Effect of Respiratory Protective Devices on Development of Antibody and Occupational Asthma to an Acid Anhydride*

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Study objectives: To determine whether the use of respiratory protective equipment would reduce the incidence of occupational asthma due to exposure to hexahydrophthalic anhydride (HHPA).

Design: Prospective cohort study.

Setting: A facility that makes an epoxy resin product requiring HHPA for its manufacture.

Participants: Sixty-six individuals newly hired at a facility that makes an epoxy resin product requiring HHPA for its manufacture.

Intervention: Employees who wished to use respiratory protective equipment could choose from three types of masks: dust mask, half-face organic vapor respirator, or full-face organic vapor respirator.

Measurements: Workers were evaluated annually for development of positive antibody to HHPA and occupational, immunologic respiratory disease, including occupational asthma.

Results: With use of respiratory protective equipment, the rate of developing an occupational immunologic respiratory disease was reduced from approximately 10 to 2% per year. Occupational asthma developed in only three individuals, and they were all in the higher exposure category. Statistically, one respirator was not superior to the others.

Conclusion: Respiratory protective equipment can reduce the incidence of occupational immunologic respiratory disease, including occupational asthma, in employees exposed to HHPA.

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Key words: acid anhydrides; antibody; IgE; IgG; occupational asthma; occupational disease; respiratory protective equipment

Abbreviations: ELISA = enzyme-linked immunosorbent assay; HHPA = hexahydrophthalic anhydride; HHP-HSA = hexahydrophthalic anhydride conjugated to human serum albumin; OD = optical density

Occasional asthma occurs as a result of the inhalation of an occupational antigen to which an individual has been sensitized. A wide variety of high- and low-molecular-weight substances have been reported to cause occupational asthma. One such low-molecular-weight substance is hexahydrophthalic anhydride (HHPA). In most cases, specific IgE against the inciting agent can be detected using either in vitro or in vivo techniques.

Respiratory protective devices that alter the composition of inspired air are commonly used in many industries to reduce exposure to a variety of noxious...
materials, including irritants and carcinogens. There are relatively few publications on use of respiratory protective equipment in an attempt to reduce exposure to respiratory sensitizers and, thus, prevent the development of immunologic sensitization as well as clinical disease.

Prevention is described to have three components: primary, secondary, and tertiary. Primary prevention means preventing a disease such as occupational asthma. Secondary prevention means early detection and intervention, such as identifying individuals who had occupational asthma develop within a short period of time of symptom onset, and removing them from further exposure. Finally, tertiary prevention means minimizing the effects of an established disease such as occupational asthma by administering medications and reducing further exposure to minimize additional loss of lung function. Obviously, primary prevention is preferable; it has been suggested that use of respiratory protective equipment might result in primary prevention of occupational asthma. Currently, most preventive strategies in occupational asthma are secondary or tertiary prevention methods.

Occupational asthma has been reported as being caused by sensitization to a variety of acid anhydrides. Use of respiratory protective equipment has been reported to prevent occupational asthma among dairy farmers. Most studies of respiratory protective equipment in occupational asthma or hypersensitivity pneumonitis involve individuals who are already sensitized. The utility of respirators in preventing respiratory symptoms in sensitized individuals has been effective in some reports but not in others.

Some type of respiratory protective equipment is generally worn by individuals with exposure to acid anhydrides such as HHPA. The use of respirators to reduce the incidence of occupational asthma has not been well studied, but most consider these devices beneficial. We evaluated a group of individuals exposed to HHPA to assess the effect of wearing respiratory protective equipment on development of specific antibody and on development of occupational asthma due to HHPA.

**Materials and Methods**

**Study Population**

The study population was composed of 66 individuals hired at a facility that makes an epoxy resin product requiring HHPA for its manufacture. In this work process, there are many curing oven machines. Part A and part B of an epoxy resin are piped into the mold of the curing oven machine in proper proportions. The mixture is heated for a predetermined amount of time. At the time when the mixture should be a solid epoxy resin product, the operator opens the mold. Rarely, the mixture does not cure properly; then, when the mold is opened, HHPA fumes emanate.

At their date of hire, none of the study population had previous exposure to acid anhydrides, and none had antibody against HHPA conjugated to human serum albumin (HHP-HSA). Each individual was annually evaluated with a questionnaire (developed jointly by the National Institute of Occupational Safety and Health and the University of Cincinnati), spirometry, and serology for IgG and IgE against HHP-HSA. The study was approved by the Institutional Review Board of Northwestern University, and informed consent was obtained from the participants.

**Industrial Hygiene Sampling and Analytical Methods**

Air was drawn through the sample media by Escort ELF (MSA; Pittsburgh, PA), Airchek Model 224–52 (SKC; Eighty Four, PA), or GilAir (Gilian, Sensidyne; Clearwater, FL) sampling pumps. For flow rates \( \geq 1.0 \text{ L/min} \), the Escort ELF pumps control the flow rate within \( \pm 5\% \) of the set point. For the Airchek and GilAir sampling pumps, the flow rate of air through the sample media was adjusted before sampling and was checked periodically using a precision rotometer (type 1355–00A1FAA; Brooks Instruments; Hatfield, PA). The pumps and rotometers are calibrated monthly against a Gilian Calibrator-2 Primary Flow Calibrator, with Bubble Generator part number DS00286 (Sensidyne).

The NATLSCO (Long Grove, IL) environmental sciences laboratory analyzed the samples. The laboratory is fully accredited by the American Industrial Hygiene Association. Breathing zone samples were collected by attaching the sampler in the “breathing zone” of the employees who were monitored. The breathing zone is defined as a hemisphere, centered on the nose, forward of the shoulders, with a radius of approximately 12 inches. Employee exposure is defined as a concentration of an airborne substance or a physical agent to which an employee would be exposed without benefit of a respirator.

**Exposure Classification**

An exposure classification was assigned to each employee in the study population by a senior management chemical engineer. The classification was based on job description and associated industrial hygiene data, which included air sampling at various work stations in each stage of manufacture of the epoxy resin product. Exposure classifications ranged from 1 to 6, with class 1 being the lowest and class 6 being the highest. In order to obtain sufficient numbers for group-wise analysis in the intermediate-risk group, we combined classes 1 and 2, classes 3 and 4, and classes 5 and 6. When the molding process is running smoothly, HHPA air concentration is below detectable levels. Only with a spill or when an epoxy resin product does not cure properly is there detectable HHPA exposure.

**Clinical Evaluation**

Any individuals who had abnormal spirometry, respiratory symptoms on questionnaire, or positive serologic findings were interviewed, examined, and skin tested with HHP-HSA. Criteria for diagnosis of immunologic respiratory disease due to HHPA are shown in Table 1. Spirometry was performed annually for all exposed employees and as needed to evaluate employees who developed respiratory symptoms related to work. The spirometry equipment used was the Satellite/Base Station system (Jones Medical Instrument Company; Oak Brook, IL).
Table 1—Criteria for IgE- or IgG-Mediated Respiratory Disease due to HHPA*

<table>
<thead>
<tr>
<th>Variables</th>
<th>IgE-Associated Diseases</th>
<th>IgG-Associated Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>Compatible symptoms, including one or more of the following: nasal congestion, pruritus, rhinorrhea, sneezing</td>
<td>Compatible symptoms, including one or more of the following: cough, dyspnea, wheeze, chest tightness</td>
</tr>
<tr>
<td>Signs</td>
<td>Bogginess, edema, erythema of nasal mucosa</td>
<td>Wheeze, prolonged expiratory phase</td>
</tr>
<tr>
<td>Spirometry</td>
<td>NA</td>
<td>&gt; 15% change</td>
</tr>
<tr>
<td>Chest radiograph</td>
<td>NA</td>
<td>FEV1 at work vs away for 1 wk</td>
</tr>
<tr>
<td>Antibody</td>
<td>IgE antibody against HHP-HSA</td>
<td>IgG antibody against HHP-HSA</td>
</tr>
</tbody>
</table>

*NA = not applicable.

HHP-HSA Conjugate

HHPA and human serum albumin (AAlbumin-25; Armour Pharmaceutical, Kankakee, IL) were conjugated on a milligram-per-milliliter basis as described elsewhere.8 The stock concentration of the HHP-HSA was 5 mg/mL.

Enzyme-linked Immunosorbent Assay for IgG and IgE Against HHP-HSA

Immulon micro-enzyme-linked immunosorbent assay (ELISA) plates (C.A. Greiner and Sons; Nurtingen, Germany) were coated with 200 µL of 120 µg/mL HHP-HSA in carbonate buffer (pH 9.6) overnight at 4°C. The washing buffer between each step was phosphate-buffered saline solution with 0.05% Tween 20. Two hundred microliters of the appropriate serum dilution 1:10, 1:100, and 1:1000 for IgG, and 1:5, 1:10, and 1:50 for IgE were incubated at 37°C for 60 min. Rabbit antihuman IgE was incubated for 45 min at 37°C, followed by alkaline phosphatase-conjugated goat antirabbit IgG (Sigma Chemical; St. Louis, MO). For IgG only, alkaline phosphatase-conjugated goat antirabbit IgG (Sigma Chemical; St. Louis, MO) was phosphate-buffered saline solution with 0.05% Tween 20. Two hundred microliters of the appropriate serum dilution 5 mg/mL HHP-HSA in carbonate buffer (pH 9.6) was added and incubated at 37°C until a positive control reached a predetermined optical density (OD). The OD at 405 nm was determined on a Bio-Tek automated model 312 Micro ELISA reader (Bio-Tek Instruments; Winooski, VT).

A serum finding was considered to be positive if it had an OD greater than or equal to twice the mean of the negative controls. Assays were performed on each sample on 2 different days. If results were divergent (one positive, one negative), a third assay was performed and the results were averaged. To demonstrate specificity of binding, select sera were preincubated for 60 min at 37°C with varying concentrations of HHP-HSA.

Immunologic Evaluation

During this surveillance study that spanned a 7-year period, immunologic assays were performed using ELISA methodology described in detail above. Annually, blood was drawn from each employee exposed to HHPA. Serum was separated from cells and stored at −20°C until the assays were performed. Both IgE and IgG antibody against HHP-HSA were evaluated. The reported titer was the lowest dilution of test serum that was greater than twice the mean of negative controls.

Respiratory Protective Equipment

Employees were provided with three different choices of respiratory protection: a disposable dust and mist respirator (model 2350; Moldex; Culver City, CA), an East-Care Dual Cartridge half-face respirator (model 7000; 3M; Minneapolis, MN), or an organic vapor full-face respirator (Racal/3M Super Airlite Powered Respirator; 3M). Respirators were fit tested using Occupational Safety and Health Administration criteria.

Statistical Analysis

The associations between exposure classifications (classes 1 and 2, classes 3 and 4, and classes 5 and 6) and age (years), time in surveillance study (years), smoking status (never, ex-smoker, and current smoker), and type of respiratory protection (dust mask, half-face respirator, full-face respirator, and none), and between antibody status (present, absent), and both exposure class and type of respiratory protection were evaluated using nonparametric optimal discriminant analysis.18,19 Due to small sample size, experiment-wise a correction via the Bonferroni procedure was not performed; effects were considered statistically significant if they had a generalized type I error rate of p < 0.05. For all statistically significant effects that were identified, jackknife validity analysis was used to estimate the potential generalizability of the effect to an independent random sample of employees.20

RESULTS

Study Population

The demographic data of the 66 employees are listed in Table 2. Subjects in the three different exposure classes did not differ statistically in terms of the number of years they had been in the surveillance study (p < 0.62), their smoking status (p < 0.99), or the type of respiratory protection they used (p < 0.15). Although not statistically different, more employees in the higher exposure class (70%) and the moderate exposure class (73%) wore half-face or full-face organic vapor respirators as compared to individuals in the lower exposure class (39%). Employees in the lower exposure class were...
the oldest, while those in the higher exposure group were the youngest (p < 0.016). However, this finding disappeared in validity analysis, indicating that it may not generalize to an independent random sample.

**Industrial Hygiene Data**

Table 2 lists results from air sampling and analysis. The employees began to wear respiratory protection in 1993. The industrial hygiene data are from 1993, 1995, and 1997, during which time the levels were similar.

**Clinical and Immunologic Data**

The clinical and immunologic characteristics of the study population, stratified by exposure class and type of respiratory protective device worn, are tabulated in Table 3. These data were aggregated in order to assess the association between presence/absence of antibody and both the exposure class (p < 0.03) and type of respiratory protection (p < 0.65). A total of 49% of employees in the higher exposure class had positive serologic findings, compared with 15% of employees in the mid- and low-exposure classes. This effect did not diminish in validity analysis, suggesting that it may generalize to an independent random sample. The antibody was primarily IgG. The time for development of antibody in the workers ranged from 1 to 6 years.

Table 4 lists annual rates of occupational respiratory disease. The top portion of Table 4 includes all employees; on the bottom portion, there are separate rows for those hired after the institution of respiratory protective equipment. The eight employees who had occupational asthma develop in 1993 had not been wearing respiratory protective equipment prior to 1993. These are incident cases, not prevalent cases. Only three newly hired individuals had occupational asthma develop due to HHPA; all were in the higher exposure group. None of the 13 individuals who used full-face respirators and neither of the 2 individuals who did not use respirators had asthma develop, while 1 of 9 employees using the dust mask and 2 of 13 employees using half-face respirators had occupational asthma develop. The time to development of occupational asthma in these three individuals was 3, 4, and 5 years, respectively. The three employees who had occupational asthma develop had worn respirators ever since they started their employment. This was the case for all individuals

<table>
<thead>
<tr>
<th>Exposure Class</th>
<th>Mean HHPA Levels (Range), mg/m³</th>
<th>Age Range (Median), yr</th>
<th>Surveillance Time (Median), yr</th>
<th>Never-Smokers, Ex-smokers, Current Smokers, No.</th>
<th>Dust Mask, Half-Face Respiratory, Full-Face Respirator, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>0.0062 (&lt; 0.0022–0.00180)</td>
<td>29–61 (46)</td>
<td>1–7 (5)</td>
<td>12/3/5</td>
<td>9/6/1/2</td>
</tr>
<tr>
<td>3–4</td>
<td>0.0190 (0.0110–0.0310)</td>
<td>27–64 (31)</td>
<td>1–7 (2)</td>
<td>6/2/3</td>
<td>3/5/3/0</td>
</tr>
<tr>
<td>5–6</td>
<td>0.635 (0.0028–0.2500)</td>
<td>23–58 (28)</td>
<td>1–7 (4)</td>
<td>23/6/8</td>
<td>9/13/13/2</td>
</tr>
</tbody>
</table>

*See Table 1 for expansion of abbreviation.*
hired from 1993 through 1999, the 7-year study period. All three of those employees had positive skin test results. Employees who developed occupational asthma as defined in Table 1 were moved to another building at the work site where HHPA was not used. Those employees who had antibody develop but did not have symptoms of an immunologic respiratory disease were not relocated.

**Discussion**

When a respiratory sensitizer cannot be controlled by changes in engineering and ventilation, some form of respiratory protection may be useful. Some employees exposed to sensitizers find it difficult to wear a respirator at all times. The pressure of a tight-fitting mask and the resistance to air flow, especially in strenuous work situations, can make employees somewhat noncompliant in the use of respiratory protection. Most of the individuals in this study wore some respiratory protection when epoxy resin fumes were present, such as with a spill or when an epoxy resin product failed to cure properly. Most of the employees who developed occupational asthma were in the higher exposure group. It would be reasonable to conclude that protective respiratory devices should be recommended in that exposure group.

Clearly, adding respiratory protection was associated with a reduction in the incidence of newly diagnosed occupational asthma. In this study, we sought to determine the effect different respirators had on different exposure levels. Though the small sample size produced a study with limited statistical power, we nonetheless obtained significant findings when predicting antibody or occupational asthma on the basis of exposure level. Our failure to obtain statistically significant effects for respirator type in predicting antibody level or development of occupational asthma was probably due to the small sample size. Since the surveillance study evaluation with spirometry questionnaire and serology is only performed annually, there may be an underestimation of occupational asthma in this population.

In a MEDLINE search, we found seven articles about asthma due to HHPA. In three of the articles, IgG was not measured. In the other articles, the prevalence of positive IgE and IgG was similar, whereas IgG was more prevalent in our study. The amount of inhalational exposure in those studies may have been different than in our population, resulting in more IgG than IgE in our study. We suspect the exposure in those studies was greater, inasmuch as there is no indication of use of respiratory protection in the publications. It must be emphasized that a full-face respirator may help to reduce the number of new cases of occupational asthma, but cannot be relied on to protect individuals who already have occupational asthma.

When we initiated the study, approximately 10% of employees developed an occupational respiratory disease due to HHPA. At least half of those employees developed asthma. Now, after institution of respiratory protection for exposure to epoxy fumes, the number of individuals who develop any occupational respiratory disease to HHPA is only 2% per year, with approximately half of the individuals having occupational asthma develop. Table 4 lists annual rates of occupational respiratory disease. Table 4 includes all employees and a separate account of those hired after the institution of respiratory protective equipment. If the 80% reduction in

<table>
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<tr>
<th>Table 4—Annual Rates of Occupational Asthma*</th>
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<tbody>
<tr>
<td>Total employees</td>
</tr>
<tr>
<td>Employees who developed occupational immunologic respiratory diseases</td>
</tr>
<tr>
<td>Employees with occupational asthma</td>
</tr>
<tr>
<td>Employees with occupational asthma, %</td>
</tr>
<tr>
<td>Employees hired after 1992</td>
</tr>
<tr>
<td>Study employees with occupational asthma</td>
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<tr>
<td>Employees with occupational asthma, %</td>
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*Data are presented as No. unless otherwise indicated.
occupational respiratory disease due to HHPA noted presently, attributable to use of respiratory protection, generalizes to the entire population, then this finding has strong clinical significance when considered from a societal perspective.

In summary, employees in the higher exposure category were more likely to have antibody and occupational asthma develop, as defined by criteria in Table 1, due to HHPA. While use of respirators during exposure to epoxy fumes reduced the incidence of immunologic respiratory disease in newly exposed employees, we were unable to definitively report that the use of organic vapor respirators, either half-face or full-face respirators, were more protective than the dust mask. None of the individuals who wore a full-face respirator developed occupational asthma, even in the higher exposure class. It could be argued that protection with a full-face respirator might be the best respiratory protection for employees in this exposure class.

REFERENCES
16 Muller-Wening D, Repp H. Investigation on the protective value of breathing masks in farmer’s lung using an inhalation provocation test. Chest 1989; 95:100–105