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Mdi-Specific Igg Exposure Biomarker And Role Of Intervention

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MDI-specific IgG Exposure Biomarker and Role of Intervention

Ann Y. Teng, DO, MA

Abstract

Background: *Diisocyanates are sensitizing agents that are used in many industries in the production of polyurethane foams, coatings and other products. Methylene diphenyl diisocyanate (MDI) exposure can occur through dermal and inhalation routes resulting in sensitization and leading to isocyanate asthma. MDI-specific IgG (MDI-IgG) bio-monitoring has been proposed to be used as a predictor of exposure.*

Methods: *A prospective longitudinal study was used to assess annual MDI- IgG serum markers in 223 workers in a fabric-coating company. Kaplan Meir Survival Analysis determined whether there was a difference in seroconversion based on exposure level. A Cox proportional hazard model was used to see if covariates such as total IgE level, cough, rash, allergies, and smoking contributed to time to change. Data from 2012 and 2013 were used to compare MDI-IgG level after the implementation of new safety measures.*

Results: *MDI- IgG seroconversion from negative to positive was statistically significant for a difference in the survival curves (p= 0.0076) with respect to job classification. No statistical difference was found among workers for time to seroconversion from MDI-specific IgG positive to negative (p= 0.9232). A non-parametric sign test was used to evaluate the paired serology results for individuals between 2012 and 2013, indicating a significant decrease in MDI-specific IgG levels (p=0.0001).*

Discussion: *There is a significant correlation between job classification and time to positive seroconversion. Biomonitoring may track and trend changes that reflect worker exposures. Data suggest that safety interventions could play an important role in preventing MDI asthma and ensure worker safety.*

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Introduction

Diisocyanates are potent sensitizing agents that have been identified as a substantial source of occupational asthma.[1-3] The main commercial isocyanates of concern are methylene diphenyl diisocyanate (MDI), toluene diisocyanate (TDI), and hexamethylene diisocyanate (HDI).[4,5] Monomeric and polymeric methylene diphenyl diisocyanate (MDI) are used to make polyurethane foams, coated fabrics adhesives and other products .[6]

The prevalence of asthma due to isocyanates is estimated to be around 5-20% of those with prior exposure.[7] Workers in end-user polyurethane work settings may be at greater risk for developing isocyanate asthma than in primary production facilities as exposures in end-user settings may be harder to monitor and control. Additionally the workers may be unaware of the chemical composition of the products that they are working with.[8] Worker exposure can occur with various forms—vapor, aerosol,—and the risk of exposure can depend on factors such as temperature, mixture variability, and intrinsic physical properties.[4,9-11] In recent years, MDI has been increasingly used in industry due to its low volatility, which decreases the risk of inhalation exposure.[4] Despite the development of less volatile and "safer" forms, asthma due to isocyanates still occurs.[8,12] In the production of MDI fabric-coating products, the dermal and inhalational routes of exposure are both possible; however, skin exposures may play a greater role than in spray operations where there is more opportunity for inhalational exposures.[13-15] Skin exposure is difficult to quantitate, highlighting the need for MDI exposure biomarkers. Few studies have monitored MDI worker exposures over prolonged periods of time.[5,16]

Similarities in clinical presentation and symptoms of MDI asthma suggest a comparable mechanism to atopic asthma (i.e. allergic immune, type I hypersensitivity reaction). Generally, immune sensitization to isocyanates is presumed based on features such as a latency period, recurrent reactions after exposures to low concentration, and variable individual susceptibility.[10,17] Once sensitized, even low levels of exposure may elicit an asthma response.[7,10]

The pathogenic mechanisms to exposure and host response are poorly understood.[4,18] Diisocyanates react with proteins (antibodies) and hydrolyze in solutions which complicates in vitro analysis.[11] The current hypothesis of pathogenicity suggests a chemical reaction with self-proteins that alters their confirmation and causes the host immune system to recognize it as foreign. This triggers airway inflammation and asthma.[11,19] However, there may be a crucial difference in the mechanism of asthma induced by MDI and other allergens: antigen specific IgE responses, a hallmark of common atopic asthma, are often not observed in isocyanate asthmatics.[4]

Due to uncertainties in pathogenesis, persistence away from exposure, and poor long-term prognosis of isocyanate asthmatics, major efforts at disease control have shifted to prevention.[20,21] Exposure is the best recognized risk factor, so hygiene, including reduction and minimization of exposure, is considered the major strategy for prevention. Exposure surveillance is crucial to ensure adequate hygiene and properly functioning PPE.[4,22]

The current primary approach to surveillance of isocyanate exposure is air monitoring; however, problems exist with personal air sampling.[10,11] In addition to missing skin exposure, air vapor sampling may underestimate the potential inhalational

exposures in workers due to the low volatility of MDI.[3,7] Additionally, the cost of

quantitative air sampling makes this approach infeasible.[3] Previous studies have shown

that despite negligible airborne MDI measurement, positive serum antibody markers can

still exist.[4,23,24]

An alternative approach to exposure surveillance is biomonitoring based on

biomarkers of exposure.[1,25] One such biomarker is MDI-specific IgG (MDI-IgG).[19] MDI

is not normally found in nature, so antibodies are not usually found in human serum.[11]

Exposure is known to trigger IgG subclass antibodies that recognize reaction products of

MDI attached to autologous albumin.[11,19] Serum antibodies allow for investigation of

exposure levels when direct measuring methods for a particular substance in the body

does not exist. The use of serology to biomonitor chemical exposure is a novel strategy,

but similar sero-epidemiology is a well-established approach for following vaccine trends

and outbreaks of infectious diseases.[11,26]

In this study, we assessed the potential utility of MDI-specific IgG for exposure

monitoring and evaluated the kinetics of this immunologic response among workers with

varying levels of potential for exposure. Participants were workers at a fabric coating

plant, which provided prevalent opportunities for both dermal and inhalation exposure to

MDI. We followed the serological immunologic markers of these workers over time to

determine whether exposure would result in seroconversion (positive MDI-IgG) and what

risk characteristics would alter the likelihood of a person to develop immunologic

sensitivity to MDI-Specific IgG.

Materials and Methods

Overall Study Design

The epidemiological study was designed as a prospective longitudinal study to assess MDI exposure among workers in a fabric-coating company. Data were collected from a fabric-coating company that had previously established a contractual agreement for annual surveillance and physicals with Yale Occupational and Environmental Medicine Program (YOEMP). Annual physicals included symptom surveillance, self-reported questionnaires, physical exam, spirometry and blood draws for immunologic and other assays. Particular to this study, serology results for total IgE and MDI-specific IgG as well as worker self-reported general health questionnaires were analyzed from a de-identified database. Informed consent was obtained from each participant and the protocol was approved by the Yale University Human Investigations Committee.

Over 90% of the workers who underwent the physicals had antibody testing. Data were collected for a total of 366 subjects, and 223 subjects were considered eligible for this analysis. As the intention was to assess changes in antibody level and symptomatology over time, inclusion requirements included completion of a health questionnaire and existence of two or more recorded values of the studied outcome variable (specific IgG) between the years of 2004 and 2012. Additionally, in order to determine if any differences existed in the work environment, a three-question safety culture survey was offered for employees to complete in 2013 regarding their personal perception of workplace safety within the past year.

Questionnaire

The subject questionnaire was self-reported and included demographic information and medical history. Questions elicited concerned specific respiratory symptoms (e.g. shortness of breath or chest tightness), non-specific respiratory symptoms (e.g. cough or wheeze), temporal relationship of symptoms, occupational history, host factors, and use of personal protective equipment (e.g. respirator, gloves, and mask).

MDI Exposure Assessment

Exposure information obtained from employee questionnaires was used to assign each worker into one of three job categories (Table 1). These categories were assigned and defined by the industrial hygienist depending on the potential for and the variability in length of time of exposure. The classification scheme was constructed using previous air sampling and isocyanate exposure data available from historical records performed at the facility between 1987 and 2000. Although the amount of production has changed, process changes were not determined to be so significant as to alter the exposure group classifications.

Antibody studies

Serum was analyzed for MDI-IgG by an enzyme-linked immunosorbent assay (ELISA), using Nunc MaxiSorp™ microtiter plates (VWR International, West Chester, PA) coated with 1 μg per well of albumin that was conjugated with MDI.[27,28] MDI-albumin was prepared by reacting a 5 mg*ml^{-1} solution of human albumin (Sigma Chemical Company, St Louis, MO), with 10% (w/v) MDI dissolved in acetone so that the final reaction mixture contained 0.1% (w/v) MDI. MDI-albumin reactions were

performed for 2 hours at 37° C with mixing, and subsequently dialyzed against PBS using dialysis tubing with a 10 kDa molecular weight cut-off (MWCO).[19]

For detection of MDI-specific human IgG from serum samples, which recognized any bound MDI-albumin on the ELISA plates, a peroxidase-conjugated anti-human IgG secondary and the substrate 3,3′,5,5′ tetramethyl benzidine were used both from Pharmingen (San Diego, CA).[19] The amount of MDI-IgG present in serum samples was quantitated as an end-titer, which is the maximal dilution that yields a positive ELISA test result. This is defined as an optical density value greater than three standard deviations above the mean value obtained with pooled sera of unexposed subjects (n = 12). An MDI-IgG titer ratio equal to or greater than 1:20 was considered significantly elevated based upon previously published studies with more than 1000 different individuals.[19,21,27,28]

Statistical analysis

Analysis was done using SAS v.9.2 (SAS Institute, Cary, NC). The outcome variable studied was MDI-specific IgG. Predictor variables included job classification,. Hispanic ethnicity, gender, age, history of asthma, previous or current smoking history, symptom report of shortness of breath, cough, symptoms of rash, total IgE level, allergies, years working at the company, and use of personal protective equipment (specific for gloves, mask, and respirators). Data were collected from 2004 to 2012.

Two different outcomes were modeled using survival analysis. For the first set, time from first test until MDI-IgG changed from negative to positive was modeled. Interval censoring occurred in this set of data because some employees never became IgG-positive and others were lost to follow-up. For the second set, the time origin was

the date of the first positive MDI-IgG, and the event of interest was the change of MDI-IgG from positive back to negative. This set of data contained right-censoring due to a loss to follow-up and a lack of changing from positive to negative.

To examine the time required for IgG to convert from negative to positive, as well as positive to negative, Kaplan Meier Survival curves were constructed evaluated using the Log-Rank Test. This analysis allows determination as to whether any of the three job classifications significantly differed with respect to changing IgG levels. The assumption of proportional hazard was evaluated based upon a visual inspection of ln(ln(survival(time))) versus ln (time) curve noting that parallel lines imply proportional hazards. Cox Proportional Hazards models were conducted to determine the effect of job classification on changing IgG levels adjusting for covariates.

Additionally, MDI-IgG were measured for current workers in 2012 and again in 2013. Since the serologic data for the workers was not normally distributed, a sign test was used to determine whether there was a difference in mean MDI-IgG values.

Finally, participants were also given a three-question workplace safety survey to determine worker perception of safety changes in their work environment in the last year. These surveys were recorded on a Likert analog scale ranging from 1 through 7. Simple counts and histograms were used to evaluate workplace safety interventions over the last year.

Results

Characteristics of Cohort

The number of employees used in the study was 223 (those who fulfilled the criteria of completing a patient questionnaire and having more than one serology test). The

majority of the population was male (87.89%) and Hispanic (56.95%) as reflective of the composition of the company as a whole. An overview of the characteristics of the population is summarized in Table 2.

Specific to the biomarkers, it is evident that those workers in wet production were more likely to have a MDI-specific IgG $\geq$ 1:20 titer (Table 3). A fewer percentage of administrative workers were found to have total IgE > 100.

Analysis of MDI-IgG Conversion: Negative to Positive

A subgroup of 161 workers who was MDI-IgG negative in their first test was analyzed to examine time they became positive (titer $\geq$1:20). Examination of the Kaplan Meier curve (Figure 1) for the time required for MDI-IgG to convert from negative to positive demonstrates that at day number 1096 (3 years), 35.8% had converted in wet production (X, where n = 97) compared to 15.5% in dry production (Y, where n = 38), and 8.2% in administrative jobs (Z, where n = 26). The Kaplan Meier Log-Rank Test indicates a statistically significant difference in the survival curves (p = 0.0076).

Next, a Cox Proportional Hazard model was performed in order to examine for covariates. With respect to the conversion of MDI-IgG from negative to positive, the following variables were statistically significant: job class (wet production versus dry production), history of asthma, current smoking, presence of shortness of breath (SOB), and use of personal protective equipment (PPE).

This model provides estimates for the hazard function $h_i(t) = C_i \times h(t)$:

$$\log(C_i) = \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_6 x_6$$

Model estimates with p-values and corresponding hazard ratios are included in Table 4.

When looking at these variables graphically (Figure 1), the conversion from negative to positive IgG clearly occurs more rapidly in the group of wet production jobs. It is of note that the estimate involving the difference between administrative jobs and wet production jobs is not significant. This is likely due to the small number of data points associated with administrative jobs (n = 26), leading to a large standard error (SE = 1.03). On the other hand, there is a significantly lower hazard associated with dry production than wet production tasks. The hazard of converting from negative to positive MDI-IgG of an employee in dry production is only one quarter that of an employee working in wet production.

The data also demonstrate that a history of asthma and current smoking increased the hazard of MDI-IgG conversion, whereas use of personal protective equipment is protective of this conversion. Interestingly, shortness of breath is also statistically significantly protective of the MDI-IgG conversion from negative to positive.

Analysis of IgG Conversion: Positive to Negative

MDI-IgG conversion was then examined for 85 participants who had a positive test and at least one more test. The Kaplan Meier curve (Figure 2) examined for time required for MDI specific-IgG to convert from positive to negative demonstrates that at day number 1096 (3 years), 55.4% converted in wet production (X, n = 66) whereas 42.7% converted in dry production (Y, n = 16) and 50.0% converted in administrative jobs (Z, n = 3). A Kaplan Meier survival analysis evaluated using the Log-Rank Test indicates that without adjusting for covariates, there is no significant difference by job in time for an individual to convert from positive to negative MDI-specific IgG (p = 0.9232).

Similarly to the analysis of MDI-specific IgG conversion from negative to positive, subjects whose specific IgG converted from positive to negative were selected. Next, a Cox Proportional Hazard regression model was applied to adjust for covariates. The only variable determined to be statistically significant was the use of personal protective equipment.

In this case, the model provides the following estimate of the hazard function:

$$h_i(t) = e^{\beta_1 \cdot x_1} \times h(t)$$

In this case, $\beta_1 = 1.19$ with standard error 0.53 (p = 0.026). The hazard ratio is 3.28, which means that using personal protective equipment provides a more than three-fold increase in the likelihood of IgG converting from positive to negative.

Comparison of 2012 and 2013

At a later stage in the process of analysis, serology data from 2013 became available. Although these data were not able to be included in the core data set, they were briefly examined to have a basis of comparison. Several safety and intervention programs were implemented in 2013, and the changes in MDI-IgG levels were studied. Interventions included greater use of gloves, protective clothing and respirators. In order to accomplish this, the 2013 data were matched directly to the 2012 data. A sign test was used to evaluate the paired serology results for individuals between 2012 and 2013. A significant decrease was demonstrated between the 2012 and 2013 data (p = 0.0001).

2013 Worker Safety Survey

The three-question survey completed by workers who participated in physicals in 2013 provided information on perceived safety factors and accessibility to resources.

Accordingly, 72.8% of the workers who participated in the annual exam and survey at the fabric-coating company felt that there was to some degree an improvement in workplace safety over the past year (Figure 3), but only 60.2% of workers felt at least somewhat safe at work (Figure 4). 82.3% of the workers felt at least somewhat likely to find the personal protective equipment that they needed to do their jobs (Figure 5).

Discussion

Overall Findings

Serology studies of a workforce at a fabric-coating factory where MDI is used have revealed new insight into the kinetics of the development and resolution of MDI-specific immune response among exposed workers and provided further evidence to support a role for biomonitoring. Surveillance of immunologic biomarkers can offer both benefits for individual protection and target engineering improvements for the company as a whole.

The data also suggest that MDI-specific serology could play an important role in preventing MDI asthma by serving as a biomarker of exposure, thus confirming effective hygiene and providing feedback on the effectiveness of PPE and engineering controls. These findings emphasize the need for surveillance and intervention in industry to support the safety of workers.

MDI- Specific IgG Conversion from Negative to positive

Our analysis of all eligible employees at the fabric coating company indicates that those in wet production are more likely to turn MDI-specific IgG positive at an earlier date than those in dry production. Data are consistent with a higher potential for exposure among wet workers.[11] Despite a distinct visual difference among job

classifications on the Kaplan Meir curve, analysis regarding exposure magnitude of administrative tasks versus wet production tasks demonstrates no statistically significant difference. This is most likely due to a large standard error propagated by a small sample size in administration jobs.

Modeling of the covariates allowed us to explore which characteristics of isocyanate exposure determined a risk for seroconversion. Our data showed increased risk associated with previous diagnosis of asthma. However, our analysis does not address when the asthma was first diagnosed, so we are unable to comment on whether the MDI-IgG relates to current (MDI) asthma or prior disease (childhood asthma).[28] A history of smoking appeared to be associated with an increased risk of developing MDI-specific IgG; however, neither high total IgE > 100 nor > 500 were found to be significant. The possibility of an independent mechanism of IgE may explain why smoking and atopy are not exclusive determinants of asthma.[29] As expected, wearing gloves, mask, or respirators provided a protective factor against individual immunologic conversion.[25]

Contrary to our a priori assumptions, shortness of breath appeared to be protective for the development of MDI-specific IgG conversion.[30] The explanation for this remains unclear, but one possibility is that those who are symptomatic with cardiac or respiratory conditions were more likely to be conscientious of staying away from exposure and wearing PPE, thereby decreasing exposure. Alternatively, it is possible the finding may be artificial due to an imprecise definition of shortness of breath in patient questionnaire: neither etiology nor degree of severity was assessed within the database.

This study confirms previous studies demonstrating that isocyanate specific-IgG antibodies appear to correlate with exposure. Evaluation of immunologic response to a surrogate marker of exposure based on job classification may serve as confirmation that a dose-response relationship does exist and that immunologic conversion may be prevented with the appropriate interventions.[4]

MDI- Specific IgG Conversion from Positive to Negative

Conversion of MDI-IgG from a positive to negative immunologic response does not seem to be dependent on surrogate job classifications. While interpretation of this factor has to be taken cautiously due to the scarcity of previous data to suggest the time interval needed for MDI- specific IgG to become undetectable, this finding may serve as the impetus for further research in this area. Based on an annual surveillance model, it appears that conversion back to negative serological marker after being positive is not different among the job tasks, even when temporal contact and level of exposure remains constant.

As previously seen in the first model (negative to positive specific-IgG conversion), after controlling for covariates, it appeared that wearing PPE was a significant protective factor.[25,31] The use of PPE also afforded a three-fold increase in the likelihood of MDI-IgG converting from positive to negative. While using PPE is the lowest on the hierarchy of controls due to compliance variability, this finding that PPE protects workers should underscore the importance of interventions in protecting workers exposed to isocyanates.[15,31]

Intervention Studies

Preliminary data to determine workers perception of safety within the fabric coating plant was positive overall. The majority of workers felt there was at least a measure of improvement in safety compared to previous years. This correlated well with a comparison of exposure levels based on MDI- specific IgG tests of the 2012 and 2013 data. The data reveals a significant improvement between the 2012 and 2013 immunologic results.

It is our impression that interventions for other exposures and hazards during the course of the last year at this fabric-coating company has brought added attention to the need for overall safety interventions and may have contributed to the decline in the exposure biomarker (MDI-specific IgG). The data strongly support the use of PPE as a protective factor in serologic conversion. While this is not to assert that most PPE can eliminate isocyanate exposure—glove breakthroughs and contaminations certainly occur and masks may not be sufficient—simple maneuvers clearly decrease the risk.[15,32]

Exposure elimination is one of the most strongly proven and the preferred primary preventive approach to reduce occupational asthma; however, since elimination is not always possible, exposure reduction is considered the second best option for primary prevention.[22] While incomplete and still in transition, the fabric company employed a varied systematic approach to improving safety over the last year, including First Aid/CPR classes for supervisors, safety focus group discussions, and overt display of safety changes (including respirator use by some wet production workers, mandated static grounder requirements, and new safety hoods over production machines). These changes

may explain the recent decline in MDI-IgG markers.

Limitations

While not the initial intention, the most recent data from 2013 was not included in our core data set. In retrospect, this omission may have provided a better assessment and overall reflection of the company prior to implementation of safety changes. Multiple safety and intervention programs were being implemented in 2013, and inclusion of the 2013 data may have affected the time to seroconversion. The safety programs would be related to exposure and process changes and would obscure the true endpoints.

Due to our rigid inclusion criteria, the database consisted of a total of 366 individuals, but only 223 were accounted for in our analysis. Most of the exclusion was due to limited number of data points (e.g. individuals who had only one previous test for the immunologic marker), which prohibited a longitudinal analysis.

In principle, the use of questionnaires in studies may pose a selection bias determined by who chose to fill out the questionnaire; however, this was not the case in our study. These questionnaires were completed as part of a health assessment survey for annual surveillance exams. While not every question was complete (two individuals had missing date of birth), the surveys were integrated into the annual exams and were completed by all workers that had annual physicals.

Because data were only collected once a year, it is possible that this annual serology is not entirely reflective of the actual daily or weekly trend related to exposure. As outlined previously, this may have led to concluding insignificant relationships in the data where a significant relationship truly exists. As there are minimal studies looking at

longitudinal biomarkers, the findings in this study are novel and should serve to motivate and spur future studies.

The study was unable to account for individual time since last exposure and nature of the exposures. Additionally, the study was unable to sort out the impact of individual components in the interventions. We believe the surrogate job classifications identified by the industrial hygienist accounted for the nature of exposures. This study was not intended to be a series of case-reports, but rather an epidemiological overview on the sample population. The goal was to determine the overall risk characteristics associated with seroconversion of IgG.

Summary

Longitudinal data was available from annual surveillance conducted on a cohort of workers from a polyurethane fabric-coating company. Previous data reflected that by using MDI- albumin assays, MDI-IgG titers could be used to monitor exposure among workers. Long-term follow up is needed to identify the potential association of developing immunologic response and the corresponding sequelae (e.g. asthma). Our study confirmed that the use of MDI-specific IgG biomarkers can predict level of occupational exposure to MDI. Using the specific IgG biomarkers has allowed for insights into understanding dose-dependent exposure and risk. We also note that trend changes to IgG levels can be altered by occupational safety interventions. A preliminary study among workers revealed that effective workplace intervention decreased exposure and subsequently MDI IgG titer level. Further study is needed to determine whether decreasing exposure eventually leads to improved overall health of workers.

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Appendix A: TABLES

Appendix A-1:

Table 1: Exposure Groups based on job title/task

Exposure Group	Job Title/Task
Wet Production	*Machine operators/helpers, mixing shed workers, maintenance, hazardous waste workers, production floor supervisors*
Dry Production	*Quality control, trimming, inspector, slitter, custodial, laboratory workers*
Administrative	*Office, sales, clerical, shipping receiving.*

Table 2: Characteristics of the Population

	Tot. Freq.	Tot. %	Wet Prod. % (n=144)	Dry Prod. % (n=48)	Admin. % (n=29)
Race/Ethnicity					
Hispanic	127	57.0	70.8	37.5	20.7
Asian	8	3.6	0.7	8.3	6.9
Black	36	16.1	15.3	29.2	0.0
White	47	21.1	11.1	20.8	72.4
Other	5	2.2	2.1	4.2	0.0
Age Class					
≤ 25 years old	26	11.8	13.9	8.3	6.9
> 25 – 35	65	29.4	30.6	25.0	31.0
> 35 – 45	64	28.9	29.2	29.2	27.6
> 45 – 55	51	23.1	20.8	25.0	31.0
> 55 – 65	15	6.8	5.6	12.5	3.5
Gender					
Female	27	12.1	2.1	18.8	51.7
Male	196	87.9	97.9	81.3	48.3
Characteristics					
Current Smoker	66	30.8	33.3	34.0	11.5
History of Smoking	118	55.4	63.1	50.0	23.1
Asthma (including childhood)	46	21.5	20.6	29.8	11.5
Allergies	72	33.6	31.9	31.9	46.2
Rash	22	10.3	13.6	6.4	0.0
Shortness of Breath	40	18.8	17.9	21.3	19.2
Use of PPE	181	93.8	97.8	88.6	71.4

Table 3: Biomarkers based on exposure classification

Exposure Classification	Total IgE > 100		Total IgE > 500		+ Specific IgG	
	Freq	%	Freq	%	Freq	%
Wet Production	49	34.0	7	4.9	82	56.9
Dry Production	16	33.3	4	8.3	16	33.3
Administrative	6	20.7	0	0.0	5	17.2
Total	71	32.1	11	5.0	103	46.6

Table 4: Analysis of Estimates for IgG Conversion from Negative to Positive

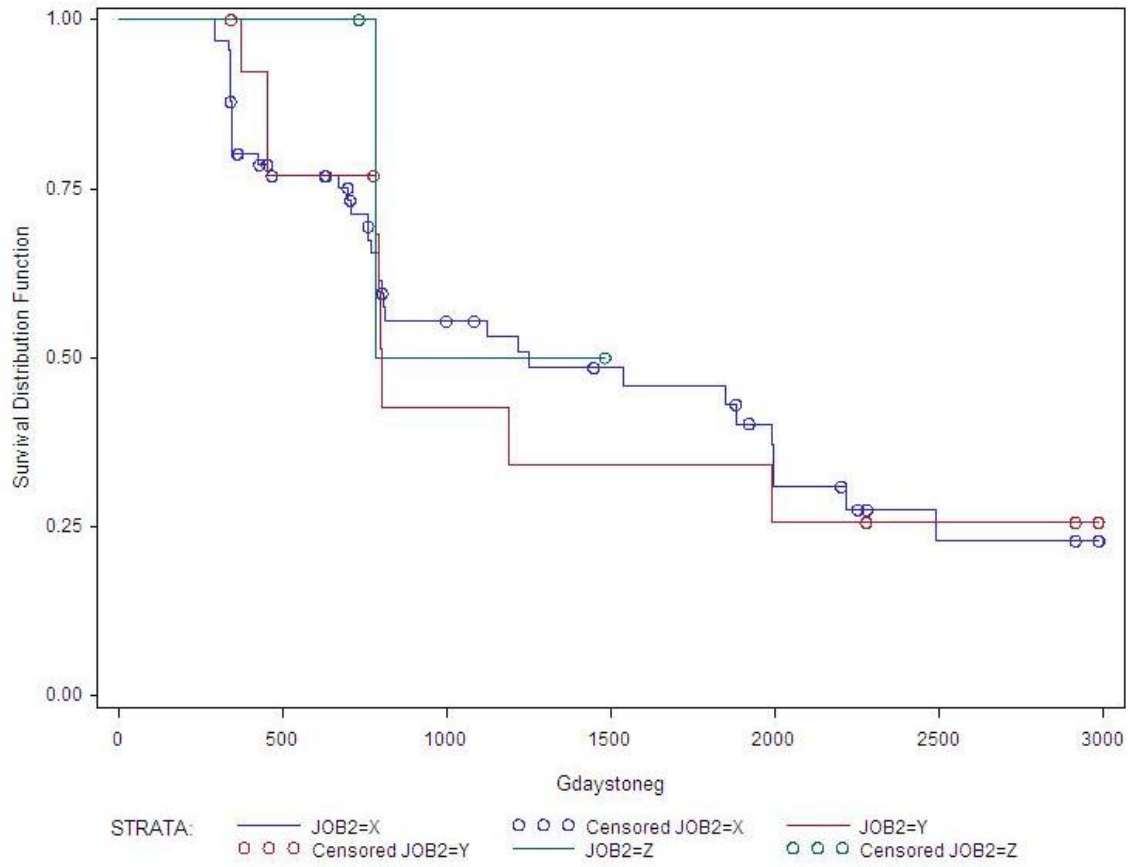| Variable	Estimate (β_i)	Std Error	P-Value	Hazard Ratio
1. Administrative (vs. Wet Production)	-1.39	1.03	0.180	0.25
2. Dry Production (vs. Wet Production)	-1.45	0.49	0.003	0.24
3. Asthma	-0.81	0.39	0.040	0.45
4. Smoking	-0.68	0.34	0.044	0.51
5. SOB	1.81	0.76	0.017	6.10
6. PPE	1.57	0.53	0.003	4.80

Appendix B- FIGURES

Figure 1:MDI-IgG Conversion from Negative to Positive by exposure classification
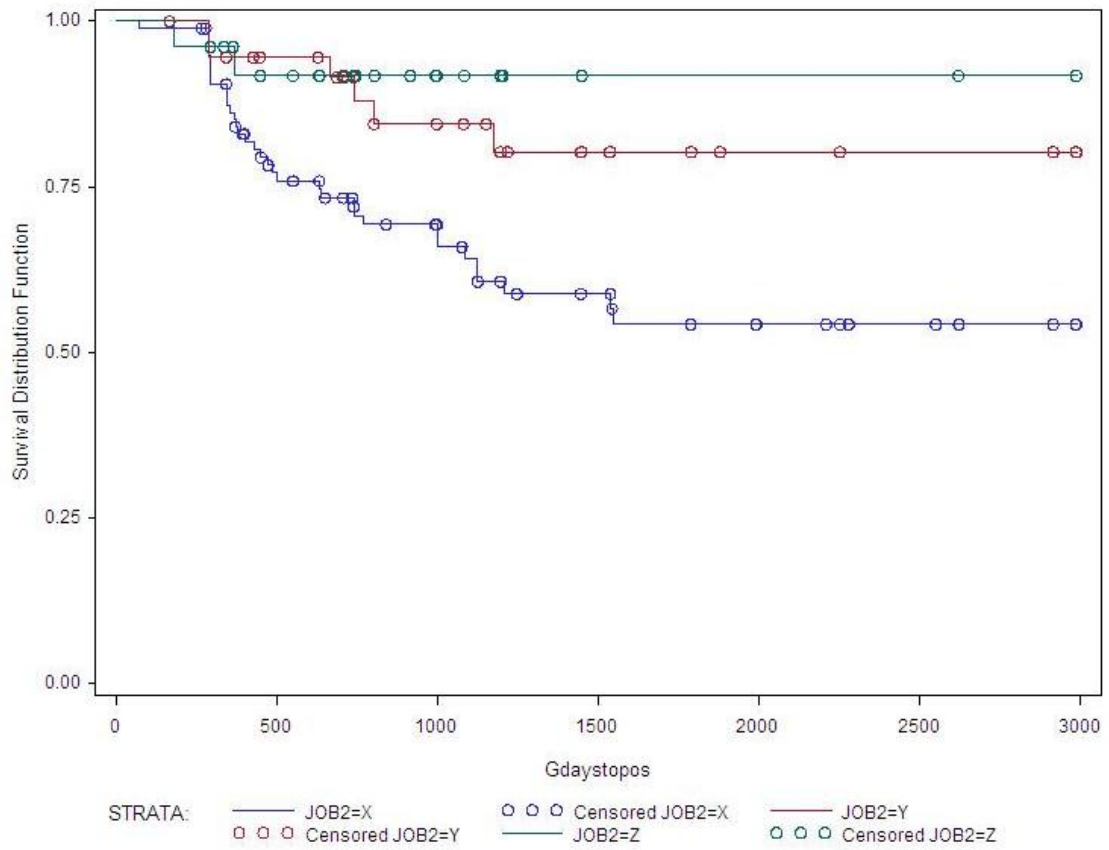
(Job "X" = Wet Production; Job "Y" = Dry Production; Job "Z" = Administrative)

Appendix B-2

Figure 2: MDI-IgG Conversion from Positive to Negative by exposure classification

(Job "X" = Wet Production; Job "Y" = Dry Production; Job "Z" = Administrative)
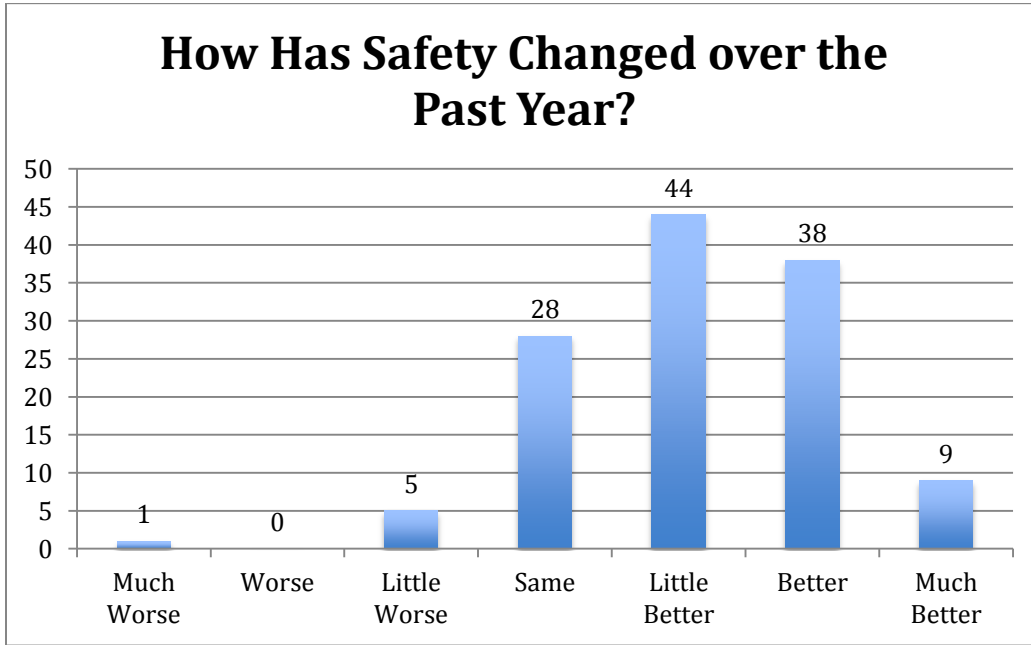
Figure 3: Survey on workplace safety question 1

How Has Safety Changed over the Past Year?

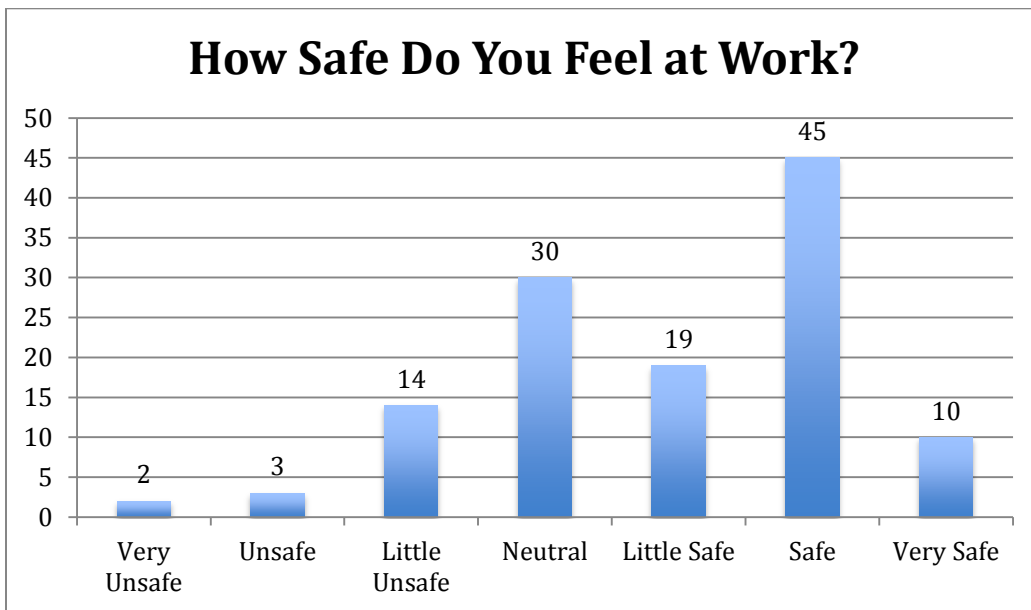*Figure 4: Survey on workplace safety question 2*

How Safe Do You Feel at Work?

Figure 5: Survey on workplace safety question 3

How Likely to Find Needed PPE?

Category	Value
Very Unlikely	1
Unlikely	1
Little Unlikely	1
Neutral	19
Little Likely	9
Likely	53
Very Likely	40