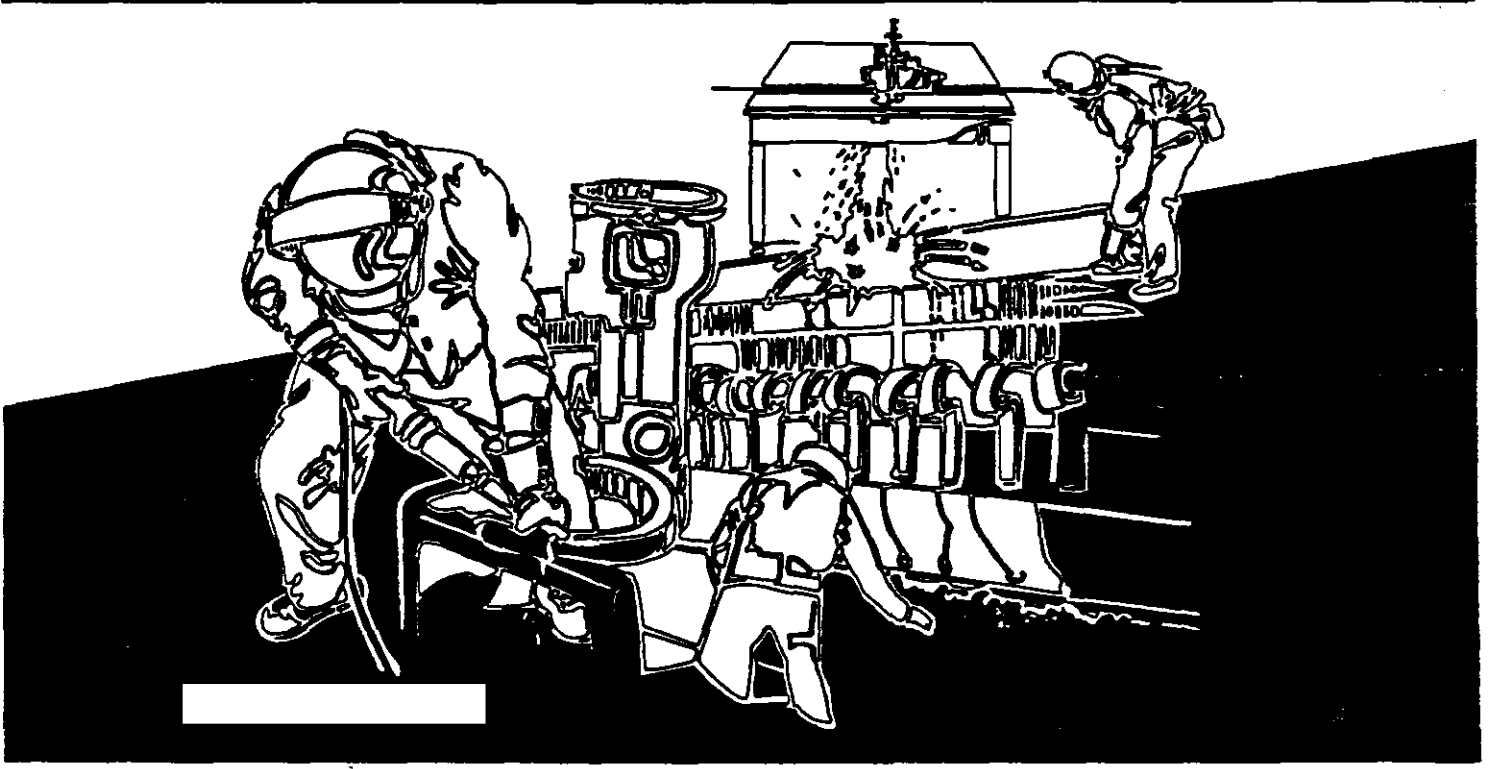
CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

NIOSH HEALTH HAZARD EVALUATION REPORT

HETA 88-267-2276
MILES, INC.
ELKHART, INDIANA



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

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 and 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, medical, nursing, and industrial hygiene technical and consultative assistance (TA) 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.

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I. SUMMARY

On May 18, 1988, the National Institute for Occupational Safety and Health (NIOSH) received a request from the United Steel Workers of America to evaluate worker health problems at the Miles Inc. enzyme plant in Elkhart, Indiana. Employees were complaining of arthritis aggravation, irritated eyes, skin, flu-like symptoms, and respiratory tract symptoms when working with specific product enzymes.

On August 25, 1988, a walk-through of the enzyme production facility was performed and confidential interviews were conducted with employees. Because workers reported shortness of breath, chest tightness, and flu-like sensations when working with two process enzymes, *Taka-Therm®* and *Milezyme®*, a medical and industrial hygiene evaluation was performed during the week of October 12, 1989. Workers participated in a medical evaluation which included pulmonary function tests, serial peak flow measurements, specific serum antibody determinations, skin prick testing using process materials, and a questionnaire detailing medical and work history. Industrial hygiene sampling to characterize worker exposures included high volume general area (GA) air samples located at selected work stations and personal breathing zone (PBZ) air samples collected on workers engaged in process activities.

Most of the environmental samples taken did not detect the presence of the enzymes in production at the time of the environmental survey. This included 24 of 26 PBZ and GA air samples collected for amyloglucosidase which were non-detectable. Results from the remaining two samples were between the limit of detection (LOD = 0.16 diazyme units [DU]/sample) and the limit of quantitation (LOQ = 0.48 DU/sample) for this sample set. Thirty-four of the 38 samples collected for alkaline protease, expressed in Detergent Alkaline Protease Units (DAPU) per sample, had levels below the LOQ of 2.0×10^{-3} DAPU/sample for this sample set. The concentration of alkaline protease in the 4 GA air samples which exceeded the LOQ for this sample set ranged from 5.9×10^{-4} DAPUs/m^3 to 13.6×10^{-4} DAPUs/m^3. The American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) for subtilisins (proteolytic enzymes as 100% pure crystalline enzyme) is 6×10^{-5} milligrams per cubic meter (mg/m^3), a level equivalent to 8.0×10^{-4} DAPU/m^3. Twenty-three of the 24 air samples collected for total protein had amounts below the LOQ for this sample set of 100 micrograms (μg) of total nitrogen per sample. There are no exposure criteria for total protein (expressed as total nitrogen).

Eight persons (22%) were classified as immunologically sensitized to one or more tested antigens (defined as a positive skin test and a significantly elevated matching specific Immunoglobulin E [IgE] antibody level). Four workers were found to be sensitized to amyloglucosidase, two persons each were sensitized to *Diazyme®* and *α*-amylase (source organism *Aspergillus*), and one worker was sensitized to *α*-amylase (source organism *Bacillus*).

When compared to all other workers, process operators were four times more likely to be immunologically sensitized to one or more of the materials tested (Prevalence Ratio [PR] 4.04, 95% Confidence Interval [CI] 1.04,15.38), and 10 times more likely to have a mild obstruction and/or mild restriction (4 operators, PR=10.00, 95% CI:1.31-76.31).

No association could be shown between job category, years worked in enzyme development, or personal air sample results, and either symptoms reported on questionnaire or specific IgG or IgE results.

The industrial hygiene sampling showed low exposures to the workplace substances evaluated on the days of testing; however, several workers had evidence of immunologic sensitization. The medical evaluation found that over 20% of employees tested had positive skin tests to *Diazyme*, 17% to *α*-amylase (source organism *Bacillus*), and that up to eight percent had positive skin tests to other substances made or used in the production process (such as *Takatherm, Takatex*, or the proprietary nutrients.) In addition, exposed workers as a group were found to have significantly elevated levels of IgE antibody to *Diazyme*, and its purified enzyme counterpart amyloglucosidase, when compared to a non-exposed control population. Three workers were identified who had elevated IgE antibody levels and positive skin tests to process substances, and who experienced significant allergic symptoms while at work. These employees were advised that continued exposure to those process materials could result in increasing severity of symptoms. The majority of employees with positive skin or IgE blood tests, while they were not symptomatic at the time of the survey, may be at increased risk of becoming symptomatic over time. Recommendations for reducing workplace exposure to allergenic substances and medical monitoring are made in Section VIII.

Keywords: SIC 2869 (Industrial Organic Chemicals, Not Elsewhere Classified), enzymes, *Taka-Therm®*, *Milezyme®*, pulmonary function tests, *Diazyme®*, Alkaline-Protease, *α*-Amylase 1,4-*α*-D-Glucan glucanohydrolase, Amyloglucosidase 1,4-*α*-D-Glucan glucohydrolase, *Takatex®*, *Tenase®*, *α*-Amylase, detergents, allergy, skin-prick tests, IgE, peak expiratory flow, Enzyme Linked Immuno-sorbency Assay (ELISA), Radioimmunosorbent Analysis (RIST).

II. INTRODUCTION

Employee complaints of irritated eyes, skin, and respiratory tract, when working with certain enzymes, prompted the Director of Health Services for the United Steel Workers of America (USWA), and the USWA Local 11273 president, to request that investigators from the National Institute for Occupational Safety and Health (NIOSH) conduct a health hazard evaluation at the Miles, Inc. enzyme plant located in Elkhart, Indiana.

On August 25, 1988, NIOSH representatives met with representatives from the USWA International Union, USWA Local 12273, and Miles to discuss worker health concerns and the specific conditions causing employees to become symptomatic. Union officials voiced concerns to NIOSH investigators that workers became ill when the plant produced *Taka-Therm®* (chemical name: *α*-amylase) or *Milezyme®* (chemical name: alkaline protease). Workers were especially concerned about potential exposures to *Milezyme®*, which was a new product at this plant, having been only recently introduced in two pre-production trial runs. Both pre-production runs resulted in workers becoming symptomatic. Worker representatives indicated that the employees found relief from their symptoms by using a non-prescription aspirin-based product provided at the work site. Following the opening conference, a walk-through tour of the enzyme production plant was completed, and confidential interviews were conducted with employees.

On January 26, 1989, a second meeting was held with union and management officials, followed by a meeting with employees to describe the scope of the NIOSH health hazard evaluation.

During the week of October 12, 1989, NIOSH personnel conducted an industrial hygiene and medical survey at the enzyme plant. Individual medical results were conveyed to each participant in writing and in person during private meetings on June 6 and 7, 1990.

III. BACKGROUND

The Miles Enzyme Plant produces industrial/commercial grade enzymes that have been cultivated from selected strains of *Bacillus licheniformis, Bacillus subtilis,* and *Aspergillus niger*. Enzymes produced at this plant are packaged in liquid formulations of varying strengths that are destined for use in detergents, food processing, and textile manufacturing.

The enzymes produced and/or handled at Miles are listed in Table 1. While the exact process and nutrients used by Miles in the production of these enzymes is proprietary, the following process description is a generalized overview of the production steps used at this plant.

Enzymes are a natural by-product of the growth and development of the parent microorganisms. Pure strains of microorganisms are combined with specially selected sterile nutrient media (selected to provide protein and nitrogen sources) in the "seed tank" where, with the aid of aeration and controlled temperatures, biologic amplification can occur.

Once sufficient biomass has accumulated in the seed tank, the culture is aseptically transferred to a large fermentation vessel, again containing pre-sterilized nutrients. The mixture of nutrients and microorganism is allowed to undergo fermentation and enzyme production.

Process operations are continuously monitored during the fermentation process to check specific parameters of the biomass broth such as temperature, pH, nutrient addition, anti-foaming agent addition, air flow rate, back pressure in the vessel, etc. Manual samples are extracted periodically from a port valve on the fermentation tank for analysis in the laboratory.

Enzymes are separated from the biomass through a series of filtration steps. The enzyme slurry is pumped to the filter system where a major portion of the suspended solids are separated from the enzyme liquid. The solid wastes from these operations are discharged in to dumpsters and transported to landfills. The enzyme liquid is concentrated with an evaporator and refiltered to remove unwanted bacterial contamination.

Following filtration, enzyme activity is stabilized, and preservative materials are added to the product during the last phase of production. The final product is shipped in headpacks, drums, or bulk tank trucks. Approximately 55 workers are employed over four work shifts at the Miles enzyme plant.

IV. EVALUATION CRITERIA

As a guide to the evaluation of the hazards posed by work place 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 or 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 if 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 work place exposures, the general environment, or with medications or personal habits of the worker to produce health effects even if the occupational exposures are controlled to the level set by the evaluation criterion. These combined effects are not often considered by 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 work place are: 1) NIOSH Criteria Documents and Recommended Exposure Limits (RELs), 2) the American Conference of Governmental Industrial Hygienists' (ACGIH) Threshold Limit Values (TLVs), and 3) the U.S. Department of Labor (OSHA) Permissible Exposure Limits (PELs).[1-3] The OSHA PELs may be required to take into account the feasibility of controlling exposures in various industries where the agents are used; the NIOSH RELs by contrast, are based primarily on concerns relating to the prevention of occupational disease. When considering the exposure levels and the recommendations for reducing these levels found in this report, it should be noted that industry is legally required to meet those levels specified by an OSHA PEL.

A time-weighted average (TWA) exposure refers to the average airborne concentration of a substance during a normal 8- to 10-hour workday. Some substances have recommended short-term exposure limits or ceiling values which are intended to supplement the TWA where there are recognized toxic effects from high, short-term exposures.

Enzymes

Within the enzyme industry, as within the overall fermentation industry, workers may be exposed to potentially hazardous microorganisms and biologically active products or intermediates.

Microorganisms

The microorganisms currently used by the enzyme industry for fermentation operations are thought to be non-infectious. However, the potential for infection by a microbe, innate or genetically modified, is not the only occupational health concern. Increasing attention is being focused upon the potential for immunologic response, after repeated inhalation, to a variety of organic materials. Cases of hypersensitivity pneumonitis have been documented in individuals exposed, in the occupational environment, to fungi, thermophilic actinomycetes, and animal proteins.[4-8]

Products or Intermediates

The biological activity of the final or intermediate products of fermentation processes is presently the primary occupational health concern within the enzyme industry. The enzyme molecule consists of a chain of amino acids arranged in a specific geometric

configuration. This protein structure, as is the case with many proteinaceous materials, will cause immunologic responses in susceptible persons if these antigens are inhaled. Inhalation of enzyme dust may provoke allergic reactions (hay fever, asthma, or rhinitis) in individuals who have become sensitized to a specific protein structure of an enzyme. Flindt was among the first to investigate chest illness in certain workers exposed to preparations containing proteolytic enzymes, derived from *Bacillus subtilis*, in the manufacture of detergents.[10] In another study of two detergent manufacturing plants, common sensitization reactions among exposed workers included cough, wheezing, chest tightness, and dyspnea (shortness of breath).[11] Numerous other reports implicate the potential respiratory hazards occurring from exposure to enzymes.[12-15] Sensitization reactions may vary from mild to severe, depending upon the particular individual exposed. Some enzymes (proteolytic, for example), have been shown to cause contact dermatitis to exposed areas of moist skin as well as irritant conjunctivitis and rhinitis.

Exposure Criteria

The ACGIH recommends a TLV of 0.00006 milligrams of subtilisins (a proteolytic enzyme produced by the bacteria *Bacillus subtilis*) per cubic meter of air averaged over an 8-hour work shift.[16] This TLV, expressed as 100% pure crystalline enzyme, is believed to be sufficiently low to prevent allergic sensitization and to prevent skin irritation.[16] There are no OSHA or NIOSH exposure criteria for proteolytic enzymes.

V. EVALUATION METHODS

Medical

A cross-sectional study design was used to evaluate the effects of employee exposure to enzymes, nutrients, and parent microorganisms. All workers at the Miles enzyme plant were invited to participate.

Pulmonary Function Tests

Pulmonary function tests were conducted using Ohio Medical Model 822 dry rolling seal spirometers, equipped with a direct reading Codonics Graphics Terminal 1550 and a HF4 dedicated microprocessor (used to record results). Pulmonary function testing procedures conformed with the American Thoracic Society's criteria for screening spirometry.[17] One-second forced expiratory volume (FEV_1) and forced vital capacity (FVC) were measured, and the ratio FEV_1/FVC was calculated for each participant. Predicted

values were calculated using Knudson's equations, as described by Hankinson, and 0.85% was used to as a correction factor for evaluating predicted FEV_1 and FVC values for black participants.[18]

Pre-shift pulmonary function tests were offered to each participant. In addition, process operators and foremen who were exposed to enzymes were selected to receive post-shift pulmonary function tests.

Peak Flow Evaluation

A Mini-Wright portable peak flow device was distributed to each participant following a demonstration of proper use and accurate measurement and recording of results. Each participant was then asked to show an understanding of the proper technique for measuring peak flow by completing one measurement and recording the result prior to leaving the testing area. Additionally, each participant received a sheet of written instructions as a reminder of proper technique. Workers were asked to record the results of three attempts, every three hours while awake, for seven days.

Skin Prick Testing

Skin prick testing materials consisted of dilute solutions of 5 process enzymes supplied from Miles, Inc. (*Milezyme®, Diazyme, Taka-Therm®, Takatex®, and Tenase®*); matching purified enzymes from Sigma Chemical Company, St. Louis, Mo. (alkaline protease [Milezyme]), (amyloglucosidase [Diazyme]), (*α*-amylase [source organisms *Bacillus* and *Aspergillus*]) for the remaining three enzymes; three confidential process nutrients (nutrient X, nutrient Y, and nutrient Z); and an atopy screen using five common aeroallergens (cat dander, blue grass, dust mites, *Alternaria*, and short ragweed).

Skin test antigens to all but the common aeroallergens (atopy screen) were tested on 14 persons (controls). These 14 controls for the skin test antigens were individuals selected by an allergy research laboratory in Cincinnati, Ohio from persons who were not Miles employees and had no known occupational exposure to enzymes. Final test dilutions were the lowest dilution that failed to elicit a positive wheal in control subjects (positive skin reaction defined as a wheal of 3 millimeters (mm) or greater in the longest diameter). Enzymes supplied by Miles were tested using 5 milligrams per milliliter (mg/ml) solutions. One exception was *Diazyme*, which was tested using a 3.4 mg/ml solution. Purified

enzymes were tested at 5.00, 1.00, 0.100, 0.010, and 0.001 mg/ml dilutions.

Test solutions (100 microliter [µl] drops) were placed on marked areas of forearms and individually pricked with a different 26-gauge hypodermic needle. Forearms of tested workers were observed for signs of wheal and/or flare reactions after an interval of 30 minutes. Histamine (0.1% histamine diphosphate, supplier: Eli Lilly, Indianapolis, IN) was used as a positive control. A phosphate buffered saline solution (PBS, 0.2 M phosphate buffer, pH 7.4, containing 0.9% NaCl) was used as a negative control for all participants.

Specific Antibody (IgG and IgE)

Specific Immunoglobulin G and Immunoglobulin E (IgG and IgE) antibodies to each protein were measured by modified indirect Enzyme Linked Immuno-sorbency Assay (ELISA) methods. Five micrograms (µg) of each enzyme were diluted and incubated in a refrigerator overnight. Each enzyme sample was then washed three times, and aliquots (200 µl) of each diluted serum sample (1:10 mixture of 5% bovine serum albumin and deionized water) were added in triplicate to the wells and allowed to incubate at room temperature for two hours. After further washing, 100 µl of goat anti-human IgG alkaline phosphatase conjugate (for IgG analysis, incubated for one hr.) or goat anti-human IgE (one hour incubation, then wash, followed by rabbit anti-goat IgG alkaline phosphatase conjugate, 1.25 hour incubation) was added. The plates were washed and 100 µl of 0.6 mM p-nitrophenyl phosphate disodium (supplier: Sigma Chemical Co.) substrate solution, diluted in alkaline glycerine buffer (0.05 M glycine and 0.5 mM magnesium chloride, pH 10.4), was added. After 30 minutes, the reactions were terminated with 50 µl of 2 N NaOH. Optical density at 410 nanometers (nm) with reference to 490 nm was read on an automated ELISA plate reader.

Questionnaire

Past employment information, medical history, use of medications, and the presence and frequency of symptoms, were all determined through use of a self-administered questionnaire.

Environmental

Exposures were characterized by collecting air samples for sensitizing agents, including alkaline protease and amyloglucosidase.

Amyloglucosidase (Diazyme®)

Air samples were collected on Teflon® filters (37-mm, 1.0-micrometer [μm] pore size) using a flow rate of 3 liters per minute (lpm) on October 12-13, 1989. All samples were analyzed by a combination and modification of methods used by Novo Biochemical Industries, Inc. and Miles Inc., for the determination of amyloglucosidase activity.[19]

This method is based on the principle that amyloglucosidase hydrolyzes p-nitrophenyl-alpha-D-glucopyranoside, a synthetic molecule, to p-nitrophenol and glucose when incubated at 55° and a pH of 4.3. The liberated p-nitrophenol is measured by adjusting the incubated solution to a pH of 8 and measuring the absorbance at 400 nanometers (nm). The amount of p-nitrophenol liberated is directed proportional to the amount of amyloglucosidase present.

Alkaline Protease (Milezyme®)

Air samples were collected using 37-mm, 1.0-μm pore size, Teflon® filters at a flow rate of 3 lpm and analyzed according to the Miles Inc., Automated Microanalysis of Alkaline Protease method (modified).[20] This method is based on the principle that protease cleaves the peptide bonds of an N,N,-dimethylcasein substrate when incubated at 55° and a pH of 8.5. Primary amino groups are produced which are reacted with trinitrobenzenesulfonic acid at 55° to produce a yellow color. The intensity of the color change is measured at 450 nm and is proportional to the amount of protease activity present.

Total Protein

Samples were collected on 37-mm glass fiber filters at a flow rate of 3 lpm and submitted to Hazelton Laboratories America, Inc. for determination of micro Kjeldahl nitrogen as a measure of total protein.* Analytical methods nos. 33.051 and 33.052 were selected from the 14th Edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC).

α-Amylase (Taka-Therm®, Takatex®, Tenase®)

No conventional personal breathing-zone (PBZ) or general area (GA) air samples were collected specifically for α-amylase. This decision

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* Kjeldahl nitrogen refers to the nitrogen present in certain organic compounds.

was based on earlier research by NIOSH investigators which found that air passing across α-amylase on a filter, or air passing through an impinger containing α-amylase in a liquid, could deactivate this enzyme. It is unclear if deactivation of the α-amylase results from a chemical alteration upon sampling.

Environmental Levels of Enzymes

Radioimmunosorbent (RIST) analysis was used in an attempt to measure airborne environmental levels of four enzymes in the plant (*Diazyme*, *Milezyme*, *Takatex*, and α-amylase [source organism *Bacillus*]). Area air samples were collected on 0.3 μm pore size polytetrafluorethylene (Teflon®) filters. The particulate collected on these filters was extracted using a phosphate buffered saline solution to solubilize the enzymes, and RIST analysis was performed using sera from workers known to have a high binding capacity for a particular enzyme (based on ELISA data). Unfortunately, the sampling flow rate for most of the air samples was inaccurate, rendering a quantitative analysis impossible to perform since the sample volume could not be calculated. The relative concentrations of these four enzymes are reported, however.

VI. RESULTS

Medical

Thirty-six workers (72% men, 26% women), representing 65% of the available work force, participated in the medical component of the health hazard evaluation. Participants had a mean age of 43 years (range: 24-59) and worked at Miles for a mean of 17 years (range: seven months to 38 years). These workers had been employed in enzyme production for an average of 5.2 years (range nine months to 17 years).

The number of participants and the disciplines they represented included the following: 12 process operators (33% of the study participants), seven laboratory technicians (19%), four maintenance workers (11%), four supervisors/foremen (11%), three secretaries (8%), two electricians (6%), two shipping and receiving (6%), and two other (6%).

Questionnaire

The 36 participants were asked to respond to questions about work history, pre-existing medical conditions, and symptoms reported to be occurring among production workers. Table 2 lists symptoms reported by the 36 participants and the frequency of positive responses.

Eighteen percent reported five or more symptoms. One worker reported experiencing 11 of the 13 symptoms listed in Table 2.

Itchy, watery eyes; sneezing; chest tightness; runny nose; and rash were combined to form an "allergic symptom group." A person was considered to meet the case definition for the allergic symptom group (ASG) if he or she responded positively to two or more ASG symptoms. In addition, sweating, muscle aches, fever, chills, and flu-like sensation were grouped to form a "flu-like symptom group" (FLSG). A person was considered to meet the case definition for FLSG if he or she responded positively to two or more of FLSG symptoms.

Fifteen persons (42%) had two or more ASG symptoms and nine persons (25%) had two or more FLSG symptoms. No association was found between meeting the case definition for either ASG or FLSG symptom groups and the following:

- Job description
- Industrial hygiene personal exposure results
- Respiratory symptoms
- Years worked in enzyme manufacturing
- Years worked at Miles Inc.

Three persons reported having asthma. Two of the individuals with asthma reported having had a physician diagnose their condition.

Skin Prick Tests

Thirty-eight workers were evaluated for allergic skin reactions. Skin test results from two workers were excluded from analysis because they did not develop a skin reaction when tested with histamine (the positive control). Three of the participants wished to participate exclusively in this phase of the medical evaluation.

Table 3 lists the antigens applied and the number of workers with positive results (defined as a wheal, which is a slightly elevated/raised area of skin that is redder or paler than surrounding skin surface, of 3 mm or greater in the longest diameter).

Eighteen persons (50%) had positive skin reactions to alkaline protease (Sigma), six persons (17%) had a positive reaction to α-amylase (source organism *Bacillus*, supplied by Sigma), eight persons reacted to amyloglucosidase (22%), eight persons (22%) reacted to *Diazyme®*, and three persons (8%) reacted

to *Takatex®*, *Taka-Therm®*, Nutrient X, and Nutrient Z. Nine persons had reactions to two or more common aeroallergens and were thus considered atopic. Those with atopy had a greater likelihood of reacting positively to alkaline protease (Prevalence Ratio [PR]=2.14, 95% Confidence Interval [CI]:1.08-4.27), α-amylase (source organism *Bacillus*), (PR=8.57, 95% CI:1.94-37.83), and both nutrient X and nutrient Z (PR=8.57, 95% CI:0.90-81.74).

Specific Immunoglobulins (IgG and IgE)

Blood samples were collected from 35 participants and analyzed using the ELISA technique. Group mean antibody levels were determined for each antigen and compared with values obtained from non-occupationally exposed laboratory controls (controls were immunology laboratory personnel with no known prior occupational exposure to enzymes). Mean IgG specific antibody levels were significantly higher for *Takatherm*, *Takatex, Tenase*, α-amylase (source organism *Aspergillus*), *Diazyme*, and amyloglucosidase among Miles workers than controls (Figure I). Specific IgE antibodies for α-Amylase (source organism *Bacillus*), *Diazyme* and amyloglucosidase were significantly higher among Miles workers than controls (Figure II).

To ascertain if elevated specific antibody levels were associated with certain job titles, job classification was examined for the process workers and seven lab workers. (Such a comparison could not be done for other job categories because they had too few participants in this study.) We found no statistically significant association between job and elevated IgG or IgE levels. In addition, there was no association observed between elevated specific antibody levels and the following.

- Years of employment
- Years worked in enzyme production
- Positive skin prick test results
- Symptoms reported on questionnaire
- Personal exposure results

Pulmonary Function Tests

Thirty-five persons (97%) completed pre-shift pulmonary function tests (PFTs). In addition, 12 process operators and foremen also completed post-shift PFTs. Pulmonary function results in 30 workers (86%) were normal and were abnormal in five workers (14%). Three persons had a mild obstructive pattern, and two persons had a mild restrictive pattern. None of the

workers participating in both pre- and post-shift PFTs showed a significant decrease in Forced Expiratory Volume in 1 Second (FEV_1), Forced Vital Capacity (FVC), or the ratio FEV_1/FVC. All decreases were less than 5% across the shift.

Each of the three workers with an obstructive pattern were process operators ($p=0.030$, 2-tailed Fishers Exact test). A mild restrictive pattern was recorded for one process operator and one maintenance worker. Thus, work as a process worker entailed an increased risk for having mild obstructive or restrictive results on lung function testing compared to all other job classifications. (PR=10.00, 95% CI:1.31, 76.31).

Since smoking is the most common cause of lung obstruction, we reviewed the smoking status of each worker with an obstructive pattern. Two of the three process workers with mild obstruction are current smokers; the third, a non-smoker, had previously been diagnosed with asthma.

Peak Flow Determination

Thirty-five of 36 persons (97%) agreed to measure and record peak flow results for a one-week period. Twenty-four workers had normal peak expiratory flow patterns (normal defined as less than a 20% difference between maximum and minimum PEFR on any day); the other 11 (31%) failed to chart results. One worker was noted to have experienced a 20% PEFR decline on the first day; however, this worker was one of the 11 employees who failed to chart for more than one day.

Environmental

Table 4 lists the summarized results for amyloglucosidase, alkaline protease, and total protein from the PBZ and GA air samples collected during this evaluation. The following sections discuss the units used to report the concentrations of these materials. The ACGIH TLV® (expressed in equivalent terms) are listed, where applicable. There are no OSHA or NIOSH exposure criteria for these materials.

Amyloglucosidase

Results for amyloglucosidase activity were reported in terms of Diazyme Units (DU) per sample.[b] In 24 of the 26 personal and general area air samples collected, no amyloglucosidase was detected. Results from the remaining two samples were between the limit of detection (LOD) and limit of quantitation (LOQ) for this sample set. The desorption efficiency was determined and corrections applied to the results. The LOD and LOQ for this sample set were 0.16 and 0.48 DU/sample, respectively. There is no equivalent ACGIH TLV expression for comparison to amyloglucosidase activity in Diazyme Units.

Alkaline Protease

Results for alkaline protease activity (*Milezyme*) are reported in terms of Detergent Alkaline Protease Units (DAPU) per sample.[c] Thirty-four of the 38 samples collected had levels below the LOQ of 2.0×10^{-3} DAPU/sample for this sample set. The concentration of alkaline protease in the four general area air samples which exceeded the LOQ for this sample set ranged from 5.9×10^{-4} DAPUs/m^3 to 13.6×10^{-4} DAPUs/m^3. For comparison, the ACGIH TLV for subtilisins (proteolytic enzymes as 100% pure crystalline enzyme) is 6×10^{-5} milligrams per cubic meter (mg/m^3), a level equivalent to 8.0×10^{-4} DAPU/m^3.[d] The two highest area air samples were collected from the press filter area and from the precoat filter area. While the levels measured in both of these areas exceed the ACGIH TLV® for subtilisins, they are not representative of typical PBZ exposures. However, these concentrations suggest that employee exposures could, at times, be excessive, depending on the length of time the worker spends in these areas.

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[b] One Diazyme Unit is that activity which will catalyze the production of one gram of glucose in one hour under the conditions of this Diazyme assay. This definition is based a Miles Inc. Product Information sheet for Diazyme L-200, 1984.

[c] One Detergent Alkaline Protease Unit (DAPU) is that activity which will liberate four micromoles of tyrosine per minute under the conditions of the assay.

[d] Conversion from DAPU to mg/m^3 obtained from a Miles, Inc. memorandum No. KVD88.026, dated August 29, 1988, entitled "Elkhart enzyme plant-alkaline protease exposure survey.

Total Protein Analysis

Twenty-three of the 24 air samples results were below the LOQ for this sample set of 100 µg of total nitrogen per sample. A full-shift PBZ air sample collected on a maintenance employee contained 425 µg of total nitrogen, equivalent to 2.7 mg of total protein. There are no corresponding ACGIH, NIOSH, or OSHA exposure level established for total protein (expressed as total nitrogen).

RIST Analysis

Air levels of *Diazyme* in this enzyme plant appeared elevated when compared to levels of *Milezyme*, *Takatex*, and *Takatherm*. No quantitation was possible using this experimental method, however, due to flow rate problems.

VII. DISCUSSION

The majority of the PBZ and GA air samples collected to measure amyloglucosidase (*Diazyme*), alkaline protease (*Milezyme*), and total protein yielded no detectable amounts of these substances. Of the enzymes tested, *Milezyme* accounted for some of the highest airborne exposures. However, only two employees had positive skin tests to *Milezyme*. Results obtained from the RIST analysis suggest that air levels of *Diazyme* appeared elevated when compared to levels of *Milezyme*, *Takatex*, and *Takatherm*. This finding is consistent with the observation by NIOSH investigators during this evaluation that *Diazyme* was handled differently than the other enzymes and there was a greater opportunity for this material to become airborne.

Testing for specific IgG antibodies in the worker's blood showed that mean IgG specific antibody levels were significantly higher for *Takatherm*, *Takatex*, *Tenase*, α-amylase (source organism *Aspergillus*), *Diazyme*, and amyloglucosidase among Miles workers as a group than in non-exposed laboratory controls. The formation of IgG antibodies to a given substance is far more common than is the production of IgE antibodies. In most instances, IgG antibodies do not elicit harmful physiologic responses and are a sign that the person has been exposed to the substance in sufficient amounts to have generated an immunologic response. On occasion, however, IgG antibodies can be involved in immune complexes such as those seen in the illness hypersensitivity pneumonitis, a form of allergic response that can cause chronic lung impairment.

Fifty-percent of the workers whose skin was tested had positive tests to alkaline protease. However, only two individuals had positive skin tests to its process enzyme counterpart, *Milezyme*. Furthermore, serum antibody IgE

levels to *Milezyme* and alkaline protease were the lowest of all enzymes tested. A subsequent report from the allergy lab responsible for preparing the test dilutions indicated that two of the 14 controls had positive skin tests to the test strength dilution of alkaline protease. Thus, the degree to which employees reacted to alkaline protease may be due to the irritant effect of concentration of alkaline protease used and not necessarily due to an allergic sensitization.

In addition to the skin testing with *Milezyme* and alkaline protease, the skin prick testing among 36 employees showed 33 positive reactions to the other substances tested. Eight workers demonstrated a positive skin test and significantly elevated matching specific IgE to one or more of the substances tested. IgE antibodies are associated with the immediate hypersensitivity reaction wherein substance specific antibodies interact with that substance and certain cells in the skin or mucous membranes to produce the symptoms of "hay fever" (itching eyes, nasal discharge) or potentially more serious symptoms like asthma, angioedema or urticaria. The majority of people do not develop IgE antibodies to a given substance even after prolonged exposure (unless that substance is an extremely strong allergen). Those that do, while they may not experience allergic symptoms after first developing an IgE response to a given substance, are at increased risk of eventually experiencing allergic symptoms when exposed to that substance. The skin prick testing is thought to be mediated by IgE antibodies and is considered to be more sensitive than the IgE blood testing, in that the skin test response can be positive before there is sufficient substance specific IgE in the blood to be detectable.

Workers had the highest mean concentrations of specific IgG and IgE antibodies to *Diazyme* and its corresponding "purified enzyme" amyloglucosidase (purchased from Sigma). In addition, eight workers had positive skin tests to *Diazyme*, and eight workers had positive skin tests to amyloglucosidase. As mentioned before, a review of the production process indicated that *Diazyme* is transported through processing stages on open conveyors rather than totally enclosed (as is the case with *Takatherm* and *Milezyme*). Transporting *Diazyme* filtrate through the plant in open conveyors may increase the chance for *Diazyme* dust becoming airborne and thereby sensitizing workers.

VIII. CONCLUSIONS

This survey was done in response to reports of work-related symptoms (e.g., nasal congestion, eye itching, shortness of breath) that suggested the possibility or IgE-mediated allergic reactions to enzymes or other workplace substances. In addition, some workers reported experiencing a work-related symptoms complex consisting of shortness of breath (SOB), chest tightness, aching muscles, and fever that was compatible with a toxic organic dust syndrome or with extrinsic allergic alveolitis (hypersensitivity pneumonitis).

Eighteen percent of the 36 employees who completed the questionnaire reported five or more of the symptoms listed in Table 2. Fifteen persons reported two or more of the symptoms in the allergic group (e.g., itchy, watery eyes; chest tightness; runny nose) that in many instances can be mediated by IgE antibody. Nine persons reported two or more symptoms in the FLSG that can be seen in people experiencing hypersensitivity pneumonitis or the toxic organic dust syndrome (e.g., sweating, muscle aches, or a flu-like sensation).

Most of the environmental air samples taken were not able to detect the presence of the enzymes in production at the time of the environmental survey. These "negative" findings indicate that on the days of testing, the production processes controlled enzyme concentrations to levels below the limit of detection of the sampling tests employed. However, the antibody testing data show that workers are getting sufficient exposure (at least on occasion) to elicit and sustain an antibody response, and the work-related symptoms of some of the workers with elevated IgE antibodies and positive skin prick reactions to one or more enzymes present in the plant are likely to be due to exposure to those enzymes.

The significance of the flu-like symptoms associated by the workers with the production of Milezyme and Takatherm is unclear at this time. There were no significant differences in IgE antibody levels between the three laboratory controls and Miles workers, but there was a significant increase in the workers' mean level of IgG antibody to Takatherm. At the time of the follow-up visit in June 1989, to inform workers of their results, interviews with the participants indicated that these symptoms had decreased greatly in frequency and severity. However, there were three employees with positive allergy test results and workplace-related allergic symptoms who were advised that continued exposure to workplace allergens could result in increasingly severe symptoms and that they should consider transfer to work not involving enzyme exposure.

The pre/post pulmonary function tests (12 employees) and peak flow testing (35 employees) did not show any worker with a significant obstructive change during the test period except for one worker, but since this worker performed the peak flow tests for only part the first day, the meaning of his test results is unclear. There were five employees whose baseline PFT tests were slightly outside the expected range (three mild obstruction, two mild restriction). (Four of these five employees were process operators.) While these results may be due to tobacco use, to other non-occupational factors or to chance, given the known potential for enzyme exposure to cause adverse respiratory effects and other allergic symptoms, it is advisable that all workers should have annual medical monitoring to help prevent the development of severe allergic symptoms and chronic adverse health effects due to enzyme exposure.

IX. RECOMMENDATIONS

1. Employees should be informed about the possibility of developing allergic reactions to enzymes and nutrient materials.

2. Engineering control techniques, such as enclosing the *Diazyme* conveyor belts, should be considered to reduce airborne enzyme levels at this facility. Additionally, there may be times when elevated enzyme exposures could occur to enzymes and nutrient materials due to equipment malfunction, or the cleaning and maintenance of enzyme processing equipment. To minimize worker exposure during these episodes, employee use of appropriate protective clothing and respirators is recommended when environmental monitoring indicates that exposures may exist.

3. Employees should be offered preplacement and periodic medical evaluations, including a medical and occupational history, physical examination and pulmonary function testing. If workers become symptomatically sensitized to workplace enzymes or nutrients, they should be offered transfer to jobs that do not entail exposure to those substances.

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Copies of this report have been sent to:

1. Miles, Inc.
2. United Steel Workers of America Local 11273
3. OSHA, Region V

For the purpose of informing affected workers, 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.

TABLE 1.
ENZYMES PRODUCTS FROM THE MILES INC. PLANT, ELKHART INDIANA

Miles Inc., Elkhart Indiana
HETA 88-267

Miles Product Name and Parent Microorganism	Chemical Name	Use
Milezyme® *Bacillus licheniformis*	Alkaline-Protease	Heavy-duty laundry detergents Presoaks Commercial laundry detergent Special purpose cleaners
Taka-Therm® *Bacillus licheniformis*	α-Amylase	Food products Paper sizing De-sizing textiles
Diazyme® *Aspergillus niger*	Amyloglucosidase 1,4-α-D-Glucan gluconohydrolase	Glucose production Alcohol production Brewing Yeast and vinegar production
Takatex® **Tenase®** *Bacillus subtilis*	α-Amylase	De-sizing fabric

TABLE 2.
QUESTIONNAIRE RESULTS: MOST COMMONLY REPORTED SYMPTOMS

Miles Inc., Elkhart Indiana
HETA 88-267

Symptom	Number Reporting	% of 36 Participants
Itchy Watery Eyes	13	36%
Sneezing	12	33%
Chest Tightness	11	31%
Muscle Aches	11	31%
Cough	10	28%
Flu-Like Sensation	10	28%
Runny Nose	9	25%
Sweating	8	22%
Fever	6	17%
Shortness of Breath	5	14%
Chills	4	11%
Rash	4	11%
Wheezing	2	6%

TABLE 3.
SKIN TEST RESULTS

Miles Inc., Elkhart Indiana
HETA 88-267

Antigen	Employees with Positive Skin Reaction	Participant Percentage (36 Workers tested)
Alkaline Protease	18	50%
α-Amylase/ Bacillus	6	17%
Diazyme®	8	22%
Amyloglucosidase	8	22%
Takatex®	3	8%
Taka-Therm®	3	8%
Nutrient X	3	8%
Nutrient Z	3	8%
α-Amylase/ Aspergillus	2	6%
Milezyme®	2	6%
Nutrient Y	2	6%
Tenase®	1	3%
2 or more common aeroallergens (ATOPY)	9	25%

TABLE 4.
GENERAL AREA AND PERSONAL BREATHING-ZONE AIR SAMPLE RESULTS

Miles Inc., Elkhart Indiana
HETA 88-267

SUBSTANCE	NUMBER COLLECTED	RESULTS		
Amyloglucosidase (Diazyme®)	26	1. In 24 of 26 air samples no enzymes were detected. 2. Two sample results were between the LOD (0.16 DU/sample) and the LOQ (0.48 DU/sample) of this method. 3. Results were reported in Diazyme Units (DU) per sample. 4. There is no equivalent ACGIH permissible exposure expression for Diazyme Units.		
Total Protein Analysis	24	1. Results from 23 of 24 air samples were below the LOQ of 100 µg of total nitrogen per sample. 2. Sample No. GF-24 (PBZ, full-shift air sample) collected on a maintenance employee contained 425 µg of total nitrogen (equivalent to 2.7 mg of total protein).		
Alkaline Protease (Milezyme®)	38	1. Results from 34 of 38 air samples were below the LOQ of 2.0×10^{-3} DAPU/sample for this sample set. 2. The chart below summarizes the results from the 4 general area air sample results which were above the LOQ.		
	Area Sample No.	Date	Operation	Enzyme Activity
	Sample No. 16	10/13/89	Ultra filtration	6.9×10^{-4} DAPUs/m³
	Sample No. 18	10/12/89	Press filtration	13.6×10^{-4} DAPUs/m³
	Sample No. 24	10/13/89	Precoat filters	5.9×10^{-4} DAPUs/m³
	Sample No. 1003	10/13/89	Precoat filters	7.7×10^{-4} DAPUs/m³

ACGIH TLV for proteolytic enzymes (as 100% pure crystalline enzyme) is 0.00006 mg/m³. This is equivalent to: 8.0×10^{-4} DAPUs/m³

Abbreviations:

LOD = Limit of Detection
LOQ = Limit of Quantitation
DAPU = Detergent Alkaline Protease Units
m³ = Cubic meter of air
µg = Micrograms
DU = Diazyme Units

Figure I
Serum IgG ELISA Results for Industrial and Purified Enzymes

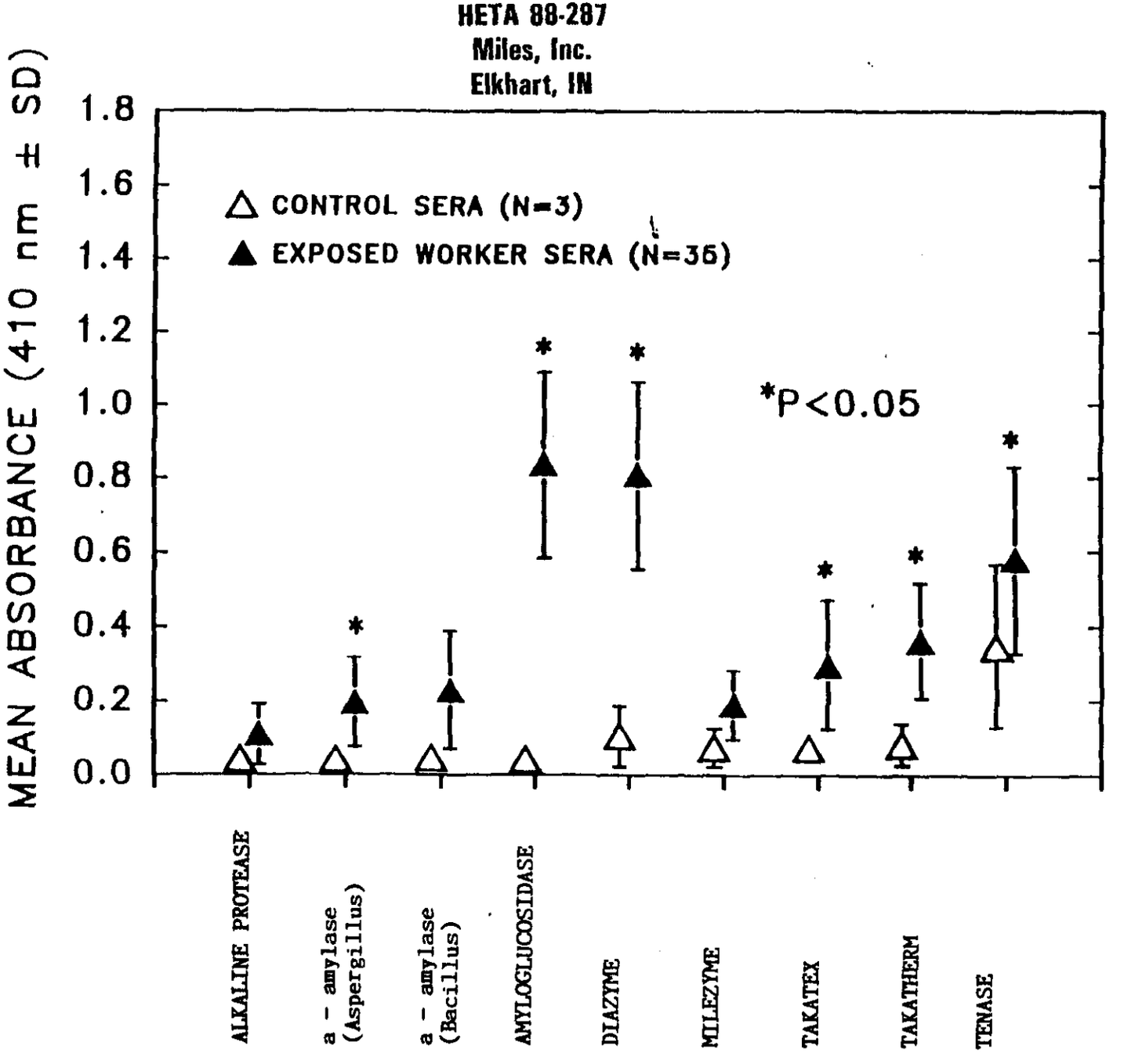

Figure II
Serum IgE ELISA Results for Industrial and Purified Enzymes

HETA 88-287
Miles, Inc.
Elkhart, IN