NIOSH HEALTH HAZARD EVALUATION REPORT:

HETA #2000-0096-2876
ChemDesign Corporation
Fitchburg, Massachusetts

May 2003

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

PREFACE

The Field Studies Branch (FSB) National Institute for Occupational Safety and Health (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 (OSHA) 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.

HETAB 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 NIOSH.

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Eva Hnizdo of the Division of Respiratory Disease Studies (DRDS), Field Studies Branch (FSB), and David Sylvain of the Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS), HETAB. Field assistance was provided by Feroza Daroowalla, Ahmed Gomaa, Diana Freeland, David Spainhour, and Jim Taylor. Analytical support for data management was provided by Kathy Fedan and Barbara Bonnett. Analytical support for laboratory investigation of the antibodies to AMT was provided by Erika Janotka and Toni Bledsoe. Research on sensitization by AMT in humans was done by Paul Siegel and Gary Depree. Research on sensitization by AMT in animal models was done by Kim Klink and Jean Meade. Analytical support for environmental sampling was provided by Ardith A. Grote, Charles E. Neumeister, and James E. Arnold of the Division of Applied Research and Technology (DART). Desktop publishing was performed by Terry Rooney. Review and preparation for printing were performed by Penny Arthur.

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Highlights of the NIOSH Health Hazard Evaluation at ChemDesign Corporation

In December 1999, the Massachusetts Department of Public Health asked NIOSH to 1) investigate the production and handling of chemicals which led to asthma symptoms in six or more employees, 2) identify the causal agents, and 3) disseminate this information to others who may be similarly affected.

What NIOSH Did

- Examined medical records, interviewed employees, administered a questionnaire, and obtained medical histories.
- Conducted medical examinations.
- Conducted a laboratory investigation of sensitization to 3-amino-5-mercapto-1,2,4-triazole (AMT) and DE-498.
- Performed task-based environmental monitoring during production of DE-498.

What NIOSH Found

- Quantifiable concentrations of AMT and DE-498 in personal breathing zone air samples.
- That there is a probable association between exposure to AMT and occupational asthma.

What ChemDesign Corporation Managers Can Do

- Install improved engineering controls at the AMT charge.
- Develop standardized, improved work methods and practices to minimize exposure to AMT during all tasks involving AMT.
- Ensure that prescribed personal protective equipment is used by all workers on all crews.
- Inform workers of respiratory hazards associated with AMT.
- Conduct effective medical screening for early detection and prevention of acute and chronic respiratory effects due to respiratory hazards.
- Educate employees regarding recognition of respiratory symptoms and the importance of early reporting of symptoms.

What the ChemDesign Employees Can Do

- Use proper personal protective equipment and work practices to minimize exposures.
- Report respiratory symptoms and potential causal situations and agents as soon as symptoms are noticed.
- Have symptoms evaluated by a health care provider familiar with occupational health issues.

CDC Workplace Safety and Health

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National Institute for Occupational Safety and Health NIOSH

Health Hazard Evaluation Report 2000-0096-2876
ChemDesign Corporation
Fitchburg, Massachusetts
May 2003

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SUMMARY

On December 13, 1999, the National Institute for Occupational Safety and Health (NIOSH) received a request for Technical Assistance (TA) from the Massachusetts Department of Public Health, Occupational Health Surveillance Program (OHSP). OHSP asked NIOSH to conduct a health hazard evaluation (HHE) at ChemDesign Corporation in Fitchburg, Massachusetts, to investigate a cluster of eight occupational asthma cases which had been reported to OHSP. The chemicals associated with the cases were identified as AMT (3-amino-5-mercapto-1,2,4-triazole) and DE-498 (Flumetsulam). AMT was raw material used in the production of DE-498 and AMT-based product two (AMTBP2).

In response to the request, NIOSH investigators, accompanied by an OHSP industrial hygienist, conducted an initial site visit to ChemDesign on February 1-3, 2000. The NIOSH industrial hygienist returned on June 5-9 to conduct air sampling at four routine operations where employees could be exposed to AMT or DE-498: (1) charging AMT powder into a reactor vessel, (2) discharging DE-498 "wet cake" from a centrifuge, (3) charging DE-498 into the dryer, and (4) discharging DE-498 from the dryer. On July 6-7, the NIOSH Project Officer and medical team visited ChemDesign to recruit workers for participation in a medical survey. On June 12-16, the team conducted a medical evaluation of volunteer production workers. The onsite medical evaluation consisted of a questionnaire interview, lung function testing, and a blood sample collection. A methacholine challenge test was administered at Burbank Hospital in Fitchburg, Massachusetts. In August 2000, NIOSH obtained copies of company medical records for the workers who signed a medical records release form.

Environmental monitoring found quantifiable concentrations of AMT or DE-498 in personal breathing zone (PBZ) air samples during tasks where these materials were manually added to, or discharged from the closed system in Building 16. The greatest potential for exposure to these materials existed during these specific tasks. Although use of respiratory protection and other personal protective equipment (PPE) appeared to provide substantial protection, reports of upper respiratory symptoms by several employees with occupational asthma (OA) indicate that PPE may not provide adequate protection for these individuals. Visible airborne dust during AMT and dryer charges, indicates a need for improved engineering controls (local exhaust ventilation) to reduce the potential for worker exposures. AMT and DE-498 in area air samples collected at the boundaries of restricted areas established during reactor and dryer charging, ranged from below the limit of detection to barely quantifiable levels. Changes in work practices, PPE, and engineering controls during the various production campaigns preclude assessment of the nature and extent of previous exposures to AMT and DE-498.

A total of 41 employees and four former employees participated in the medical survey; the participation rate was 41% in production workers with a potential for AMT exposure. The medical survey identified 12 cases of physician-diagnosed asthma that were diagnosed after the cases started working at ChemDesign. In 11 of these, the onset corresponded with periods when AMT was used in the company. The physician's diagnosis of OA was

mostly made on the basis of the presence of nonspecific bronchial hyperreactivity (NSBH), work-related respiratory symptoms, and in some cases work-related serial peak flow changes. The NSBH occurred after a latency period; allergy to common allergens was not a risk factor for the development of OA in these cases.

Laboratory studies were undertaken to assess whether the respiratory symptoms observed in ChemDesign workers could be due to an allergic response to AMT and DE-498. Studies done on human blood of employees exposed to AMT were not able to clearly demonstrate that AMT or DE-498 exposures were associated with an allergic response to those agents. However, animal studies clearly show that AMT, but not DE-498, is capable of causing an allergic response. The results from the animal studies support the original complaints that AMT caused occupational asthma. However, the possible role of DE-498 cannot be excluded from negative animal studies.

A large percentage of employees reported respiratory symptoms that started during 1998, when two new campaigns using AMT were started in ChemDesign. However, apart from AMT, other agents were reported as causing or making the respiratory symptoms worse. Chronic lung function effects were also found. A high percentage of the participants (18%) had mild airflow obstruction according to the American Thoracic Society (ATS) criteria. The cross-sectional analysis of the lung function measurements done by NIOSH showed significant decrease in Forced Expiratory Volume in one second (FEV1) and in the ratio of FEV1 and Forced Vital Capacity (FVC), FEV1/FVC: this pattern is suggestive of airway obstruction. The data analysis of company yearly lung function data confirmed that the study participants had a higher mean decline in FEV1 and FEV1/FVC with age, than would be expected from the reference equations.

In summary, the results of the medical and laboratory investigation provide evidence that the incidence of OA was associated with exposure to AMT. There was an association between AMT exposure and asthma onset, NSBH associated with AMT exposure improved after withdrawal from AMT exposure, and AMT was found to be a sensitizer in animal studies. The findings show that ChemDesign employees are exposed to agents that can lead to occupational asthma and steeper decline in lung function with age than would be expected. Respiratory symptoms and lung function monitoring currently done at ChemDesign provide an opportunity to utilize the data for the protection of employees' respiratory health. Active workers' participation in the respiratory health protection program should be encouraged.

Investigators examined changes in yearly thyroid hormone (T_4) measurements in relation to working on the AMT campaigns. The possibility that AMT was associated with the decrease in thyroid hormone production could not be ruled out because of insufficient data.

The investigation provides strong evidence that AMT (3-Amino–5-mercapto-1,2,4-triazole) was the causal agent responsible for the cluster of occupational asthma that occurred in ChemDesign. AMT has a potential for causing allergic response in experimental animals. Environmental monitoring found quantifiable concentrations of AMT in personal breathing zone air samples collected during routine production. Study participants had a high frequency of work-related respiratory symptoms whose onset corresponded with the use of AMT. The group of study participants had decreased mean pulmonary function values suggestive of airflow obstruction, identified from cross-sectional and longitudinal data. The findings of this study show that ChemDesign employees are exposed to chemical agents that can lead to occupational asthma and to COPD. Therefore effective exposure controls and a pulmonary function monitoring program need to be implemented and maintained to prevent further occurrence of respiratory disease in the employees.

Keywords: SIC 2879 (Pesticides and Agricultural Chemicals, Not Elsewhere Classified), 3-Amino–5-mercapto-1,2,4-triazole (CAS 16691-43-3), Flumetsulam (CAS 98967-40-9), DE-498, occupational asthma, nonspecific bronchial hyperreactivity, chronic airflow obstruction.

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INTRODUCTION

On December 13, 1999, the National Institute for Occupational Safety and Health (NIOSH) received a request for Technical Assistance (TA) from the Massachusetts Department of Public Health, Occupational Health Surveillance Program (OHSP). OHSP asked NIOSH to conduct a health hazard evaluation (HHE) at ChemDesign Corporation in Fitchburg, Massachusetts, to investigate a cluster of occupational asthma (OA) cases that had been reported to OHSP. The chemicals associated with the cases were said to be DE-498 (later identified as Flumetsulam), AMT (later identified as 3-amino-5-mercapto-1,2,4-triazole) and/or dusty residue from processing or centrifuging the product. AMT was raw material used in the production of DE-498 and another AMT based product (AMTBP2). No additional information about these materials was available.

In response to the request, NIOSH investigators, accompanied by an OHSP industrial hygienist, conducted an initial site visit to ChemDesign on February 1-3, 2000. The site visit consisted of an opening conference, and a walk-through inspection of the building where the occupational asthma cases worked at the time of disease onset (Building 16). Prior to the walk-through inspection, plant management presented an overview of the DE-498 and AMTBP2 production processes. DE-498 was identified as a systemic herbicide. On February 2, 2000, NIOSH investigators conducted confidential, voluntary interviews of Building 16 employees.

The NIOSH industrial hygienist returned on June 5-8, 2000 to conduct air sampling at the operations in Building 16 which appeared to present the greatest risk of exposure to DE-498 and/or AMT. The OHSP industrial hygienist participated in air sampling on June 5-6. On June 6-7, a team consisting of the project officer and two medical doctors visited ChemDesign to recruit workers for participation in a medical survey. On June 12-16, NIOSH investigators conducted a medical evaluation of employee volunteers from Building 16. The onsite medical evaluation consisted of a questionnaire interview, lung function testing, and drawing of blood samples. A methacholine challenge test was administered at Burbank Hospital in Fitchburg, Massachusetts. A total of 45 employees participated in the medical survey. In August 2000, NIOSH obtained copies of company medical records for the workers who participated in the study.

BACKGROUND

Massachusetts is one of three states that receive funding from the NIOSH's Sentinel Event Notification Surveillance for Occupational Risks (SENSOR) Program to conduct surveillance of work-related asthma. As part of this program, OHSP receives reports of persons with occupational asthma from physicians as mandated by Massachusetts Public Law 105 CMR 300. OHSP collects and summarizes this information, and in some cases refers cases to another agency for follow-up. Some cases may be selected for a worksite investigation to identify the conditions that contributed to worker illness, to evaluate the risk to other employees, and to recommend control measures.

In February 1999, a pulmonologist at the University of Massachusetts Memorial Hospital (UMass Memorial) reported OA in an individual who was working at ChemDesign Corporation. The physician noted that at least two other ChemDesign employees were being evaluated for asthma. From January 1998 through December 1999, eight employees had been referred to the pulmonary clinic at UMass Memorial by the company's consulting physician. OHSP learned that the chemicals associated with the cases were AMT and DE-498, which were processed in Building 16.

ChemDesign is a specialty chemical manufacturer. In February 2000, at the time of the first site visit, the company employed 250 employees. There were several production lines located in separate buildings. AMT was used in the production of AMTBP2 in Building 2, in addition to the production of DE-498 in Building 16. Table 1 provides a summary of the production campaigns and their duration. The production of AMTBP2

began in 1991, and was conducted during five campaigns; the production of DE-498 began in 1993, and was conducted during two campaigns.

The most recent DE-498 production campaign ran from November 1998 to July 2000. DE-498 was produced in Building 16 using a batch process in a closed system of reactor vessels. Although reactants, intermediates, and product were contained within the closed system during production, there were release points at the start of the process, where AMT was charged into a reactor, and at the end, where DE-498 was charged into and discharged from a rotary dryer. AMT and DE-498 are white powders which can become airborne, thus creating the possibility of respiratory and dermal exposures.

There is little information in the literature on health effects of AMT, a derivative of 1,2,4-triazole. Of the other 1,2,4-triazole derivatives, aminotriazole (2-amino-1,3,4-triazole) is best documented. Aminotriazole is used as a nonselective systemic herbicide. The International Agency for Research on Cancer (IARC) has designated aminotriazole as a Group 2B carcinogen, i.e., it is possibly carcinogenic to humans.[1] Other listed hazards include dermal irritation, muscle spasms, shortness-of-breath, anorexia, and antithyroid activity.[2] Aminotriazole is a well-known goiterogen that has been shown to lead to thyroid gland enlargement by inhibiting peroxide activity. Aminotriazole also causes histological changes in the thyroid glands.[3] In comparison to aminotriazole, the antithyroid effect of the other triazole derivatives, such as 5-Mercapto-1,2,4-triazole was found to be even stronger.[3] In that study, the physiological markers of hormone activity were 3,5,3'-triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH). Based on these results, the Toxic Substance Control Act (TSCA) Interagency Testing Committee (ITC) added AMT to its Priority Testing List.[4] Currently, ITC is collecting information on all known adverse health effects associated with exposure to AMT. There is also suspicion that changes in thyroid hormone activity can be related to the functioning of the lungs through thyroid hormone receptors in the lungs.

During interviews on February 2, 2000, employees who were diagnosed with occupational asthma reported that they developed severe upper and lower respiratory symptoms while working on the most recent DE-498 campaign. The symptoms developed progressively within 1-6 months after starting the DE-498 production. Employees who developed respiratory symptoms were referred to UMass Memorial. Medical records of seven patients who were diagnosed with OA at UMass Memorial were reviewed by the NIOSH physician. In most cases, the diagnosis of OA was based on a positive methacholine test and work-related changes in asthma-like symptoms. In some employees, serial peak flow tests were conducted which showed apparent work-related changes.

METHODS

Medical

The medical investigation involved a medical survey, and an analysis of annual lung function and thyroid hormone (T_4) data obtained from the company medical records.

Medical survey

The main objectives of the medical survey were to identify employees with adverse respiratory effects due to exposure to potential asthmagenic agent(s), and to evaluate the work-relatedness of respiratory symptoms in employees. We also studied the natural history of physician-diagnosed occupational asthma in the employees.

In total there were 126 current production workers in the plant at the time of the medical evaluation. The employees worked in several production buildings. Thirty-nine people worked in Building 8 (formerly Buildings 1 and 2) where AMTBP2 was produced, and 34 worked in Building 16 where DE-498 was manufactured. Other production areas consisted of the Pilot Plant (3 employees), Research & Development (13 employees), Quality Control (13 employees), Warehouse (5 employees), and Maintenance (19 employees). All production

workers were invited to participate in the study by a personal letter, and in a meeting conducted at ChemDesign one-week prior to the survey. We also invited ex-workers who worked on the most recent DE-498 production campaign.

During the medical survey we administered a questionnaire on respiratory symptoms and exposure history, collected blood samples for allergy tests, and conducted lung function testing. Workers who volunteered to participate in the study signed a consent form for all tests. Information obtained from the interviews and walk-through conducted during the initial visit was used to develop the questionnaire.

Questionnaire. An adapted European Community Respiratory Health Survey questionnaire was used.[5] The questionnaire was designed to establish: (1) the frequency of asthma-like respiratory symptoms since November 1998, (2) the onset of asthma and asthma-like symptoms in relation to starting work at ChemDesign, and in relation to starting work with AMT in the different campaigns, and (3) the history of exposure to AMTBP2 and DE-498, and to other potential respiratory irritants.

Occupational asthma definition was based on the following criteria: 1) occupational asthma diagnosis was made by a pulmonary physician and the disease onset was related to AMT exposure (a cluster of eight cases); or 2) asthma diagnosis was made by a medical doctor and the disease onset was related to AMT exposure, as reported during the survey on a questionnaire. Occupational asthma onset was considered to be related to exposure to AMT if the diagnosis was made during the period when DE-498 or AMTBP2 were being produced at ChemDesign.

Lung function tests. Spirometry testing was conducted to evaluate the presence of obstructive or restrictive lung function impairment in the participants. The spirometry tests included Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1), and the FEV1/FVC ratio. Functional impairment was identified by comparing the individual's measurements with reference values for a normal healthy U.S. male population.[6] For each test we calculated the percent predicted values as a ratio between the observed and predicted lung function values. Standardized differences were calculated as (% predicted-100) and tested for a deviation from zero by a t-test.

We conducted methacholine tests to identify undiagnosed cases with work-related nonspecific bronchial hypereactivity (NSBH), and, secondly, to determine the natural history of NSBH in the previously-diagnosed OA cases. Assessment of NSBH by methacholine test is recommended as an objective test for the diagnosis of OA, and for the assessment of the effect of irritant or chemical agents on NSBH itself.[7,8,9,10] The Evaluation Criteria Section (Appendix I) describes the lung function definitions and methods.

Allergy tests. Because the reported health conditions were suggestive of a sensitization reaction, we collected a blood sample to test whether symptoms were due to an allergic reaction to AMT or DE-498. To learn whether AMT exposure can initiate an IgE mediated respiratory response, or another immunological response, in exposed workers, we conducted an experimental test to detect antibodies to AMT in blood, using radioallergosorbent (RAST) techniques (See Appendix II for laboratory methods.) We also tested for allergy to common allergens, as this may increase the risk of respiratory sensitization from agents in the workplace, and may increase the risk of NSBH.

Personal medical records for OA cases. We obtained medical records from UMass Memorial on seven of the OA cases in the original cluster. From these records we extracted the date of diagnosis of OA, the upper and lower respiratory symptoms reported at the time of diagnosis of OA, the response to allergy questions, lung function results (FVC, FEV1, FEV1/FVC), and a methacholine test result. To establish the natural history of the OA, we compared the results at diagnosis of OA with those from the NIOSH survey, for the six cases who participated in the survey.

Analysis of company medical records

Copies of company medical files were obtained from UMass Memorial for 45 participants who signed the medical records release form. We extracted yearly lung function and thyroid hormone results from the medical files.

Lung function data. First, the quality of lung function data was assessed by a NIOSH pulmonologist according to the American Thoracic Society criteria.[11] The ATS grading was primarily based on reproducibility within the testing sessions for FEV1 and FVC. All tests achieved good end-of-test plateau. The spirometry tests were categorized into three categories: A=exceeds all ATS criteria; B=meets 80% of ATS criteria; and C=meets less than 80% of ATS criteria.

Next, the company and NIOSH lung function tests were combined to determine the decline in lung function with age. In the employees with more than three years of testing, we estimated a slope of decline with age for each employee for FEV1, FVC, and FEV1/FVC by the linear regression model. The mean slopes of decline were calculated as the mean of the individual slopes. Because we do not have an effective unexposed comparison group, we compared the decline in lung function with an expected decline estimated from reference equations. For convenience, we used equations estimated from a cross-sectional study of a general white U.S. male population.[6] The cross-sectional reference equations assume a constant (linear) decline with age. An alternative is to use reference values estimated from longitudinal studies, which assumes non-linear decline with age; i.e., lower decline up to about age 40 and higher decline from 40 years of age. Since many young workers (<40 years of age) had steep decline with age, the longitudinal reference values would show even bigger differences. We evaluated also whether duration of exposure to AMT in the DE-498 or AMTBP2 productions, was associated with steeper decline in lung function.

Thyroid hormone data. The annual measurements for T_4 were analyzed to examine the quality of the data and to determine whether there is a statistically significant short term decrease of thyroid hormone due to current exposure to AMT. Because of the experimental evidence described in the Background section (above), we hypothesized that AMT exposure can lead to decreased T_4 values.

Laboratory Investigation of Sensitization

Human studies. Laboratory studies were undertaken to determine if exposed workers had any serologic evidence of allergic disease. To evaluate whether the symptoms were due to an allergic reaction, the NIOSH Health Effects Laboratory Division (HELD) initiated a project to develop assays to determine if the exposed employees had antibodies to the suspect compound, and to analyze the sera for markers of allergy. In addition, studies were undertaken to develop assays that would detect AMT in serum. Appendix II - Human studies, describes the methods used.

Animal studies. Because little toxicological information is available on either AMT or DE-498, animal studies were conducted to evaluate the sensitization potential of both chemicals. Irritancy was evaluated using a mouse ear-swelling assay, and the potential to induce sensitization was first evaluated using the murine Local Lymph Node Assay (LLNA). The LLNA evaluates the induction phase of sensitization by quantitating DNA replication in the cells of lymph nodes draining the site of dermal exposure to the chemical. Because the assay does not clearly differentiate respiratory from dermal sensitizers, additional endpoints including phenotypic analysis of lymph node cells, evaluation of serum IgE levels, and cytokine production by draining lymphocytes were evaluated to better understand the mechanism of sensitization. Appendix II - Animal studies, describes the methods.

Industrial Hygiene

On June 5-8, 2000, personal and area air samples were collected to characterize chemical operator exposures to AMT and DE-498 during the production of DE-498. Personal breathing zone (PBZ) samples were collected during specific tasks in Building 16, where AMT and/or DE-498 were

charged into or discharged from chemical processing vessels or apparatus. Area samples were collected at the boundary of restricted areas which were established by the company around AMT charging and DE-498 discharging operations. During discharge of the rotary dyer, where no restricted area was established, area samples were collected in the vicinity of the operation.

Each sample was collected on a 37-millimeter (mm) polytetrafluoroethylene (PTFE) filter (0.2 micrometer [µm] pore size) using a calibrated, battery-operated sampling pump to draw air through the filter at a nominal flow rate of 2.0 liters per minute (lpm). Air sampling pumps were calibrated with an in-line PTFE filter before and after each monitoring period. Field blanks were collected and submitted to the laboratory for each sampled task. Quantitative sample concentrations were calculated based on the actual monitoring time (time-weighted average [TWA-actual] concentrations).

Analysis was performed according to a high-pressure liquid chromatography (HPLC) procedure developed by the NIOSH Division of Applied Research and Technology (DART). AMT/DE-498 was extracted from each filter with 4 milliliters (mL) of 1:1 acetonitrile/water solution. Separation was achieved using a C18 column at 1 mL/minute with a mobile phase of 75% di-butyl amine phosphate buffer and 25% acetonitrile. AMT was identified by monitoring the eluent at a retention time of 1.6 minutes using a UV detector set at 253 nanometers (nm). DE-498 was identified at a retention time of 6.5 minutes using a UV detector set at 215 nm. Quantitation was achieved by comparing sample responses to calibration curves established using standards prepared from 95% pure commercial AMT, or 99% pure commercial DE-498, dissolved in 1:1 acetonitrile/water.

RESULTS

Medical

Medical survey

Forty-one employees and four former employees participated in the medical survey. In Table 1, column 'Number exposed' shows the number of participants who reported exposure during production campaigns in which AMT was used. For example, two of the 45 participants worked on the AMTBP2 campaign from January 1991 to December 1991, and their mean exposure duration was eight months. The column for 'Cumulative number exposed' shows that 42 participants were exposed to AMT in any of the campaigns, most of them since June 1998. Thus, we do not have an effective unexposed comparison group. The median duration of exposure corresponds closely with the duration of the campaigns.

Of the 41 current workers, 38 were from the buildings where AMTBP2 and DE-498 were produced (in total 39 and 34 worked in those buildings), or they were maintenance workers (in total there were 19 maintenance workers). Participation was mainly from workplaces where exposure to AMT occurred; thus 92 (current production workers) was an appropriate denominator. Although we invited ex-workers and workers from other areas to participate, only three participants from the Quality Control section, and four ex-workers participated in the study.

Frequency of respiratory symptoms and respiratory conditions. Table 2 shows the frequency of reported respiratory symptoms that occurred since November 1998 (wheezing, chest tightness, shortness of breath, cough, and other upper respiratory symptoms) for the 45 participants. The table also shows how many participants developed the symptoms (a) after starting to work at the company, and (b) after starting to work on the campaigns where AMT was used, and whether the symptoms were still present at the time of the survey. There were 19 participants who reported wheezing or whistling in their chest at any time since November 1998. In 16 of these participants, wheezing began after they started to work at the company. In 15 of these, wheezing started around the time of the beginning of the campaigns listed in Table 1. On average, wheezing started 5.3 months after participants started working with AMT. In 9 of the

15 participants, wheezing was better on days away from work; in 13 participants wheezing still persisted at the time of the survey. Similar statistics are shown in Table 2 for chest tightness, shortness of breath, cough, and other symptoms.

There were 27 participants who developed any of the upper respiratory symptoms or eyes irritation listed in Table 2 after they started working on the campaigns where AMT was used. On average it took 13.5 months before the symptoms developed. Most participants who developed AMT associated respiratory symptoms still had some of those symptoms during the survey.

Table 3 lists campaigns, tasks, and specific materials reported by the participants as causing or exacerbating respiratory symptoms. Thus, there were other potential respiratory irritants apart from AMT and DE-498.

Occupational asthma. In total we identified 12 cases of physician-diagnosed work-related asthma. Of these, 11 were occupational asthma cases whose diagnosis was related to AMT exposure. Eight of the 11 cases came from the original cluster of OA cases that were diagnosed by the UMass Memorial and reported to OHSP. Three OA cases were newly identified by the questionnaire survey. During the medical survey, 10 participants reported ever having physician-diagnosed asthma (Table 4). Of these 10, one case had a childhood onset of asthma and in nine the diagnosis of asthma was made after starting to work at ChemDesign. In eight cases, the diagnosis was AMT-related (five were from the original cluster of eight and three were newly identified cases). On average, the onset occurred 10 months after they started working with AMT. The original cluster of eight cases developed the OA after November 1998 on average within 5 months. Thus, the questionnaire survey identified three new cases not included in the original cluster, making the total number of OA cases related to AMT exposure to be eleven.

For one OA case, not included in the original cluster, the medical records indicated that on June 18, 1998, a diagnosis of asthmatic bronchitis was made. According to the medical record, the employee had a runny nose, chest congestion, shortness of breath with worsening symptoms, and was feeling feverish for several weeks. The employee had bilateral wheezes, and was febrile at medical examination. Peak flow rates were around 150 milliliters per second (ml/sec); chest x-ray was clear. Treatment helped, but he still continued to have occasional wheezing and did not feel quite normal. Wheezing responded promptly to an inhaler. The patient stated that despite wearing protective gear, the symptoms got worse during the three days of shift work while working with AMT, and seemed to get better when away from work.

At the time of the survey, all six OA cases for whom we had medical records from UMass Memorial, and who participated in the survey, reported still having some upper respiratory symptoms, especially irritation in the nose, and stuffy and runny nose. Five of the six OA cases had NSBH present at diagnosis, but at the time of the survey only one employee still had NSBH present, and four participants no longer had NSBH. Only two participants with the diagnosis of OA had a positive allergy test for common allergens, and one of these participants remained positive for the methacholine test. Thus, in most participants, the NSBH associated with AMT exposure improved after removal from exposure. The NSBH was not associated with an allergy to common allergens.

There were five cases with NSBH identified during the survey. Of these, one was diagnosed with OA by the UMass Memorial, but we could not obtain the medical records. This OA case had a positive allergy test. Thus, in total there were two OA cases with NSBH present at the time of the survey. The three not diagnosed with OA had a positive allergy test for common allergens, but none reported work-related symptoms; suggesting that their NSBH may not be work-related.

Lung function results. Of the 45 participants, 43 had lung function tests done by NIOSH. All the participants were males, and the average age of the group was 42.2 years (S.E., 0.11). Table 5 shows the means for the observed and predicted values for FVC, FEV1, and the FEV1/FVC ratio, and the

standardized differences (% predicted-100). The t-values and P-values in Table 5 show that the % predicted-100 values for FEV1 and the FEV1/FVC ratio are significantly different from zero. This indicates an increase in airflow obstruction in this group of participants. On evaluation of individual spirometry curves according to ATS criteria, there were eight participants with mild airflow obstruction (18%), and one employee with restriction (2%). There was no association between lung function values and duration of exposure to AMT.

Analysis of company medical records

Pulmonary function data. There were 34 participants who had three or more years of testing and for whom we could estimate the slope of decline of lung function with age. Assessment of lung function data quality showed that in 15 participants the test quality exceeded the ATS criteria, in 16 participants the tests met 80% of the ATS criteria, and in 3 participants the tests met less than 80% of the ATS criteria. On visual inspection of the individual data points plotted against age, we established that the NIOSH data points, which were the last points on the regression line, were mostly in line with the company data. The average differences between the company lung function values obtained in 1999 and the NIOSH values obtained in June 2000 for 26 participants were -20 ml (S.D. 35) for FEV1, -8 ml (S.D. 46) for FVC, and 2.4% (S.D. 3.32) for FEV1/FVC. These differences are within the limits of expected annual decline. Thus, according to our evaluation, the company lung function data are of a good quality and suitable for estimating the slope of decline of lung function with age.

Table 6 shows the frequency distribution for the number of periodical tests, including the NIOSH tests, available for individual participants. Table 7 shows the years of follow-up and number of tests by the year of the follow-up, and the average current age of the participants.

According to the cross-sectional reference equations of Knudson *et al*,[6] the general population estimates of the slopes of decline with age are -29 ml/yr for FEV1, -30 ml/yr for FVC, and -1.1 %/yr for FEV1/FVC, starting from about 25 years of age. These estimates represent annual rates of decline in lung function. In the 34 participants with three or more years of testing, the mean annual rates of decline were -45 ml/yr (S.E., 7) for FEV1, -30 ml/yr (S.E., 8) for FVC and -4.4 %/yr (S.E., 0.7) for the FEV1/FVC ratio. Figures 1, 2, and 3 show the predicted curves for decline in lung function with age, based on the observed average slope of decline calculated on the 34 participants. Superimposed are the Knudson's prediction curves. The observed curves have steeper decline for FEV1 and for FEV1/FVC, but not for FVC. The dotted lines are the predicted lower 95% confidence intervals for Knudson's curves.

Because age may be associated with an increased decline in lung function, the average rates of decline in ml/yr, according to four categories of age when the first lung function test was done, are shown in Table 8. The results show that the decline is higher then expected across all age categories for FEV1 and FEV1/FVC, but not for FVC. Thus, the group of employees that we investigated had an accelerated decline in FEV1 and FEV1/FVC, suggesting an increase in an obstructive type of lung function decline across all age groups. These results are in agreement with those from the cross-sectional data analysis in which we used the NIOSH measurements only and support increased occurrence of chronic airflow obstruction in the study participants.

Because of a small sample size and a lack of a comparison group, we could not effectively estimate associations between the slope of decline of lung function and the AMT exposure or personal factors. The limited analysis did not show an association with duration of AMT exposure.

Thyroid hormone data. There were 37 participants for whom we had yearly thyroid hormone data for years 1988 through 1999. To evaluate the effect of AMT exposure on yearly T_4 values, we divided the tests for each employee into: (1) 'Unexposed tests', i.e., tests done during the period when the employee was not exposed to AMT, and (2) 'Exposed tests', i.e, tests done during the period when the employee worked on an AMT campaign (see Table 1). Table

9 shows the number of tests, N, the mean T_4 value in micrograms per deciliter (µg/dl), and the standard deviation (S.D.) for each year and the two types of tests. There were no statistically significant differences between the exposed and unexposed tests within the years where sufficient number of tests allowed comparison (1995, 1998, 1999). Note that most of the exposed tests occurred in 1999, i.e., during the last DE-498 campaign.

To take into account the variability in T_4 within individual participants, we also calculated the difference between the means for exposed and unexposed tests for each employee. We then tested whether the mean difference between the exposed and unexposed tests, calculated over all the participants, was statistically different from zero. There were 26 participants who had both types of tests, i.e., exposed and unexposed. The mean T_4 values were 7.46 µg/dl for the unexposed tests and 6.96 µg/dl for the exposed tests. The mean difference between exposed and unexposed tests was 0.50 µg/dl (S.D. 0.12); this value was statistically significant from zero ($t=-4.1$; $p<0.001$). This analysis indicates that tests taken while participants were exposed to AMT had significantly lower values. There were, however, two factors that could have contributed to this difference; these were age and time trend in the T_4 measurement. We noted that in 1997 a new laboratory started doing the T_4 tests. To take into account the potential effect of age and laboratory, we used the multivariate analysis of covariance (Mixed Effect Models) to test the effect of exposure to AMT. After adjustment for age, the AMT exposure was still associated with a statistically significant decrease in T_4 of 0.39 µg/dl ($t=-3.4$; $p<0.02$). However, after the variable LABORATORY, that represented the two laboratories that conducted the testing, was included in the model, the effect of AMT exposure was no longer statistically significant, but there was still a decrease of 0.28 µg/dl in T_4 ($p=0.14$). Unfortunately, the change in the laboratory also coincided with the high AMT exposure episodes from 1998-2000 that were associated with the occurrence of occupational asthma cases. To resolve this issue, we also included an interaction between variables for the LABORATORY and the AMT exposure in the model (both variables had a value of 0 or 1) and this was not statistically significant ($p=0.61$), suggesting that the difference was independent of AMT exposure.

Laboratory Investigation of Sensitization

Human studies. Of the 44 participants tested for allergy to common allergens, 16 had a positive test. The test for sensitization to AMT did not show an association with having been previously exposed to AMT or duration of exposure. However, the methods are still being developed and the current results should be regarded as inconclusive.

Animal studies. Using the irritancy assay, no signs of systemic toxicity (as measured by body weight gain) or irritancy (as measured by ear swelling) were observed following exposure at concentrations up to 25% AMT and 40% DE-498 (the limits of solubility for each chemical). DE-498 was negative in the LLNA at concentrations up to 40% (Appendix II, Fig. 1A) indicating a lack of sensitization potential. However, a dose dependent increase in lymphocyte proliferation was observed following exposure to AMT, reaching a three-fold increase over control, indicating a positive response, at a concentration of 25% (Appendix II, Fig. 1B). The results of the phenotypic analysis assay supported the findings of the LLNA in that exposure to AMT induced a significant increase in the percent of B220+ cells at all concentrations of AMT tested. Additionally the 25% dose group exhibited a significant increase in IgE+/B220+ cells (Appendix II, Fig. 2) indicating that AMT may induce the production of IgE. The mouse ear swelling test (MEST) was used to further evaluate the potential for AMT to induce contact sensitization. This assay was negative following induction at concentrations up to 25% and challenge with 25% AMT. To further evaluate the mechanisms of sensitization following AMT exposure, a time course study for the induction of IgE was conducted. Levels of IgE were found to increase in animals dermally exposed 5 days a week for up to 77 days (Appendix II, Fig. 3). Preliminary evaluation of the cytokine mRNA expression profile

of lymphocytes from AMT-exposed animals demonstrated an up-regulation of interleukins 2,4,5, 9,10,13,and 15. This cytokine expression pattern is consistent with the induction of a TH2 response favoring IgE production, and includes cytokines that influence mast cell growth and differentiation.

Industrial Hygiene

Observations. Four routine operations where employees could be exposed to airborne concentrations of AMT or DE-498 were observed: (1) charging AMT powder into a reactor vessel, (2) discharging DE-498 "wet cake" from a centrifuge, (3) charging DE-498 into the dryer, and (4) discharging DE-498 from the dryer. While performing these tasks, operators wore disposable coveralls, disposable boot covers, rubber gloves (taped at the wrist), and respiratory protection (described below).

The AMT charge consisted of charging AMT into a reactor from a flexible textile tote container ("Supersac"). One or two operators, wearing personal protective equipment (PPE), used an electric hoist to suspend the tote above the open charging port (manway) of the reactor. An operator placed the end of the tote in the manway and opened the tote to release the AMT into the reactor. The operator(s) shook, pushed, and otherwise manipulated the tote to empty AMT from the Supersac into the reactor. After the tote was empty, the operator tied-off the open end of the tote, and removed it from the manway. The operator used a hose to rinse AMT from the reactor and surrounding area, and then sealed the manway. The empty tote was then inserted into a 55-gallon drum prior to removal of PPE by the operator(s). Disposable PPE was also placed in the drum. The respirator was the last PPE item to be removed at the conclusion of this task. With the exception of one operator who wore a full-facepiece air-purifying respirator (FF APR), operators wore FF supplied-air respirators (FF SARs) or supplied-air (SA) hoods while charging AMT into the reactor. Local exhaust ventilation (LEV) at the AMT charge was provided by a length of flexible duct (with no exhaust hood) which the operator placed near the manway. Despite the use of the flexible exhaust duct, airborne dust was visible during two of the three AMT charges that were observed during the survey.

DE-498 was discharged ("dropped") from a centrifuge located on the ground floor. This task involved plowdown of the centrifuge, discharge of the product into a Supersac (in a bin) beneath the centrifuge, tying-off and weighing the filled sac, placing an empty Supersac in a bin on a pallet, hosing down the area, and moving the palletized bin and sac beneath the centrifuge to receive the next batch. Disposable coveralls, boot covers, gloves, and a FF APR were worn by the operator during this task.

After being discharged from the centrifuge, DE-498 was brought upstairs where it was charged into the dryer. At the dryer, the operator used a hoist to suspend several Supersacs (one at a time) above the charging port where the operator opened the sac into the port. Pneumatic "paddles" were used to compress the opening of each sac to help unload the product (DE-498) into the dryer. The paddles were a relatively new addition to the dryer, being added sometime in mid-1999. A small hood attached to a flexible duct was suspended near the charging port to serve as local exhaust ventilation. While unloading a Supersac, an operator would sometimes push on the sides of the Supersac to facilitate the flow of product into the dryer. When empty, the operator manually compressed the sac, and tied the opening before removing it from the charging port. Disposable coveralls, boot covers, gloves, and a FF SAR or SA hood was worn by each operator who charged the dryer.

The dryer was discharged through a tube which ran from the bottom of the dryer into a Supersac on the lower level of the building. The Supersac was attached to the tube, thus creating an enclosed discharge system. An auger in the tube propelled the DE-498 from the dryer, through the tube, and into the Supersac. On the two occasions when dryer discharge was observed, the operator wore a SA hood or FF SAR, coveralls, and latex gloves while replacing the full Supersac and taking samples.

(Note: one of these operators had initially donned an APR. The operator changed to an SAR upon instruction by a management representative who was present at the time.) No respiratory protection or coveralls were worn at other times during this operation. The operator is free to move throughout the area while the dryer is being discharged. Upon completion, the filled Supersacs are weighed and taken to the warehouse. There were no visible emissions during dryer discharge. It appeared that exposure could be expected to be low, with the possible exception of an unusual event which might cause dust to be released.

Air sampling. The results of air samples collected during specific tasks are shown in Table 10. The reported values are TWA concentrations for the duration of each monitoring period. Each sample was analyzed for AMT and DE-498 regardless of task.

DE-498 was not detected in samples collected during the AMT charge; similarly, AMT was not detected in samples collected during centrifuge or dryer tasks. Quantifiable concentrations of AMT or DE-498 were detected in all personal breathing zones (PBZ) samples. AMT and DE-498 in area samples collected at the boundries of restricted areas ranged from below the limit of detection to barely quantifiable levels; thus, it appears that concentrations in these locations are likely to be very low under routine conditions.

AMT concentrations measured in four PBZ samples ranged from 1.5 to 5.6 milligrams per cubic meter (mg/m^3) during the 8 to 13 minute sampling periods. Three PBZ samples collected at the dryer charge found DE-498 concentrations of 0.37 to 5.8 mg/m^3 during 28 to 41 minute sampling periods (5.8 mg/m^3 was measured during the 28 minute period). At the dryer discharge, DE-498 concentrations of 0.011 mg/m^3 and 0.14 mg/m^3 were measured during sampling periods of 286 and 170 minutes respectively. A PBZ sample collected during centrifuge discharge measured 0.56 mg/m^3 of DE-498 during an 18 minute period.

DISCUSSION

Medical

One of the main objectives of the medical investigation was to identify the agent that caused the cluster of OA in the ChemDesign plant. To demonstrate the causative association, we first present the American College of Chest Physician (ACCP) definition of occupational asthma[8] as this provided a basis for making our decision.

According to ACCP occupational asthma is defined as a disease characterized by variable airflow limitation and/or bronchial hyperresponsiveness due to causes and conditions attributable to a particular working environment, and not to stimuli encountered outside the workplace. Two types of asthma are distinguished according to whether they appear after a latency period: (a) asthma with latency – encompasses all instances of immunological asthma for which an immunological mechanism has been identified; and (b) asthma without a latency period – irritant induced asthma such as reactive airways dysfunction syndrome (RADS).[8] American College of Chest Physicians (ACCP) definition of OA requires a physician diagnosis of asthma, an onset of asthma after entering the workplace, association between symptoms of asthma and work, and at least one of the four findings: (1) workplace exposure to an agent known to give rise to OA; or (2) work-related changes in FEV1 or peak expiratory flow; or (3) work-related changes in airways responsiveness as measured by nonspecific inhalation challenge; or (4) positive response to inhalation provocation testing with an agent to which the patient is exposed at work; or (5) an onset of asthma with a clear association with a symptomatic exposure to an inhaled irritant agent in the workplace.[8]

In total, we identified 11 cases of occupational asthma related to exposure to AMT. In addition to the eight cases of OA reported in the original cluster, the medical survey identified three other cases of asthma related to exposure to AMT. All the 11 cases

had physician-diagnosed asthma. In the cluster of eight OA cases, the physician diagnosis of occupational asthma was made on the basis of the presence of NSBH; the presence of work-related respiratory symptoms; or work-related serial peak flow tests in some employees. In these cases the onset of asthma was associated with starting to work on the DE-498 production campaign. The disease onset was after a latency period of 1 to 9 months from first exposure to AMT. Allergy to common allergens was not a risk factor for the development of OA in these cases. A further proof of work-relatedness of the NSBH is that in most of the cases for whom we had medical records and survey methacholine test, the NSBH was no longer present during the survey. Most of the OA cases had been removed from the exposure to AMT after they were diagnosed with OA.

Occupational asthma with latency is usually associated with immunological sensitization to the agent. The results from tests of sensitization to AMT and DE-498 done on human blood of employees exposed to AMT did not show conclusively that respiratory symptoms associated with AMT or DE-498 exposure were associated with IgE mediated sensitization (see Appendix II). However, the laboratory findings on animals show that AMT, but not DE-498, is capable of causing IgE sensitization (i.e., an allergic response).

Apart from the cases of OA, other employees were also affected by the exposure to AMT. Respiratory symptoms were highly prevalent in this group of workers. Results from the respiratory symptom questionnaire show that 42% reported having wheezing, 18% chest tightness, 22% shortness of breath, and 22% reported cough, at any time since November 1998. In a large percentage of the participants, symptoms were reported to have started after starting to work at ChemDesign, or after the onset of AMT usage in the company in 1998. The symptoms were reported to persist at the time of the survey.

To rule out the possibility that the production of materials other than DE-498 or AMTBP2 might be associated with OA, NIOSH investigators questioned management and employees to determine if onset or occurrence of OA coincided with other production processes. NIOSH investigators were told that, while other products were being manufactured in Building 16 during the time of the DE-498 campaigns, the startup and conclusion of other product campaigns did not correspond to the onset of asthma-like respiratory symptoms.

In summary, the evidence on AMT being the causative agent for OA is as follows. There was strong suspicion among the OA cases diagnosed at UMass Memorial that AMT caused their disease. In most cases the onset of OA corresponded with starting to work with AMT. The NSBH found at diagnosis of OA improved after removal from exposure to AMT. Some OA cases reported that their serial peak expiratory flow measurements showed decline when they worked on the DE-498 campaign. The results of the medical survey show high prevalence of respiratory symptoms with onset which was related to AMT exposure. The results of the immunological investigation show that AMT has a potential to cause immunological sensitization in animals, and thus support the hypothesis that AMT is capable of causing OA with latency due to immunological mechanism. Although there is little information on AMT in the literature, adverse health effects attributed to another 1,2,4- triazole derivative, the herbicide 3-Amino-1,2,4-triazole also known as aminotriazole, such as dermal irritation, muscle spasms, shortness of breath, anorexia, antithyroid activity, and possible carcinogenicity, are not inconsistent with the above findings.

To evaluate the respiratory health of ChemDesign employees, we evaluated also their pulmonary function in a cross-sectional study using NIOSH measurements, and in a longitudinal study using both NIOSH and the company's yearly measurements. Results of both of these studies show that the participants had increased loss of lung function consistent with airflow obstruction. In the cross-sectional study, eight out of 45 (18%) had mild airflow obstruction according to ATS criteria. The average age of those with airflow obstruction was 48 years. The cross-sectional data analysis also showed that the group had significantly lower FEV1 and the

FEV1/FVC ratio, but not FVC, in comparison with reference values. In the longitudinal data analysis we found that the group as a whole had a steeper decline in FEV1 and the FEV1/FVC ratio with age, but not in FVC. The lack of association between AMT exposure and loss of pulmonary function may be due to the fact that the number of employees tested was small and almost all employees were exposed to AMT.

The findings of the medical investigation show that ChemDesign employees are exposed to chemical agents that can lead to occupational asthma and to chronic obstructive pulmonary disease (COPD). Thus, an effective pulmonary function monitoring program needs to be implemented to prevent further occurrence of respiratory disease in the workers. ChemDesign employees have annual pulmonary function tests which appear to be of good quality. However, it is our impression from talking to the workers, that the results of lung function testing are not used effectively to increase workers' awareness of their respiratory health, and for disease prevention. In general, monitoring of pulmonary function and respiratory symptoms in workers exposed to respiratory hazards could play an important role in the prevention of respiratory disease from occupational causes as well as from tobacco smoking.[12,13] Monitoring of respiratory health by a short questionnaire and lung function testing can be useful for the following purposes: (1) detection of cases with work-related respiratory effects, (2) identification of respiratory hazards by examining groups of workers with specific exposures, (3) health promotion by fostering workers' participation in their respiratory health through smoking cessation and workplace risk awareness, and (4) pre-placement testing (there are however ethical and legal issues to be considered when this is implemented).[13] Interpretation of lung function tests can be facilitated by plotting individual worker's test results on a chart kept in the employee's medical file and by discussing the chart with the employee. At least five years of testing is required to establish the average annual rate of decline in individual employees with some reliability. However, discussing unusual pulmonary function decline with a worker can help to make the worker aware of the effect of hazardous exposures, as well as tobacco smoking, on pulmonary function. Many studies have shown that tobacco smoking can potentiate the effect of workplace exposure on pulmonary function decline. Evaluating the yearly rates of decline in groups of workers known to be exposed to a potentially hazardous agent can also help to identify hazardous conditions. Pre- and- post shift changes in lung function can also provide an indication on the effect of working with potentially dangerous agents.

A potential adverse health effect associated with exposure to AMT is a decrease in thyroid hormone production. Animal studies showed that most of the derivatives of 1,2,4-triazole have an depressing effect on thyroid hormone through antiperoxidase action.[3] To assess the possibility of thyroid effects, we obtained the company annual medical records for thyroid hormone (T_4) measurements to examine changes in T_4 in relation to working on the AMT campaigns. The yearly data on T_4 for 37 employees show a decrease in the mean T_4 values during the years 1997-1999. However, after we adjusted for the years when a new laboratory started doing the tests (1997-1999), the effect of AMT exposure was no longer statistically significant, but there was still a decrease of 0.28 μg/dl ($p=0.14$) in the exposed tests. The possibility that AMT was associated with some decrease in T_4 could not be completely ruled out.

Industrial Hygiene

Environmental monitoring during this investigation found quantifiable concentrations of AMT or DE-498 in breathing zone samples during tasks where AMT or DE-498 were manually added to, or discharged from the closed system of reactor vessels in Building 16. The greatest potential for exposure to the agents existed during these specific tasks. Although use of supplied-air respiratory protection, full-body coveralls, and gloves appeared to provide substantial protection from most airborne dust, reports of upper respiratory symptoms by several employees with OA may indicate that PPE did not afford adequate protection for these individuals.

PBZ and area sampling during this HHE provide an estimate of worker exposure while performing selected tasks under current conditions; however, changes in methods and equipment at AMT and dryer charge operations may have resulted in corresponding changes in airborne concentrations and potential worker exposures. For example, AMT charges have been performed in various ways, including hoisting the Supersacs above the manway, shoveling AMT from Supersacs into the reactor, and charging from fiber drums (Building 2). Several employees reported that some workers did not use respiratory protection during the initial months of a previous DE-498 production campaign (March 1993 - June 1995) because the material safety data sheets (MSDSs) for AMT and DE-498 did not indicate that the use of respiratory protective equipment was required. One employee stated that previous batch sheets, which specified PPE requirements at each point of the process, did not require or mention the use of respiratory protection; however, current batch sheets state that disciplinary action will be taken if respiratory protection and other PPE are not used. Thus, it is apparent that: (1) the PPE practices which were observed during this investigation were instituted at some point after unprotected exposures to these materials had occurred, and (2) the results of samples collected during this HHE should not be regarded as representative of worker exposures under previous workplace conditions.

Exposures during quality control procedures, housekeeping, spill cleanup, and warehousing were not monitored or observed during this evaluation. It is likely that the workers who performed these tasks were exposed to undetermined airborne concentrations of various powdered materials, including AMT and/or DE-498. Furthermore, there is evidence that at least some work practices have changed since the start of the most recent campaign. For example, the corporate industrial hygiene report contained a recommendation that surface contamination be removed from work areas with a HEPA vacuum (reportedly the current practice) rather than by sweeping with brooms.

As noted above, airborne dust was visible during two of the three AMT charges observed during the industrial hygiene investigation. The release of airborne dust indicated that the flexible exhaust duct, which was placed near the charging port, did not provide effective control of potential exposures to AMT. An unhooded circular duct, as used during the AMT charge, has a capture velocity at a point one duct diameter from the duct opening that is approximately 7% of the velocity at the face of the duct.[14] Although the capture velocity could not be measured during this HHE, the release of visible airborne dust served as an indication of inadequate capture velocity at short distances from the duct. Thus, AMT that was released beyond the immediate vicinity of the duct opening was likely to escape into the workplace. This situation could be corrected through the installation and use of a properly designed LEV system at reactors or other equipment where powdered materials are charged. Information on the design of local exhaust ventilation systems can be found in the American Conference of Governmental Industrial Hygienists (ACGIH) publication, Industrial Ventilation: A Manual of Recommended Practice, 24th edition.

During observed dryer charges, pneumatic paddles and LEV did not eliminate potential exposure to DE-498. The potential for exposure to DE-498 was made apparent in the form of visible airborne dust when a full sac was hoisted (June 5, 2000), and when an operator pushed on the side of a sac during dryer charge (June 7, 2000). It should noted that a horizontal section of the flexible LEV ductwork above the charging station sagged a bit, which could result in an accumulation of DE-498 in the duct if an adequate duct velocity is not maintained. Where feasible, the flexible duct should be replaced with metal ductwork, and a minimum duct velocity should be maintained at approximately 3000 feet per minute.[14] In addition, the capture velocity could be increased by placing a flanged hood on a flat work surface at the charging port, and drawing air along the work surface. This would decrease the airflow area by approximately 50%, and would result in an increased capture velocity.[14] Although improvements in ventilation efficiency would help reduce the likelihood of exposure to DE-498 at the charging port, LEV would not substantially reduce potential exposure to DE-498 particulate during tasks

beyond the immediate area of the duct opening, e.g., surface contamination released from Supersacs and pallets.

Even though "wet cake" at the centrifuge might be expected to be relatively dust-free due its damp condition, white dust was visible in the empty bins, on horizontal and vertical surfaces beneath the centrifuge, and on the shovel and scraper which are used to help unload product. A layer of white dust, approximately 1/8- 1/4 inch thick, was observed in the bottom of one of the bins. This coating of dust could become airborne when disturbed, thus serving as an additional source of exposure to DE-498. Also, since the Supersacs are reused repeatedly for this part of the operation, dust is likely to be released from the Supersacs when they are handled and placed in the bins. Greater attention to housekeeping in this area could reduce the potential for generating airborne dust while working around the centrifuge, as well as other locations where powdered materials may accumulate.

During dryer discharge, DE-498 was contained within an enclosed system, except for brief periods when the operator obtained a quality control (QC) sample and tied the neck of each Supersac. The potential for exposure to DE-498 appeared to be lower during routine discharge of the dryer than during the other operations which were observed during this HHE. Nevertheless, the potential for exposure still exists, as was observed when a worker used his gloved hand to brush powder (presumably DE-498) from a sheet of cardboard on a pallet near the dryer discharge.

CONCLUSIONS

A. The results of the medical and laboratory investigation show that the incidence of occupational asthma was probably associated with exposure to AMT.
   The supporting evidence includes:
   (i) A strong association between the AMT exposure pattern and the diagnosis of OA.
   (ii) Employees who developed occupational asthma while working with AMT had a positive NSBH and work-related respiratory symptoms. In some employees work-related serial peak flow changes were observed while they worked on the DE-498 production campaign.
   (iii) Though AMT was not yet found to be associated with sensitization in human blood, it was found to be a sensitizer in animal studies. The laboratory assay for human sensitization is still being developed.

B. In most employees who were diagnosed with occupational asthma, the NSBH was present at diagnosis. NSBH was no longer present at the time of the NIOSH survey in most of the cases. Removal from exposure lessened NSBH in previously exposed individuals. This is a further proof that the asthma was caused by workplace exposure. The presence of NSBH was not associated with allergic sensitization to common allergens.

C. In comparison with the general population of white US males, the participants had significantly decreased lung function suggestive of airflow obstruction and a higher rate of decline of FEV1 and FEV1/FVC, but not the FVC ratio.

D. Though the possibility that AMT was associated with a decrease in thyroid hormone (T_4) could not be completely ruled out because of insufficient data.

E. AMT or DE-498 were released into workers' breathing zones during tasks where AMT or DE-498 were manually added to, or discharged from the closed system of reactor vessels. Although use of respiratory protection and other PPE appeared to provide substantial protection, reports of upper respiratory symptoms by several employees with OA indicate that PPE may not afford adequate protection for these individuals.

F. Improved engineering controls (local exhaust ventilation) should be installed to reduce the potential for future worker exposures. Changes in work practices, PPE, and engineering controls

preclude NIOSH from inferring the extent of previous exposures from data collected during this evaluation. Although the significance of occupational exposures is unclear from an industrial hygiene perspective, it is clear that AMT and DE-498 were released into the workplace during the production of DE-4989, and presumably AMTBP2.

RECOMMENDATIONS

The following recommendations are based on observations and findings during this survey, laboratory studies of the sensitization potential of AMT and DE-498, and a review of the current scientific literature. These recommendations are intended to reduce the potential for occupational asthma among chemical operators who work with AMT.

1. A medical monitoring program should be designed for early detection and prevention of the acute and chronic effects of exposure to potentially hazardous chemicals. Pre- and post-shift peak expiratory flow (PEFR) testing should be conducted to evaluate acute changes in lung function. PEFR testing can provide an indication of adverse effects resulting from working with potentially dangerous agents.

   Management should provide specific education and information to the employees regarding recognition of respiratory symptoms and the importance of early reporting of symptoms. Workers reporting wheezing, chest tightness, shortness of breath, cough, and other upper respiratory symptoms should be identified as soon as possible through self-referral. They should be evaluated in a timely manner by a health care provider familiar with occupational health issues, particularly respiratory conditions. We recommend that an occupational, medical, and smoking history be taken during the initial visit.

   The annual lung function testing (spirometry tests [FEV1 and FVC]) should be utilized for disease prevention. Test results should be plotted on a personal chart to identify any unusual decrease in lung function. All results, especially those showing decreased lung function, should be discussed with tested employee(s).

2. Workers should be informed of the nature of health hazards which appear to be associated with airborne, and possibly dermal exposure to AMT. Employees should be made aware that AMT is a likely asthmagen.

3. Engineering controls such as closed systems and local exhaust ventilation should be the principal method for minimizing exposure to chemical agents in this workplace. A well-designed exhaust ventilation hood should be installed to capture AMT particulates released during AMT charge. Information on the design of local exhaust ventilation systems can be found in the American Conference of Governmental Industrial Hygienists (ACGIH) publication, Industrial Ventilation: A Manual of Recommended Practice, 24th edition.

4. Housekeeping should be improved at the centrifuge where white dust, presumably from product, was visible in bins, on surfaces, and on hand tools. Wet methods, or a HEPA-filtered vacuum cleaner should be used in all areas, where reactants, intermediates, or products accumulate on surfaces or equipment.

5. On two occasions during the NIOSH industrial hygiene investigation, employees used, or attempted to use, air-purifying respirators to perform activities for which supplied-air respiratory protection was specified by the company. Management needs to ensure that all required PPE is used properly on all shifts by all crews. In light of evidence indicating that AMT may be an asthmagen, the importance of using of supplied-air respiratory protection should be stressed to all employees who perform or assist in the AMT charge.

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Table 1. Campaigns in which AMT was used, the number of participants exposed to AMT during the listed campaigns, and duration of exposure

Campaign	Periods	Duration (months)	Number exposed	Cumulative number exposed‡	Mean exposure duration (months)	Median exposure duration (months)	Exposure range (months)
AMTBP2 †	1/1991 to 12/1991	11	2	2	8.0	8.0	5 to 11
AMTBP2	6/1994 to 11/1994	5	1	2	5.0	5.0	5
AMTBP2	6/1995 to 8/1995	2	3	4	2.0	2.0	2
AMTBP2	3/1997 to 6/1997	3	5	6	3.0	3.0	3
AMTBP2	6/1998 to 8/1999	14	22	26	9.0	11.5	1 to14
DE-498	3/1993 to 6/1995	27	8	28	16.1	15.5	7 to 27
DE-498	11/1998 to 6/2000	19	23	42	13.1	18.0	2 to 19

† AMTBP2= AMT-based product produced in Buildings 1 and 2.‡ Cumulative count of distinct individuals.

Table 2. Frequency of respiratory symptoms and their onset in relation to starting work at ChemDesign and working with AMT (N=45)

Wheezing	N	%
Have you had wheezing or whistling in your chest at any time since Nov. 1998?	19	42
Wheezing onset after started working at ChemDesign.	16	84
Wheezing onset after started working with AMT.	15	79
Wheezing was better on days away from work?	9	47
Do you still have this wheezing occasionally?	13	68
Onset months after first exposure to AMT (mean, range).	5.3	1-14
Chest Tightness		
Have you woken up with a feeling of tightness in your chest first thing in the morning, at any time since November 1998?	8	18
Chest tightness onset after started working at ChemDesign.	8	100
Chest tightness onset after started working with AMT.	8	100
Chest tightness was better on days away from work.	3	38
Do you still have chest tightness occasionally?	5	63
Onset months after first exposure to AMT (mean, range).	4.6	1-13
Shortness of Breath		
Have you at any time since November 1998 had an attack of shortness of breath that came on when you were not doing anything strenuous?	10	22
Have you at any time since November 1998 been awakened at night by an attack of shortness of breath?	3	7
Shortness of breath onset after started working at ChemDesign.	10	100
Shortness of breath onset after started working with AMT.	10	100
Shortness of breath was better on days away from work.	5	50
Do you still have shortness of breath occasionally?	6	60
Onset months after first exposure to AMT (mean, range).	7.2	4-14
Cough	Yes	%

Have you at any time since November 1998 been awakened at night by an attack of coughing?	10	22
Do you usually cough first thing in the morning?	9	20
Cough onset after started working at ChemDesign.	13/15	88
Cough onset after started working with AMT.	9/15	60
Cough better on days away from work.	3	33
Do you still have this cough occasionally?	11	56
Onset months after first exposure to AMT (mean, range).	10.8	3-19
Upper respiratory symptoms and eye irritation		
During your working hours have you had the following symptoms apart from colds, at any time since November 1998?		
Irritation of the nose	21	47
Stuffy or runny nose	22	49
Sneezing	26	58
Irritation of the eyes	18	40
Irritation of the throat	15	33
Any symptoms onset after started working at ChemDesign.	27/33	82
Any symptoms onset after started working with AMT.	17/33	52
Symptoms better on days away from work.	19/33	57
Do you still have any of these symptoms occasionally?	30/33	91
Onset after months of exposure to AMT (mean, range).	13.5	1-47

Table 3. Campaigns, tasks, and agents listed by employees as causing or making respiratory symptoms worse

Campaigns	**Tasks**	**Specific materials**
DE-498	Multiple	AMT
AMTBP2	Recording weights	SB1
DPE	Supervisor/doing rounds	THBP
Pack Products	AMT charge	BBT
PDR14	Centrifuge	PDR
Thaizpore	Discharging the rosenmund	MCB
AS2000	Troubleshooting	Dust
TAP	Strips	DE-498
Sodium HP	Reactions/Reactor	Powder
K5	Dropping the bud	Ventilation
BAPO	Building16 top floor	HCL
PAC	Maintenance	Butanol
CL215	Dryer	Methylene chloride
Clinda	Charging chemicals to vessels	
All campaigns	Charging VR09	
FH-132	GC	
BRDMAC	HDLC	
Butinol	Wet chemical analysis	
	Operator	
	Pumping methylene chloride into vessels	

Table 4. Response to questions on work-related asthma in 45 employees

Asthma Questions	**N**	**%**
Have you ever had asthma?	10	22
Was your asthma diagnosed by a doctor?	10	100
Did you ever take medication for your asthma?	10	100
Do you still take medication for your asthma?	6	60
Do you still have asthma?	7	70
Adult onset asthma (at 30+ years).	9	90
Asthma diagnosed after started working at ChemDesign.	9	90
Asthma diagnosed after started working with AMT.	8	80
Asthma better on days away from work.	6	60
Are/were there materials or conditions at work that make asthma worse?	6	60
Has a doctor ever told you that your asthma is related to your work?	5	50
Onset after months of AMT exposure (mean, range in months).	10.1	(1-33)

Table 5. Cross-sectional lung function tests for 43 employees

Variable	Mean	S.E.	Range	t-test	P-value
FVC	4.87	0.13	3.2-6.9		
Predicted FVC	4.78	0.13	2.9-6.6		
%predicted	103.0	2.21	62-133		
%predicted-100	2.9	2.21		1.4	0.18
FEV1	3.71	0.11	2.5-5.8		
Predicted FEV1	3.93	0.11	2.3-5.4		
%predicted	95.4	2.12	57.7-122.1		
%predicted-100	-4.56	2.12		-2.2	0.04
FEV1/FVC	76.4	7.0	60.4-88.5		
Predicted FEV1/FVC	82.3	1.2	79.4-85.4		
%predicted	92.8	8.0	73.8-105.0		
%predicted-100	-7.2	1.2		-5.9	0.0001

Table 6. Frequency distribution for the number of periodical lung function tests available per employee

Number of tests	Frequency	Cumulative frequency
0-1	10	10
2	2	12
3	4	16
4	4	20
5	4	24
6	6	30
7	5	35
8	4	39
9	1	40
10	5	45
12	1	46

Table 7. Number of periodical tests by the year of follow-up

Year of test	Number of tests	Average age
1987	1	42
1988	5	35.6
1989	8	34.5
1990	13	36.7
1991	11	39.3
1992	14	37.1
1993	17	41.9
1994	26	39.5
1995	26	40.6
1996	26	39.4
1997	31	41.6
1998	24	44.4
1999	29	41.7
2000	43	42.2

Table 8. The average slopes of decline in lung function tests, according to the age at the start of follow-up

Age category	**N**	**Mean age at start of follow-up**	**Rate of decline in lung function**		
			FEV1 (s.e.) (ml/yr)	**FVC (s.e.) (ml/yr)**	**Ratio (s.e.) (%/yr)**
<30	10	25.6	- 51 (11)	- 35 (12)	-5.0 (1.7)
30-39	11	33.1	- 44 (12)	- 34 (12)	-3.5 (0.9)
40-49	10	42.8	- 39 (14)	- 20 (19)	-4.6 (1.2)
50+	3	56.0	- 48 (8)	- 33 (31)	-5.1 (3.9)
Total	34	35.8	- 45 (7)	- 30 (8)	-4.4 (0.7)
Expected		for ≥25 yrs	- 29	-30	-1.1

Table 9. The frequency distribution for the 'exposed' and 'unexposed' thyroid hormone tests and the mean T_4 values according to year of test

Year of test	Exposure to AMT						t-test; P-value
	Unexposed tests			Exposed tests			
	N	Mean	S.D.	N	Mean	S.D.	
1988	4	7.7	1.2				
1989	7	7.2	0.5				
1990	11	7.8	1.0				
1991	7	6.9	1.4	1	5		
1992	14	6.8	0.8				
1993	18	8.2	2.1	1	6.7		
1994	21	7.8	1.2	1	8.3		
1995	22	7.8	1.5	4	7.5	0.6	0.36; 0.72
1996	29	7.5	1.5				
1997	24	6.8	1.8	2	7.8	0.8	
1998	26	6.7	1.1	7	7.5	2.3	-0.9; 0.40
1999	10	7.1	2.3	20	6.8	1.2	0.6; 0.63
Total	193	7.3	1.5	36	7.1	1.4	1.0;0.30

Table 10. Air Sampling, ChemDesign Corporation, HETA 2000-0096					
Sample No.	**Description**	**Sampling Period (minutes)**	**Sample Volume (liters)**	**Concentration[1] (mg/m^3)**	
				AMT	**DE-498**
AMT Charge					
6/5/00					
5	PBZ; FF SAR; Pro/Shield®2 suit; latex gloves.	2022-2032 (10)	19.7	5.6	–
6	Area sample at boundary of restricted area near end of aisle.	2024-2036 (12)	23.8	(0.01)	–
7	Area sample at boundary of restricted area near overhead door.	2023-2035 (12)	24.0	<0.004	–
6/6/00					
10	PBZ; FF APR; Pro/Shield®2 suit; gloves.	1411-1420 (9)	17.7	2.5	–
11	PBZ; FF SAR; Pro/Shield®2 suit; gloves.	1410-1418 (8)	15.7	1.5	–
12	Area sample at boundary of restricted area near overhead door, ≈22' from vessel.	1411-1421 (10)	19.9	<0.005	–
13	Area sample at boundary of restricted area near end of aisle; ≈40' from vessel.	1413-1423 (10)	19.5	(0.02)	–
6/7/00					
22	PBZ; Hood w/ SAR; Pro/Shield®2 suit; latex gloves	0722-0735 (13)	26.0	3.5	–
23	Area sample at boundary of restricted area near overhead door; ≈22' from vessel.	0723-0736 (13)	25.6	0.039	–
24	Area sample at boundary of restricted area near end of aisle; ≈40' from vessel.	0722-0738 (16)	31.8	<0.003	–

Table 10. Air Sampling, ChemDesign Corporation, HETA 2000-0096					
Sample No.	Description	Sampling Period (minutes)	Sample Volume (liters)	Concentration[1] (mg/m^3)	
				AMT	DE-498
Dryer Charge					
6/5/00					
2	PBZ; supplied-air hood, Pro/Shield®2 suit; five supersacs charged into dryer.	1932-2000 (28)	55.2	–	5.8
3	Area sample at boundary of restricted area;≈40' from operator.	1935-2002 (27)	54.0	–	0.022
4	Area sample at boundary of restricted area;≈30' from operator.	1938-2005 (27)	53.6	–	(0.004)
6/7/00					
19	PBZ; six- supersacs charged.	0132-0211 (39)	76.8	–	0.53
20	Area sample at boundary of restricted area;≈40' from operator.	0135-0213 (38)	75.5	–	<0.0007
21	Area sample at boundary of restricted area;≈30' from operator.	0137-0216 (39)	76.1	–	0.0034
6/8/00					
30	PBZ; FF SAR; Pro/Shield®2 suit. Six supersacs charged.	0044-0125 (41)	82.7	–	0.37
31	Area sample at boundary of restricted area;≈40' from operator.	0050-0126 (36)	72.4	–	<0.0007
32	Area sample at boundary of restricted area;≈30' from operator.	0051-0129 (38)	76.3	–	<0.0007

Table 10. Air Sampling, ChemDesign Corporation, HETA 2000-0096					
Sample No.	Description	Sampling Period (minutes)	Sample Volume (liters)	Concentration[1] (mg/m^3)	
				AMT	DE-498
Dryer Discharge					
6/6/00					
14	PBZ; latex gloves worn throughout operation; Supplied-air hood & Pro/Shield®2 suit worn while collecting QC sample, closing & removing full super sacs (2). Operator remained in general vicinity of dryer.	1817-2001 2109-0011 (286)	560.	–	0.011
15	Area, ≈4' from super sac.	1819-1957 2111-0014 (279)	554.	–	0.0034
16	Area, ≈24' from super sac near writing stand.	1822-2000 2110-0013 (281)	548.	–	0.0073
6/7/00					
25	PBZ; FF SAR, Pro/Shield®2 suit, & latex gloves worn while unclogging flex duct and removing full supersacs at 1825 & 2100; Operator was elsewhere in facility during much of sampling period.	1816-2106 (170)	343.	–	0.14
26	Area, ≈24' from supersac near writing stand.	1818-2109 (171)	344.	–	0.0014
27	Area, ≈4' from supersac.	1741-1752 1818-2108 (181)	363.	–	<0.0001
Centrifuge Discharge (FC 246) – 6/5/00					
1	PBZ; FF APR; gloves; Pro/Shield®2 suit;	1401-1419 (18)	35.4	–	0.56

- "<" indicates a value less than the minimum detectable concentration (MDC).
- Values within parentheses are between the MDC and the minimum quantifiable concentration (MQC).

PBZ = Personal breathing zone sample
FF SAR = Full facepiece supplied-air respirator
FF APR = Full facepiece air-purifying respirator

1. Each sample was analyzed for AMT and DE-498. No DE-498 was detected in samples collected during the AMT charge; no AMT was detected in samples collected during the dryer charge, dryer discharge, and centrifuge discharge. The analytical limit of detection (LOD) for AMT and DE-498 is 0.1 µg/sample and 0.05 µg/sample respectively.

Figure 1. Predicted decline in FEV_1 with age for workers, in comparison to Knudsons's reference curve

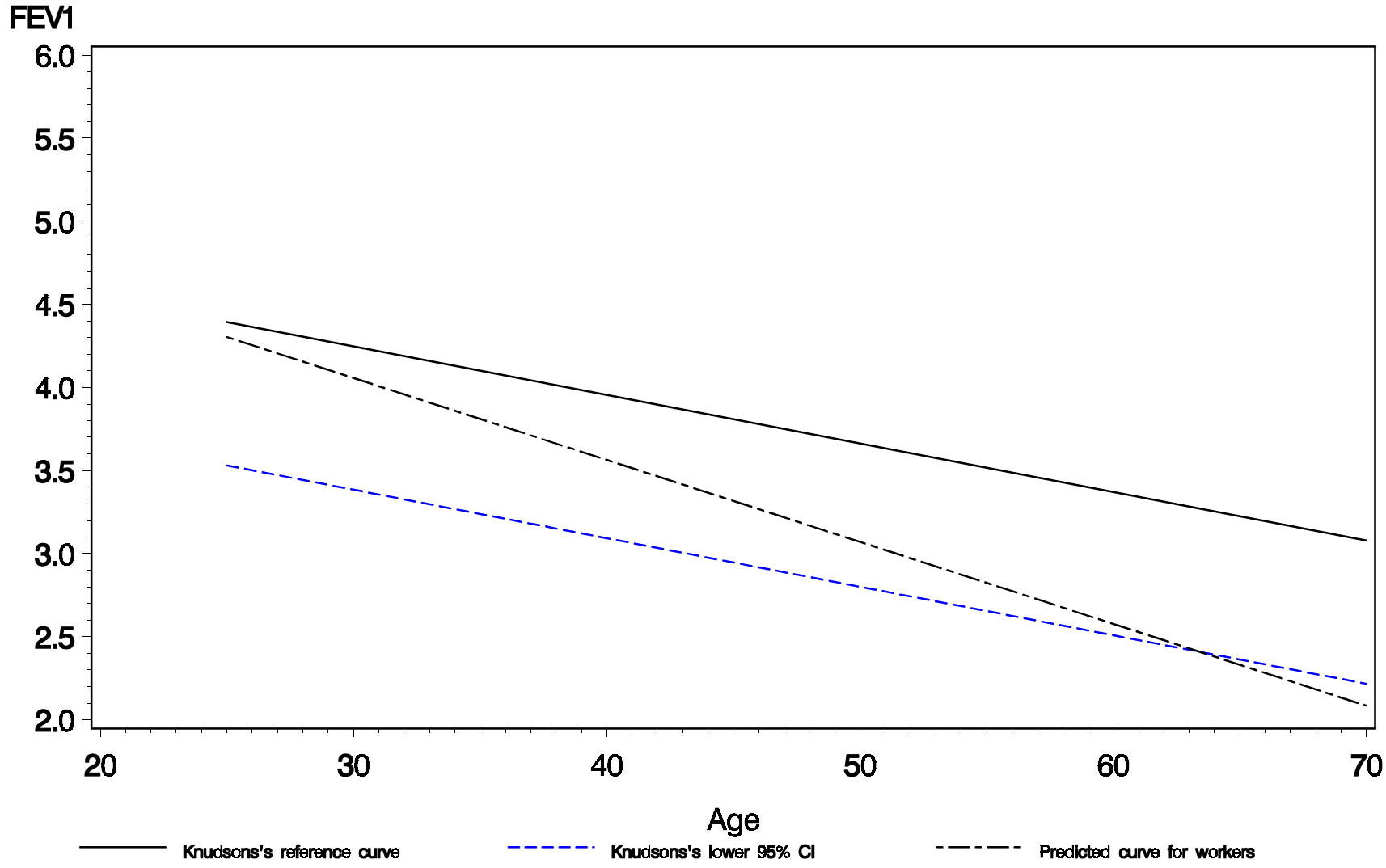


Figure 2. Predicted decline in FVC with age for workers, in comparison to Knudsons's reference curve

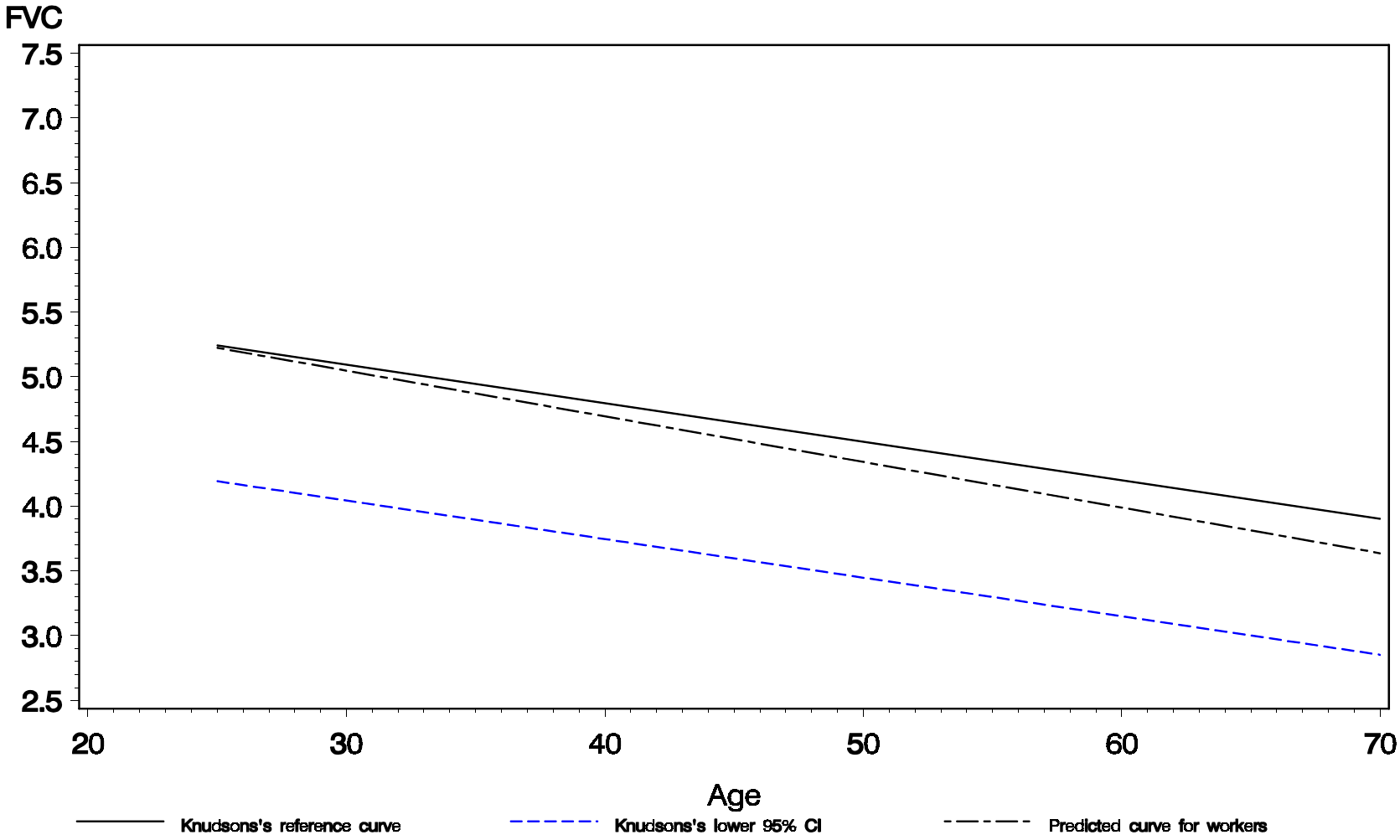
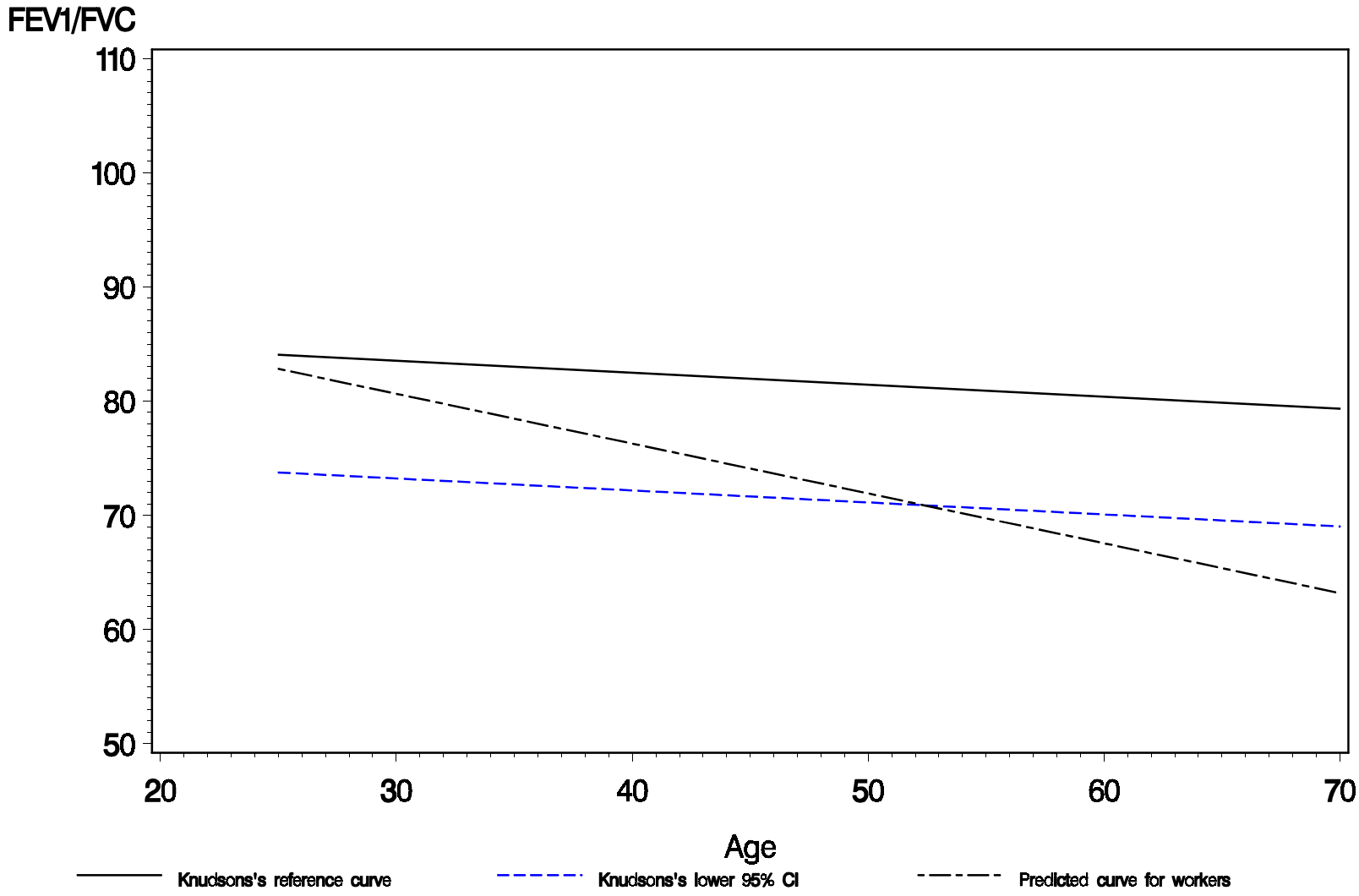


Figure 3. Predicted decline in FEV_1 /FVC with age for workers in comparison to Knudsons's reference curve



Appendix I - Evaluation Criteria

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 increases 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),[12] (2) the American Conference of Governmental Industrial Hygienists' (ACGIH®) Threshold Limit Values (TLVs®),[13] and (3) the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs).[14] Employers are encouraged to follow the OSHA limits, the NIOSH RELs, the ACGIH TLVs, or whichever are the more protective criterion.

OSHA requires an employer to furnish employees a place of employment that is free from recognized hazards that are causing or are likely to cause death or serious physical harm [Occupational Safety and Health Act of 1970, Public Law 95–596, sec. 5.(a)(1)]. Thus, employers should understand that not all hazardous chemicals have specific OSHA exposure limits such as PELs and short-term exposure limits (STELs). An employer is still required by OSHA to protect their employees from hazards, even in the absence of a specific 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 STEL or ceiling values which are intended to supplement the TWA where there are recognized toxic effects from higher exposures over the short-term.

It should be noted that occupational exposure limits (OELs) have not been established for DE-498 or AMT. The lack of OELs for these materials does not indicate that these materials are harmless or nontoxic.

MEDICAL CRITERIA

Spirometry was performed using a dry rolling-seal spirometer interfaced to a dedicated computer. All values were corrected to BTPS (body temperature, ambient pressure saturated with water vapor). The largest forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were selected for the analysis. Testing procedures conformed to the American Thoracic Society (1995) recommendations.[4] Predicted values were calculated using the Knudson reference equations.[5] To identify employees with abnormal spirometry patterns, the 95th percentile lower limit of normal (LLN) values were used as cutoff points.[6]

Obstructive pattern is diagnosed when the observed ratio of FEV_1/FVC% is below the LLN.

Restriction is diagnosed when the observed FVC is below the LLN; and FEV_1/FVC% is above the LLN.
Criteria for the interpretation of the level of severity for obstruction and restriction, as assessed by spirometry, are based on the NIOSH classification scheme as follows:

Mild obstruction is diagnosed when FEV_1/FVC x 100 is within the range 60-80%.
Moderate obstruction is diagnosed when FEV_1/FVC x 100 is within the range 50 - 65%.
Severe obstruction is diagnosed when FEV_1/FVC x 100 is less than 50%.

Methacholine test was performed according to ATS guidelines.[7] From the baseline spirometry we selected the highest FEV1 value and used it as a baseline for the test. Concentrations of methacholine used were: 0.5 mg/ml, 2.0 mg/ml, 8.0 mg/ml, and 32.0 mg/ml.

References:

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Appendix II - Laboratory Methods

Laboratory support studies for the ChemDesign HHE Investigation

Human studies

Preliminary information indicated that exposures at a chemical manufacturing facility were associated with asthma or asthma-like symptoms. The exposure to a feedstock chemical, 3-amino-5-mercapto-1,2,4-triazole (AMT) was of particular concern. The reported symptoms were suggestive of a sensitization reaction, and so laboratory studies were undertaken to determine if exposed workers had any serologic evidence of allergic disease. To evaluate the hypothesis that the symptoms were due to an allergic reaction, the laboratory developed assays to determine if the exposed employees had antibodies to the suspect compound, and analyzed the sera for markers of allergy. In addition, studies were undertaken to develop assays that would detect AMT in serum.

Methods. A total of 44 serum samples were received in the laboratory. The sera were stored at -80C until assayed. Samples of the feedstock chemical (AMT) and the product compound (DE-498) were obtained from the plant. The serum samples were assayed for total immunoglobulin E (IgE) levels using the CAP® assay system according to manufacturer's recommended procedure (Pharmacia & Upjohn Diagnostics, Kalamazoo, Michigan). As a general screen for atopic status, the sera were assayed for IgE antibodies to common environmental allergens by the CAP® assay. Each serum sample was assayed using six allergen mixes which were selected to reflect allergens common to the geographic region where the employees lived. Results were scored as either positive or negative according to criteria in each kit, and a employee was considered atopic if they were positive to one or more of the allergen mixes. By this criteria 16 of the 44 sera tested were positive.

An assay to detect IgG antibodies to AMT was developed using AMT-protein conjugates in an ELISA assay. The protein conjugates were prepared by mixing AMT with the carrier proteins, either human serum albumin (HSA) or keyhole limpet hemocyanin (KLH), in the presence of 0.3% glutaraldehyde. The reaction was carried out using a 100 molar excess AMT to protein for two hours at room temperature in 0.1M borate buffer (pH 10.0), and followed by dialysis against phosphate buffered saline (PBS) to remove un-reacted AMT and glutaraldehyde. The degree of conjugation of the AMT to protein was monitored by measuring an increase in free thiols in the proteins. The two proteins used for carriers were chosen because the employees would be unlikely to have antibodies to the native proteins and any antibody binding would be the result of the AMT moiety carried by the protein, and the use of two protein should enhance the specificity of the results. The ELISA was performed by coating each well of 96 well plates with either the native protein or the AMT-protein conjugate (5.0 ug/ml in carbonate coating buffer, pH 9.3). The plates were reacted sequentially with employee sera (diluted 1:100), and peroxidase labeled anti-human IgG (Sigma). The plates were developed with a diaminobenezidine (DAB) substrate and the absorbency at 630 nm monitored. All sera were tested against HSA, HSA-AMT, KLH and KLH-AMT. The difference in absorbency between the native protein its AMT conjugate was considered to be related to the binding AMT specific antibodies. Because serum samples with known levels of anti-AMT antibodies were not available, the ELISA's could not be evaluated and optimized using a positive control. To estimate the range of non-specific or background binding in the ELISA assay, serum samples from 22 individuals with no known exposure to AMT were assayed. Values above the upper limits of the normal range would indicate that the employee likely had antibodies to AMT, but the results should be interpreted causiously.

Chemical Analysis for AMT: Studies have been conduct to determine optimal methods for measuring AMT in biological fluids. Whole blood samples were obtained from the exposed employees to determine if AMT or a metabolite could be detected. To date all studies have been done *in vitro* by adding known quantities of AMT to specific biological fluids, and then evaluating the sensitivity of the assay. Preliminary studies attempted to directly

measure AMT in serum using HPLC coupled to a UV detector. This method lacked sufficient sensitive and specificity due in part to the poor retention of AMT on the reverse phase column used in the HPLC. A fluorescent method was developed which involved conjugating the AMT to a fluorescent compound (monobromobimane, MBB) through the free thiol present in AMT. The AMT-MBB conjugation step improved both the chromatographic separation and the detection of the product so that adequate sensitivity and specificity were obtained. Preliminary studies using this methodology has shown that AMT can be detected in whole blood and serum, that no metabolism of the AMT by red blood cells or leukocytes was observed, and biological fluids can be concentrated by a solid phase extraction technique to further improve the sensitivity of the assay. There is some binding of the AMT with human serum albumin and other serum proteins (approximately 10% of added sample was not recovered) making accurate measurement of AMT in serum difficult. These assays continue to be developed, and will be applied to the employee samples once the assays are fully evaluated and validated.

Results. While some individuals did appear to react more strongly to the AMT protein conjugate than to the carrier protein alone, there was no concordance between the HSA-AMT and the KLH-AMT. Normal human serum proteins like HSA are commonly used as carrier proteins in immunoassays because antibodies to them are rare in human serum samples. Similarly, KLH is a protein to which most people would not be exposed and thus most people would not have antibodies to it. It was hoped that by using both proteins that the specificity of the assays would be improved. That the reactions to the two conjugates did not correlate suggests that the binding observed in the immunoassays was not due to an IgG antibody specific for AMT. Additional studies to assay for IgE antibodies to AMT are planned, but are on hold until the necessary equipment and reagents are available. The assays for total IgE and specific IgE to common environmental allergen indicate that approximately one third (16 of 44) of the employees are atopic, i.e., allergy prone. The significance of this observation is unknown, but supports the need to do the additional studies for IgE antibodies to AMT.

Animal studies

Given that there was the potential for exposure to both AMT and DE-498 and little toxicological information is available on either chemical, animal studies were conducted to evaluate the sensitization potential of both chemicals. Irritancy was evaluated using a mouse ear-swelling assay and the potential to induce sensitization was first evaluated using the murine Local Lymph Node Assay (LLNA). The LLNA evaluates the induction phase of sensitization by quantitating DNA replication in the cells of lymph nodes draining the site of dermal exposure to the chemical. The assay has recently undergone peer review through the Inter-Agency Coordinating Committee for Alternative Test Methods and has been accepted by U.S. and European regulatory agencies as an acceptable method for evaluating the contact sensitization potential of chemicals. The LLNA has also been shown to be positive when evaluating chemicals with the potential for inducing respiratory responses such as TDI, MDI, and TMA. Because the assay does not clearly differentiate respiratory from dermal sensitizers, additional endpoints including phenotypic analysis of lymph node cells, evaluation of serum IgE levels, and cytokine production by draining lymphocytes were evaluated to better understand the mechanism of sensitization

Methods. All assays were conducted using female BALB/c mice approximately 6-8 weeks of age at the start of the study. Study designs consisted of 4 groups of animals (N=5-8), a vehicle control group (VH) and 3 groups dermally exposed to increasing concentrations of the chemical to be tested. For the LLNA 30% HCA or 2.5% TDI was used as a positive control and 2.5% TDI was used as the positive control for the phenotypic analysis assay. The irritancy assay was conducted by first pre-measuring the thickness of the animals' ears, followed by dermal exposure on the ear pinna to 25 ml of VH or the test article for 3 consecutive days. Twenty-four hours following the final exposure, the animals' ears were measured and the percentage ear swelling for each animal and the group mean were calculated. Values for test groups were compared to control for significance. For the LLNA, the same dosing regime was used, however following the final exposures, animals were allowed to rest for 2 days and were then injected intravenously with [^{3}H]-thymidine. Five hours post injection, animals were sacrificed, draining lymph nodes were excised, and single cell suspensions were made and prepared for counting on a β-scintillation counter. The amount

of [^{3}H]-thymidine incorporation was used as a measure of cellular proliferation. For phenotypic analysis, animals were exposed as for the irritancy assay with the exception that exposures were for 4 days. On the 10th day following the initial exposure, animals were sacrificed, single cell suspensions made and stained with antibodies against B220 and IgE. Cells were then enumerated using a flow cytometer. Blood was collected from these animals and used for the analysis of serum IgE levels by ELISA. For the time course of IgE production study, animals were pre-bled to establish a base-line IgE level and then exposed to 50µl of 25% AMT or VH on the shaven dorsal surface of the back 5 days per week for 77 days. Animals were bled approximately every 2 weeks to evaluate IgE levels. The Mouse Ear Swelling Test (MEST) was used to further evaluate contact hypersensitivity potential. Animals were exposed to the chemical or VH on the shaven abdomen for 3 days, allowed to rest for 4 days and then challenged with the test article on the ear. Ear thickness was measured prior to challenge and 24 and 48 hours following challenge to evaluate the elicitation of a contact hypersensitivity response. To measure the induction of cytokines, mice were exposed to the chemical or VH on the ears for 3 days and were sacrificed 24 hours post final exposure. Lymph nodes were excised, a single cell suspension was prepared, and cells were stimulated with Concavalin A (ConA) for 24 hours. Messenger RNA (mRNA) was isolated from the stimulated cells, and an RNase protection assay was performed (Ambion RPA III kit). The kit includes probes for a panel of both TH1 and TH2 cytokines.

Results. Using the irritancy assay, no signs of systemic toxicity (as measured by body weight gain) or irritancy (as measured by ear swelling) were observed following exposure to either chemical at concentrations up to 25% AMT and 40% DE-498 (the limits of solubility for each chemical). DE-498 was negative in the LLNA at concentrations up to 40% (Figure 1A) indicating a lack of sensitization potential. However, a dose dependent increase in lymphocyte proliferation was observed following exposure to AMT, reaching a 3-fold increase over control, indicating a positive response, at a concentration of 25% (Figure 1B). The results of the phenotypic analysis assay supported the findings of the LLNA in that exposure to AMT induced a significant increase in the % of B220+ cells at all concentrations of AMT tested. Additionally the 25% dose group exhibited a significant increase in IgE+/B220+ cells (Figure 2) indicating that AMT may induce the production of IgE. The MEST was used to further evaluate the potential for AMT to induce contact sensitization. This assay was negative following induction at concentrations up to 25% and challenge with 25% AMT. To further evaluate the mechanisms of sensitization following AMT exposure, a time course study for the induction of IgE was conducted. Levels of IgE were found to increase in animals dermally exposed 5 days a week for up to 77 days (Fig. 3). Preliminary evaluation of the cytokine mRNA expression profile of lymphocytes from AMT exposed animals demonstrated an up-regulation of interleukins 2,4,5, 9,10,13,and 15. This cytokine expression pattern is consistent with the induction of a TH2 response favoring IgE production and includes cytokines that influence mast cell growth and differentiation.

Conclusion. Using murine models, DE-498 tested negative for dermal irritancy and sensitization potential. AMT tested negative for dermal irritancy potential but was considered positive for dermal sensitization potential in the LLNA. Further evaluation of the mechanism of sensitization of AMT using the MEST yielded negative results. In contrast to the LLNA which evaluates the induction phase of sensitization, the MEST requires both induction and the elicitation of the response and a positive response is indictative of a T-cell mediated mechanism. Phenotypic analysis of draining lymph node cells demonstrated the potential of AMT to induce an IgE mediated response which was further supported in a time course study where levels of total serum IgE increased over time. The cytokine profile of the lymph node cells draining the site of AMT exposure gave further support of the potential of AMT to induce an IgE mediated response. An up-regulation of the mRNA of TH2 cytokines involved in the production of IgE and cytokines associated with mast
cell growth and differentiation was observed. These studies indicate that DE-498 does not induce sensitization following dermal exposure, however AMT was identified as a sensitizer and may have the potential to induce IgE mediated hypersensitivity responses.

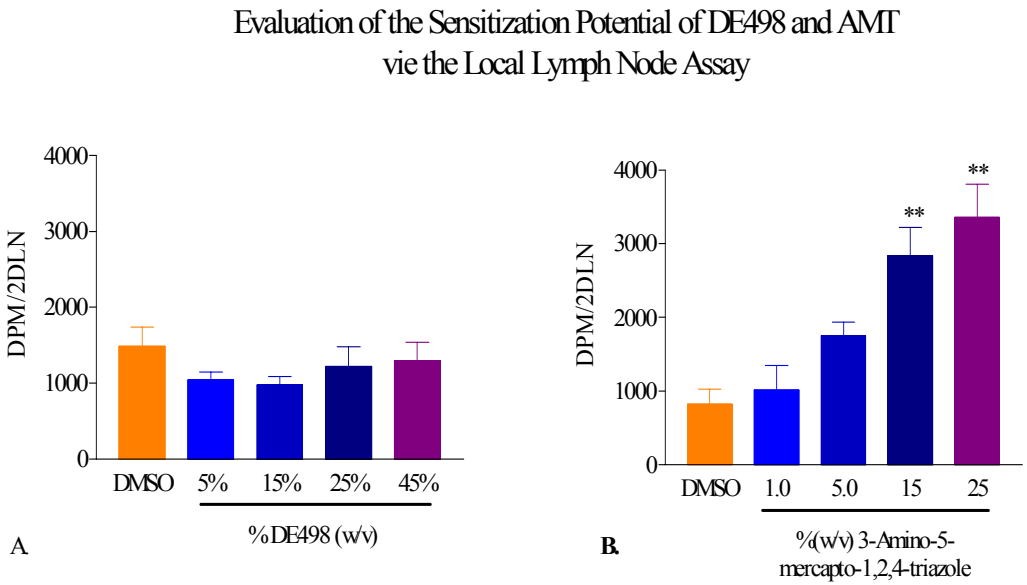

Figure 1. Bars represent cellular proliferation in left and right draining lymph nodes combined for A) DE498 exposed mice and B) AMT exposed mice. Positive controls for A) 30% HCA 6,363 +/- 717.6 DPM B) 2.5% TDI 11,010+/- 2,099 DPM. ** indicates $p < 0.01$

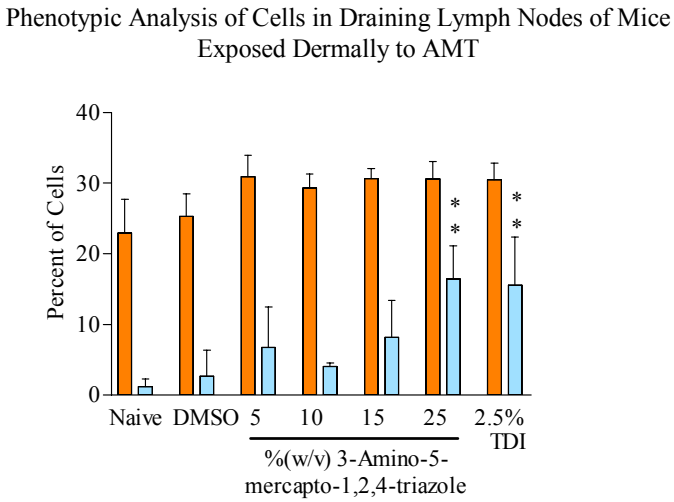

Figure 2. Orange bars represent percentage of B220+ cells and blue bars represent percentage of IgE+/B220+ cells. ** indicates $p<0.001$.

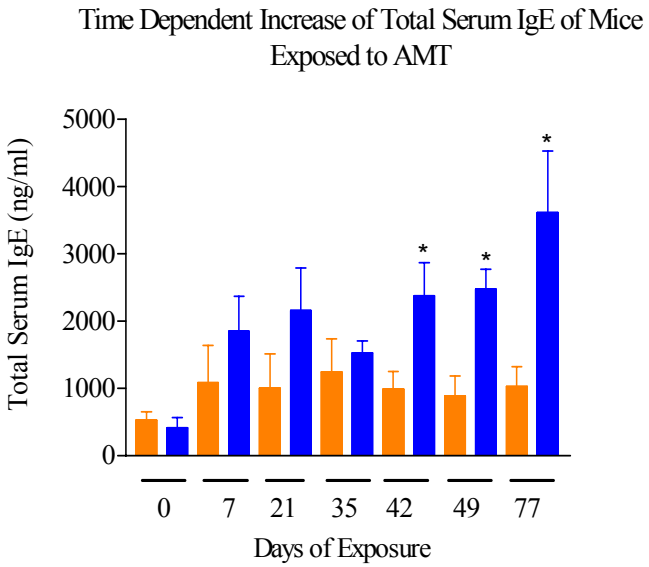

Figure 3. Orange bars represent total IgE levels of vehicle treated mice and blue bars represent 25% AMT treated animals on days indicated.
* Indicates $p<0.05$.,

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