to acrylamide or 1,3-butadiene. Of the demographic variables examined, the state of residence (Northern or Southern states) showed higher levels of the *pyr*-Val adduct lfor those study participants residing in the Southern states and the differences were statistically significant. Exposure to second hand smoke was also reported by study participants. Statistically significant differences in the geometric mean *pyr*-Val levels was also seen between study participants who rated their exposure to second hand smoke as light or moderate when compared to study participants who rated their exposure to second hand smoke as heavy.

The difference between the geometric mean acrylamide adduct levels of smokers compared to non-smokers was also statistically significant as was the difference in adduct levels between those who ever smoked versus those who never smoked. As hemoglobin adducts reflect exposures that had occurred during the past 120 days the result suggests a need for further study of this association.

There was one diet question that generated statistically significant differences for the glycidamide adduct of acrylamide, frequency of consumption of wheat, corn or millet containing products. However there was no increasing trend with increasing food consumption.

Acrylamide adduct results were compared to results of the NHANES study released in December, 2010. Although the samples were analyzed by the same laboratory the acrylamide adduct results were generally lower and the glycidamide results generally higher for the two sets of samples.

The hemoglobin adduct study did not show evidence of an association between potential occupational exposure and hemoglobin adduct levels.

The study was limited by the number of study participants and the lack of industrial monitoring information that would confirm the occupational exposures.

Section 1

Highlights/Significant Findings

The use of biomarkers of exposure as indicators of exposure continues to develop as a viable method of detection. In the biomarker study for 1,3-butadiene and acrylamide there were several significant findings. The *pyr*-Val hemoglobin adduct for 1,3-butadiene has been previously undetected in human blood samples. The 1,2,3,4-diepoxybutane metabolite is formed during 1,3-butadiene metabolism and is considered to be the most carcinogenic of the three metabolites formed. The *pyr*-Val hemoglobin adduct is an indicator of the presence of this metabolite and that exposure to 1,3-butadiene had occurred within the past four months. The hemoglobin adduct was found in all samples analyzed which indicated the improved sensitivity of analytical methods. The *pyr*-Val adduct levels were not statistically significantly different for subjects potentially exposed to 1,3-butadiene when compared to those subjects who had no occupational exposure. However, when *pyr*-Val adduct levels were compared between subjects living in Northern states to those living in Southern states a statistically significant difference was seen between the two groups with the adduct levels of subjects living in the Southern states being higher than subjects living in the Northern states. Also of note were comparisons of *pyr*-Val adduct levels for second hand smoke exposure. Subjects reporting heavy exposure to second hand smoke had statistically significant higher adduct levels than either low or moderately exposed subjects. Acrylamide hemoglobin adduct levels were also not statistically significantly different based on potential occupational exposure. However, when acrylamide hemoglobin adduct levels were compared between subjects who had ever smoked versus those who had never smoked a statistically significant result was observed. This observation deserves further study as hemoglobin adduct levels represent recent exposures.

The studies using questionnaires and self-reported work histories did not provide statistically significant differences between subject with glioma and controls. While a cumulative exposure score was calculated for those subjects who had submitted work histories the number of participants in any one industry or occupation was small. A larger study or a study of specific industries or occupations may detect the differences between cases and controls hypothesized in this study. The questionnaire data also did not identify a sufficient number of cases and controls in any one industry or occupation to observe significant findings.

Translation of Findings

Identifying chemical exposures that may result in workplace disease involves collaboration between several different disciplines, exposure assessment, industrial hygiene and epidemiology. In the studies conducted here, potential exposures to several chemicals were estimated over the course of a study subject's work history and a relative exposure score was generated for subjects with glioma and a control population. The study size was a limiting factor in that there was not a concentration of subjects in any one industry or occupation. The methodology is flexible in that variables may be added or subtracted from the formula and may prove useful for future studies. Studies of a particular industry or occupation with known exposures to a group of study chemicals may benefit from a similar study. While

not statistically significant, the odds ratios generated as a part of this study indicated that some industries and occupations may pose an increased risk of work related disease. These studies may be useful in evaluating exposure to mixtures of chemicals in the workplace.
The full benefit of biomarker research is yet to be realized. The biomarker study completed here demonstrates that there may be more than one exposure route for a chemical to caused damage at the cellular level. The study further indicates that a combination of factors may be responsible for the onset of disease. Because significant chemical exposures occur in an occupational setting the use of biomarkers to quantify exposures is a field that continues to evolve. This study will add to the body of knowledge about chemical exposures. An understanding of the cellular damage caused by chemical exposures will lead to better methods of disease prevention from workplace exposures.

Outcomes/Relevance/Impact

The studies completed under this grant did not show a statistically significant association between occupation and glioma. The use of self-reported work histories is important to gain insight into the etiology of occupational disease. The algorithm created during this study may potentially be used in other studies in conjunction with single industries or occupations and an appropriate set of chemical exposures. The addition of additional variables such as industrial hygiene monitoring records or professional judgment will enhance the usefulness of the algorithm. In addition, it should be noted that glioma is a rare cancer not currently linked to any occupational exposure.
The use of biomarkers of exposure and further studies in this area will have the greatest impact on detecting workplace disease. The results of this study will assist in the design of future biomarker studies. The prevention of chronic workplace diseases such as occupational cancers starts with the detection of an exposure. If detection of the exposure occurs at the cellular level then methods for disease prevention can be instituted before the onset of disease. The biomarker study shows that an individual's interaction with environment is not singular in nature and affected by all aspects of daily living. This was observed with regard to occupation, cigarette smoke exposure, place of residence and diet although the study population was small. Biomarker research continues to evolve and the results of this study will add to the body of literature concerning this important discipline.

Section 2

Scientific Report

Background for the project

Prevention of disease is critical if the health of the population is to be achieved. 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 research grant was that chemical exposures in the workplace 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 grant investigated potential exposures to known or suspected animal neurocarcinogens found in the workplace in a brain cancer case-control study population.

Specific Aims

There were three specific aims addressed in this grant. First, a cumulative exposure scoring method was developed to gauge potential exposure to known or suspected animal neurocarcinogens (ANCs) found in the workplace using self-reported work histories. This methodology was applied to subjects in a glioma case-control study population. Second, using a questionnaire developed to address workplace chemical exposures, responses to questions designed to assess potential exposure to 1,3-butadiene, acrylamide and polycyclic aromatic hydrocarbons (PAHs) were analyzed to examine the association between these potential exposures and glioma in a second case-control study. Third, hemoglobin adducts for acrylamide and 1,3-butadiene, as biomarkers of exposure, were assessed by laboratory analysis and compared to the subject's current work assignment to compare potential exposure to these chemicals with the laboratory findings.

Methods

Individual studies were designed to address the three specific aims listed above.

Study Subjects

Institution Review Board (IRB) approvals were received prior to the enrollment of study subjects. 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, sibling or hospital based controls who were English speaking and 18 – 80 years old. Study subjects completed an extensive questionnaire which consisted of a number of modules (e.g., demographics, family history, smoking history, medical history, and environmental, occupational, and dietary exposure histories). Study participants enrolled early in the study completed paper-based self –reported occupational work histories. These 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 and were used to calculate the

cumulative exposure scores. Study subjects either provided a blood sample at the time of enrollment or were called back to provide a blood sample for the hemoglobin adduct study.

National Occupational Exposure Survey (NOES)

The NOES completed by NIOSH during 1981-1983 was used to identify the workplaces where the ANCs of interest were found. 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.

List of Known or Suspected Animal Neurocarcinogens

A list of more than 40 ANCs was compiled by members of the research group. The list of ANCs was compared to the NIOSH-NOES and 18 ANCs were found to be common to both lists. The ANCs of interest to this study were acrylamide, acrylonitrile, bis (chloromethyl) ether, dimethyl sulfate, ethylene oxide, glycidol, 1-H-benzotriazole, bromoethane, 1,3-butadiene, chloromethane, CI Direct Blue 15, 3,3'-dimethylbenzidine dihydrochloride, 3,3'-dimethoxybenzidine dihydrochloride, ethyl methyl sulfonate, isoprene, vinyl chloride 2-acetylaminofluorene and divinyl benzene. This list of 18 chemical, the NOES survey codes and the self-reported work histories were used to generate the exposure scores.

Development of the Cumulative Exposure Score

To create the cumulative exposure score, three variables were assigned to each job reported by a study subject in the self-reported work history based on the NOES. 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 industry and occupation codes for the job. The scores for each job were totaled for each study participant. The cumulative exposure score was expressed as follows:

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

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. A score of 1.5 would indicate a potential exposure to half of the ANCs found in the SIC code for the job. An exposure score for each job was estimated by multiplying the number of ANCs times the exposure study participant and the score for each job were totaled to generate the cumulative exposure score.

Questionnaire Data

The questionnaire completed by study respondents were designed to identify potential exposure to specific chemicals based on industry, occupation or perceived exposure to fumes and dusts as reported by the study subjects. Responses to the questions related to acrylamide, 1,3-butadiene and PAHs were analyzed to evaluate the association between potential exposure to these chemicals and glioma in a case-control study.

Hemoglobin Adducts Study

Blood samples were prepared for analysis by harvesting the globin from the red blood cells. Globin samples were sent to the Centers for Disease Control and Prevention's (CDC's) National Center for Environmental Health for acrylamide hemoglobin adducts 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. The total hemoglobin in the sample was measured and results were reported as pmol adduct/g globin.

Globin samples were also analyzed for the *pyr*-Val hemoglobin adduct at Dr. Jim Swenberg's laboratories at the University of North Carolina, Chapel Hill. 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 adduct/g hemoglobin.

Statistical 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 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. Both conditional and unconditional logistic regression models were generated. Odds ratios were also calculated based on industry, occupation and ANC.

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

Hemoglobin is found in the red blood cells which have a lifespan of 120 days. Hemoglobin adduct results reflect exposures that have occurred within the prior 120 days. To evaluate the hemoglobin adduct results, an occupation 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

NOES. 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. Smoking histories and a food frequency questionnaire were also completed. Odds ratios and 95% confidence limits were calculated for these data.

Acrylamide hemoglobin adduct results were also compared to the NHANES report released in December, 2010.

Results

To evaluate the cumulative exposure score methodology, 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). 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.

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 for the NOES. 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.

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.

Odds ratios and 95% confidence intervals were also calculated by chemical, industry and occupation. Odds ratios by chemical were not statistically significant. The highest odds ratio observed was for 3,3'-dimethylbenzidine dihydrochloride (N=428, OR=1.92, 95% CI=0.94-3.92, p=0.07).

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. The highest odds ratio for industries by SIC code was calculated for SIC code 20, Food and Kindred Products (N=428, OR=2.88, 95% CI=0.80-10.38, p=0.09). 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 (N= 229, OR = 2.99, 95% CI=0.92-9.68, p=0.07).

Odds ratios were also calculated for study participants based on the SOC codes for occupation. The highest odds ratio was calculated for SOC code 49, Installation, Maintenance and Repair (N=428, 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 (N=428, OR=1.52, 95 %CI=0.93-2.48, p=0.10).

For the study evaluating the association between glioma cases and potential exposure to acrylamide, 1.3-butadiene and PAHs, responses from 412 cases and 602 hospital-based controls were analyzed. 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.

Of the questions related to type of industry, acrylamide was associated with 20 of the 39 industries, 1,3-butadiene was associated with five of the industries and PAHs were associated with 12 of the industries. 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 potentially exposed individuals working in these industries (OR < 1.0).

Of the 51 occupation questions, 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. 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.

Blood samples (N=76) were analyzed for hemoglobin adducts for acrylamide and 1,3-butadiene. Study participants were also part of the control population for one of the two studies described above and had completed several different modules of a questionnaire, in addition to providing their current job status. Not all participants completed all modules, thus the number of responses used in the analyses varied.

The results for the analysis of the *pyr*-Val adduct of 1,3-butadiene varied from 0.23-3.14 pmol/g globin. Two hemoglobin adducts were measured for acrylamide analysis, the acrylamide adduct results varied from 14.7-380 pmol/g globin and the glycidamide adduct 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.

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 (N=11) or SIC code 80, Healthcare (N=13). From this information, exposure variables were created for acrylamide and 1,3-butadiene. 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 hemoglobin adduct levels of exposed and unexposed study populations.

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. For the *pyr*-Val hemoglobin the only statistically significant difference in the geometric mean was for the state of residence. When respondents from Northern states (N= 28, Illinois and Ohio) were compared to respondents from the Southern States (N=38, Arkansas, Oklahoma, North Carolina, South Carolina, Tennessee, Texas and Virginia) a statistically significant difference was observed ($F=5.26$, $p=0.03$). For the acrylamide adducts there were no statistically significant results for the demographic responses.

As both 1,3-butadiene and acrylamide are found in cigarette smoke available data on study participant smoking history were analyzed. Study participants rated their exposure to second hand smoke as light, moderate or heavy. For the *pyr*-Val adduct results, the differences in geometric means between both light and moderate exposures were statistically significant when compared to the geometric mean for study participants who reported heavy exposure to second hand smoke. For the acrylamide adduct of acrylamide the differences in geometric means between current smokers and non-smokers and between those who had ever smoked compared to those who had never smoked were statistically significant.

Sixteen questions from the Food History Questionnaire were examined for an association with the hemoglobin adduct levels of acrylamide and 1,3-butadiene. Respondents reported the frequency of consumption of the foods in question. Responses were divided into three groups and the geometric

means of the groups were compared. 1,3-Butadiene is not normally associated with foodstuffs but the analysis was included for completeness.

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 significantly different ($F=6.58$, $p=0.004$). When the two extreme values were removed and the data re-analyzed, Question 12, Wheat, corn and millet remained statistically significant ($F=3.70$, $p=0.03$).

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 comparison category for both current smokers and non-smokers the glycidamide adduct results, the results of this study were lower than the NHANES results. For the acrylamide hemoglobin adduct results, the results of this study were higher for non-smokers and lower for current smokers than the NHANES results. The resulting ratios of GA-Hb to AA-Hb were also higher for the non-smoking study participants and comparable for smokers when compared to the NHANES results.

Discussion

The development of the cumulative exposure score evaluated 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 influence of occupations in a case-control study of glioma was evaluated 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 a matched pair analysis, although the resulting odds ratios were not statistically significant.

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, the odds ratios that were calculated were not statistically significant.

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 an occupational history of a questionnaire, and the glioma case/control status of the study participant. 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.

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.

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 a reduced risk of glioma based on the association of potential workplace exposures with glioma status.

The detection of the *pyr*-Val hemoglobin adduct 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 this adduct is significant as the *pyr*-Val adduct is an indicator of the formation of 1,2,3,4-diepoxybutane (DEB), thought to be the most carcinogenic of the three metabolites formed during 1.3-butadiene metabolism. 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.

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.

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. Glycidamide is also capable of forming DNA adducts and is considered responsible for acrylamide's genotoxic capabilities.

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 and the NOES study. 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.

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 occupational 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, thus the potential for occupational exposure could not be evaluated.

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 geometric mean 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 (geometric mean) 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 geometric means between ever smokers and never 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 results 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 ever smoked. This result and its significance cannot be explained.

Recently, the discovery of acrylamide in foods has resulted in another potential exposure to this chemical. Foods such as French fries, backed crackers and chips and other fried starch containing foods have been found to contain varying amounts of acrylamide. Some participants in the hemoglobin adduct study completed a food frequency questionnaire that asked participants to estimate the number of times they ate certain foods in a week or month. Responses to 16 questions were evaluated to determine the influence of diet on acrylamide adduct formation. For completeness, the *pyr*-Val adduct levels were also compared to diet question responses. Responses were collapsed into two or three groups and statistical comparisons of the geometric means of the adduct levels were made. These results should be interpreted with great care since there may be statistically significant differences between the geometric means; however, a linear trend upward with increased consumption may not be present. In some instances, the middle group had the highest geometric mean and in others the hemoglobin adduct levels increased as the rate of consumption decreased. When both the statistical significance and upward linear trend were considered, no association between diet and hemoglobin adduct levels was identified.

Acrylamide hemoglobin adduct results were different when compared to the NHANES results. One of the issues with biomarkers or exposure is that normal or typical adduct results have not been established. The NHANES study comes closest to establishing the average levels as the study considers the entire country and all ages. The small study completed as part of this grant show a departure from the NHANES average values. The same laboratory completed both sets of analyses but at different times. A simple laboratory calibration error might be responsible or there may be differences in the results due to demographic differences.

These studies had several limitations. The sizes of the studies limited their power. While the glioma cases in this study presented a homogeneous group, the work histories of study participants indicated that there were several different occupations within the glioma cases. A larger study of a case group based on occupation might be more useful in establishing an association between occupation and incidence of glioma.

There is a potential for recall bias for both the self-reported work histories and the responses to the questions used to identify industry and occupation. The number of questions that a study participant may be asked to answer may also limit the ability to achieve the desired detailed work history. In addition, there may be coding errors based on interpretation of the study subject responses. This was somewhat limited in that only one coder was used to assign industry and occupation codes.

The age of the NIOSH-NOES study may also be a limiting factor as the survey was conducted from 1981-1983. From this survey the presence of the chemicals of interest was established; however, the engineering controls and amounts of the chemicals in used are details that might better define the exposures. Since less than half of study participants worked in industries surveyed by the NOES, establishing potential exposures could not be completed.

The lack of industrial hygiene monitoring data was also a limitation. Even though each factory has different processes and engineering controls these data might allow for an increased probability in establishing the potential occupational exposure.

The use of biomonitoring data was confirmation that an exposure had occurred; however, the source of any one exposure could not be confirmed. Use of biomonitoring data should be interpreted with great care as many different sources may account for the results. In the hemoglobin adduct study the exposures to acrylamide and 1,3-butadiene were confirmed but the sources of the exposure could not be identified.

Conclusions

These studies demonstrated the need for collaboration between toxicology, epidemiology and exposure assessment fields of study to evaluate occupational chemical exposures. The hypothesis that a cumulative exposure score can be generated from work histories is sound; however, the addition of a variable to reflect professional judgment might be useful.

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. The difference in acrylamide hemoglobin adducts between those subject who had ever smoked versus those subject who had never smoked was also of interest and bears further investigation.

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 was not observed in this study. This may be due to the size of the study or the lack of potential exposure to acrylamide and/or 1,3-butadiene in study participants. A larger study with more focused survey questions is needed to assess the contribution of occupation to hemoglobin adduct formation. 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 association between exposure and disease. The need for further study of industries such as the printing industry, specialty contractors and food industry and the association with glioma are also suggested by the findings of these studies.

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.

Further exposure assessment studies of the chemical found in the workplace are needed to evaluate the exposure-disease relationship. The usefulness of biomarkers of exposure in evaluating these workplace exposures has yet to be determined but holds much promise. Further research is needed to evaluate the role of occupation in biomarker formation.

Publications

Shimek, JM: [2010] Occupational Chemical Exposure Assessment in a Brain Cancer Case-Control Study, Ph.D. Thesis, University of Illinois at Chicago.
Shimek, JM, McCarthy, BJ, Erdal, S, Davis, FG: [2011] Evaluating Chemical Exposures Using Hemoglobin Adducts for Acrylamide and 1,3-Butadiene, Poster Session, Society of Toxicology 50th Annual Conference, Washington, D.C., March 6-11, 2011.

Inclusion of gender and minority study subjects

Inclusion Enrollment form is attached.

Inclusion of Children

As this was a study of occupational chemical exposures, no children were included in this study.

Materials available for other investigators

Not applicable.