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OCCUPATIONAL EXPOSURE TO ACRYLAMIDE IN CLOSED SYSTEM PRODUCTION PLANTS: AIR LEVELS AND BIOMONITORING

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The aim of this study was to evaluate biomarkers of acrylamide exposure, including hemoglobin adducts and urinary metabolites in acrylamide production workers. Biomarkers are integrated measures of the internal dose, and it is *total* acrylamide dose from all routes and sources that may present health risks. Workers from three companies were studied. Workers potentially exposed to acrylamide monomer wore personal breathing-zone air samplers. Air samples and surface-wipe samples were collected and analyzed for acrylamide. General-area air samples were collected in chemical processing units and control rooms. Hemoglobin adducts were isolated from ethylenediamine teraacetic acid (EDTA)–whole blood, and adducts of acrylamide and glycidamide, at the N-terminal valines of hemoglobin, were cleaved from the protein chain by use of a modified Edman reaction. Full work-shift, personal breathing zone, and general-area air samples were collected and analyzed for particulate and acrylamide monomer vapor. The highest general-area concentration of acrylamide vapor was 350 μg/cm^3 in monomer production. Personal breathing zone and general-area concentrations of acrylamide vapor were found to be highest in monomer production operations, and lower levels were in the polymer production operations. Adduct levels varied widely among workers, with the highest in workers in the monomer and polymer production areas. The acrylamide adduct range was 15–1884 pmol/g; glycidamide adducts ranged from 17.8 to 1376 p/mol/g. The highest acrylamide and glycidamide adduct levels were found among monomer production process operators. The primary urinary metabolite *N*-acetyl-*S*-(2-carbamoylethyl) cysteine (NACEC) ranged from the limit of detection to 15.4 μg/ml. Correlation of workplace exposure and sentinel health effects is needed to determine and control safe levels of exposure for regulatory standards.

Acrylamide and its metabolic-related congeners have been identified as high-priority toxicants as a result of animal toxicity studies conducted by the National Toxicology Program. An international interest has developed as a result of the finding by Tareke et al. (2002) that exposure to acrylamide is ubiquitous and comes from sources such as tobacco smoke and diet. Research today focuses on the relationship between measures of external exposure (including air levels and contaminant levels on surfaces) and biomarkers (blood hemoglobin adducts and urinary metabolites). These exposure levels need to be related with sentinel health endpoints to evaluate risk-based exposure limits. Thousands of

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workers worldwide are occupationally exposed toacrylamide, as evidenced by the 1981–1983 National Occupational Exposure Survey, which estimated 10,500 workers exposed to acrylamide. Acrylamide production rates are increasing, raising concern about categories of workers in jobs where control of exposure may be difficult to adequately evaluate and achieve. Summarization by an independent, international panel of experts on acrylamide concluded that human studies using and correlating biomarkers (hemoglobin adducts) with conventional exposure measures constitute a critical data need (NTP-CERHR 2004).

Acrylamide exposure occurs from both occupational and nonoccupational sources (worksite, diet, and tobacco smoke), and by multiple routes (inhalation, oral, dermal), intake, metabolism, and excretion (Exon 2006). Hemoglobin adduct measurement provides an integrated assessment of internal dose from all routes and sources during the last 120 d. Thus, a worker's hemoglobin adduct levels, including acrylamide and glycidamide, which is a by-product of acrylamide metabolism shown to be cytotoxic and clastogenic, provide useful information about *total* exposure, including absorption that cannot be completely captured by workplace air monitoring alone.

ACRYLAMIDE EXPOSURE LITERATURE

A number of recent publications provide normative information on acrylamide hemoglobin adduct levels (AA) in the general population (Exon 2006). The National Health and Nutrition Examination Survey 2003–2004 (NHANES) provided general population ranges and is reported by Vesper et al. (2009). In 2004, the National Toxicology Program, Center for Evaluation of Risks to Human Reproduction (NTP-CERHR 2004), document cited norms that ranged from <12 to 50 pmol/g hemoglobin among nonsmokers ($n = 25$) and 13 to 294 pmol/g for smokers ($n = 47$) based on an earlier study of acrylamide levels among the German population (Schettgen et al. 2003). In the largest acrylamide population-based study to date, Kütting et al. (2009) measured mean acrylamide adduct levels of 27.1 pmol/g globin (ranging from 3 to 68.1 pmol/g) among 749 adult nonsmokers from the general Bavarian population. Acrylamide adduct levels among smokers averaged 83.2 pmol/g (ranging from 8.2 to331 pmol/g) in that study. Similarly, Vesper et al. (2008) measured acrylamide adduct levels of 48.4 pmol/g (88.3 pmol/g at the 95th percentile) among 255 nonsmokers and 137 pmol/g (285 at the 95th percentile) among randomly selected individuals from nine European countries. Several recent publications presented results from the multiple smaller studies of acrylamide levels among populations in other locales (Vesper et al. 2008; Hartmann et al. 2008).

Acrylamide hemoglobin adduct levels have also been measured in several occupational studies. One such small study published mean values for acrylamide adducts by smoking status for Swedish polyacrylamide gel preparation workers ($n = 22$) and controls ($n = 18$) (Bergmark 1997). Among the gel workers, nonsmokers ($n = 15$) and light smokers ($n = 7$) had average adduct levels of 54 pmol/g (ranging from 24–116 pmol/g) and 70 pmol/g (ranging from 43–85 pmol/g), respectively. Nonsmoking ($n = 8$) and smoking ($n = 10$) controls had average adduct levels of 31 pmol/g (ranging from 24 to 49 pmol/g) and 116 pmol (ranging from 27 to 148 pmol/g), respectively.

Calleman et al. (1994) published a study of relationships between total exposures, diagnostic indicators, and adverse effects in Chinese acrylamide production workers. The study examined biomarkers of acrylamide exposure and neurological effects in 41 workers employed at a plant that produced acrylamide monomers and copolymers, and 10 controls from the same city. The investigators reported peeling skin of the hands in many workers, which was cited as evidence of inadequate personal protection and dermal exposure. Acrylamide hemoglobin adduct levels as reported by Calleman et al. (1994) were as high as 13,400 pmol/g. Acrylamide adduct levels were significantly correlated with neurological effects as measured by a neurotoxicity index, but not with air concentrations in that study.

A more recent occupational hygiene survey (Jones et al. 2006) of an acrylamide production

plant in the United Kingdom found a statistically significant positive correlation among airborne exposure and acrylamide adducts. Data demonstrated that airborne exposures at the maximum allowable limits (300 $\mu g/m^3$) would project to an adduct level of 1550 pmol/g. Data indicated that acrylamide hemoglobin adduct measurement is a sensitive and specific means of assessing exposure to acrylamide that shows good correlation with environmental exposure parameters (airborne acrylamide, swab, and gloves sampling). In a study of accidentally exposed workers, Hagmar et al. (2001) reported on the extent of health effects in 210 tunnel workers in Sweden exposed to a grouting agent containing acrylamide and n-methylolacrylamide. Acrylamide adduct levels ranged from 20 to 70 pmol/g among controls and from <80 to 17,700 pmol/g among tunnel workers.

Hagmar et al. (2010) found significant dose-response associations between adducts and peripheral nervous system symptoms, irritation of the respiratory tract, eyes, and skin, and more general symptoms, such as dizziness and nausea. Based on the upper confidence limits for the threshold dose, the estimated no-effect levels were 1860 pmol/g for leg cramps, 1280 pmol/g for skin peeling, and 510 pmol/g for numbness or tingling in legs and feet. Thirty-nine percent of workers with adduct levels above 1000 pmol/g had tingling or numbness in their hands or feet. A subset of 23 workers with peripheral nervous system impairment and adduct levels of at least 300 pmol/g were followed for neurophysiologic examinations at 6, 12, and 18 mo postexposure. At 18 mo postexposure, symptoms had clinically improved in 96% of these workers. Evidence indicated that 510 pmol/g (acrylamide adducts) was the no-observed-effect level, and that 1000 pmol/g was the neurological threshold dose for the tunnel workers in the study.

REGULATORY EXPOSURE LIMITS FOR ACRYLAMIDE IN THE UNITED STATES

The current occupational exposure criteria for acrylamide monomer established by each U.S. safety and health organization are as follows (however, this is not for establishing the relationship to the biomonitoring of acrylamide):

- The current National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL) for worker exposures to acrylamide in air is 30 $\mu g/m^3$ for a 10-h time-weighted average exposure. NIOSH considers acrylamide monomer to be a potential occupational carcinogen.
  - The current OSHA permissible exposure level (PEL) for worker exposures to acrylamide in air is 300 $\mu g/m^3$ for an 8-h time-weighted average exposure.
  - ACGIH recommends a threshold limit value (TLV) for worker exposures to acrylamide in air of 30 $\mu g/m^3$ for an 8-h time-weighted, average exposure. ACGIH classifies acrylamide as a "confirmed animal carcinogen with unknown relevance to humans."

(The REL, PEL and AGGIH all carry a "skin" notation, which indicates that acrylamide may be absorbed through the skin via direct contact, and that acrylamide absorbed in this way adds to the exposure due to inhalation.)

STUDY PURPOSE

The aim of this study was to characterize acrylamide worker exposures in the breathing zone as well, as internal dose as characterized by hemoglobin adduct levels and urinary metabolite levels, among workers in several U.S. acrylamide manufacturing facilities.

STUDY METHODS

Description of Facility and Operations

Workers were studied from three companies with plant sites covering 200 to 300 acres, all located in rural areas. Besides manufacturing acrylamide and polyacrylamide, other chemicals used in water treatment were produced. Almost all of the acrylamide monomer produced at the facilities was used to produce various polyacrylamides and copolymers

at the plants. The polymers produced are used in applications across numerous industries. The major areas for this application are potable water and wastewater treatment processes for municipalities and in enhanced oil recovery, mining and mineral processing, agriculture, and in the production of steel, pulp and paper manufacturing, automotive, paints and coatings, and other industries.

Acrylamide is produced within a closed, continuous-flow reactor system by the catalytic hydration of acrylonitrile. The reactors carry hot water to a catalyst bed through which the reaction materials flow. Specifically, a water solution containing 30–40% acrylonitrile by weight reacts on a copper catalyst at an elevated temperature and pressure. The solution of acrylamide is stripped of acrylonitrile, and this is recycled to the reactor. The final product contains approximately 50% acrylamide, with less than 100 ppm acrylonitrile. All acrylamide remains in solution. No acrylamide crystal is manufactured at these plants; however, one site used the crystalline form for certain products.

Acrylamide solutions are transported within the facilities via closed systems of piping, and stored in tanks on the plant sites. The loading of trucks or rail cars is solely for shipping outside the facility. At the time of the NIOSH study, each facility employed 150 to 300 workers. The plant operations were 24-h, multiple-shift operations.

Recruitment and Sample Collection

NIOSH Human Subjects Review Board approval was obtained prior to instituting the exposure study. All workers aged 18 yr or older and in jobs identified as having the potential for acrylamide exposure were prioritized for recruitment. Workers holding jobs with low to high potential for exposure (per company health and safety staff) were invited to participate. Informed consent prior to enrollment was obtained from workers who agreed to participate. Each participant was assigned a unique identifier code. No list linking names and codes was maintained by NIOSH study investigators.

Blood and end-of-shift urine samples were collected from consenting volunteers. Biomarkers of internal dose (hemoglobin adduct and urinary metabolites levels) were measured in this study in order to characterize the potential magnitude of worker exposures in comparison to exposures among controls and other populations in preparation for the larger health effects study. Hemoglobin adducts represent exposure over the average life span of red blood cells in the body (approximately 120 d), while urinary metabolites represent recent exposure. Cotinine, a by-product of nicotine metabolism, was measured in urine to ascertain exposure to tobacco smoke, and environmental source of acrylamide. Internal dose measurements were augmented by industrial hygiene sampling that was conducted on a subset of company workers. Personal breathing zone (PBZ) air sampling, general-area air sampling, and surface-wipe sampling were conducted. A subset of company workers participated in the PBZ monitoring. In total, 160 workers participated; however, not all data endpoints were measured in all workers because some samples could not be obtained from all participants.

Air Monitoring

Workers asked to wear PBZ air samplers were those potentially exposed to acrylamide monomer through working in or near chemical processing units synthesizing or utilizing this chemical compound. Air samples and surface-wipe samples were collected and analyzed for acrylamide. All samples were found to be vapor, not particulate. General-area air samples were collected in the vicinity of potential points of exposure (e.g., process-material sampling ports) within these same chemical processing units, and in control rooms for these units. PBZ air samples were collected by attaching portable air-sampling apparatuses to workers' clothing, with the air inlets in their breathing zones, and general-area air samples were collected by affixing additional air-sampling apparatuses in stationary locations within the facility. Air samples were collected

across a relatively long time (i.e., several hours) and were intended to characterize full-shift worker exposures to, and area concentrations of, acrylamide.

Both PBZ and area air samples for acrylamide were collected in accordance with procedures of OSHA Method 21 and OSHA Method PV2004. Earlier site samplings used Method 21 and later site sampling used Method PV2004. To collect a PBZ air sample using Method PV2004, a battery-powered portable air-sampling pump was used to draw air at a measured flow rate through an OSHA Versatile Sampler (OVS-7) tube, containing a glass fiber filter and two sections of XAD-7 adsorbent (200 mg front section and 100 mg back section). The nominal air-sampling flow rate was 1 L/min. For a typical air sample collected during this survey, the air volume was about 0.5 m^3, based on a typical sampling time of 8 h and the nominal air-sampling flow rate of 1 L/min. After sampling, the OVS-7 tubes were capped and sent to the laboratory for analysis. Area air samples were also collected using the OVS-7 tubes.

In addition, area air samples for acrylamide were collected in accordance with OSHA Method 21. This method uses a 13-mm-diameter glass-fiber prefilter in a two-piece plastic cassette with a sealing gasket, followed in series by a glass tube packed with two sections of silica-gel adsorbent (150 mg front section followed by 75 mg back section). The nominal air-sampling flow rate for this method was also 1 L/min. After sampling, each filter cassette was opened, and the filter and gasket inside were transferred to a screw-cap glass vial containing 1 ml methanol, which extracts any acrylamide on the filter into solution. The vial was sealed, the ends of the silica-gel tube were capped, and the samples were sent to a laboratory for analyses.

A limited number of acrylonitrile area samples were collected on 150-mg charcoal tubes. The individual sections of the charcoal tube were removed and placed into separate vials. The individual sections were then chemically desorbed by using 1 ml of a solution consisting of 98% carbon disulfide/2% acetone. Area air samples were also collected for acrylonitrile using charcoal tubes (100 mg front section and 50 mg back section) with a nominal air-sampling flow rate of 200 ml/min.

Acrylamide and acrylonitrile air samples collected were sent to a certified industrial hygiene laboratory (Bureau Veritas North America, Inc., Novi, MI). Included with the field air samples for quality assurance purposes were several "field blank" samples, handled, stored, and shipped identically to the air samples but through which no air was drawn. In addition, unused media, OVS-7 tubes, 13-mm-diameter glass-fiber filters, silica-gel tubes, and charcoal tubes for use as "media blanks" in the analysis of the field samples were provided to Bureau Veritas North America. Media blanks were used for laboratory control spikes, blind spikes, and replicate analysis.

OVS-7 samples are desorbed with a solution of 5% methanol 95% water, agitated using a mechanical shaker for 30 min, and analyzed by high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector. Samples collected using 13-mm glass-fiber filters followed by silica gel tubes were analyzed separately, the 13-mm filters as one set and the silica gel tubes as the second set. The 13-mm filters were field desorbed in 1 ml methanol and analyzed by HPLC using the UV detector. For the silica gel tube analyses, the individual sections were removed and placed into separate test tubes. The glass wool that separated the sections was included with the front section of the tube because there was a significant amount of media adhering to the glass wool. The back foam plug and the glass wool at the front of the tube were discarded. The individual sections were then chemically desorbed using 2 ml of a 95% water/5% methanol solution. The samples were capped and placed on a mechanical shaker for 30 min. After desorption, the samples were transferred to autosampler vials and analyzed by HPLC using the UV detector.

Surface Monitoring

Surface-wipe samples were collected on surfaces in the production areas and laboratories as a surrogate measure of the

potential for dermal exposures to monomer acrylamide. Most, but not all, of these wipes were collected from surfaces that workers might commonly contact, such as desktops and doors. Each surface-wipe sample was collected by wetting a 47-mm-diameter glass fiber filter with 2 ml methanol and wiping a 100-cm^2 area using a disposable cardboard template. On one occasion, a larger area was wiped and the result was normalized to 100 cm^2. Wiping was done first by going left and right, then up and down, and then around the inside perimeter of the template. The glass fiber filter was placed in a conical tube with a screw cap and 10 ml methanol was added. The wipe samples were also sent to Bureau Veritas North America, Inc., Novi, MI, for analysis using HPLC and UV detection.

Hemoglobin Adducts

Hemoglobin adducts of acrylamide and glycidamide were determined as described previously (Vesper et al. 2005). In brief, globin was isolated from ethylenediamine tetraacetic acid (EDTA)whole blood, and the adducts of acrylamide and glycidamide, at the N-terminal valines of hemoglobin, were cleaved from the protein chain by use of a modified Edman reaction with pentafluorophenyl isothiocyanate as the Edman reagent. The resulting pentafluorophenylthiohydantoin reaction products were extracted by use of liquid–liquid extraction and analyzed by HPLC/MS/MS. The detection limits for HbAA and HbGA adducts were 3 and 4 pmol/g hemoglobin, respectively. The interday imprecision ($n = 20$ d) of this method, expressed as percent coefficient of variation, was 13% for HbAA and 19% for HbGA, on average, determined with three blood pools HbAA and HbGA concentrations in picomoles per gram hemoglobin: pool 1 (135 and 93); pool 2 (103 and 700); pool 3 (62 and 50), respectively.

Urinary Metabolites

N-Acetyl-*S*-(2-carbamoylethyl) cysteine (NACEC) was found to be the primary metabolite (80%) in human urine (Vesper et al. 2007). Analysis of urines for acrylamide (AM) and NACEC was conducted using an HPLC-MS/MS with an API 3000 triple quadrupole mass spectrometer for analysis and quantification. The analysis used selected reaction monitoring in the positive ion mode. Calibration was conducted using human urine spiked with AM and NACEC and standards to evaluate method performance of linearity, accuracy, and recovery. Addition of the internal standards resulted in dilution of the sample, increasing the volume from 65 μl to 100 μl. Using this HPLC-MS/MS approach, the following calibration ranges were achieved: AM, range 0.1–10 μg/ml, $r^2 = .998$; NACEC, range 0.5–200 μg/ml, $r^2 = .983$.

For the parent compound and the metabolite, using a 10-μl sample injection volume, a limit of quantitation (LOQ, signal/noise of 10:1) and a limit of detection (LOD, signal/noise of 3:1) corresponded to the following: NACEC, LOQ = 0.25 μg/ml, LOD = 0.08 μg/ml; AM, LOQ = 0.002 μg/ml, LOD = 0.001 μg/ml. The sampling of acrylamide in urine, possibly due to the low LOD and LOQ, resulted in most samples being below the LOQ. Therefore, it was not possible to make further comparisons of AM with other samples.

RESULTS

Air Sampling Results

Personal breathing zone air samples for acrylamide were collected on consecutive work shifts. All PBZ samples collected had detectable concentrations of acrylamide vapors ranging from 5 to 984 $\mu g/m^3$. Shown in Table 1 are summary statistics across all plants by location in acrylamide production plants for the PBZ air samples.

General area air samples for acrylamide were collected in the monomer and polymer production areas. These results are listed in Table 1. In total, 37 area air samples were collected in acrylamide monomer and polymer production areas, with samples showing an acrylamide concentration ranging from 0.23 to 349 $\mu g/m^3$.

TABLE 1. Summary Statistics for Air Sampling Samples Taken in Acrylamide Production and Polyacrylamide Operations

Plant operation	Samples	Min. ($\mu g/m^3$)	Max. ($\mu g/m^3$)	Mean ($\mu g/m^3$)	Mean s.d. ($\mu g/m^3$)	Geomean	Geom. s.d.
Acrylamide monomer production personal breathing zone	15	5.4	984	85.46	249.31	20.59	3.70
Polyacrylamide operations personal breathing zone	18	5.0	60	17.70	14.59	13.47	2.11
Acrylamide monomer production general area sampling	20	0.23	349	85.89	101.20	29.03	8.91
Polyacrylamide operations general area sampling	17	0.25	169	41.59	53.11	10.45	9.19

Note. $\mu g/m^3$ = micrograms of acrylamide per cubic meter of air. Geomean = geometric mean; Geom. s.d. = geometric standard deviation.

Area air samples were also collected for acrylonitrile, the starting material for producing acrylamide monomer. All acrylonitrile samples were collected in the monomer production area. A total of 5 acrylonitrile samples were collected. Two of the samples had nondetectable results at a detection limit of 0.002 ppm. The three detectable results for acrylonitrile ranged from 0.004 to 0.069 ppm.

Surface-Wipe Sampling

The surface-wipe sampling results did not reveal widespread contamination of surfaces with acrylamide. All wipe samples acrylamide levels were nondetectable with a detection limit of 0.1 $\mu g/cm^2$. Contaminants on surfaces are rarely found to be homogeneously distributed, so the measured acrylamide levels on particular surfaces are likely dependent on the specific location chosen to wipe for sample collection. For this reason, surface-wipe sampling results need to be considered only indicative of dermal exposure.

Biomonitoring Results

To summarize biomonitoring results, the plant operations/workers were assigned to five job classifications:

- Administrative workers (secretary, training/safety, and other administrative staff).
- Facility operations workers (shipping clerk, security guards, mechanics, and grounds workers).
- Laboratory workers (quality assurance, laboratory technical, instrument technical staff).
- Temporary workers (contractors).
- Production workers (production operators and production supervisors).

Data were pooled over all three plants. Table 2 presents the number of workers who are nonsmokers or smokers and are above the upper range of nonoccupationally exposed. Production operators demonstrated the largest

TABLE 2. Hemoglobin Acrylamide Adducts for Each Job Class and Number Exceeding Mean and Extreme (95th Percentile) Values for General Population (Not Occupationally Exposed)

	Nonsmokers			Smokers		
Job classifications	Total number	Number above population mean	%	Total number	Number above population mean	%
Administrative workers	11	2 (0)	18 (0)	2	0 (0)	0 (0)
Facility operations	14	7 (2)	50 (14)	4	1 (0)	25 (0)
Laboratory workers	13	8 (4)	62 (31)	2	2 (0)	100 (0)
Temporary workers	7	5 (2)	71 (29)	2	0 (0)	0 (0)
Production operators	64	54 (32)	84 (50)	33	20 (6)	61 (18)

Note. The population means and extreme values (in parentheses) used as cutoffs were above 48 pmol/g (95th percentile = 88 pmol/g) for non-smokers and 137 pmol/g (95th percentile = 285 pmol/g) for smokers as reported by Vesper et al. (2008).

TABLE 3. Biomonitoring—Hb Adducts (pmol/g) and Urinary Metabolite (μg/ml)

Job title	Number of workers	Average adduct levels		Minimum adduct level	Maximum adduct level	Urinary metabolite NACEC		
						Average	Minimum	Maximum
Administrative workers	13	Acrylamide[a]	50.4	25.0	97.9	0.080	0.056	0.094
		Glycidamide[a]	40.8	16.9	87.2			
Facility workers	18	Acrylamide	86.0	14.5	497.0	0.246	0.052	1.000
		Glycidamide	66.7	8.8	331.0			
Laboratory workers	15	Acrylamide	103.5	28.0	230.2	0.232	0.052	0.433
		Glycidamide	82.7	22.6	196.0			
Temporary workers	9	Acrylamide	93.9	42.6	193.2	0.211	0.095	0.566
		Glycidamide	111.2	45.7	203.5			
Production operators	97	Acrylamide	220.0	29.1	1884.0	0.804	0.008	15.400
		Glycidamide	158.3	17.8	1376.0			

[a]Significant at $p < .05$.

incidence of acrylamide adduct levels above the normal range for nonsmokers.

Based on these job classifications, Table 3 presents overall comparisons and summary statistics that were calculated for the levels of acrylamide adducts, glycidamide adducts, and urinary metabolites. A median test comparing levels of acrylamide adducts showed a significant difference between workers in different job classifications; a median test comparing glycidamide levels yielded evidence of a significant difference; a comparison of NACEC levels with a median test also indicated a significant difference in medians. Significantly lower average adduct levels between production operators and other job classifications (obtained using Fisher's exact test) are indicated by asterisks. Graphical summaries are of levels of acrylamide adducts, glycidamide adducts, and the primary urinary metabolite.

Box plots of levels of acrylamide and glycidimide hemoglobin adducts, and the urinary metabolite (NACEC) for workers in different job categories are shown in Figures 1 to 3, respectively. Figure 4 and 5 present regressions plots. Shown in Figure 4 are results of the regression of log of acrylamide adducts on log of PBZ. Figure 5 shows the regression of the urinary metabolite NACEC and PBZ.

The relationship between air levels of acrylamide in air levels (PBZ) and hemoglobin acrylamide adducts (AA) was examined through linear regression. Diagnostic tests indicated that log-transformed data yielded results for which

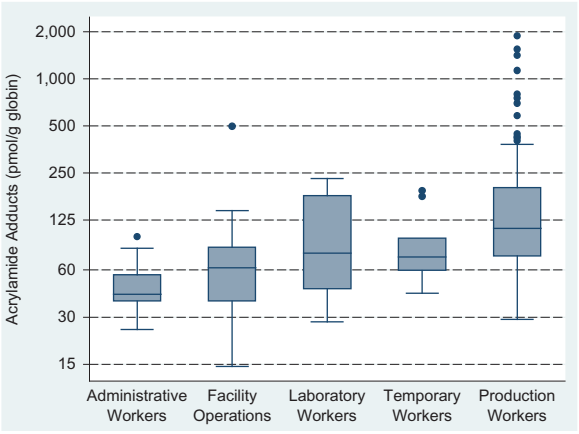

FIGURE 1. Acrylamide adducts by job title (color figure available online).

Note. Acrylamide adducts by job category – summarized in box plots. Horizontal line within each box is the median; upper and lower edges of each box are third quartile (Q_3) and first quartile (Q_1) respectively; short horizontal line at end of upper "whisker" is the largest data point $\leq Q_3+1.5^*$ (Q_3-Q_1); short horizontal line at end of lower "whisker" is the smallest data point $\geq Q_1-1.5^*$ (Q_3-Q_1). Data points outside the ends of the whiskers are plotted separately.

the assumptions of normality and constant variance were valid. Using the results from the regression of log-transformed data, estimates of mean exposures were then obtained in the original scales by exponentiation, as shown in Figure 4. Estimated means of AA were calculated using results of the regression of log(AA) on log PBZ to obtained fitted values of log(AA) and then transforming to estimated means. Confidence bands were calculated by exponentiating the confidence bands of the estimated means of log(AA).

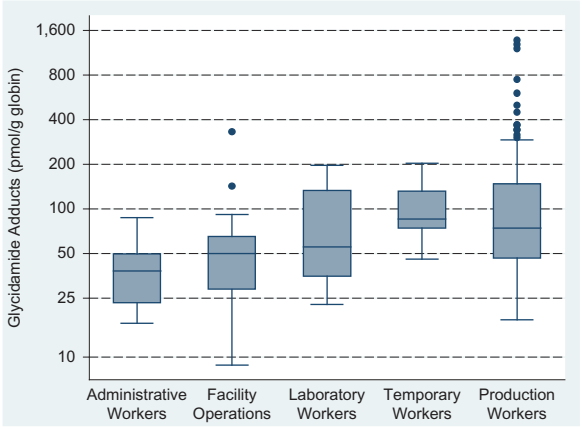

FIGURE 2. Glycidamide hemoglobin adducts by job title (color figure available online).
Note. Glycidamide adducts by job category – summarized in box plots. Each box plot shows median within the box, first and third quartiles at lower and upper ends of the box, and data outside the interquartile range (see Fig. 1 caption for details).

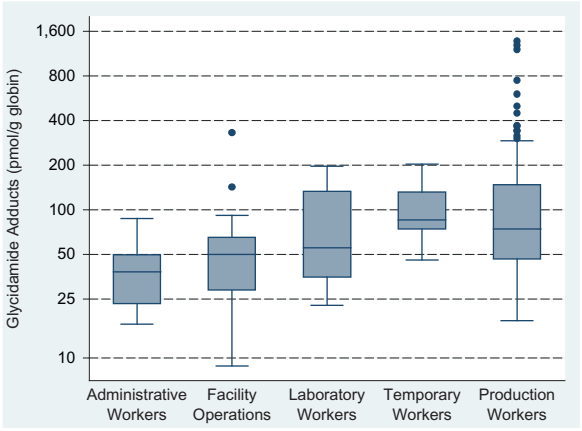

FIGURE 3. Box plots of the urinary metabolite NACEC for different job titles (color figure available online).
Note. Presents the urinary metabolite (NACEC) of acrylamide by job category summarized in box plots. Each box plot shows median within the box, first and third quartiles at lower and upper ends of the box, and data outside the interquartile range (see Fig. 1 caption for details).

Values of interest include the NIOSH REL of 30 μg/m^3, the OSHA PEL of 300 μg/m^3 for PBZ, and from the Hagmar et al. (2001) study, values of AA of 510 pmol/g (no-observed-effect level) and 1000 pmol/g (the Hagmar et al. [2001] neurological threshold dose for the tunnel workers). Relevant values of PBZ and means and standard errors of AA are presented in Table 4. The standard error of AA was obtained using the delta method (Agresti 2002).

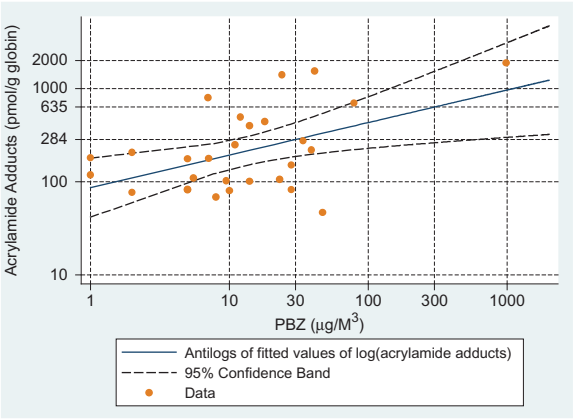

FIGURE 4. Regression of Acrylamide Adducts, and PBZ Air Sampling data with 95% confidence bands (color figure available online).
Note. Acrylamide adducts as a function of level of acrylamide vapor in personal breathing zone (PBZ). Shown are results of the regression of log of acrylamide adducts on log of PBZ.

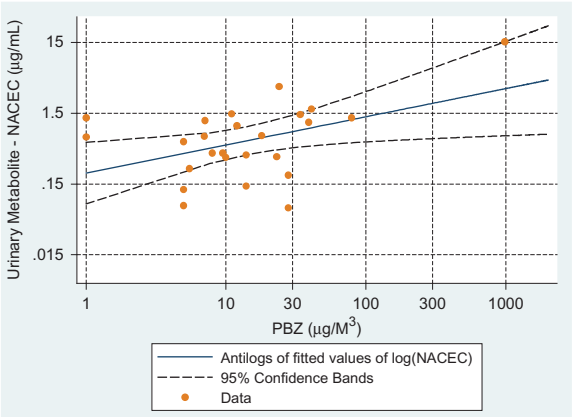

FIGURE 5. Regression of the urinary metabolite NACEC with PBZ (color figure available online).
Note. Estimated mean level of urinary metabolite (NACEC) of acrylamide as a function of level of acrylamide vapor in personal breathing zone (PBZ).

TABLE 4. Relationships of PBZ Air Samples and Hemoglobin AA Levels

PBZ (μg/m^3)	Mean AA (pmol/g)	Standard error of AA
30	284.04	57.78
150	498.49	175.94
300	635.13	273.05
1100	1000.15	580.96

Figure 5 presents the regression of the primary urinary metabolite NACEC and PBZ. Shown are exponentiated results of the

regression of log of urinary metabolite (NACEC) on log PBZ. The effect of log PBZ on NACEC was significant.

DISCUSSION

All workers sampled for exposure to acrylamide monomer vapors had detectable levels in PBZ and adducts. These workers were involved in the production of the acrylamide monomer using acrylonitrile and subsequent polymerization of the monomers in the production areas. The air sampling reflects exposure on the day of sampling and varies from day to day. This makes it difficult to project precise values from a correlation of breathing zone air levels with acrylamide adducts reflecting a 120-d integrated exposure. This needs to be understood when reviewing the correlation of PBZ air sampling with acrylamide adducts. Still, it is of interest to consider the relationships of PBZ and AA as presented in Figure 4. Further, due to individual differences in uptake, metabolism, and excretion, workers may be exposed to the same PBZ yet possess different internal doses based on AA. It also needs to be understood that control values or background levels reflect acrylamide exposures to nonoccupational (dietary/smoking) sources.

The main finding of the biomonitoring data was wide variation in adduct levels among workers, with the highest levels found in monomer and polymer production areas. Acrylamide adduct levels ranged from 15 pmol/g to 1884 pmol/g among the workers sampled, while glycidamide adducts ranged from 9 to 1376 pmol/g. The highest AA levels were found among process operators working in monomer production areas, a few with levels approximately 10- to 19-fold higher than administrative workers (consistent with control levels). Two findings indicate that occupational exposures contributed to internal acrylamide dose: (a) based on general population studies, the percentage of workers with AA levels that were above average, as well as above the 95th percentile for nonsmokers and smokers; and (b) the generally higher adduct and urinary metabolite levels among the nonadministrative acrylamide worker subgroups.

The range of adduct and urinary metabolite levels is wide in each job subgroup. The extent to which this variation reflects within-job subgroup differences in exposure levels versus individual differences in uptake, metabolism, and excretion is unclear.

Urinary metabolites offer a measure of exposure during the day when the sample was collected. This is useful when efforts are under way to evaluate and reduce exposures in certain processes within the workday. The finding of this research was that the NACEC was the most useful urinary metabolite measured and that acrylamide in urine was generally below the limit of quantitation (LOQ = 0.002 μg/ml).

Those workers who had values above the upper range for AA as seen in the general population were assumed to have occupational exposure. Eighty-five percent of the nonsmoking monomer production workers had adduct levels above the National Toxicology Program (NTP) 2005 upper limit of 46 pmol/g for normal nonsmoking populations.

The regression model between air (PBZ) and AA indicates that worker exposure to the NIOSH REL (30 $\mu g/m^{3)}$ would result in a mean AA level of 285 pmol/g and the OSHA PEL (300 $\mu g/m^3$) would result in a mean AA level of 635 pmol/g.

Jones et al. (2006) reported that exposure to air levels of 300 $\mu g/m^3$ might result in AA of 1550 pmol/g (95% confidence interval of 1150–1950). Data showed that smoking status did not significantly affect results, as this study also demonstrated. As stated earlier, the data on 28 workers from the current study, which could be used to correlate both PBZ and adduct levels, indicate that exposure to 300 $\mu g/m^3$ would result in a mean adduct level of 635 pmol/g (95% confidence interval of 262.5–1536.9) pmol/g. The explanation for this difference may relate to the range of adduct values found in the workers sampled. Jones et al. (2006) sampled workers with an adduct range from 71 to 1854 pmol/g, and this investigation sampled workers (having both AA and PBZ measurements) ranging from 15 to

1884 pmol/g, of which only three workers had values of AA greater than 800 pmol/g.

Correlation between exposure data (air and biomarker) and sentinel health effects is needed to determine safe levels of occupational exposure to acrylamide. This will require extensive cooperative partnership with industry, where the range of exposures and potential health effects to workers can be studied. An issue for investigators in this important area is how to deal with the high costs of biomonitoring and the potential liability and worker misconceptions associated with such studies. Future research is needed to determine whether traditional, lower cost exposure surveillance methods such as air sampling provide an adequate proxy estimate of a worker's internal acrylamide dose.

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