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Ford Electronics and Refrigeration Corporation
Connersville, Indiana

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PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, technical and consultative assistance to Federal, State, and local agencies; labor; industry; and other groups or individuals to control occupational health hazards and to prevent related trauma and disease. Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Douglas Trout, M.D., M.H.S., Beth Reh, M.H.S., and Angela Weber, M.S. of the Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Laboratory assistance was provided by Daniel M. Lewis, Ph.D., Division of Respiratory Disease Studies. Field assistance was provided by David Marlow, Ken Martinez, Gregory Burr, Marian Coleman, Jenise Brassell, Deborah Sammons, and Barb Mackenzie. Desktop publishing by Patricia C. McGraw. Review and preparation for printing was performed by Penny Arthur.

For the purpose of informing affected employees, copies of this report shall be posted by the employer in a prominent place accessible to the employees for a period of 30 calendar days.

Health Hazard Evaluation Report 96-0156-2712
Ford Electronics and Refrigeration Corporation
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SUMMARY

In May 1996, the National Institute for Occupational Safety and Health (NIOSH) received a request from the International Union of Electrical Workers (IUE) Local 919 to conduct a health hazard evaluation (HHE) at the Ford Electronics and Refrigeration Corporation plant in Connersville, Indiana. The request expressed concern about recurring respiratory problems, including hypersensitivity pneumonitis (HP), which were thought to be associated with exposures to metalworking fluids (MWF) in the Compressor area of the plant. In response to the HHE request, NIOSH representatives made multiple site visits to the Connersville plant and performed industrial hygiene and medical surveys over the period June 1996 - April 1998.

The industrial hygiene survey included bulk sampling of MWF (analyzed for fungi, bacteria, and endotoxins), and personal breathing zone (PBZ) and general area air sampling for total particulate. The medical survey included review of medical records, a questionnaire and serologic survey among MWF-exposed and MWF-unexposed workers, and a separate evaluation to determine whether the primary microbiological contaminant (*Mycobacterium chelonae*) cultured from the MWF at the plant may have been directly related to HP among some employees.

All of the 14 bulk MWF samples had detectable concentrations of bacteria, ranging from 1.4×10^3 to 1.0×10^7 colony-forming units per milliliter. *M. chelonae* was the predominant organism identified in all of the samples. The average PBZ exposure to MWF aerosol was 0.40 milligrams per cubic meter (mg/m^3), with a range of 0.08-1.17 mg/m^3. Three of the PBZ air samples had MWF aerosol concentrations above the NIOSH recommended exposure limit (REL) for MWFs of 0.5 mg/m^3.

Fourteen Connersville employees have been diagnosed with HP since 1993; the last diagnosis was made in December 1996. Thirteen of the 14 worked in or directly adjacent to the Compressor area; no clustering of cases around a specific type of MWF or machine was identified. Two hundred fifty-two employees participated in the questionnaire and serologic survey; the survey identified one employee who had not been previously identified by the company as having HP. All of the nine symptoms evaluated (cough [dry and productive], wheeze, chest tightness, shortness of breath, unusual tiredness, muscle or joint aches, fever and chills) and reports of "flu" (defined as fever, shivering, cough, tired, weak, and ache all over) were reported more frequently among the employees exposed to MWF compared to those not exposed. The medical evaluations conducted were not able to determine the specific component(s) or contaminant(s) of the MWF causing the HP.

The causative agent(s) for HP diagnosed among some Ford Connersville employees has not been determined; it is not known whether exposures to MWF concentrations at levels above the NIOSH REL in the Compressor area are related to the occurrence of HP. Recommendations are offered to potentially reduce the occurrence of HP among workers exposed to MWF at the Connersville plant, and also to minimize other health effects potentially related to MWF exposure. These include reducing worker exposure to MWF aerosols to levels below the NIOSH REL of 0.5 mg/m^3 as part of a comprehensive safety and health program regarding MWFs. During the period of time this HHE was being conducted, many improvements were made in the engineering and ventilation of the Compressor area in the attempt to decrease employee MWF aerosol exposures. Completion of planned changes (such as eliminating recirculation of machine tool exhaust) should be helpful toward that goal.

KEYWORDS: hypersensitivity pneumonitis, metalworking fluids, machining, *Mycobacterium chelonae*
SIC 3714 (Motor vehicle parts and accessories)

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INTRODUCTION

In May 1996, the National Institute for Occupational Safety and Health (NIOSH) received a request from the International Union of Electrical Workers (IUE) Local 919 to conduct a health hazard evaluation (HHE) at the Ford Electronics and Refrigeration Corporation plant in Connersville, Indiana. The request expressed concern about recurring respiratory problems, including hypersensitivity pneumonitis (HP), which were thought to be associated with exposures to metalworking fluids (MWF) in the Compressor area of the plant. In response to the HHE request, NIOSH representatives made multiple site visits to the Connersville plant over the period June 1996 - April 1998. Interim letters to management and union representatives summarizing ongoing HHE activities were distributed in October 1996 and January 1997.

BACKGROUND

Workplace Description

The Connersville plant is a 1.7 million square-foot facility which produces automotive climate control components. Of the five areas involved in production, the Compressor area is the only one performing machining using large quantities of MWF. Approximately 265 employees, including production and maintenance personnel, work full-time in the Compressor area. The Compressor area is divided into four phases (I-IV), each of which performs similar machining operations, with the primary difference between the phases being the age of the machines (ranging from 1986 [phase I] to 1995 [phase IV]). The primary operations involve various types of cutting of aluminum, although each phase has one grinding operation involving steel. There are 19 central MWF systems in the Compressor area, ranging in size from 1,000 to 30,000 gallons of continuously recirculated water-based MWF (semi-synthetic or soluble oil). Table 1 lists the types of fluids in use at the time of the HHE. Mist collectors in phases I-III filter the MWF with bag filters. The filtered fluid then remains in the mist collectors and is drained back into the MWF systems approximately once a day. The phase IV mist collector is newer and consists of a three-stage filter – metal mesh, cartridge, and high efficiency. The filtered fluid from this collector is not recirculated back to the systems.

At the time of the HHE, the MWF systems in the Compressor area were being maintained by an on-site employee of the MWF manufacturer. Monitoring of the fluid consisted of analysis for pH, MWF concentration, bacterial and fungal counts, and biocide concentration. Additions of MWF concentrate and two different types of biocides (one triazine-based and one isothiazolin-based) were made on an as-needed basis. Complete changes of the MWF in the central systems were made based on monitoring data; at the time of the HHE the fluids in the central systems had been in service from two weeks to nearly two years (Table 1).

During the period of time the HHE was being conducted, the plant had been making changes in the ventilation of the machine tools and in the ventilation systems supplying the Compressor area. In July 1996, the supply air to the Compressor area was increased with the addition of several rooftop ventilation units. In July 1997, the mist collection ductwork and hoods for the machines in the Compressor area were re-engineered to improve efficiency. Industrial hygiene sampling (including microbiologic air sampling) in the Compressor area was performed by Ford and their consultants after the July 1997 ventilation changes; these data, and data from previous sampling done at the same sites, were supplied to NIOSH for review. In October 1997, approximately half of the mist collectors serving machines in the Compressor area were ducted to exhaust outdoors; current plans call for the remaining mist collectors to be exhausted outdoors in the near future.

METHODS

June 1996 Site Visit

During the June 1996 site visit, NIOSH representatives held an opening conference, participated in a site walk-through, reviewed Material Safety Data Sheets (MSDSs), and performed bulk sampling of MWFs. Bulk samples were taken at the initial site visit in order to sample the fluid prior to routine cleaning operations expected to occur in the planned plant shutdown in July 1996. Fourteen bulk samples of used MWFs were collected from representative areas of all four phases of the Compressor area and analyzed for fungi, bacteria, and endotoxins. Table 1 summarizes information concerning the collection sites of the bulk fluids. All samples were collected in 20-milliliter (ml) scintillation vials with Teflon™-lined caps.

The 14 bulk samples were shipped by overnight delivery to the NIOSH contract laboratory. Sequential dilutions from each bulk sample were made in the field and then plated on either R2A agar for bacterial analysis or malt extract agar (MEA) for fungal analysis. The plates were incubated at room temperature for four to seven days, then the colony forming units (CFUs) were counted and the species were identified. Results are reported as colony-forming units per milliliter of fluid (CFU/ml). Duplicate samples of the MWFs were sent to a NIOSH laboratory for analysis of endotoxins by the Limulus amoebocyte lysate assay.

During the site visit, the current status of exposure monitoring in the Compressor area and reported illnesses among Compressor employees were discussed in detail.

July-August 1996 Site Visit

During the site visits in July-August 1996, NIOSH representatives performed further industrial hygiene sampling, conducted a medical survey, and reviewed medical records. Twenty-one personal breathing zone (PBZ) and five general area (GA) air samples were collected for total particulate according to NIOSH method 0500. In addition, 24 real-time particulate measurements were made using the Grimm Model 1105 Dust Monitor (Labortechnik GmbH & CoKG, Ainring, Germany). The Grimm Dust Monitor is a light-scattering aerosol spectrometer designed for real-time particulate measurement with particle size discrimination. For each sampling location, data were integrated for one minute and stored sequentially on the Grimm data card over the entire sampling period. The collected particle count and size information was transferred to a laptop computer at the end of the sampling day.

In addition to the air sampling reported above, NIOSH representatives also performed an initial evaluation to characterize potential exposures related to operation of the superfinisher machines. Three of the four superfinishers use a silica filtering medium (diatomaceous earth) in a self-contained filtering system; this media is changed approximately once or twice a shift in what was reported by employees to be a dusty operation. A bulk sample of the media was collected and analyzed for quartz, cristobalite, and tridymite using NIOSH method 7500.

The medical survey was approved by the NIOSH human subjects review board (HSRB) and was conducted during the week of August 5, 1996. The survey consisted of questionnaire and serologic surveys of employees working in the Compressor and Fuel Rail areas. Employees in the Fuel Rail area, who perform operations (such as assembly) which do not involve MWF, served as a comparison group. The questionnaire survey included questions about work and medical history and current respiratory and systemic symptoms. The magnitude of the association between reported symptoms and current MWF exposure was assessed by the prevalence ratio and 95% confidence intervals (CI). A 95% CI means that there is a 95% chance that the prevalence ratio for the population will be within that interval. A 95% CI which excluded 1 was used to indicate statistical significance. Analysis of reported respiratory symptoms (cough, wheeze, chest tightness, and shortness of breath) was done controlling for reported cigarette use. Five employees who had previously been diagnosed with HP and removed from MWF exposure were excluded from the analysis of the symptom data.

The purpose of the serologic survey was to evaluate employee exposure to *Mycobacterium chelonae*, the major microbial contaminant identified in all of the bulk MWF samples from the June 1996 site visit. The serum from each participant was analyzed for antibodies against *M. chelonae* by both an enzyme linked immunosorbent assay (ELISA) and a precipitin assay. In addition, the serum from each participant was analyzed by preciptin assay for antibodies against five other microorganisms which are included in standard HP testing panels (but were not identified in NIOSH industrial hygiene sampling at the plant). The ELISA method was developed and used only for *M. chelonae* because *M. chelonae* was thought to be the most likely organism to which employees were exposed, and the ELISA was felt to be a more sensitive test than the precipitin assay. The methods for this serologic survey are presented in detail in Appendix 1. Analyses were done by the Immunology Section of the NIOSH Division of Respiratory Disease Studies. Comparisons of antibody status between groups was done using reported exposure to MWF within the six months prior to the evaluation; a p value of < 0.05 was used to indicate statistically significant differences. All participants in this portion of the HHE were informed in writing of their personal results in January 1997.

During the July - August 1996 site visits, the NIOSH medical officer reviewed all available medical records at the Connersville plant for employees who, in the prior three years, had been restricted from work in the Compressor area. Medical records from private physicians were reviewed for all employees reported by the Ford Connersville medical department to have had HP or an HP-like illness (such as multiple episodes of pneumonia), and for all those employees who participated in the questionnaire survey and who reported having HP or an HP-like illness.

December 1997- April 1998

During December 1997 - April 1998, a research project was conducted to evaluate whether the *M. chelonae* cultured from the MWF at the plant may have been directly related to HP among some workers. Because previous studies have shown that alveolar macrophages from HP patients release higher levels of certain cytokines than levels found in people without HP,[1] this portion of the HHE attempted to evaluate whether a similar differential response among peripheral cells could be detected. The goal of our research was to measure the response of workers' peripheral blood mononuclear cells (PBMC) (a type of white blood cell) after the cells were exposed to a *M. chelonae* antigen preparation in the laboratory. The project was approved by the NIOSH HSRB.

We attempted to contact all Connersville employees who had been diagnosed by their physicians with HP to ask them to participate in the study; seven agreed to do so. Asymptomatic employees (both MWF-exposed and MWF-unexposed) who had taken part in the previous serologic study were identified and randomly chosen to participate in the study. The study was limited to 13 participants due to resource constraints. Four site visits were made to collect blood from participants who had provided informed consent. All participants had approximately 100 ml of blood collected which was transported immediately to a contract laboratory for analysis.[*] The PBMC were purified and exposed to antigen stimulators, and the resultant cytokine (substances produced by the cells) production was measured. The methods for this process are presented in detail in Appendix 2.

[*] Bernstein Allergy Group and Clinical Research Center, Cincinnati, OH.

By comparing the results of this testing among employees who had been diagnosed with HP and employees who were asymptomatic, we hoped to determine whether increased cellular immune reactivity to *M. chelonae* (as measured by PBMC cytokine production) in workers diagnosed with HP could be used as a surrogate for the pulmonary cell-mediated inflammatory process observed in those with HP. Test results from asymptomatic employees (both MWF-exposed and MWF-unexposed) were analyzed as a group due to the small size of the study. All participants in this portion of the HHE were informed in writing of their personal results in July 1998.

EVALUATION CRITERIA

General

As a guide to the evaluation of the hazards posed by workplace exposures, NIOSH field staff employ environmental evaluation criteria for the assessment of a number of chemical and physical agents. These criteria are intended to suggest levels of exposure to which most workers may be exposed up to 10 hours per day, 40 hours per week for a working lifetime without experiencing adverse health effects. It is, however, important to note that not all workers will be protected from adverse health effects even though their exposures are maintained below these levels. A small percentage may experience adverse health effects because of individual susceptibility, a pre-existing medical condition, and/or a hypersensitivity (allergy). In addition, some hazardous substances may act in combination with other workplace exposures, the general environment, or with medications or personal habits of the worker to produce health effects even if the occupational exposures are controlled at the level set by the criterion. These combined effects are often not considered in the evaluation criteria. Also, some substances are absorbed by direct contact with the skin and mucous membranes, and thus potentially increase the overall exposure. Finally, evaluation criteria may change over the years as new information on the toxic effects of an agent become available.

The primary sources of environmental evaluation criteria for the workplace are: (1) NIOSH Recommended Exposure Limits (RELs),[2] (2) the American Conference of Governmental Industrial Hygienists' (ACGIH®) Threshold Limit Values (TLVs®),[3] and (3) the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs).[4] In July 1992, the 11th Circuit Court of Appeals vacated the 1989 OSHA PEL Air Contaminants Standard. OSHA is currently enforcing the 1971 standards which are listed as transitional values in the current Code of Federal Regulations; however, some states operating their own OSHA-approved job safety and health programs continue to enforce the 1989 limits. NIOSH encourages employers to follow the 1989 OSHA limits, the NIOSH RELs, or the ACGIH TLVs, whichever are the more protective criterion. The OSHA PELs reflect the feasibility of controlling exposures in various industries where the agents are used, whereas NIOSH RELs are based primarily on concerns relating to the prevention of occupational disease. It should be noted when reviewing this report that employers are legally required to meet those levels specified by an OSHA standard and that the OSHA PELs included in this report reflect the 1971 values.

A time-weighted average (TWA) exposure refers to the average airborne concentration of a substance during a normal 8- to 10-hour workday.

Metal Working Fluids

MWFs are used for lubrication, cooling, and removal of metal chips during machining operations. There are four major types of MWFs – straight oils, water soluble oils, semi-synthetic, and synthetic. Straight oils (neat oils) are solvent-refined petroleum oils not designed to be mixed with water. The other three types are water-based MWFs.

Epidemiologic studies have found a number of types of cancer to be associated with past MWF exposure.[5] Acute health effects that have been associated with exposure to MWFs include dermatitis and respiratory health effects, including

HP (also called extrinsic allergic alveolitis). HP is a spectrum of granulomatous, interstitial lung diseases which occur after repeated inhalation and sensitization to a wide variety of microbial agents (bacteria, fungi, amoebae), animal proteins, and low-molecular weight chemical antigens. The time of onset of HP after initial exposure to an antigen may range from a period of weeks to years. It is marked by a pneumonitis, which is reversible if exposure to the antigen is stopped; continued exposure can lead to a chronic interstitial fibrosis or scarring of the lungs. HP associated with exposure to MWFs has been recently described in several reports.[6,7,8]

In general, HP is marked by nonspecific symptoms. Acute HP begins in the first 12 hours after exposure with cough, dyspnea (shortness of breath), chest tightness, fevers, chills, malaise, and myalgias (muscle aches). The symptoms of the subacute and chronic forms of HP include cough, dyspnea, wheezing, loss of appetite, and weight loss. The diagnosis should be considered in anyone with recurrent "pneumonia" or recurrent or persistent unexplained respiratory symptoms; suggestions for uniform criteria for the diagnosis of HP have been published.[9]

These and other health effects, and other information relevant to occupational exposure to MWF, are discussed further in the NIOSH booklet, "What You Need to Know About Occupational Exposure to Metal Working Fluids," and also in the recently published NIOSH criteria document "Occupational Exposure to Metalworking Fluid."[10,11]

To prevent or greatly reduce the risk of adverse health effects due to MWF exposure, NIOSH recommends that airborne exposures to MWF aerosols be limited to 0.4 milligrams per cubic meter of air (mg/m^3) for thoracic particulate mass[**] as a TWA for up to 10 hours per day during a 40-hour week.[11] Because of the limited availability of thoracic samplers, measurement of total particulate is an accepted substitute to measurement of thoracic particulate mass. The REL for total particulate mass of MWF aerosol is 0.5 mg/m^3. The NIOSH REL was established primarily to eliminate or reduce respiratory health effects; other considerations in developing the REL included sampling and analytical feasibility, the selection of an index for assessing MWF exposure, the applicability of the REL to all types of MWFs, and technological feasibility. Concentrations of MWFs should be kept below the REL where possible because some workers have developed work-related asthma, hypersensitivity pneumonitis, or other adverse respiratory health effects when exposed to MWF concentrations less than the REL. Neither OSHA or the ACGIH have exposure limits for all MWF aerosol, although both have an 8-hour TWA limit of 5 mg/m^3 for mineral oil mist.

In addition to the REL, NIOSH recommends that a comprehensive safety and health program be developed and implemented as part of the employer's management system. The major elements of this type of program are (1) safety and health training, (2) worksite analysis, (3) hazard prevention and control, and (4) medical monitoring of exposed workers; these are explained in detail in the NIOSH Criteria Document.[11]

Endotoxin

Endotoxin is a lipopolysaccharide (LPS) compound that is part of the outer cell wall of all gram-negative bacteria (GNB). The LPS consists of a lipid (lipid A) that is embedded in the outer cell membrane and a polysaccharide that protrudes out from the cell membrane. Portions of the LPS evoke a specific antibody response. The lipid A component is thought to be responsible for the ill effects of endotoxin exposure.[12,13,14]

GNB, and therefore endotoxins, are ubiquitous in nature. Endotoxins are released when the bacterial cell is lysed (broken down) or when it is multiplying.[12,13] They are found in water, soil, and

[**]Thoracic particulate mass is the portion of MWF aerosol that penetrates beyond the larynx and may be deposited in the lung airways and/or gas exchange region.

living organisms. Endotoxins have been found in a variety of agricultural settings in many types of agricultural materials. Endotoxins have also been quantified in machining operations where water-based MWFs are used, in waste disposal, sewage, and sewage composting operations, in biotechnology processes, and in industrial and non-industrial environments associated with cooling towers, humidifiers, air-conditioners, and other water-associated processes.[13,15,16,17]

Clinically, little is known about the response to inhaled endotoxins. Exposure of previously unexposed persons to airborne endotoxin can result in acute fever, dyspnea, coughing, and small reductions in one-second forced expiratory volume (FEV_1), although some investigators have not been able to demonstrate acute changes in FEV_1.[17] The effects of repeated exposure to aerosols of endotoxins in humans are not known, although animal studies have suggested that repeated exposure may cause a syndrome similar, if not identical, to chronic bronchitis.[17]

Occupational exposure limits have not been established for endotoxin by either OSHA, NIOSH, or ACGIH. However, Rylander has reported that sufficient toxicological data are available for establishing an occupational limit for endotoxin based on acute changes in pulmonary function.[18] Eight-hour TWA air concentrations of endotoxin have been suggested as being related to the specified health effects: (1) 200 endotoxin units (EU)/m^3 - airway inflammation with increased airway reactivity; (2) 2000 EU/m^3 - cross-shift decline in FEV_1; (3) 3000 EU/m^3 - chest tightness; and (4) 10,000-20,000 EU/m^3 - toxic pneumonitis.[18] Castellan has reported a calculated "zero pulmonary function effect" concentration of 90 EU/m^3.[19]

Microbial Growth in MWF

Microorganisms (including fungi and bacteria) are normal inhabitants of the environment. The saprophytic varieties (those utilizing non-living organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide an ample supply of a nutrient substrate. Under the appropriate conditions (optimum temperature and pH, and with sufficient moisture and available nutrients), saprophytic microorganism populations can be amplified; water-based MWF provide a suitable environment for microbial amplification. Some individuals manifest increased immunologic responses to bacteria, fungi, or their metabolites encountered in the environment. Although microbial contamination of MWFs poses a potential occupational hazard, there are insufficient data to determine acceptable levels of microbial growth in MWF or in the air. In addition, allergic or hypersensitivity reactions can occur even with relatively low air concentrations of allergens (such as microorganisms), and individuals differ with respect to immuno genic susceptibilities.

It has been suggested that well-maintained MWF systems should have bacterial concentrations of less than 10^6 CFU/ml.[20] Although the acid-fast organism *M. chelonae* has been found to be present in MWF associated with outbreaks of hypersensitivity pneumonitis,[7] the significance of finding any particular fungal or bacterial species in MWF is not clear at this time.

RESULTS

June 1996 Site Visit

Results of the bulk sample analysis are reported in Table 2. Fungi were recovered from three of the 14 samples. Sample #2 and #8 had 10 CFUs/ml each, and sample #14 had 40 CFUs/ml. The other 11 samples had no detectable fungi (less than one CFU/ml). These concentrations of fungi are considered to be very low. All 14 samples had quantifiable concentrations of bacteria, ranging from 1.4×10^3 to 1.0×10^7 CFUs/ml. *M. chelonae* was the predominant organism identified in all of the samples. Sample #8 (from coolant system #9, serving the grinder in Phase II) contained the highest levels of bacteria. The limit of detection (LOD) for the bacteria samples was one CFU/ml. The results of the endotoxin analysis are also presented in Table 2. The concentrations ranged from not detected (less than 0.05 endotoxin units per milliliter [EU/ml]) to 44,375 EU/ml. The highest concentration was found in sample #5 (collected from coolant pit #6, serving the traubs in Phase II). Although microbial growth was observed in the drain pans of the mist collectors, no samples were taken for identification.

July-August 1996 Site Visits

Industrial Hygiene Sampling

Results of the air sampling for particulate during the July-August 1996 site visits are presented in Table 3. The average PBZ exposure was 0.40 mg/m^3, with a range of 0.08-1.17 mg/m^3. Three of the PBZ air samples had concentrations above the NIOSH REL for MWFs of 0.5 mg/m^3. The GA air sample average was 0.60 mg/m^3, with a range of 0.24-1.1 mg/m^3. The real-time sampling average mass, using the Grimm, was 0.85 mg/m^3, with a range of 0.25-3.70 mg/m^3. Since this instrument measures particles based on their refractive index, it is not appropriate to compare these measurements to the REL.

During one of the follow-up visits, a strong odor was detected in one of the Tocco machines. The Tocco machines contain a synthetic fluid (CX-MO5A) which reportedly is stagnant at times, a condition which may promote microbial growth. Therefore, a bulk sample was collected from each Tocco machine (4 samples, 1 in each phase) and analyzed for bacterial contamination. The Tocco machines from three phases (I, III, and IV) had relatively high concentrations of bacterial contamination, ranging from 1.2×10^8 to 3.9×10^8 CFU/mL. All the species identified from these three samples were gram negative, and *Alcaligenes faecalis* comprised about half of the total count of each sample. The Phase I sample also contained *Psychrobacter immobilis* and CDC group E species; the Phase III sample also contained *Pseudomonas fragi*; and the Phase IV sample also contained *Pseudomonas diminuta*. The Phase II sample had 1.5×10^7 CFU/mL total bacteria, half *Morganella morganii*, a gram negative species, and half *Corynebacterium nitrilophilus*, a weakly acid-fast, gram positive species.

The bulk sample of the silica media used in the superfinisher machines was found to contain 38% cristobalite and trace levels of quartz. Tridymite was not detected in the sample. The LOD and the limit of quantification (LOQ) for this analysis were 0.8% and 1.5%, respectively, for all three analytes.

Symptom and Serologic Survey

Two hundred fifty-two employees participated in the questionnaire and serologic survey. One hundred and sixty-five (62%) of 265 employees from the machining area participated; 87 (98%) of 89 employees from the comparison area (Fuel Rail) of the plant participated. The mean age of participants was 43 years; the mean time of employment at the plant was 10 years. Six of the 14 employees who had been diagnosed with HP participated in the survey. One of those six had not been previously identified by the company as having HP. The other eight persons previously diagnosed with HP not participating in the survey either refused to participate or were not present at work during the week of the survey. Information on MWF exposure within the six months prior to survey was collected in the questionnaire; this information was not available for 15 persons and those 15 were excluded from the analyses regarding antibody status.

All symptoms included in the questionnaire were reported more frequently among those employees exposed to MWF (Table 4), with prevalence ratios ranging from 1.3 - 3.5. CI for the prevalence ratios for two of the symptoms included 1. The most frequently reported symptom was 'unusual tiredness or fatigue,' which was reported by 134 (54% of the total participants). Episodes of 'flu' (defined as fever, coughs, and aches) and pneumonia in the two months prior to the survey were also more commonly reported among the MWF-exposed workers.

The percentage of workers with positive antibody tests was higher in the MWF-exposed group (compared to the MWF-unexposed) for all organisms tested except *Micropolyspora faeni* (see Table 5). The differences were statistically significant for *M. chelonae* (ELISA only), *Aureobasidium pullulans*, and *Thermoactinomyces vulgaris*. When evaluating only those study participants who reported exposure to MWF, the six study participants who had physician-diagnosed HP (compared to those who had not been diagnosed with HP) had a higher percentage of positive antibody tests for all organisms tested except *T. vulgaris* (see Table 5). The differences were statistically significant for *M. chelonae* (ELISA only), *Aspergillus fumigatus* 1, and *M. faeni*.

Medical records from employees' personal physicians were reviewed for 14 employees with HP, with dates of symptom onset ranging from January 1993 to April 1996. The age of the employees diagnosed with HP ranged from 33 - 62. With one exception, all persons diagnosed with HP worked in, or directly adjacent to, the Compressor area. The one employee who did not work in the Compressor area reportedly occasionally visited co-workers in the Compressor area; no other potential risk factors for HP were identified for that individual. No specific area, machine, or MWF in the Compressor area was identified as being related to the diagnosis of HP. According to records available to NIOSH, the last person diagnosed with HP was diagnosed in December 1996; that person's symptoms began in February 1996.

No single 'case definition' was used in diagnosing HP among these individuals; a combination of symptoms, exposure history, and test results was used to make the diagnosis of HP. Table 6 summarizes some of the clinical findings from the record review. Shortness of breath and cough were the most common symptoms reported at presentation, and all but two of the 14 individuals had abnormal crackles heard on auscultation of the lungs. Although three individuals had normal chest x-rays at the time of diagnosis, high resolution computed tomography scans (HRCT) of the chest were abnormal in all 10 persons who had this test (including 2 persons who had normal chest x-rays). Eight persons had lung biopsies performed; six of these biopsies revealed noncaseating granulomas and two revealed lymphocytic alveolitis. Pulmonary function abnormalities among the 14 workers included combinations of decreased diffusion capacity for carbon monoxide (DLCO) and spirometric patterns consistent with restrictive defects and mixed restrictive/obstructive defects. Four of the employees were hospitalized prior to or during the diagnosis of HP, and seven were treated with steroids during the illness.

December 1997 - April 1998

Industrial Hygiene Review

Ford performed air sampling in all four phases both before and after the July 1997 ventilation changes (in May and July 1997). At both times, nine PBZ and three area samples were taken for oil mist and total particulate. These samples were taken from identical operations or areas before and after the changes (in many cases the same employees were sampled) so that comparisons could be made. Of the nine PBZ samples from July 1997, six had concentrations of total particulate which were above the NIOSH REL for MWF of 0.5 mg/m^3 (median value 0.64 mg/m^3, range 0.44 - 1.40 mg/m^3). None of the three area samples had concentrations of total particulate above the NIOSH REL (values of 0.42, 0.43, and 0.32 mg/m^3). One of the PBZ samples, taken from Phase I, revealed a substantial decline in total particulate level from May to July (2.60 to 0.74 mg/m^3). None of the other pairs of samples revealed a consistent change in the magnitude or the direction (increase or decrease) of the total particulate concentrations from May to July 1997. The microbiologic air sampling done by Ford's consultant in 1997 revealed a substantial reduction in absolute number of microbes per cubic foot of air (ft^3) sampled when compared with 1996 air sampling from the same locations in the Compressor area. The mean number of microbes detected among 23 samples in 1996 was 232 microbes/ft^3 (range 26 - 363 microbes/ft^3), while the mean number in 1997 was 54 microbes/ft^3 (range 1 - 370 microbes/ft^3). The predominant organism detected in the air sampling in both 1996 and 1997 was *Mycobacterium abscessus* (identified in 18 of the 23 air samples from 1997).

Study of Cellular Immune Reactivity

Thirteen employees (seven with HP diagnosed in the past, 6 without) participated in the medical survey evaluating cytokine production after in vitro stimulation of PBMCs. No statistically significant differences in PBMC cytokine production were found between the HP and the comparison group for any of the antigens tested.

DISCUSSION AND CONCLUSIONS

Our evaluation found that 14 Connersville employees have been diagnosed with HP since 1993; the last diagnosis was made in December 1996. Although 13 of the 14 worked in or directly adjacent to the Compressor area, no clustering of cases around a specific type of MWF or machine was identified. We also found that exposures to MWF at concentrations above the NIOSH REL are occurring in certain parts of the Compressor area. Due to our limited knowledge concerning MWF-related HP, we cannot determine at this time whether the overexposures to MWF in the Compressor area are related to the occurrence of HP.

The diagnostic criteria for patients with suspected HP have been reviewed in the literature;[9,21,22,23,24] they often involve a combination of HRCT of the chest, bronchoscopy with bronchoalveolar lavage, and transbronchial or thoracoscopic biopsy in patients with the appropriate history, physical findings, pulmonary function studies, and chest radiography. As was observed in this survey, the medical testing performed in the evaluation of a person with suspected HP often varies depending on each specific clinical situation. Specific broncho-provocation studies can be diagnostic, but are infrequently performed in clinical settings, and were not used in the diagnosis of any of the Ford employees.

The causative agent(s) for the HP that was diagnosed among Ford Connersville employees is not known. The concentrations of bacteria and fungi found in the MWF at this plant are similar to those seen in other evaluations of water-based MWF.[7,8,25] Most water-based fluids have low concentrations of fungi, except when a bloom (which is often caused by a dramatic decrease in bacterial contamination) occurs.[26,27] Bacterial concentrations in MWFs often range from 10^5 to 10^8 CFU/mL, but they can be as high as 10^9 CFU/mL, with the predominant bacterial species typically being GNB bacteria.[27,28,29,30,31,32] Only recently have gram-positive and *Mycobacterium* species been identified as predominant species in the MWF of some plants.[7] This difference may be an artifact resulting from not looking for *Mycobacterium* species in the past, or it may be due to a real change in the micro ecology of the MWFs. Due to the limited nature of microbial sampling, these results may only represent a portion of the micro ecology; we may not know what the true predominant species are in these MWFs.

Nevertheless, several machining plants (including the one evaluated here) that have had documented cases of HP among employees have also identified *Mycobacterium* species in their MWFs.[7] Because the ecology of MWF systems fluctuates, documenting the microbial exposures at the time of disease (or symptom) onset is difficult. In evaluations of symptoms or illness associated with MWF exposure, bulk fluid samples collected to identify potential etiologic agents are typically collected after the onset of the health effect being studied. This points to the need for ongoing evaluations of the MWF environment, which may be able to be correlated with ongoing surveillance of health effects among exposed workers.

M. chelonae, which has been classified as a group 4 (rapid grower) nontuberculous mycobacteria (NTM),[33] is found in water supplies.[34] Disease secondary to infection with NTM is unusual and has been grouped into four primary categories of illness: pulmonary disease, lymphadenitis, skin lesions, and (usually in persons with advanced human immunodeficiency virus infection) disseminated disease.[33] *M. chelonae* has also been associated with cases of corneal ulcer and keratitis.[35] It has been estimated that 192 cases of disease, due to *M. chelonae*, occurred in the United States from 1981-1983.[33] A recent workshop summarizing information available concerning outbreaks of HP among workers exposed to MWF reported that *M. chelonae* was isolated from the MWF in four of the eight industrial sites which had reported HP cases,[7] although no conclusions regarding etiology of the HP could be made for those outbreaks. *M. chelonae* was suspected as potentially causing HP among the employees at the Ford plant, but it is possible that other microbes related to the HP may not have been isolated in our bulk sampling due to a variety of factors, including normal sampling variability, recent additions of biocidal agents to MWF systems, and growth requirements which differed from those used in the sampling we performed.

This evaluation highlights the need for, and the limitations of, environmental testing as a tool to be used in the proper interpretation of precipitating antibody testing in the clinical evaluation of patients with HP. Precipitating antibodies, primarily reflecting past exposure to the corresponding antigens, offer supporting evidence in cases where a specific causal antigen is suspected. In this evaluation, antibodies to five of the six tested microorganisms were found more commonly among the MWF-exposed group of workers, although only *M. chelonae* had been identified in cultures of the MWF used in the machining areas. A number of factors could explain these findings, including: (1) other microorganisms may have been growing in areas of the Compressor area that were not sampled; or (2) the antibody responses may reflect past occupational exposure. Precipitin testing is only useful in the diagnosis of HP if a positive precipitin test can be correlated with exposure to that antigen.

This evaluation also highlights the differences in the sensitivity of the standard precipitating antibody testing as used in this survey when compared to the ELISA. The *M. chelonae* antigen was prepared from organisms isolated from MWF used in the Compressor area, yet only 24% of the employees who reported past or current MWF exposure had a positive precipitating antibody test. This compares to a 42% positive rate for the ELISA test, confirming that the ELISA is likely a more sensitive test than the precipitating antibody test as used in this HHE.

The goal of this evaluation with regards to the testing of PBMCs was to determine if in vitro cellular immune responses could be demonstrated to *M. chelonae* in PBMCs of workers with HP. If the PBMCs of workers with HP had a measurably different immune response after exposure to the *M. chelonae*, that would have provided some evidence that *M. chelonae* was causing HP. The lack of a significant difference in cytokine production between the two groups (those diagnosed with HP and an asymptomatic comparison group) could be explained in a number of ways: (1) The small number of samples may have made it difficult to detect small differences between the groups; (2) The employees diagnosed with HP in the past had been removed from active exposure at work to potential causative microbial antigens for a variable amount of time; therefore, PBMCs may not have been as 'immunologically reactive;' (3) PBMCs may not react to antigen exposure by increasing production of the cytokines which were tested for; and (4) *M. chelonae* may not be the antigen responsible for the HP among those employees.

In addition to decreases in pulmonary function over a work shift and the occurrence of occupational asthma and hypersensitivity pneumonitis, exposure to MWF is known to be associated with increased prevalence of respiratory symptoms.[11] This survey found a small but consistent increase in reporting of respiratory and irritant symptoms among those employees who worked with MWFs, and is thus consistent with findings of previous studies.[25,36] The significance of reported respiratory and irritant symptoms in relationship to loss of pulmonary funtion or illnesses such as asthma or HP is unclear at this time.

Although we did not document the specific cause of HP among employees, it is clear from this evaluation, and from HP observed among employees in other machining environments, that HP is occurring among some employees working with or around MWFs. Proper management of the MWF and ventilation systems in those areas plays an important part in reducing exposures to substances which could potentially be the cause of the HP among employees in those areas.

RECOMMENDATIONS

The following recommendations are offered to potentially reduce the occurrence of HP among workers exposed to MWF at the Connersville plant, and also to minimize other respiratory and dermatologic health effects potentially related to MWF exposure. Some of these recommendations have been presented in interim reports distributed during the course of this HHE.

1. Fluid from the mist collectors should not be recirculated into the MWF systems because, although partially filtered, it still contains microbes and their metabolites, and stagnation of the fluid further encourages microbial growth.

2. Exposures to MWF should be reduced to levels below the NIOSH REL of 0.5 mg/m^3 (total particulate). Completion of planned changes to eliminate recirculation of machine tool exhaust should be helpful toward that goal. The American National Standards Institute Technical Report B11 TR-2-1997 contains guidelines for ventilating machining and grinding operations.[37] To document the effectiveness of engineering or ventilation changes in the Compressor area, industrial hygiene monitoring should be performed.

3. Until exposures can be reduced through engineering or administrative measures, workers exposed to MWF at concentrations above the REL should have respiratory protection. An air-purifying respirator equipped with an R- or P-series filter would be appropriate. Because respiratory protection is usually the least desirable method of reducing exposures, the use of respiratory protection should not be considered a permanent solution. Respirators should only be used within the constraints of a comprehensive respiratory protection program (29 CFR Part 1910.134). Users must be medically cleared, trained, and fit-tested for their assigned respirator.

4. Machines and machine sumps found to be contaminated with microbes should be appropriately cleaned. Appropriate precautions should be taken to protect the health of workers performing the cleaning. This should include personal protective equipment to minimize skin contact with MWF and contaminants. If there is the potential to generate aerosols during the cleaning process, respirators should be worn to minimize inhalation of those aerosols. Respirators which should be considered for use in this type of work include the R-series or P-series NIOSH-certified particulate respirators. Increased levels of respiratory protection (e.g., half-face respirators equipped with high-efficiency particulate filters, powered air-purifying respirators equipped with HEPA filters, etc.) may be required, depending on the level of visible contamination and/or the nature of the microorganisms present.

5. Although not the focus of this HHE, prevention of skin contact should be a primary focus of a MWF safety and health program. Skin contact with MWFs should be reduced as much as possible by the use of engineering controls and modification of work practices, and lastly, by the use of appropriate personal protective equipment.

6. A comprehensive safety and health program regarding MWFs (including engineering controls, fluid maintenance, environmental surveillance, and medical monitoring) following recommendations published in the NIOSH Criteria Document "Occupational Exposure to Metalworking Fluids"[11] should be implemented to minimize health effects related to MWF exposure in the machining environment. Although portions of this type of program have been in place at Ford Connersville, particular emphasis should be placed on the following:

A. The employee in charge of MWF management should be given the authority to ensure that fluids are not tampered with, that additions are made appropriately, and that systems are routinely cleaned. No unauthorized additions should be made.

B. Employees and management should be educated about the MWF systems and the importance of proper fluid management.

C. The mist collectors and washers are part of the MWF systems and should also undergo routine cleaning and maintenance.

D. Individuals with definite or possible occupational respiratory diseases should be protected from exposures to presumed causes or exacerbators of the disease. In some cases, reassignment to areas where exposure is minimized or nonexistent may be medically advisable. In such cases, the reassigned worker should retain wages, seniority, and other benefits that might otherwise be lost by such a job transfer.

7. Employees should be encouraged to report all potential work-related health symptoms to the medical department at the plant. Ford should continue to monitor reported health problems in a systematic manner designed to identify particular job duties, work materials (such as particular MWFs), machines, or areas of the plant which may be associated with particular health effects.

8. The company should conduct PBZ air sampling during the process of changing the silica filter media in the superfinisher machines. Based on the bulk sample, the filter material used in the superfinisher machines contains 38% crystalline silica. The MSDS for this material lists amorphous silica and flux-calcined diatomaceous earth, the latter of which usually contains cristobalite. Although changing this filter material occurs only once or twice a shift, it is performed without any controls or respiratory protection. NIOSH considers crystalline silica to be an occupational carcinogen and has established an REL (based on preventing silicosis) of 0.05 mg/m^3 (10-hour time weighted average).

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Table 1
Collection Locations for Bulk Samples of MWFs
HETA 96-0156, Connersville, Indiana

Sample #	Pit #	Phase	Operation	MWF	Type	Age of Fluid (months)[1]
1	1	1	Grinder	Cimstar 50B	Semi-synthetic	11
2	2	1	Piston	Cimstar 60	Semi-synthetic	21
3	3&4	1	Kingsbury/Cargill	Cimstar 60	Semi-synthetic	15
4	5	1	Traubs	FQ #2	Soluble Oil	7
5	6	2	Traubs	FQ #2	Soluble Oil	9
6	7	2	Piston	Cimstar 60	Semi-synthetic	0.5
7	8	2	Cross/Cargill	Cimstar 60	Semi-synthetic	7
8	9	2	Grinder	Cimstar 50B	Semi-synthetic	22
9	11	3	Traubs	Cimstar 60	Semi-synthetic	4
10	13	3	Cross	Cimstar 60	Semi-synthetic	11
11	15	4	Cargill	Cimstar 60	Semi-synthetic	17
12	16	4	Cross	Cimstar 60	Semi-synthetic	13
13	17	4	Piston	Cimstar 60	Semi-synthetic	11.5
14	18	4	Traubs	FQ #2	Soluble Oil	13

[1]Reported number of months since last complete MWF dump (as of June 15, 1996)

Table 2
Fungi, Bacteria, and Endotoxin Concentrations in Metal Working Fluids, June 1996
HETA 96-0156, Connersville, Indiana

Sample Number‡	Fungi (CFU/mL)	Bacteria (CFU/mL)	Endotoxins (EU/mL)
1	ND	3.1×10^{5}	1.2×10^{3}
2	1×10^{1}	2.0×10^{6}	1.3×10^{3}
3	ND	6.2×10^{5}	8.0×10^{2}
4	ND	6.2×10^{5}	6.8×10^{3}
5	ND	1.3×10^{6}	4.4×10^{4}
6	ND	2.5×10^{5}	2.7×10^{3}
7	ND	8.3×10^{5}	ND
8	1×10^{1}	1.0×10^{7}	5.2×10^{3}
9	ND	3.3×10^{6}	ND
10	ND	5.0×10^{5}	ND
11	ND	1.2×10^{6}	ND
12	ND	1.6×10^{5}	ND
13	ND	1.4×10^{3}	ND
14	4×10^{1}	1.3×10^{5}	3.4×10^{2}

‡ See Table 1 for the location of the coolant pits.
CFU/mL =Colony Forming Units per milliliter
EU/mL = Endotoxin Units per milliliter
ND = Not Detected

Table 3
Air Sampling Results for Total Particulate Concentrations, July-August 1996
HETA 96-0156, Connersville, Indiana

Sample # ‡	Phase	Machining Operation	Grimm (mg/m^3)	Total Particulate (mg/m^3)	
				Personal	Area
1	1	Cargill	0.28	0.44	NS
2	1	Kingsbury	2.60	**1.2***	**0.65**
3	1	Piston	0.76	**0.63**	NS
4	1	Swashplate	0.30	0.2	NS
5	1	Shaft	0.32	0.35	NS
6	1	Superfinisher	0.31	NS	0.24
7	2	Cargill	0.48	0.34	NS
8	2	Cross	3.70	0.38	**1.1**
9	2	Piston	1.10	**0.65**	**0.77**
10	2	Swashplate	0.58	0.21	NS
11	2	Shaft	0.70	0.39	NS
12	2	Superfinisher	0.47	0.34	NS
13	3	Cargill	1.00	0.08	NS
14	3	Cross	1.20	0.31	**0.56**
15	3	Piston	0.47	0.43	NS
16	3	Swashplate	0.58	0.28	NS
17	3	Shaft	0.55	0.35	NS
18	3	Superfinisher	0.58	0.35	NS
19	4	Cargill	2.00	NS	NS
20	4	Cross	0.54	NS	NS
21	4	Piston	0.81	0.42	NS
22	4	Swashplate	0.29	0.36	NS
23	4	Shaft	0.25	0.3	NS
24	4	Superfinisher	0.42	0.28	NS

‡ See Table 1 for the location of the coolant pits.
NS= No sample collected.
* Bolded numbers are greater than the REL for MWF of 0.5 mg/m^3.

TABLE 4
Reported Symptoms/Illnesses Among Employees Exposed and Unexposed to MWF[1]
HETA 96-0156, Connersville, IN

Symptom/Illness	Number of Exposed (% of 163) reporting symptom/illness	Number of Unexposed (% of 84) reporting symptom/illness	Prevalence Ratio[2] [95% Confidence Interval]
Unusual shortness of breath	76 (47)	11 (13)	3.5 [2.1 - 5.7]
Tightness in chest	67 (41)	13 (15)	2.5 [1.5 - 3.9]
Chills or shivering	25 (15)	6 (7)	2.1 [0.92 - 5.0]
Wheezing or whistling in chest	74 (45)	22 (26)	1.9 [1.3 - 2.8]
Ache all over	52 (32)	14 (17)	1.9 [1.1 - 3.2]
Unusual tiredness or fatigue	104 (64)	30 (36)	1.8 [1.3 - 2.4]
Dry cough	83 (51)	26 (31)	1.7 [1.2 - 2.3]
Cough with phlegm	102 (63)	32 (38)	1.7 [1.3 - 2.2]
Fever or sweats	40 (25)	16 (19)	1.3 [0.77 - 2.16]
Pneumonia	4 (2)	0	Not calculated
Chest flu[3]	93 (57)	23 (28)	2.0 [1.4 - 2.9]

1 Five participants excluded due to previous diagnosis with HP and removal from MWF areas.
2 Prevalence ratio for the reporting of the symptom among the MWF-exposed group compared with the MWF-unexposed group.
3 Chest flu defined as fever, cough, and aches.

Table 5
Antibody Test Results for Study Participants, By Exposure Status and By Past Diagnosed with HP
HETA 96-0156, Connersville, Indiana

		# (%) of employees with positive antibody test						
Exposure Status - exposure to MWF within the 6 months prior to the survey		MC[1]		AF1[4]	AF6[5]	AP[6]	MF[7]	TV[8]
		ELISA[2]	Prec[3]					
Exposed to MWF in Past 6 Months	Diagnosis of HP (N=6)	6(100%)*	3(50%)	6(100%)*	2(33%)	2(33%)	5(83%)*	3(50%)
	No Diagnosis of HP (N=171)	68 (40%)	40 (23%)	102 (60%)	16 (9%)	17 (10%)	47 (27%)	89 (52%)
Exposed to MWF in Past 6 Months- **Total** (N=177) (**Total of Previous Two Rows**)		74 (42%)**	43 (24%)	108 (61%)	18 (10%)	19 (11%)**	52 (29%)	92 (52)**
No Exposure to MWF in Past 6 Months (N=60)		11 (18%)	14 (23%)	33 (55%)	3 (5%)	0 (0%)	20 (33%)	17 (28%)

[1] *Mycobacterium chelonae*
[2] Enzyme linked immuno-sorbent assay (ELISA) for antibodies to *Mycobacterium chelonae*
[3] Precipitin assay for antibodies to *Mycobacterium chelonae*
[4] Precipitin assay for antibodies to *Aspergillus fumigatus* 1
[5] Precipitin assay for antibodies to *Aspergillus fumigatus* 6
[6] Precipitin assay for antibodies to *Aureobasidium pullulans*
[7] Precipitin assay for antibodies to *Micropolyspora faeni*
[8] Precipitin assay for antibodies to *Thermoactinomyces vulgaris*
[*] Statistically significant difference between MWF-exposed participants with and without past diagnosis of HP ($p<0.05$)
[**] Statistically significant difference between MWF-exposed and MWF-unexposed participants ($p<0.05$)

TABLE 6
Clinical findings for 14 patients with physician diagnosis of hypersensitivity pneumonitis
HETA 96-0156, Connersville, Indiana

Case #	Symptoms	Chest Exam	CXR[1]	HRCT[2]	PFTs (% Predicted)[3]				Biopsy
					FEV1	FVC	TLC	DLCO	
1	Fatigue, cough, dyspnea, 'flu'	Basilar crackles	Bibasilar interstitial infiltrates	Interstitial infiltrates	NA[4]	77%	72%	Normal	Noncaseating granulomas
2	Dyspnea, productive cough, wheeze	Basilar crackles	Normal	ND[5]	67%	64%	75%	68%	ND
3	Dyspnea, productive cough	Basilar crackles	Diffuse interstitial infiltrates	ND	57%	68%	78%	54%	ND
4	Cough	Clear	Normal	Ground glass opacification	NA	NA	NA	NA	Noncaseating granulomas
5	Weight loss, dyspnea, cough, fatigue	Basilar crackles	Interstitial infiltrates	Ground glass opacification	66%	66%	79%	81%	ND
6	Dyspnea, productive cough	Basilar crackles	Bibasilar interstitial infiltrates	Basilar fibrosis	NA	NA	83%	73%	Noncaseating granulomas
7	Dyspnea, cough	Basilar crackles	Normal	Ground glass opacification	74%	76%	70%	86%	NA
8	Fatigue, dyspnea, productive cough	Basilar crackles	Bibasilar interstitial infiltrates	Ground glass opacification	61%	59%	63%	127%	ND
9	Prod. cough, weeze	Basilar crackles	Reticulonodular pattern	Ground glass opacification	NA[6]	NA[6]	NA[6]	NA[6]	Mild pulmonary fibrosis with lymphocytic alveolitis
10	Dyspnea, productive cough, chills	Basilar crackles	Bilateral interstitial infiltrates	Ground glass opacification	59%	56%	70%	66%	Noncaseating granulomas
11	Dyspnea, productive cough	Basilar crackles	NA	Ground glass opacification	61%	61%	NA	NA	ND
12	Dyspnea	Crackles	Patchy infiltrates	Diffuse infiltrates	75%	70%	70%	82%	Noncaseating granulomas
13	Myalgia, cough, dyspnea	Clear	Bibasilar interstitial infiltrates	ND	53%	56%	59%	77%	Lymphocytic alveolitis with interstitial inflammatory infiltrates
14	Fever, dyspnea, productive cough	Diffuse crackles	Bilateral interstitial infiltrates	ND	64%[7]	81%[7]	128%[7]	65%[7]	Noncaseating granulomas

TABLE 6 Continued
Clinical findings for 14 patients with physician diagnosis of hypersensitivity pneumonitis
HETA 96-0156, Connersville, Indiana

[1] Chest x-ray
[2] High resolution computed tomography of the chest
[3] Initial pulmonary function studies: FEV_1= forced expiratory volume at 1 second (normal:>80% predicted); FVC=forced vital capacity (normal:>80% predicted); TLC=total lung capacity (normal:>80% predicted); DLCO=diffusion capacity for carbon monoxide (normal:>80% predicted).
[4] NA= test results not available
[5] ND= test not done
[6] PFTs reported in record as mild restrictive ventilatory defect with marked decrease in diffusion capacity.
[7] PFTs done 2 months after diagnosis

APPENDIX 1

Serologic Survey of Machining Workers Using Precipitin Assay and Elisa - Methods

Serologic Studies

Six isolates of Mycobacterium chelonae (*M. chelonae*) were obtained from the contract microbiology laboratory. The six isolates were identified according to standard microbiological techniques and represented the predominant microbial contaminant found in all of the MWF bulk samples from Ford Connersville. Isolates were grown in trypicase soy broth (TSB) or R2A broth at 30°Centigrade (C) with constant stirring in a rotatory incubator set at 80 revolutions per minute (rpm) for four to six weeks. The purity of the cultures was confirmed by acid-fast staining at the beginning and end of the incubation period. The bacterial cells were recovered by centrifugation (2500 rpm for 15 min.), washed twice with sterile saline, and resuspended in saline as a 10% volume/volume suspension. The bacterial suspensions were sonicated for one minute using a Branson Model 350 sonifier set at 40% output, 50% duty cycle pulse. The sonicates were clarified by centrifugation at 3500 rpm for 20 minutes, and the supernatant fluid was recovered, stored at -20°C, and used as the source of antigen for all subsequent studies.

The protein content of bacterial sonicate was determined using a modified Lowry method (BioRad) according to the manufacturer's recommendations. The bacterial sonicates were diluted with saline to comparable protein levels and tested by both precipitin and western blotting techniques with a commercial antisera to mycobacteria (Bio-Genesis) and found antigenically identical. Based on these results, all immunoassays were carried out using a sonicate of the isolates that yielded the largest volume of antigen extract.

The presence of precipitating antibodies to the *M. chelonae* extracts and to a panel of microbial antigens associated with HP (from a standard HP panel) was detected using a counter immuno-electrophoresis (CIEP) technique as previously described (Gordon et al. Am. J. Clin. Pathol. 56:471-474, 1971). The *M. chelonae* extract (200 microgram protein/ml) was tested against rabbit antisera to mycobacteria to confirm that the extract contained sufficient antigen to precipitate with antibodies in the CIEP assay. The other microbial extracts (from standard HP panel antigens) used for the precipitin analysis were purchased from Greer Laboratories (Lenior, NC). Antigens used were Aspergillus fumigatus #1 and #6, Aureobaccidium pullulans #1, Micropolyspora faeni (Faeni rectivirgula), and Thermoactinomycetes vulgaris #1. Rabbit antisera to A. pullulans was purchased and used as a positive control in all the precipitin analyses. Following the electrophoresis, the slides were washed, stained with Commassi blue, and were read by two individuals blinded to the exposure status of the subjects.

A direct enzyme linked immunosorbent assay (ELISA) for antibodies to *M. chelonae* was developed using the procedures described by Voller and Bidwell (Manual of Clinical Immunology, 4th Edition, ASM Press, Washington, D.C.). ELISA plates were coated with the *M. chelonae* extract (3 to 5 ug protein/ml) in carbonate coating buffer overnight at 4°C, blocked with 1% human serum albumin, and stored at 4°C until used but for no more than one week. The subject sera were initially tested in duplicate at a 1:80 dilution, and antibody binding was detected using peroxidase labeled anti-human immunoglobulins (Sigma) and developed with TMB substrate. Appropriate positive and negative controls were performed with each plate, including (as a positive control) rabbit antisera to mycobacterial antigen, a primary and a secondard antibody control, and an antigen blank. To determine the background, or non-specific binding levels, a set of sera were pre-incubated with soluble antigen and then assayed as usual. An ELISA positive reaction was defined as the mean plus two standard deviations of the absorbance of 32 inhibition assays. All ELISA-positive sera were then titered by testing two-fold dilutions beginning at 1:80, and a titer was defined as the last dilution with absorbance readings above the background level.

APPENDIX 2

In Vitro Cytokine Response of PBMC from Machining Workers - Methods

Preparation of PBMCs

Approximately 100 milliliters (ml) blood was collected in siliconized Vacutainer tubes containing acid citrate phosphate dextrose (ACPD) anticoagulant. Peripheral blood mononuclear cells (PBMCs) were purified using Histopaque-1077 and endotoxin tested (Sigma Product # H8889) according to the manufacturer's instructions (Procedure AST- 1) except that Hank's Balanced Salt Solution (BioWhittaker, Walkersville, MD), without calcium chloride (CaCl) and magnesium chloride (MgCl), was substituted for phosphate buffered saline as the wash solution.

The cells were resuspended in RPMI medium (BioWhittaker, Walkersville, MD) containing 5% fetal bovine serum (FBS) (Sigma Hybrimax™), penicillin-streptomycin, glutamine, and pyruvate, and counted by Coulter Counter. Cells were adjusted to a concentration of 5 x 10^6/ml in RPMI medium, and one ml/well of the cell suspension was distributed into 24 well tissue culture plates (Corning 25820). Stimulators were added to duplicate wells, according to the protocol given below, and plates were incubated 24 h, 37^{N}C, in an atmosphere of 5% carbon dioxide ($C0_2$).

Cell supernatants were removed and frozen at -80 ¨C until assayed for cytokines. Quantikine assay kits for cytokines ® & D Systems, Minneapolis, MN) were used to assay supernatants for human MCP-1, MIP- lα, IL-2, IL-4, IL-5, IL-8, TNF-α, and IFN-γ, according to the manufacturer's instructions.

Antigen Stimulators

The following antigen extracts of *M. chelonae* and dialyzed metal working fluid (MWF) were supplied by Dr. Dan Lewis (NIOSH):

1. *M. chelonae* XT #1 (not dialyzed)
2. *M. chelonae* XT #2 (dialyzed)
3. Metal Working Fluid (MWF)
4. Tryptic Soy broth (*M. chelonae* growth medium)
5. Middlebrook broth (*M. chelonae* growth medium)

Antigens were tested for sterility by plating on petri plates containing blood agar (BA), Sabarouds media, R2A, brain heart infusion broth, Mycobacterial media 7HI 1, and standard methods agar. One tube of MWF and one tube of *M. chelonae* XT #2 showed microbial growth on BA (semi-smooth small colonies). These tubes were used for preparing detoxified antigen and subsequently passaged through a 0.22 µm membrane filter.

Endotoxin was removed from antigen extracts by column detoxification, using Detoxi-Gel Endotoxin Removing Affinity Gels (Product # 20344) supplied by Pierce (P.O. Box 117, Rockford, IL 61105), according to the manufacturer's instructions.

Standardized PPD antigen produced by Pasteur Merieux Cannaught was obtained from the research division of Connaught Laboratories, Inc. (Swiftwater, PA 18370). This product was required in order to achieve a sufficiently high concentration of antigen for testing (100 microgram/milliliter).

A mycobacterial antigen extract was prepared in-house. M. Tuberculosis H37Ra desiccated cells were obtained from Difco. A 100 milligram/milliliter suspension was prepared in sterile, nonpyrogenic saline, sonicated at 50 watts for 5 min, then centrifuged, and the supernatant was removed, sterilized by 0.2 micrometer membrane filtration, and stored frozen for testing.

Phytohemagglutin-P (PHA-P), cell culture tested, Sigma Product # L9132, was used as the mitogen positive control stimulator.

All antigen extracts were tested for protein content by the Bicinchinonic Acid (BCA) test, standard protocol as supplied by the manufacturer (Pierce Chemical Co.) and an appropriate volume was added to cells as shown below in the protocol.

In preliminary studies, the growth media (Middlebrook or Tryptic Soy) did not show stimulation of cytokine synthesis by normal PBMCs, and the use of these materials was discontinued.

The panel of 8 antigens used for in vitro stimulation of PBMCs are listed below:

1. pyrogen free saline (medium control)
2. PHA-P (500 µg/ml)
3. *M. chelonae* XT #2 (dialyzed) 100 µg/rnl
4. Metal Working Fluid 180 µg/ml
5. *M. chelonae* detoxified (60 µg/ml)
6. MWF detoxified (100 µg/ml)
7. Standardized PPD (100 µg/ml)
8. M. Tb H37Ra, (280 µg/rnl)

Purification of PBMCs.

1. Prepare 50 ml radiation sterilized Sigma Accuspin tubes (Sigma Product # A2055) by pipetting 15 ml of Estopaque-1077 (Sigma Product # H8889, endotoxin tested for cell culture use), or equivalent Ficol product into the upper chamber of the tube.
2. Centrifuge at 1000 x g for 30 seconds at room temperature. Ficol will now be below the "frit". Pour whole undiluted blood onto top of the frit. Distribute 25-30 ml blood to 3 or 4 50 ml tubes.
3. Centrifuge the tubes at room temperature.
4. A layer of white cells (mononuclear cells) is under the top (blood plasma) layer. With a sterile pipet, carefully aspirate the plasma layer.
5. Use a sterile transfer pipet to collect the PBMCs which can be seen as an opaque whitish band about 1 centimeter above the frit. The less fluid collected below this band, the less contamination with other white blood cells.
6. Dilute the cells to about 40 ml with cold HBSS, Ca++ and Mg++ free, containing .0 1 M EDTA. Resuspend cells by pipetting or vortex. Centrifuge cells 250 x g for 10 min at 4°C. Discard the supernatant. Repeat wash steps twice. Resuspend the pellet in 5 ml of complete RPMI, containing 5% heat inactivated FBS, pen-strep, glutamine, pyruvate). Place the tube on ice.
7. Remove 10 ul of cell suspension and add to a tube containing 90 ul 0. 1% trypan blue. In a hemacytometer counting chamber, count at least 100 cells to determine % viability. To use this count of the cells diluted 1:10 in trypan blue to determine cell concentration in the original 2.5 ml cell suspension, count

all the mononuclear cells (ignore any red cells) in all of the four large outer squares, divide by 4 and multiply the number counted per square (N) by 1 x 10^5 (equals #cells/ml in the original undiluted cell suspension).
8. Dilute cells to 5 x 10^6/ml in cold RPMI. Add 1.0 ml of cell suspension to each of 24 wells of a 2 ml well, cell culture plate. Add 100 ul of each stimulator (.22 µ filter-sterilized) to cell wells. Incubate plates 24 hr, 37°, 5% carbon dioxide. Remove supernatants and store at -80° .

Cytokines

Cytokines were assayed from 24 hour cell supernatants using commercial kits according to methods protocols provided by the manufacturer (R&D Systems, Minneapolis, MN). Cytokines assayed were MCP-1, MIP-1α, IL-8, TNF-α, IL-Iβ, IFNγ, IL-2, IL-4, and IL-5. Results were expressed as nanogram/ml for MCP-1, MIP-1α, and IL-8, and in picogram/ml for the other cytokines.

Data analysis

The quantitative cytokine response to all stimulators was compared between the HP group (7 machining workers in whom the diagnosis of HP had previously been confirmed by biopsy and/or clinical criteria) and the comparison group (6 control subjects including 2 asymptomatic workers exposed to MWF in the facility and 4 non-exposed asymptomatic persons). Groups were compared by the Mann-Whitney rank sum test.