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UniSea, Inc.
Dutch Harbor, Alaska

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PREFACE

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UniSea, Inc.
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SUMMARY

On December 19, 1997, the National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation (HHE) from the management of the UniSea shore-based crab processing facility in Dutch Harbor, Alaska. They were concerned about respiratory illness among workers including bronchitis and asthma. The NIOSH study consisted of early-season and late-season medical and environmental surveys during the 1998 opilio snow crab season.

The objectives of this investigation were to understand the nature of respiratory illness observed in crab processing workers, to identify areas and sources of exposure, to identify any relationships between crab processing exposures and respiratory health outcomes, and to develop strategies to prevent illness in crab-processing workers.

The two surveys included a symptoms questionnaire, lung function testing, and blood collection. The environmental evaluation consisted of air sampling for aerosolized protein, crab allergens, endotoxin and microscopic analysis of materials splashed on workers and breathed by workers.

An over-all participation rate of 76% of workers was attained for completion of both the early and late-season questionnaires. At the early-season survey, five individuals noted a previous doctor-diagnosis of asthma. Over the course of the season, one of the participants with a previous doctor-diagnosis of asthma experienced significant work-related worsening of asthma. In addition, one individual working in crab processing and one individual engaged in related activities acquired a new doctor-diagnosis of asthma during the season.

In this investigation, combination of symptoms were used to define specific health outcomes. The percent incidence of new cases of the upper respiratory outcome was 56%; of the asthma-like outcome 26%; and of the bronchitic outcome 19%. Workers with a positive family history of allergies or working in the butchering area had a significantly increased risk for development of the upper respiratory outcome. For the asthma-like outcome, male gender, family history of allergies, elevated ECP (a protein in the blood indicating eosinophilic inflammation), butchering activities and degilling activities were risk factors significantly related with the outcome. For the bronchitic outcome, significant association was found among workers with

age less than 35 years, male gender, elevated antibodies to crab (serum anti-kanimiso IgE and anti-Pagurus crab IgE,) elevated serum ECP and the task of degilling.

Although this investigation was limited due to the relatively small size of this population, data showed development of new respiratory symptoms and asthma among crab processing workers over the six weeks of crab processing. These problems appeared to be occupationally related. Higher prevalence of IgE-sensitization to crab among new or "naive" workers suggests that workers susceptible to respiratory illness related to crab processing may be more likely to leave their job (" healthy worker effect"). In part because the precise etiological agent causing respiratory symptoms was not fully characterized, exposure assessment did not allow evaluation of dose-response relationships.

During the course of this investigation data indicated that a health hazard existed from exposures related to crab processing. Both new onset of asthma and increased burden of respiratory symptoms relative to the early-season survey were observed. Recommendations to limit the impact of respiratory problems are described in the report.

Keywords: SIC Code 0104 (Crab related work), asthma, allergy, crab, sea food, IgE, microscopy, spirometry, environmental assessment.

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INTRODUCTION

On December 19, 1997, the National Institute for Occupational Safety and Health (NIOSH) received a management request for a health hazard evaluation (HHE) at the UniSea shore-based crab processing facility in Dutch Harbor, Alaska. Reported respiratory illness among workers included colds, pneumonia, bronchitis, and asthma. The evaluation consisted of early-season and late-season medical and environmental surveys during the 1998 opilio snow crab season.

The objectives of this investigation were to understand the nature of respiratory illness observed in crab processing workers, to characterize workplace exposures, identify areas and sources of antigenic exposure, determine the relative degrees of exposure in different areas of the plant and among the various groups of processing workers, assess any potentially adverse exposures associated with the work environment, identify any relationships between crab processing exposures and respiratory health outcomes, and develop prevention strategies.

On January 23 to 28, 1998, the early-season survey was conducted at the facility. The medical evaluation of the crab processing workers consisted of a symptoms and occupational history questionnaire, spirometry (lung function testing), and venipuncture (blood collection). The environmental evaluation consisted of air sampling for aerosolized protein, crab allergens, and endotoxin. Bulk samples of snow crab products were collected for allergen extract preparation.

On March 5 to 10, 1998, the late-season portion of the survey was conducted at the facility. The medical evaluation consisted of follow-up testing and questionnaire of the same group of crab processing workers who completed the early-season survey. The environmental evaluation consisted of more air samples for aerosolized protein, crab allergens, and endotoxin. Air samples were also collected for microscopic particulate analysis, volatile organic compounds (VOCs), carbon monoxide, and ammonia. Bulk samples from the crab processing tanks were collected for microbiological and elemental metals analysis. In addition, direct reading aerosol measurements were made.

BACKGROUND

The UniSea Dutch Harbor Facility

The UniSea facility is located in Dutch Harbor, Alaska, which is on the Aleutian island chain in the Bering Sea. The entire UniSea complex covers 44 acres, with a separate building dedicated to crab processing. Opilio snow crab (*Chionoecetes opilio*) is processed from January through March and the brown king crab (*Lithodes aequispinus*) from September to November. The crab workers do different job tasks at various work stations in a large open facility. Other buildings of the facility are dedicated to fish processing (pollock, cod, and roe). Two production plants in the facility process pollock into imitation crab meat or surimi. An additional facility, known as the "meal plant," is located in a separate building. In this area, crab shells are crushed, processed, and bagged for use in fillers, fertilizers, etc.

Crab processing workers are usually hired seasonally and sign contracts for 3 to 4 month periods. Many are non-English speaking immigrants to the U.S. Approximately 160 crab processing employees work 12-hour shifts, 7 days a week, for nearly 60 days during the crab harvesting season. The day shift runs from 6:00 a.m. to 6:00 p.m. and the evening shift from 6:00 p.m. to 6:00 a.m. Workers have two 15-minute breaks and a 30-minute meal break during each shift. During the processing season, workers live in bunk houses provided by the company.

Crab Processing and Potential Exposures

The process begins with commercially harvested male opilio snow crabs that arrive live in the holds of ships (See Figure 1. Diagram of Snow Crab Processing at UniSea). The snow crabs are transported from the ship holds onto the plant dock by UniSea employees called offloaders. It takes up to three (12-hour) work shifts for 6 to10 unloaders to empty each ship, which can hold 40,000 to 60,000 pounds of crab. From the dock, forklifts transport the crabs to a hopper that supplies 10 butchering work stations. Butchering employees grab each crab by the legs and then using a semi-rigid plate around their abdomen, push the crab's body shell against a fixed dull metal blade. The body splits open and the leg clusters separate from the shell. Shells from the body fall away and are ground up in a separate process (meal plant). The internal organs or kanimiso are collected and flash frozen for the Asian market.

Crab legs are transported from the butchers via conveyor belt to 25 degilling employees, who remove the gills on a rotating blade. The degilled crab leg clusters are conveyed to the packing and sorting line. Approximately 40 employees called packers sort the crab legs into three grades (based on appearance, size, and condition) and pack the clusters into 30-pound stainless steel tubs. The tubs are weighed and sent to the cooking tank. An overhead conveyor lowers a stack of tubs into the cooking tank, where they are cooked in fresh water at 210°F for about 15 minutes. A worker called a cooker is stationed at each end of the cooking tank.

After cooking, the tubs are transferred to an ambient water tank (fresh water at room temperature) for rinsing and cooling. The crab tubs are then transported to a tank of water chilled to 33°F to further reduce their temperature. Finally, the crab tubs are sent to a brine tank (with salt [NaCl] added in a saturated solution for freezing point depression) at -3°F. The crab legs are frozen in the brine tank. After the brine tank, the crab tubs are rinsed in a fresh water glaze tank to remove excess brine. Eight employees work at the cooking and cooling tanks. Ten workers in the case-up and shipping area transfer the crab legs from the tubs into cardboard boxes and place them in a large freezer (-40°F) until shipment to wholesalers. Ammonia is used as the refrigerant for the freezer, chiller and brine tanks.

In addition, 10 to 12 employees work in sanitation, 4-6 in quality control, 10 in maintenance, and 2 in laundry service. There are 5 supervisors called team leaders working each shift. During the processing season, workers sometimes rotate among the different work stations. Workers in the crab processing plant wear personal protective equipment that includes hard hats, safety glasses, ear plugs, rain suits, and insulated rubber gloves and boots.

Workers are potentially exposed to spray and crab particulate generated during the butchering, degilling and cooking processes. Any workers engaged in tasks in the vicinity of these processes may also be exposed (Figure 1). In addition, crab workers could also be involved in the surimi or meal plant operations, depending on UniSea's production needs.

Occupational Respiratory Disease Associated with Crab Processing

Asthma is a chronic inflammatory disorder of the airways. Inflammation makes the airways chronically sensitive, or hyperresponsive. When these hyperresponsive airways are irritated, airflow is limited and exacerbations cause intermittent respiratory symptoms, including shortness of breath, wheezing, chest tightness, and cough. Making the diagnosis of asthma can be both difficult and challenging. Diagnosing asthma requires appreciating the underlying disorder that leads to asthma symptoms and understanding how to recognize the condition through information gathered from the patient's history, physical examination, measurements of lung function, and allergic status.[3] In occupational asthma, airway obstruction is caused

or made worse by workplace exposure to dusts, fumes, gases, or vapors.

Asthma or asthma-like illness has been identified among crab processing workers,[2-4] but the incidence rates, host risk factors, and course of disease have yet not been fully described. The mechanism is most likely to be mediated by IgE allergic response. In 1982, NIOSH reported a survey of 46 symptomatic crab-processing workers visiting a health clinic in Dutch Harbor, Alaska. The investigation concluded that during the crab processing season, the estimated rate of onset of dyspnea with wheeze was 80 times the monthly incidence of asthma for similarly aged Americans.[2]

Snow crab, in particular, has been implicated in cases of occupational asthma among processing workers.[3-4] A prevalence rate of 15.6% for occupational asthma (OA) was reported. Early reports suggested that asthma in crab processing workers is an immunologically mediated process with a latency period resulting in both immediate and late symptoms. An IgE-mediated response to a high molecular weight antigen liberated during the crushing of shells, boiling of whole crab, cutting and separating legs and claws was the proposed mechanism. In workers with asthma, an association was found between skin test reactivity (IgE) to crab cooking water and three commercial crab extracts. In this study, 12 of 46 subjects with occupational asthma (confirmed by immunologic and inhalation challenge tests) also manifested signs and symptoms of allergy after eating crab.[3]

While studies have suggested that IgE-mediated respiratory illness is caused by exposure and sensitization to airborne antigen in crab processing facilities, the involved antigen has not been fully described. One of the major protein groups speculated to be involved in allergic reactions to crab and other *crustacea* are the tropomyosins. The tropomyosin family consists of heat-stable highly homologous proteins, some of which have allergenic properties. The structure of tropomyosins from various organisms, including arthropoda (*crustacea, insecta, arachnida*) and mammalia (humans) has been previously defined. A recent report described the molecular characteristics of the major IgE-reactive molecule in crab, called Cha f 1, with a 34-kD (kilodaltons) molecular weight.[5] This investigation was based on sera reactivity (IgE) of individuals with an allergic reaction to crab after ingestion.

Summary of Environmental Characterizations of Crab Processing

Several environmental evaluations conducted at crab processing facilities have been reported in the literature (see Table 1. Summary of Environmental Evaluations). In response to an outbreak of crab asthma, the Washington Industrial Safety and Health Administration conducted a case study in a crab processing facility where crab legs were split with a band saw and scored using a rotating blade.[6] The environmental assessment included five total particulate air samples collected over a 3-hour period to determine "crab dust" exposures. On the splits line, employee exposures were 0.11 and 0.16 mg/m^3 (milligrams of dust per cubic meter of air). On the scoring line, employee exposures were 0.03 mg/m^3 or less.

An environmental study was conducted aboard a tanner crab processing ship in the Bering Sea.[7] Total airborne particulate collected during butchering and packing ranged from 0.14 to 0.68 mg/m^3 for four samples. Fourier-transform infrared microspectroscopy (FT-IR) revealed a mix of protein, cellulosic and synthetic fibers, silicates, and pigment constituent particles. Energy-dispersive X-ray analysis (SEM-EDX) of inorganic particles revealed silicon, aluminum, and iron. Area samples collected with a Burkard Sampler near steam cookers yielded an array of fluorescent particles (205,600/m^3), crystalline structures (98,000-392,000/m^3), and other materials.

A study conducted at a Canadian snow crab processing plant identified the presence of aerosolized snow crab allergens through

radioallergosorbent test (RAST) inhibition assays of air samples.[8] Samples were analyzed by RAST-inhibition for snow crab allergen, first using cooked snow crab meat or cooking water extract, and then using cooking water extract only. The highest protein concentration and the highest RAST-inhibition activity (13% inhibition with crab meat and 23 to 28% inhibition with cooking water) were found in the cooking area. The snow-crab allergen in this area was approximately 1.7 µg/filter (micrograms of allergen per filter). Samples from other processing stations within the facility were characterized by low protein concentrations and minimal inhibition activity. Conclusions of the study suggested that snow crab processing workers become sensitized through airborne dispersion of snow crab antigen during the cooking process. The airborne crab-derived proteins were suggested as the cause of allergic reactions and occupational asthma.

More recently a study conducted by the same group of investigators at a snow crab processing facility focused on identifying areas and tasks associated with high snow crab allergen exposure.[9] Area and personal samples were taken to determine problem tasks and areas. Samples were analyzed by RAST inhibition assays, measured against a snow crab cooking water allergen standard. Personal samples measured higher crab allergen exposures than area samples. Of all the processing locations, the crab cracking section (where the body of crab is broken and legs are brushed) had the highest allergen concentration. The cooling basin exit had the second highest allergen concentration, followed by workers cleaning (under a water jet) and sorting crabs for packaging. A personal sample at the end of the production line had the lowest antigen exposure measured.

METHODS

Environmental Methods

Personal and area air samples were collected at the facility during opilio snow crab processing to measure concentrations of aerosolized protein (using two different methods), crab allergens, and endotoxin - both the total and respirable fractions. The respirable fraction are particulates that may penetrate deep to the gas exchange region of the lungs. The total fraction are particulates of a size range that may deposit anywhere within the respiratory tract. The total particulate fraction includes the respirable fraction.

Bulk samples of the processing tank water were also collected for microbial and elemental metals analysis. Real-time direct reading instruments were used to measure ammonia and carbon monoxide concentrations. The plant operations were evaluated at full production during the air sampling periods (approximately 8 to 9 hours). A summary of the various environmental methods is shown in Table 2.

Microscopy Analysis

Air samples were collected for microscopic analysis by drawing air at a flow rate of 2.0 liters per minute (L/min) through polycarbonate filters. An open face cassette was used to help insure an even density of particles across the filter. Settled dust sampling was conducted by placing glass microscope slides at various points throughout the facility. For certain jobs, the surface of new safety glasses, worn for a specified period, was used as a surface sampling and analysis device. Surface and filter samples were first examined with a stereomicroscope, followed by light and scanning electron microscopy. Prior to examination of the field samples, bulk samples of crab were obtained and individual fragments of meat, gill, and shell were dissected out and studied using a variety of microscopic techniques.

Direct Reading Aerosol Measurements

Real-time measurements of aerosol exposure were made using a Miniram aerosol photometer. The meter was operated in the passive mode and one-second averages were stored on a Metrosonics data logger. Both personal and area samples were collected. In order to relate periodicity of Miniram data to workplace dynamics, video images were collected during area and personal sampling segments in the cooking area. Dynamic graphs of aerosol data were created using LabView data acquisition software.

Aerosolized Protein Analysis

It was presumed that measurement of aerosolized crab protein could be used as a surrogate measure for exposure to unknown crab constituents causing respiratory symptoms and illness; and also could be used to determine relative degrees of employee exposure in the various job classifications and plant locations. Samples were collected on 37mm glass fiber filters and analyzed for total (collected at 2.0 L/min) and respirable (at 1.7 L/min) protein aerosol. All area samples were collected with open face cassettes to maximize uniform aerosol loading and exposed filtration area. Based on the splatter associated with plant processing conditions, personal sampling was collected with closed face cassettes. Samples were analyzed by the micro-Kjeldahl method. The limit of detection (LOD) for this analysis was 0.01 milligram (mg) of total Kjeldahl nitrogen per sample and the limit of quantification (LOQ) was 0.04 mg. The minimum detectable concentration (MDC) was 10 $\mu g/m^3$, based on an air sampling volume of 960 liters. Samples for the micro-Kjeldahl analysis were only collected during the early-season survey.

Microbiological Analysis

Microbiological sampling was conducted to determine the extent of any potential contamination. Bulk samples of the plant processing water were collected and analyzed qualitatively for bacteria, fungi, and algae. The liquid samples were collected in sterile containers from the various processing tanks. Samples were collected from the kanimiso water tray, cooking tank, ambient water tank, two chiller tanks, two brine tanks, two glazing tanks, and the pan washing tank. Sea water was collected as a control sample. Bacterial cultures were grown in tryptic soy agar (TSA) and incubated at 5, 25, 35, or 55°C. Fungal cultures were grown in 2% malt extract agar (MEA) and incubated at 5, 25, 35, or 55°C. The samples were incubated at temperatures consistent with the plant processing tank conditions. After incubation, the predominant genera growing in each sample were identified and counted as colony forming units (CFUs). Algae cultures were examined to identify *Hematodinium*-like dinoflagellates.

Volatile Organic Compounds Analysis

Samples were collected from various areas within the processing plant during both production and non-production shifts. Samples were also collected outside and in two other facilities buildings. Samples were submitted for qualitative analyses of VOCs using gas chromatography/mass spectrometry (GC/MS).

Area air samples for VOCs were collected on thermal desorption tubes. Thermal desorption tubes are extremely sensitive for sampling VOCs when concentrations are expected to be low. Each thermal desorption tube contained 3 beds of sorbent material, a front layer of Carbotrap Y (90mg), a middle layer of Carbotrap B (115 mg), and a back layer of Carboxen 1003 (150 mg), all housed in a stainless steel tube. Prior to field sampling, each tube was conditioned for 2 hours at 375°C. Samples were dry purged with helium for 30 minutes at 100 cc/min to remove water. During the environmental survey, air was drawn through each thermal desorption tube at a constant flow rate of 50 ml/min for a maximum of two hours. Samples were analyzed with a Perkin-Elmer automatic thermal desorption system

interfaced with a gas chromatograph with a mass selective detector.

Elemental Metals Analysis

Elemental metals analyses were conducted on bulk samples from the plant processing tanks to determine if any heavy metals were present that would interfere with the immunological testing. The water samples were collected in containers from the various processing tanks. Samples were collected from the kanimiso water tray, cooking tank, ambient water tank, two chiller tanks, two brine tanks, two glazing tanks, and the pan washing tank. Sea water was collected as a control sample. The samples were digested and analyzed by inductively coupled plasma emission spectroscopy according to NIOSH Method 7300.[10]

Carbon Monoxide Analysis

Potential sources of carbon monoxide (CO) were from the oil-fired boiler on the loading dock and from propane-fueled forklifts used throughout the plant. Sampling of CO was conducted in the bunk house (dormitory) area above the processing facility. Area concentrations of CO were determined with a direct-reading Industrial Scientific STX70 gas monitoring instrument. Measurements were collected via a datalogger over a 2-day period. The measuring range of the CO monitor was 0 to 999 ppm, in 1 ppm increments. The accuracy of this instrument was ±10%. The instrument was calibrated in accordance with the manufacturer's instructions prior to use.

Ammonia Analysis

Ammonia (NH_3) sampling was conducted near the chiller and brine tanks where ammonia is used as a refrigerant. Area concentrations of ammonia were determined with a direct-reading Industrial Scientific STX70 gas monitoring instrument. Measurements were collected via a datalogger over a 2-day period. The measuring range of the NH_3 monitor was 0 to 99 ppm, in 1 ppm increments. The accuracy of this instrument is ±10%. The instrument was calibrated in accordance with the manufacturer's instructions prior to use.

Immunological Methods - Development and Analysis

Serological Studies

Blood samples collected from study participants were analyzed for total and specific immunoglobulin E (IgE) antibodies and eosinophil cationic protein (ECP) using a standard commercial assay (Pharmacia/Upjohn, Kalamazoo, MI). Blood was centrifuged, and serum was collected and stored at -20°C until analyzed. Sera were also analyzed for specific IgE antibodies to common aeroallergens using commercial standard methods and to snow crab with assays prepared at the NIOSH laboratory (see below). All samples were tested in duplicate. Serological methods used in this study are summarized in Table 3.

Total IgE Antibodies and Eosinophil Cationic Protein

Total IgE and ECP sera levels were measured using the CAP® method (Pharmacia/Upjohn, Kalamazoo, MI), following the manufacturer's recommended procedure. In this investigation, ECP levels of 23 µg/l or greater were considered suggestive of an active inflammatory process.[11] In the adult population, total IgE levels greater than 100 kU/L are considered abnormally elevated.[12] Increases in total serum IgE levels may be noted in allergic diseases, parasitic infestations and other systemic conditions. The great majority of patients in the general population with elevated serum IgE have atopic disorders such as allergic rhinitis, atopic dermatitis or asthma. As with other laboratory tests, the total serum IgE can be properly interpreted only in reference to pertinent clinical information. Thus, a low serum total IgE level does not rule out serious allergic potential; similarly, asymptomatic

individuals with high serum IgE levels are also encountered.

Specific Antibodies

To assess atopic status, common environmental allergens were also evaluated using the CAP® method (Pharmacia & Upjohn, Kalamazoo, MI). Antibodies to a house dust mite mix (*Dermatophagoides pteronyssinus, D. farinae* and German cockroach), a mold mix (*Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus,* and *Alternaria alternata*), a weed mix (ragweed, mugwort, English plantain, Russian thistle, and lamb's quarters), and an epidermal mix (cat, dog, horse, and cow dander) were measured, following the manufacturer's recommended procedure. Pharmacia CAP results ranged from class 0 (negative) to class IV (strongly positive). Results were expressed as positive if class II or greater. Atopy was defined as having one or more positive tests.

Allergen Extract Preparation

Four types of allergen extracts of snow crab were prepared from bulk samples collected during the early-season survey. Extracts were prepared with water from the tank used to cook the crab legs, cooked and uncooked crab meat, and kanimiso. For details refer to Appendix 1.

Crab-Specific IgE Antibodies

All sera were initially assayed for IgE antibodies to crab, using a commercial Pagurus crab CAP® assay. A standard curve was constructed for each assay and appropriate positive and negative reference samples were used as recommended by the manufacturer. Test results were expressed depending upon binding relative to known positive standards provided with the commercial assay. A score equal to or greater than 2+ was considered positive. Sera initially screened using this method were characterized as strongly positive, weakly positive, or negative.

In addition to the CAP® Pagurus crab assay, RAST assays were developed at the NIOSH Immunology laboratory for the detection of specific IgE antibodies to allergens in snow crab cooking water and snow crab kanimiso, as previously described.[13] A known pool of sera positive to snow crab (provided by Dr. Samuel Lehrer, Tulane University) was used as a positive control for allergen preparations. The optimal concentrations of four allergen extracts (cooking water, uncooked meat, cooked meat, and kanimiso) were determined. Binding of IgE antibodies was detected using an I^{125}-labeled anti-human IgE (Diagnostic Products Corporations, Los Angeles, CA). RASTs performed using allergens derived from cooking water, uncooked meat, and cooked meat gave nearly identical results. Allergen from crab cooking water was chosen to be used based on results of sodium dodecylsulphate (SDS) - polyacrylamide gel electrophoresis studies (see Appendix 2). Results were expressed as a RAST score determined by dividing the counts bound by allergen-coated beads by counts bound by human serum albumin (HSA)-coated beads. Positive tests were defined as a score equal to or greater than the 90th percentile among non-exposed naive workers in the early-season survey.

Environmental Samples for Allergen Content

Air samples for snow crab allergens, total aerosolized protein, and endotoxin were collected on 37mm glass fiber filters. From one air sampling filter, results were reported for crab cooking water allergen, kanimiso allergen, protein, and endotoxin. Area samples were collected at 2.0, 4.0, and 10 L/min. Personal samples were collected at 2.0 and 4.0 L/min for the total fraction and at 1.7 L/min for the respirable fraction. Area samples were collected open- face and personal samples were collected closed-face. For additional details refer to Appendix 2.

Protein Analysis of Environmental Samples

The total protein content of the filter extracts was determined by a modification of the Lowry assay by Bio-Rad Laboratories (Hercules, CA) adapted for low concentrations of protein. The LOD for the assay as performed was 5.0 µg/ml or 50 µg/filter using bovine serum albumin (BSA) as the standard. The MDC was 52 µg/m^3, based on an air sampling volume of 960 liters. The protein content of the crab meat extracts and concentrated cooking water was determined by the Bio-Rad DC Protein assay for high protein content samples which had a 100 µg/ml limit of detection using BSA as the standard.

Endotoxin Analysis of Environmental Samples

The endotoxin content of the extracts of air sample filters was determined by the Kinetic-QLC Limulus Amebocyte Lysate assay (BioWhittaker, Inc., Walkersville, MD), using the manufacturer's recommended procedure. The results are reported in terms of endotoxin units per cubic meter (EU/m^3) and were corrected for field blanks. The LOD for the assay was 0.05 EU/ml or 0.5 EU/filter. The MDC was 0.52 EU/m^3, based on an air sampling volume of 960 liters.

Medical Methods

The medical surveys were conducted near the beginning and end of the 1998 opilio snow crab season in January and March. Advance announcement of the NIOSH medical surveys was distributed to all crab processing workers by UniSea management. UniSea scheduled willing participants for these medical evaluations during their work shifts.

Study Participants

All workers identified by management as "crab-processing workers" were eligible for entry into the study. Exclusions from the subsequent group analysis were as follows: 1) Failure to complete both early and late season questionnaires; 2) Failure to meet the definition of being a "crab processing worker" (at least 4 weeks of documented time in crab-processing job categories; see "job categories" below).

Questionnaire

Trained interviewers administered questions modified from the British Medical Research Council respiratory questionnaire[14] and the International Union Against Tuberculosis and Lung Disease questionnaire.[15] Participants were asked about upper and lower respiratory symptoms, skin symptoms, personal and family health history, work history, work activities, tenure in the crab industry, housing location, and tobacco use. Spanish and Tagalog translations of the questionnaires were used when appropriate.

Case Definitions

Changes in individual self-reported symptoms over time were evaluated among participants completing both the early and late-season survey questionnaires. Aggregates of symptoms were classified in the following categories: upper respiratory outcome, asthma-like outcome, bronchitic outcome and functional impairment.

Health outcomes were defined based on self-reported symptoms (see below). Please note that questions marked with an asterisk (*) at the early-season represent 'the last 12 months' and for those in the late-season questionnaire represent 'the last 6 weeks - during the crab season'.

Upper Respiratory Outcome: Was defined as a "yes" answer to any of the following questions: 1) do you usually have stuffy nose, or drainage at the back of your nose?; or 2) have you had two or more episodes of blocked, itchy, or runny nose*?

Asthma-like Outcome: Was defined as a "yes" answer to at least two of the following questions: 1) does your chest ever sound wheezing or whistling apart from colds or most days or nights each week?; 2) do you ever have attacks of shortness of breath (SOB) with wheezing or whistling and your breathing was absolutely normal between attacks?; 3) have you been bothered by tightness in your chest*; 4) have you been awakened by an attack of coughing, wheezing, or SOB?; and 5) have you had an attack of SOB or coughing that came on during or shortly after you stopped exercising?

Bronchitic Outcome: Was defined as a "yes" answer to both of the following questions: 1) do you usually cough on getting up, or first thing in the morning?; 2) do you usually bring up any phlegm from your chest on getting up, or first thing in the morning?

Functional impairment: Was defined as a "yes" answer to any of the following questions: 1) are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?; 2) do you get short of breath walking with other people of your own age on level ground?; 3) do you have to stop for breath when walking at your own pace on level ground?

Other factors

Tenure Status: Participants were divided into two groups based on their tenure in the crab industry. Those with four weeks or less work experience in the crab industry at the early-season survey were considered naive. The remaining participants were considered "experienced" workers. This period of time was selected because the initial survey began after the first week of the season and lasted two weeks. Therefore, a "naive" worker could be working in crab processing for up to three weeks at the end of the early-season survey.

Housing Status: Participants were classified into two groups according to the place where they were housed. Those that lived in buildings away from the processing plant were considered "off-site". The remaining participants were considered "on-site".

Job Categories: Although job categories changed during the crab season for most of the workers, workers were classified into one of the following to facilitate the understanding of health outcomes associated with job category: unloading, butchering, degilling, packing, case-up, mixed/dirty and mixed/clean. To be included in the analysis, a worker must have spent at least four weeks in crab processing. Workers were grouped in each of the job categories if they had worked in that job for three weeks or more and time spent in that job exceeded time spent in all other jobs. Workers were categorized as having a mixed job type if they had not spent at least three weeks in a single job or if time spent in a job did not exceed time spent in other jobs. The mixed/dirty group included workers with at least three weeks total in butchering, degilling or cooking. The mixed/clean group included workers with less than three weeks in those activities.

Potential high exposures: Workers involved in job categories where greater aerosol generation was observed (visually) were also grouped. These jobs included butchering, degilling and cooking.

Spirometry

Spirometry was performed following the standard American Thoracic Society (ATS) guidelines.[16] Spirometry was performed using a dry rolling-seal spirometer interfaced to a dedicated computer. Tests were conducted in a standing position and administered by trained technicians. At least five maximal expiratory blows were recorded for each test. All measured volumes 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 analysis, regardless of the blows in which they occurred.

Predicted values were calculated using published reference equations.[17] Predicted values for blacks were determined by multiplying the predicted value by 0.85 to correct for chest size as compared to Caucasians and native populations.[18] All spirometry tests that resulted in interpretable results were used in the analysis. Abnormal tests were defined as results lower than the 95th percentile limit of the predicted value (i.e., the lower limit of normal [LLN]). Five percent of a large population of asymptomatic nonsmokers would be expected to have test results that fall below the LLN, while 95% would have test results above the lower limit.[18] Abnormal test results were categorized as having a pattern suggesting obstruction or restriction as follows:

Obstruction:	Observed ratio of FEV_1/FVC below the LLN for ratio.
Restriction:	Observed FVC below the LLN and the FEV_1/FVC ratio > LLN.

Bronchodilator Response

Bronchodilator response was determined using the change in FEV_1 from the first test (baseline) 30 min. after administration of two puffs of Albuterol by metered dose inhaler. A significant change with bronchodilator was defined as a greater than 8% increase in FEV_1 from pre-bronchodilator FEV_1. This level of change is thought to represent a true FEV_1 change in airways that have an underlying bronchoconstriction and undergo dilation after bronchodilator delivery.[19] Although less stringent than the 12% threshold recommended by the ATS, this more sensitive threshold has been used in epidemiological studies.[19]

Statistical Analysis

The medical data were examined cross-sectionally at early and late-season surveys and longitudinally by examining the changes over time. These longitudinal changes were evaluated using McNemar's test. Descriptive statistics and tables were produced with Statistical Analysis System (SAS), version 6.12.[20] Univariate analyses were performed with Mantel-Haenszel chi-square test and Fisher's exact test (for data in 2x2 tables with less than five subjects per cell) using Epi Info version 6.04b. Any risk ratio (RR) higher than 1.0 was considered to be statistically significant if the lower bound of the 95% confidence interval (CI) for the RR estimate was ≥ 1.0 or if the P value was ≤ 0.05. An arbitrary risk ratio higher than 1.5 but 95% CI lower bound included one, suggested an association or trend without reaching statistical significance.

In addition, stratified analysis was conducted. For the multivariate analysis, models were fitted to the data in order to determine whether potential risk factors (tenure, job category, early season outcome, and potential high exposures) would be modified by age, race, smoking, atopy, family history of allergy, or housing.

RESULTS

Environmental Results

Workplace Observations

During the butchering process, workers were exposed to large amounts of crab meat, shell, and kanimiso which were propelled onto their rain suits, safety glasses, and exposed sections of their faces. Similar observations were made at the degilling stations, where gill tissue and shell particulate were also propelled onto the worker. Workers routinely removed the crab splatter from their safety glasses, and crab particulate was observed on employee

faces. Before taking breaks, butchering and degilling employees washed their rain suits with warm soapy water from a large bucket or stood under an overhead shower. Potential employee exposures during crab processing are summarized in Table 4.

Visible exposure to crab meat, kanimiso, and shell was less at packing, cooking tank, and case-up work stations. Employee exposure to cooking steam was observed at the entrance and exit of the cooking tank. In addition, there was condensation on the ceiling above the cooking tank. The cooking tank was provided with a local exhaust ventilation system to remove cooking steam. The exhaust for the ventilation system was located on the roof in close proximity to the crab hopper and entrance to the butchering workstations. Intermittently, reentrainment of the cooking tank exhaust into the facility thru the butchering work area was observed.

Cleaning of the processing line and tanks with high-pressured water, was another potential source of exposure to crab meat and kanimiso from underneath the conveyor belts, on the floor, and around the butchering, degilling, and packing/sorting workstations. Case-up workers were also potentially exposed during the cleaning of used crab cooking pans and kanimiso trays.

Temperature and Relative Humidity

During the January 1998 survey, the temperature inside the crab processing plant ranged from 29-47°F, with an average plant temperature of 42°F. Relative humidity (RH) ranged from 43-88%, with the average plant RH of 67%. During the March 1998 survey the temperature ranged from 45-46°F in the plant, with an average temperature of 46°F. Relative humidity ranged from 66-80%, with an average RH of 75%. Outside, the temperature ranged from 23-40°F with 43-61% RH.

Microscopy Analysis

Scanning electron microscope images of surface and filter samples collected in various areas of the plant are provided in Figure 2. Image A is of a particle isolated from a glass slide sampler from the degilling area. It was not uncommon to see this "stonehenge" arrangement of particles around the periphery of a deposit. This evaporated deposit was the liquid particle at the time of sampling. Elemental analysis on the individual particles revealed predominant sodium and chloride peaks providing evidence that the liquid was a sodium chloride (NaCl) solution (probably sea water).

Similar particles were seen which also contained various fragments of crab (shell, meat and gill) within the larger deposit. Image B is a closer view of salt crystals isolated from a glass slide sampler from the butchering area. Image C is an image of a fragment of crab meat detected on an air sample collected in the degilling area and Image D shows a particle of crab shell from an air sample taken in the butchering area. The meat particles tended to be elongated, sometimes striated and often showed evidence of tearing at the ends. Shell fragments appeared as equant flakes, often layered.

Image E is a portion of the surface of an employee's safety glasses worn in the degilling area. A predominance of large circular deposits is seen with a background of small white specks. Many of these specks were identified as individual sodium chloride crystals. Many of the particles from degilling had a gelatinous texture. On some of the larger viscous particles, selective staining confirmed the presence of protein.

Image F is from an air sample from degilling, and again evidence of a liquid aerosol is seen with a deposit pattern suggesting higher viscosity. This deposit is more subtle, appearing as a splattering which seems to radiate from a point center left on the print. The circular features on this image and also on images C and D are the holes in the polycarbonate

filter. Note that the observation of a gelatinous character to many particles observed in the degilling area is consistent with the nature of this operation which is essentially grinding away the gills from the crab legs. Although dissection showed that the gills had a geometry consisting of many thin plates, overall texture was gel-like. Slow motion close up video of the degilling area suggests that in addition to large particles, a spray of aerosol is sometimes created.

Direct Reading Aerosol Measurements

Direct reading aerosol measurements for an area sample collected in the cooking area is shown in Figure 3. The response of such monitors is affected by various physical features of a given particle including size, shape, refractive index and absorptivity. The raw output cannot therefore be related directly to more fundamental measures of particulate and the ordinate is thus labeled simply as Miniram units. The spectrum is clearly periodic and remarkable in how quickly the peaks rise and fall. Peaks were also seen in other areas of the plant, but frequency and intensity were lower. Time synchronized video overlay onto dynamic graphs of aerosol concentration from both personal and area monitors, indicated that these peaks were associated with visible clouds of condensed water vapor periodically hovering in the neighborhood of the meter inlet.

Aerosolized Protein Analysis

Personal Sampling Results

On January 23, 1998, five personal samples were collected open-face for total aerosolized protein (Table A-1). Samples were collected from one butchering and four degilling employees. Results ranged from 34 to 1500 $\mu g/m^3$ of total protein. Observation of these filters showed the presence of relatively large fragments of crab meat and gills. Subsequent sampling was done closed-faced to minimize collection of such particles. These results were used as a positive control indicating that the micro-Kjeldahl method detects crab protein effectively.

Area Sampling Results

During the January survey, 28 area samples were collected for total aerosolized protein (Table A-2). Total protein ranged from not detected for most samples, 20 of 28 (71%), to 6400 $\mu g/m^3$ at the cooking tank. Protein was detected on one sample collected from the butchering area (32 $\mu g/m^3$), two samples collected from the packing line (9.6 and 20 $\mu g/m^3$), three samples at the cooking tank (10, 11, and 6400 $\mu g/m^3$), one sample from the shipping department (39 $\mu g/m^3$), and one sample from outside on the loading dock (11 $\mu g/m^3$). The three samples collected outside on the loading dock were intended to be negative controls and were not expected to yield any aerosolized protein.

Respirable protein was not detected on any of the 12 area samples collected (Table A-3). See Table 5 for a summary of the aerosolized protein sampling results.

Microbiological Analysis

Bulk samples of the processing tank water were analyzed for bacteria, fungi, and algae. Processing tank water is from a municipal source that is disinfected with chlorination treatment. No sanitizing chemicals, biocides or other reagents are added to the processing water except for the brine tanks where salt is added.

Bacteria

Bacterial levels ranged from 110 to >37 million CFU/ml. Gram-negative bacteria were the dominant group identified. Mesophilic bacteria (at 25°C) were detected in the ambient water

tank, chiller tanks 1 & 2, glaze tanks 1 & 2, and in the pan wash tank. The chiller tank samples (>37 million CFU/ml) were both overloaded at 74,000X dilution.

Fungi

Fungal levels ranged from <110 CFU/ml (below detection) in Brine Tank 1 to 7260 CFU/ml in the Glaze Tank. Yeasts and Rhodotorula (a pink yeast) were the only fungi detected in four samples. Fungi were not detected in seawater, the kanimiso water tray, the ambient water tank, in chiller tank 2, in brine tank 2, or in the pan wash tank.

Algae

Samples were analyzed for dinoflagellates that can infect the snow crab. Many live *Hematodinium* spp. dinoflagellates were identified in the kanimiso water tray, the ambient water tank, and the two chiller tanks. A few live dinoflagellates were identified in seawater obtained dockside. Dead *Hematodinium* spp. dinoflagellates were also found in chiller tank 1.

Volatile Organic Compounds Analysis

Ten samples were collected for VOCs: Four samples were collected during crab production: one sample was collected in the adjacent screening plant where crab shells are ground, four samples were collected in the crab plant when the process was down, and one sample was collected outside. Only trace levels of contaminants were detected on any of the samples, but dimethyl sulfide was detected in the screening plant. Based on these results, crab processing employees exposure to volatile organics was minimal.

Elemental Metals Analysis

Analysis of bulk water samples from the plant processing tanks did not indicate any appreciable concentrations of toxic elemental metals.

Carbon Monoxide

Real-time carbon monoxide sampling was conducted in the employee bunkhouse above the crab processing plant where the only potential CO exposure was from employee smoking. CO levels were determined from March 6 to 8, 1998. Levels ranged from 0 to15 ppm which indicated that CO concentrations were below all occupational exposure criteria. Carbon monoxide was not detected within the processing plant or outside.

Ammonia

Real-time ammonia sampling was conducted at the chiller tank cooling unit which uses ammonia as the refrigerant. On March 6 to 8, 1998, ammonia concentrations ranged from 0 to10 ppm with a 6.5 ppm TWA, indicating that ammonia levels were below occupational exposure criteria.

Environmental Samples for Allergen Content

Each filter was analyzed for Lowry protein, snow crab cooking water allergen, kanimiso allergen, and endotoxin. Samples were collected in the butchering, degilling, packing, cooking tank, and case-up areas. Samples were also collected in non-crab exposed and comparison areas, such as outside, the employee break room, and in other production areas within the facility including the surimi plant. The environmental sampling results for allergen content are summarized in Table 6.

Personal Sampling

During both surveys, 43 personal samples were collected for immunological analysis. All samples for the total aerosol fraction were collected closed-face (see Table A-4). In addition, 12 personal samples were collected for the respirable aerosol during the early-season survey (see Table A-5).

Lowry Protein

Aerosolized protein was detected on 2 of 43 (5%) of the samples collected (Table A-4). Total aerosolized protein was only detected from a cooling tank employee (69 $\mu g/m^3$) and from a shipping employee (63 $\mu g/m^3$). Respirable protein was only detected on 1 of 12 (8%) of the samples collected. This was collected from a cooking tank operator (56 $\mu g/m^3$).

Crab Cooking Water Allergen

Crab cooking water allergen was only detected on 8 of 43 (19%) of the samples collected. The single highest measured exposure to crab allergen was for one packing employee (150 RAU/m^3). Differences in level of exposure between the job categories were not clearly demonstrated.

Respirable crab water allergen levels ranged from not detected to 58 RAU/m^3 from a cooking tank employee. Crab allergen was detected on 3 of the 12 samples collected (25%). Results did not clearly differentiate between task categories.

Kanimiso Allergen

Kanimiso allergen was detected on 26 of 43 (60%) samples collected. The highest kanimiso level was 830 RAU/m^3 for the freezer forklift driver. Although statistically significant differences in exposure levels were not demonstrated between the various task categories, the highest individual measurements were collected from degilling and case-up/shipping employees.

Respirable kanimiso allergen was only detected on three of the 12 samples collected. All three samples were collected from butchering employees with levels of 844, 1420 and 2270 RAU/m^3. These results are suspect, as every one of these "respirable" measurements markedly exceeds those measured for the 43 total kanimiso allergen levels noted above. We speculate that butchering process may be associated with contamination of the filters in the cyclones used for sampling through droplet or particulate splatter, and that these high levels are not truly reflective of aerosolized kanimiso allergen in the respirable size range.

Personal sampling results for kanimiso and snow crab allergen for each task are displayed in Figure 4. Only samples for which detectable levels were obtained are presented in the figure. The results indicate that there is great variability in allergen exposure among employee tasks. Unlike previous reports in the literature, no single job title appeared to be associated with the highest allergen exposures.

Endotoxin

Although large quantities of Gram-negative bacteria were found in bulk samples of the plant processing tanks, personal levels of endotoxin were generally very low.

Total endotoxin concentration for the 43 samples collected ranged from 1.1 EU/m^3 for an employee at the exit of the cooking tank to 949 EU/m^3 for a shipping employee (see Table A-4). This single level was more than 10-fold greater than any other measurement. Only 4 samples exceeded 25 EU/m^3 of which 3 exceeded 50 EU/m^3. The mean exposure was 32.6 EU/m^3 with a standard deviation of 143.9 EU/m^3.

In the 12 respirable samples collected, endotoxin ranged from not detected for two butchering employees to 39.0 EU/m³ for a shipping employee. The mean exposure was 15.6 EU/m³ with a standard deviation of 11.8 EU/m³.

Area Sampling

Sampling data were also collected in control locations outside the crab plant, in low exposure areas within the crab plant (i.e. washroom, break room and production office) and other production areas not associated with crab production (see Table A-6). The immunological data collected in the surimi plant indicated the

average Lowry protein concentration was 34 µg/m³ with a standard deviation of 3.8 µg/m³. Surimi was a different allergenic exposure (fish) and crab and kanimiso allergens were not detected.

Medical Evaluation Results

Study Participation

During the early-season survey, 95% (152 of 160) of the potential study participants completed the questionnaire. During the late-season survey, questionnaire results were obtained from 113 participants. Nineteen of the early-season participants had left the facility; nine of these subsequently completed follow-up questionnaires by telephone. Thus, an over-all participation rate of 76% (122 of 160) was attained for completion of both the early and late-season questionnaires.

Study Exclusions

Of the 122 participants completing both questionnaires, 15 were excluded from the subsequent group analysis, although individual health outcomes were noted and are documented as appropriate later in the report. Eleven of these individuals were excluded because it was found that they had spent most of their time in activities other than crab processing, primarily surimi processing. One individual could not be classified as a crab processing worker due to lack of information about his occupational activities. Three additional participants were excluded because they left the facility prior to spending four weeks in crab processing, and thus were not defined as crab processing workers. Of these three, one left due to a death in the family, one left to have surgery related to a musculoskeletal problem, and one left after one week due to pre-existing asthma that was markedly exacerbated by his job in case-up. Thus, the group analysis focuses on a total of 107 participants who completed both early- and late-season questionnaires and met the definition for "crab-processing worker."

Demographic Information

Table 7 shows demographic characteristics of 107 workers completing both surveys, 15 workers completing both surveys but excluded from the group analysis because they did not meet the definition for "crab processing worker," and 30 workers completing only one survey. The mean age for these workers ranged between 35 and 40 years. The majority of participants were males, except for the group of 15 workers failing to meet the definition of "crab processing worker." The percentage of white participants ranged from 37 to 53%. The longest place of residence for most workers was outside the United States. Nearly half of the workers had worked more than four weeks in the crab industry. About half of the workers had never smoked cigarettes.

The percentage with a family history of allergies among all workers ranged between 21 to 33%. Total level of IgE higher than 100 kU/ml ranged from 27 to 42%. The percent with elevated total IgE was very similar to the percent with atopy in the group of 107 crab processing workers that completed both surveys (Table 7).

Doctor-Diagnosed Asthma

At the early-season baseline survey, five individuals noted a previous doctor-diagnosis of asthma. Three indicated that their asthma was inactive, and they were not taking medications for the condition. Two indicated that their asthma was active and they were using medications for the condition. One of these had onset of asthma in childhood. The other had onset of asthma at the age of 58, several years after entering employment in the seafood industry. Of the five individuals with a doctor-diagnosis of asthma in the early-season survey, four participated in the late-season survey.

Over the course of the season, one of the participants with a previous doctor-diagnosis of asthma experienced significant work-related worsening of asthma. This was the individual with active asthma at the baseline evaluation and adult-onset as already described. The individual worked in case-up for only one week prior to leaving the facility due to work-related exacerbation of asthma. This participant had a positive family history of allergies, met the criteria for atopy, had levels of serum total IgE higher than 100 kU/ml, and was demonstrated to have serum specific IgE antibodies to crab water and kanimiso by RAST. IgE antibodies to crab Pagurus by CAP® method were negative at the early-season survey. Spirometry was abnormal relative to the predicted values: FEV_1 47%, FVC 69% and FEV_1/FVC ratio 56%. Bronchodilator response was not assessed.

In addition to those with a doctor-diagnosis of asthma during the baseline evaluation, two individuals were noted to have acquired new doctor diagnoses of asthma over the course of the crab processing season.

One of these individuals worked in the "meal plant." This individual had worked in crab processing for a period of one month at the end of the previous crab processing season. Despite acquiring a new doctor-diagnosis of asthma, the individual remained employed by the facility and the asthma did not worsen over the course of the season. There was a positive family history of allergies. The individual was demonstrated to be atopic by detection of serum specific IgE antibodies to common inhalants by CAP® method. However, the level of serum total IgE was less than 100 kU/ml. Serum specific IgE antibodies to crab kanimiso and crab water measured by RAST became positive at the end of the season. Specific IgE antibodies to crab Pagurus detected by CAP® were present at both the early and late season. Spirometry was normal.

The second individual with new doctor-diagnosed asthma during the course of the crab processing season worked for 2 weeks in packing and 4 weeks in sanitation. The individual had no previous experience working in the crab processing industry. This individual was unable to finish the crab processing season because of the severity of the respiratory symptoms, thus was not available for blood collection or spirometry in the late-season survey. In the early-season the participant had wheezing, shortness of breath and chest tightness; he did not know whether there was a family history of allergies. He was not atopic, had serum levels of total IgE that were less than 100 kU/ml, and did not have serum specific IgE antibodies to crab detectable by RAST and CAP® methods. Spirometry in the early-season was compatible with mild obstruction, with FEV_1 69%, FVC 86% and the FEV_1/FVC ratio 69% of predicted values. FEV_1 improved by 11% after the administration of bronchodilator.

Respiratory Symptoms

Results shown in Table 8 describe findings among the 107 crab processing workers who completed both the early and late season surveys. Overall, increases in the prevalence of reported upper respiratory symptoms were observed during the season. New onset of nasal symptoms associated with stuffy nose was reported in 37% and nasal obstruction or runny nose in 45% of the participants without these symptoms in the early-season. Changes were also

observed in asthma-like symptoms. New onset of being awakened by an attack of cough, wheezing or SOB was reported in 32%, and chest tightness in 19% of the participants. Increase in bronchitic symptoms was also reported over the season. The incidence of usual morning cough was 26% and usual morning phlegm was 30%. In contrast to upper respiratory, asthma-like, and bronchitic symptoms, there was only a minor change in the functional impairment (see Methods for the definitions) over the course of the season.

Health Outcomes

All respiratory health outcomes except for functional impairment were present in significantly greater proportion of workers in the late season, as compared to the early season. Incidence (new cases) of the upper respiratory outcome in those without it at baseline was 56%, incidence of the asthma-like outcome was 26%, and incidence of the bronchitic outcome was 19% (Table 9).

Spirometry and Bronchodilator response

Spirometry was available for 91 (85%) of crab processing workers completing the early and late-season surveys. The mean values for the group of all workers of FEV_1, FVC and the FEV_1/FVC ratio did not change substantially over the crab season. Overall, a pattern of obstruction was observed in 4% and restriction in 3% of crab processing workers in the late-season survey. Post-bronchodilator change in FEV_1 were seen in 7% of workers in the early-season and in10% in the late-season evaluation (Table 10). Of those who had positive bronchodilator response at the late-season, 2 were positive at both early and late-seasons, and 8 developed "new" bronchodilator response. The profile of these 8 workers includes family history of allergies present in all of them, atopy present in 3, specific IgE antibodies to crab Pagurus and kanimiso present in 2, all 8 worked in various indoor job categories (none worked in unloading), 4 were current smokers, 2 developed new onset of asthma-like symptoms (one had IgE antibodies to crab), and 4 developed bronchitic symptoms.

IgE Antibodies

Serum levels of IgE were determined for 96 crab processing workers; the median level of total IgE for all workers was 84 kU/l at the early-season. Early-season levels stratified by tenure status showed a non-statistically significant median difference of 91 kU/l among naive workers and 64 kU/l among experienced workers (Table 11).

The presence of serum antigen-specific IgE was detected using the CAP®method for Pagurus crab and by RAST for allergens derived from opilio snow crab kanimiso or cooking water (Table 11). Twelve percent of naive workers were sensitized to each of the allergens derived from opilio snow crab in the early-season. Lower percentages of experienced workers were sensitized to these allergens in the early season (kanimiso 5%; crab water 2%). In contrast, similar proportions of naive and experienced workers were sensitized to the Pagurus crab allergen in the early-season (12% and 14%, respectively). The proportion of workers sensitized to each of the allergenic preparations did not increase over the course of the crab processing season.

Most of the doctor-diagnosed asthma cases were demonstrated to have serum IgE specific antibodies to crab. In those with specific IgE antibodies to crab, 50% developed asthma-like symptoms in contrast to 25% of those without specific antibodies. Although, these information is limited to the small number of subjects, these data suggest an association between allergy sensitization and health outcomes. Moreover, two of three workers with doctor-diagnosed asthma affected by work had elevated serum levels of anti-crab IgE. This suggests a role of IgE-sensitization in their disease. The remaining worker did not have blood drawn in the late-season, so it is unclear if IgE-sensitization played a role in his disease.

Risk Factors Associated with Upper Respiratory Outcomes

Among the crab processing workers, 69% (74 of 107) did not have upper respiratory symptoms at early-season and therefore were at risk for subsequent development of the upper respiratory outcome during the crab season. In the univariate analysis, workers with a positive family history of allergies or working in butchering had a statistically significant increased risk for development of the upper respiratory outcome (Table 12). In addition, a trend was noted for increased risk in workers less than 35 years of age. It was noted that butchering and degilling operations were carried out by males. Further stratified analysis by gender was not different from the univariate analysis. Additionally, multivariate analysis was performed controlling for age, smoking, and family history of allergies. Results from this analysis were similar to the univariate analysis.

Risk Factors Associated with Asthma-like Outcomes

Among the crab processing workers, 86% (92 of 107) did not satisfy the criteria for asthma-like outcome in the early season and therefore had the potential to develop this outcome during the subsequent season. Univariate analysis for risk factors associated with incidence of this outcome showed male gender, family history of allergies, elevated ECP, butchering, and degilling to be significantly associated (Table 13). An increased risk for the asthma-like outcome was also noted in association with age < 35 years, smoking, naive tenure status, case-up and packing, although these associations did not attain statistical significance. Multivariate analysis of risk factors for the asthma-like outcome did not show any additional associations or changes other than those demonstrated by the univariate analysis.

Risk Factors Associated with Bronchitic Outcomes

At the start of the crab processing season, 98% (105 of 107) of the participants did not have bronchitic outcome. Univariate analysis of risk factors potentially associated with the bronchitic outcome showed significant associations with age < 35 years, male gender, elevated serum anti-kanimiso IgE and anti-Pagurus crab IgE, elevated serum ECP and degilling. Increased risks that were not statistically significant were observed for smoking, atopy, family history of allergies, naive tenure status, and "high exposure jobs" (Table 14). Multivariate analysis controlling for age, smoking, and family history of allergies yielded adjusted relative risk ratios similar to those noted in the univariate analysis.

DISCUSSION

The environmental survey at UniSea was the most extensive characterization of exposures in the crab processing industry. It revealed that processing workers are exposed to a predominantly liquid aerosol. The viscosity of the liquid can vary, as can individual components (sea minerals, shell, meat, gill, etc.) during different job tasks. Further, the aerosols have been shown to be polydisperse. Microscopy revealed particles as small as a few micrometers while some particles observed on the surface of worker's safety glasses were in the millimeter size range.

Slow motion close-up video of employees performing their tasks indicated that aerosols are formed through mechanical action in the butchering and degilling areas. The direct reading aerosol Miniram data indicate that although steam peaks were observed throughout the processing plant, the frequency and intensity of these peaks were highest in the cooking area. Although steam peaks are dramatic in both visual impact and meter response, it is unclear whether this would be expected to contribute to adverse health outcomes. Steam is

being generated within an environment where we clearly have the potential for aerosolized protein through other forms of generation, and the predominant effect of steam may be to influence the size, lifetime or other dynamics of these existing particles.

The reason the aerosolized protein and allergen data were variable and largely below limits of detection throughout the plant is perhaps related to particle size and sampling inlet geometry. On the first sampling day, personal samples were collected with an open-faced inlet. At the end of sampling, visible pieces of crab were observed on the filter. For this reason subsequent personal samples were collected with a closed-faced inlet. For open-faced samples, all collected quantifiable protein. On the other hand, only a few of the personal closed-faced samples were above the limit of detection. Although microscopic analysis confirmed the presence of small particles, it may be that the majority of aerosolized protein is in the form of large localized particles accelerated away from various processing points. If true, the sampling efficiency of such particles would be largely influenced by the area of the inlet opening. Examination of the surface of employees' safety glasses shows that in certain tasks, large particles are accelerated in the direction of the workers. Further, we know that these particles can contain fragments of crab. Although there is some probability that the trajectories of these particles could result in their entering a small inlet, the probability is much lower relative to the open-faced configuration. This could also explain the occasional high value for certain assays mixed in among the many non-detectable levels within the same job or area.

Water is a major feature of this work environment. Since water provides general aerosol suppression, this may also have some influence on the control of small particles. Microscopy analysis did, however, confirm the presence of small particles. This suggests the possibility that our limits of detection for protein and allergen may have been only sufficiently sensitive to occasionally quantify large, localized aerosol, but inadequate for quantifying the small particle component. The fact that our highest volume sampler results were all below our limits of detection for both protein and allergen would support this. Note that these samplers, being physically large, required that they be placed away from the immediate work area and thus away from direct splash. Higher flow rates are advised for any future study to improve assay sensitivity to crab allergen.

This investigation documented several cases of work-related asthma and confirmed high prevalence and incidence rates of respiratory symptoms among crab processing workers. These findings are consistent with those noted in the literature. [2,3] Studies in the crab processing industry conducted by other groups also document similar findings to those noted in this study.[3,4] For example, Cartier and co-workers reported 19% (57 of 303) of crab processing workers suffered with productive cough and dyspnea and 21% (64 of 303) had symptoms suggestive of occupational asthma. Occupational asthma was confirmed in 50% of these cases.[3] Snow crab allergen liberated during the crushing of shells, boiling of whole crab, and cutting and separating of legs was the proposed asthma-inducing exposure as there was an association between skin test reactivity and symptoms. In our study, the 21% prevalence of productive cough (bronchitic outcome) was very similar to that reported by Cartier. However, the prevalence of asthma-like symptoms in our study was 32%, higher than that reported by Cartier. Differences in the case definition or exposures might be a factor(s) influencing such difference.

A study among king crab processing workers from a factory in the state of Washington also yielded results similar to those of this study. An increased prevalence of respiratory symptoms in 15 randomly selected workers, as compared to fish processors not exposed to crab, was noted. In addition, skin prick test using crab meat, dust from the machines, and crab shell resulted in immediate skin reactions in some of these workers and in none of the controls. However, skin test positivity did not correlate with symptoms. Precipitin (IgG) antibody reactions to all

three components (meat, shell, and dust) were found in serum from 60% of the crab workers compared to none of the control workers (non-crab processors). IgG antibodies did not correlate with the respiratory symptoms.[21] The predominant symptoms included productive cough and dyspnea. As was the case in the current investigation, no major changes in spirometry were observed among participants.

Doctor-Diagnosed Asthma

In our study, two incident cases of adult-onset of asthma were documented among 107 participating workers without a previous history of asthma over the approximately 41-day period of observation. A population-based study in the U.S. [22] suggests an asthma incidence of 2.1 cases/1000 person-years, in the general population. More recently,[23] a random sample of nearly 16,000 European subjects reported an incidence of adult-onset of asthma (defined as a positive response to a question about the presence of physician-diagnosed asthma) of 1.3 cases/1000 person-years for women and 1.0/1000 person-years for men.

Spirometry and Bronchodilator Response

Bronchodilator response was used to assess the effect of crab processing work on airways reactivity. During the early-season, 7% of study participants had a bronchodilator response and 10% (10 of 91) did at the end of the season. Eight were new cases. Our results differ from a previous study among crab processing workers in which only 1.4% (4 of 290) workers had a change in FEV_1 (between 15 to 20%) after bronchodilator administration.[3] This difference might be due to the threshold FEV_1 response used to define a bronchodilator response in our study. Other studies have used histamine challenge or specific allergen challenge to assess airway hyperreactivity in workers with diagnosis of occupational asthma among crab processing workers.[2,24] These methods offer a more precise means to demonstrate specific airway responsiveness, but were not feasible in this investigation.

IgE Antibodies

In our study, participants' median level for total IgE at the early-season was 84 kU/l with the 25th and 75th percentiles at 25 kU/l and 273 kU/l, respectively. IgE is a marker of allergic diseases; high serum levels are present in individuals with asthma, allergic rhinitis, or atopic dermatitis. Gender, age, and tobacco smoke may affect the levels of IgE. Increased levels could be present in parasitic infestation and bronchopulmonary mycosis.[25] In this investigation, high levels of IgE due to parasitic infestation were not ruled out.

The levels of total and specific IgE antibodies were higher among naive workers in relation to the experienced workers. This may suggest that workers from previous seasons who became sensitized (produced specific IgE), and possibly symptomatic, were less likely to return for work in the current season. This is consistent with a 'healthy worker effect', with the experienced workers who do return being healthier than those who do not return. This selection out of work of less healthy workers had not occurred yet with the naive participants. A healthy worker effect in experienced workers is supported by the observation that naive workers were more likely to develop symptoms and adverse health outcomes in the three categories analyzed.

In this investigation and in previous reports among workers in the crab industry,[3] personal atopy, or the tendency to mount IgE responses after antigenic exposure, was not noted to be significantly associated with symptom status. However, family history of allergy did show significant associations with the presence of symptoms. This is perhaps due to a family history of allergies being a marker for genetic predispositions with greater impact on symptom status in crab workers than on the development of IgE responses.

Limitations on exposures and health effects

A limitation of this study is the use of non standardized sera for the development of the immunological assays. This might explain some of the discrepancies between this work and other published work. Thus, potential technical problems related with assay sensitivity or inappropriate solid-phase allergen exist. In addition, symptoms being triggered by materials of crab origin through a mechanism other than IgE sensitization, or triggered by exposures other than those of crab origin (e.g., hypertonic saline aerosols, cold air, or exercise) should be considered.

In our study, workers involved in unloading activities outside the facility appeared to have fewer symptoms relative to workers inside the facility. This suggests that air quality inside the facility is associated with the development of respiratory symptoms. Several factors might non-specifically trigger such symptoms in susceptible individuals, including salt-containing aerosols and low temperatures. In addition, associations of symptoms with particular operations such as butchering and degilling suggests that the aerosols generated in these operations are able to trigger respiratory symptoms even in the absence of allergic sensitization.

Workers at the plant currently are completely covered except for certain areas of the face (safety glasses are worn). Past work has demonstrated the role of mucosal surfaces in initiation of an immune response.[27] Since safety glasses leave the nose and mouth exposed, face shields could be considered as an alternative to glasses in these areas. However for asthma, we do not know the full significance of particle size and the possibility that the small particles observed in this environment, even if they represent only a small portion of the total airborne mass, may be most important in the sensitization or development of an allergic reaction. Face shields would do little to prevent respiratory system exposure to these particles.

An important determinant of occupational asthma is exposure. Several studies have shown that the degree of exposure determines the likelihood of immunological sensitization and asthma. In different studies, personal samples have been useful to quantify individual exposure to biologic substances in the workplace using immunochemical methods.[26] In this investigation we were unable to document a quantitative exposure-response relationship leading to respiratory illness in crab processors. However, we documented qualitative exposure-response relationships in the process-related risks of respiratory outcomes in relation to degilling and butchering. Thus, further studies are needed in a industry wide to better understand the magnitude of the problem and to improve current methodology for the assessment of environmental allergens in the workplace.

CONCLUSIONS AND RECOMMENDATIONS

- Crab processing workers in butchering, degilling and other indoor work had excess incidence of respiratory symptoms over the six weeks of crab processing in comparison to outdoor unloaders. This excess of persistent and new onset respiratory symptoms appears to be occupationally related.

- Cases of new and exacerbated doctor-diagnosed asthma occurred during the season, most of whom had evidence of immunologic reactivity to crab-derived antigens.

- Although we were unsuccessful in quantifying protein and allergen exposures, the qualitative exposures in specific crab processing jobs were related to symptom risks.

The following are specific recommendations for this workplace:

1. Medical Screening: A medical monitoring program should be in place for the early detection and prevention of acute and chronic work-related adverse health effects:

   To identify those at particular risk, new employees should have their medical history taken including questions regarding pre-existing respiratory symptoms and disease, asthma, and history of crab allergy. Physical examination with particular attention to the respiratory system should also be done. These exams provide a baseline for comparison and an opportunity for education. It should be noted that under the Americans with Disabilities Act (Public Law 1-1-336 (S. 993); July 26,1990), unless these examinations reveal a disabling condition which would prevent the applicant from performing the essential functions of the job, if "reasonable accommodations" were made, the applicant may not be refused employment. Thus, a history of pre-existing asthma would not be grounds for refusing an individual employment.

   During the season, workers with clinical evidence of asthma-like symptoms (e.g. wheeze, cough, chest tightness, awakened by attacks of cough, or shortness of breath) should be referred for a more thorough medical evaluation. This might include lung function testing to document asthma presence and severity, such as spirometry, bronchodilator testing, and methacholine challenge. Testing should conform to recent National Asthma Education and Prevention Program Guidelines for diagnosis and management of asthma.[1] Also, testing for IgE-sensitization to crab using available allergy skin tests or blood tests should be considered. Detection of specific IgE antibodies is a suggestive, but not completely specific marker for allergy; thus, clinical judgment must be considered for its interpretation.

   Individuals who develop work-related asthma should be offered work assignments that will minimize their inhaled exposure to crab proteins. Workers would have less incentive to conceal work-related health problems or to continue working in areas of potential exposure if, after job transfer, they retained all wages and benefits associated with their previous job.

   Employees should receive written reports of all medical surveillance tests performed by the company or by a physician to whom the company makes referral, regardless of the results of such tests.

2. Worker Education: Equipment maintenance and worker education are vital aspects of a good occupational health and safety program. Workers must be informed of (1) any potentially hazardous materials and (2) the nature of the potential hazard. Workers should be instructed to follow safe work practices to help protect their health and safety and that of their fellow workers. This information should be transmitted by means of a hazard communication program, which is to include container labeling, MSDSs, and worker training. With regard to working in crab processing, workers should be instructed in the risk of occupational asthma, and other respiratory symptoms, the type and timing of symptoms, the importance of early detection and intervention, and actions (e.g. personal protection) the worker can take to prevent developing an immunologic response to crab proteins.

3. Engineering Controls: In other industries, reduction in allergen exposure decreases risk of IgE-sensitization and respiratory illness in those already sensitized.[28] To decrease these exposures, spray-generating tasks such as butchering, degilling, and cooking, should be enclosed if possible. Ventilation should be

modified as possible in areas where these tasks are conducted to direct airflow away from workers.

4. Administrative Controls: Presence in the crab processing facility should be restricted to workers who are essential to its operation. Smoking should be prohibited inside the facility. It should only be allowed outside or in designated areas with independent exhaust ventilation such that smoke is not re-circulated within the building. Similar regulation should be applied to dormitories. Employees who continue to smoke should be counseled on how smoking may exacerbate the adverse health effects of occupational respiratory hazards.

5. Personal Protection: The use of respirators is the least preferred method of controlling worker exposures. Respirators should not be used as the only control for routine operations. However, a trial of using approved particulate respirators should be considered for symptomatic workers if the controls already noted are inadequate to reduce symptoms. This trial should be followed by further evaluation of its effectiveness in reducing the rate of respiratory symptoms. Respirators should be used as part of a comprehensive respiratory protection program. This program should include regular training and medical evaluation of personnel and fit testing, periodic environmental monitoring, and maintenance, inspection, cleaning and storage of equipment. The program should be evaluated regularly. For specific information and decision logic refer to the NIOSH guide to industrial respiratory protection.[29] Because of the potential ability of crab allergen to induce sensitization by skin contact, workers should continue to be protected from contact through the use of impervious protective clothing such as gloves, aprons, rainsuits, and protective footwear. In addition, workers engaged in activities exposed to particle spray such as butchering, degilling or cooking should be protected using face shields.

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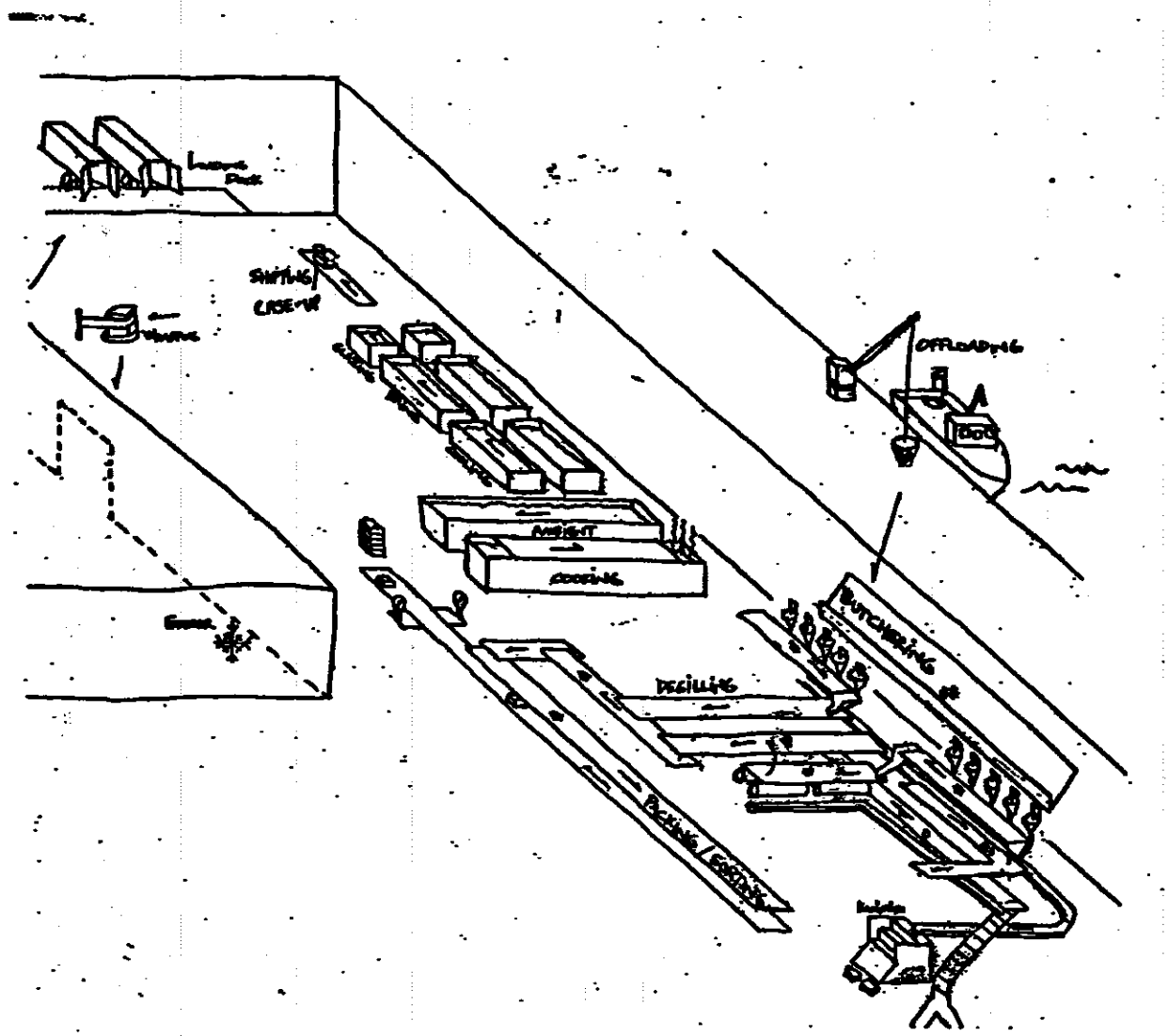
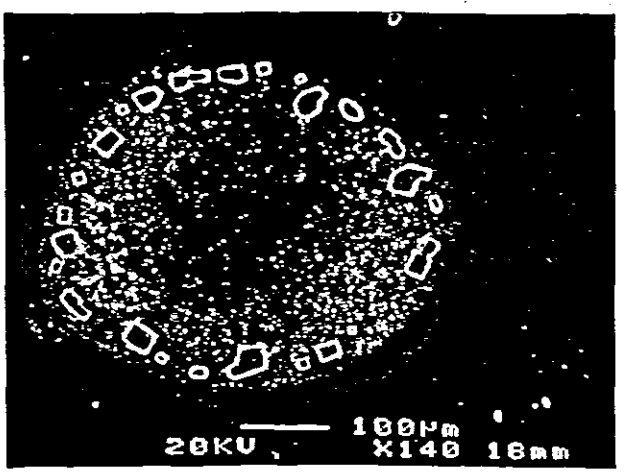
Figure 1. Diagram of snow crab processing at UniSea Inc., Alaska

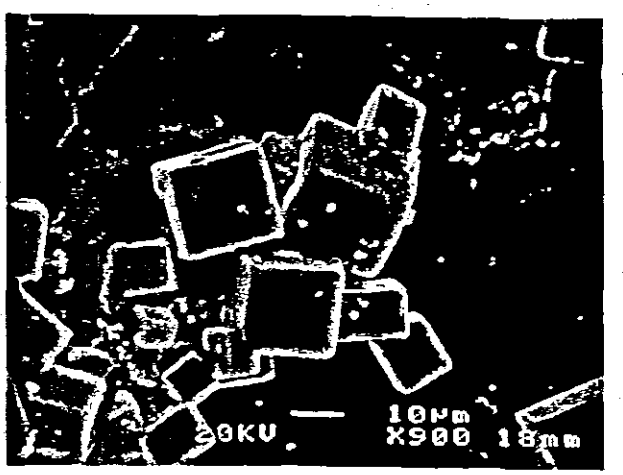


Initially crabs are transported from the ship holds to the plant dock; at the butchering station crab legs are separated and transferred to degilling station. The degilled crab leg clusters are conveyed to the packing and sorting line. Leg clusters are sent to the cooking tank, followed by rinsing and cooling. Finally, crab legs are frozen and packed at the case-up station.

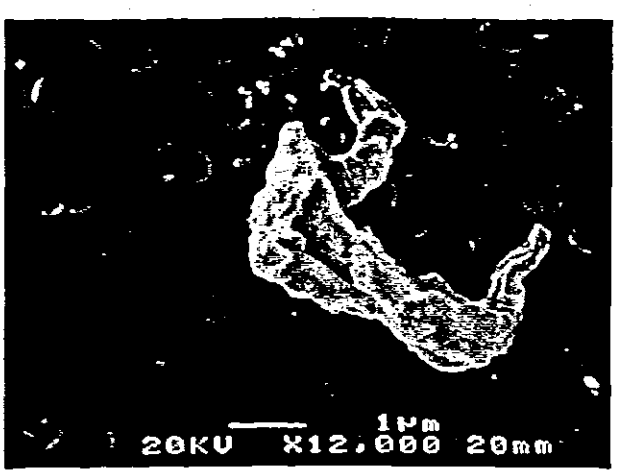
Figure 2. Scanning Electron Microscope Images of Various Particles Isolated from Crab Processing Environment



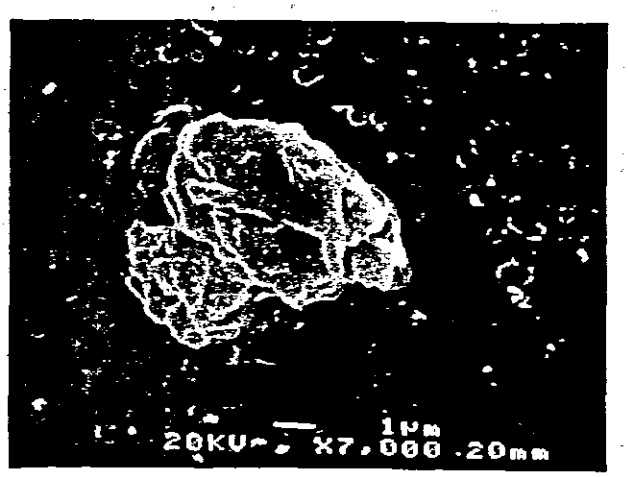
A) Evaporated particle observed on glass slide sampler placed in degilling area - note arrangement of particles at the periphery



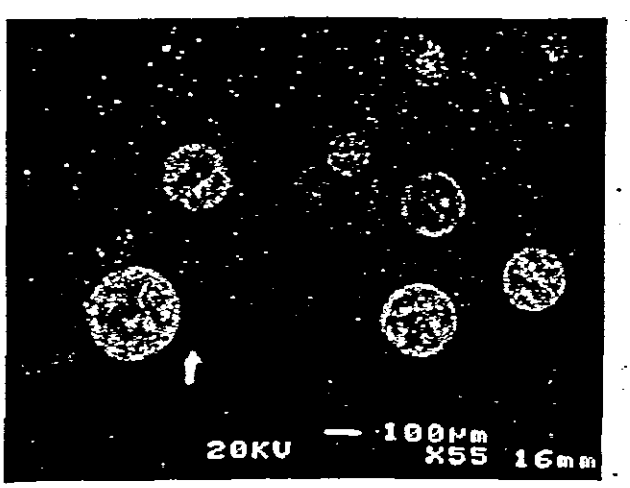
B) Sodium chloride crystals isolated from a glass slide sampler set out in the butchering area



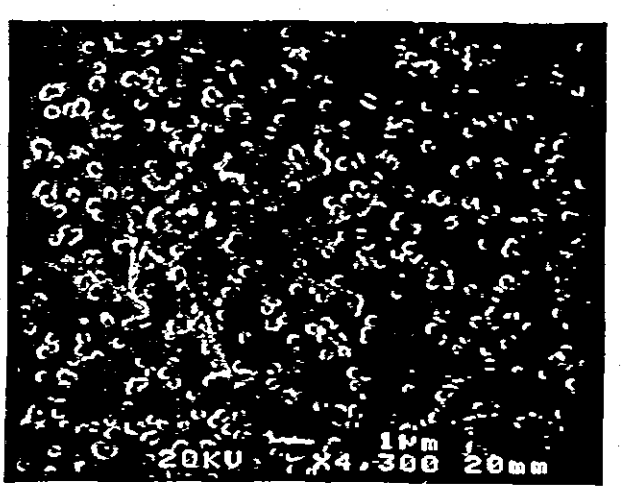
C) Fragment of crab meat from filter sample collected in the degilling area



D) Particle of crab shell from filter sample collected in the butchering area



E) Particles observed on surface of safety glasses of worker from degilling area



F) Particle deposit (center left) observed on filter sample from degilling area

Figure 3. Direct Reading Aerosol Measurements

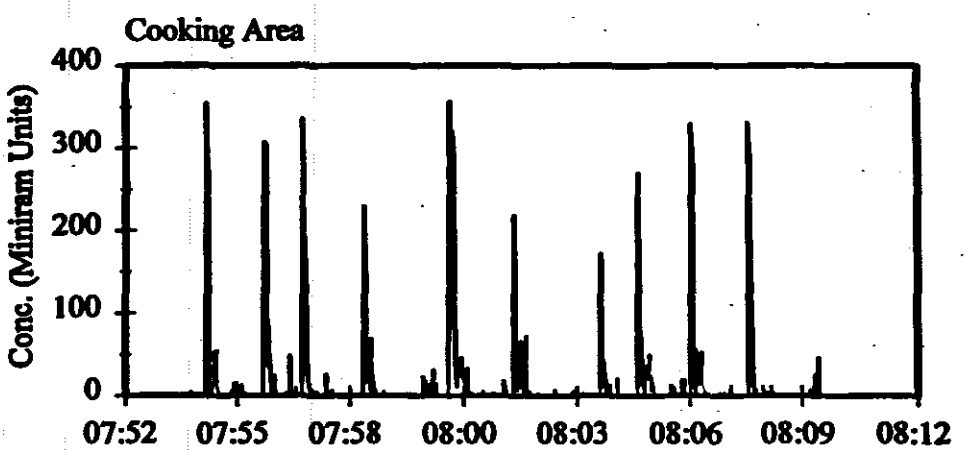
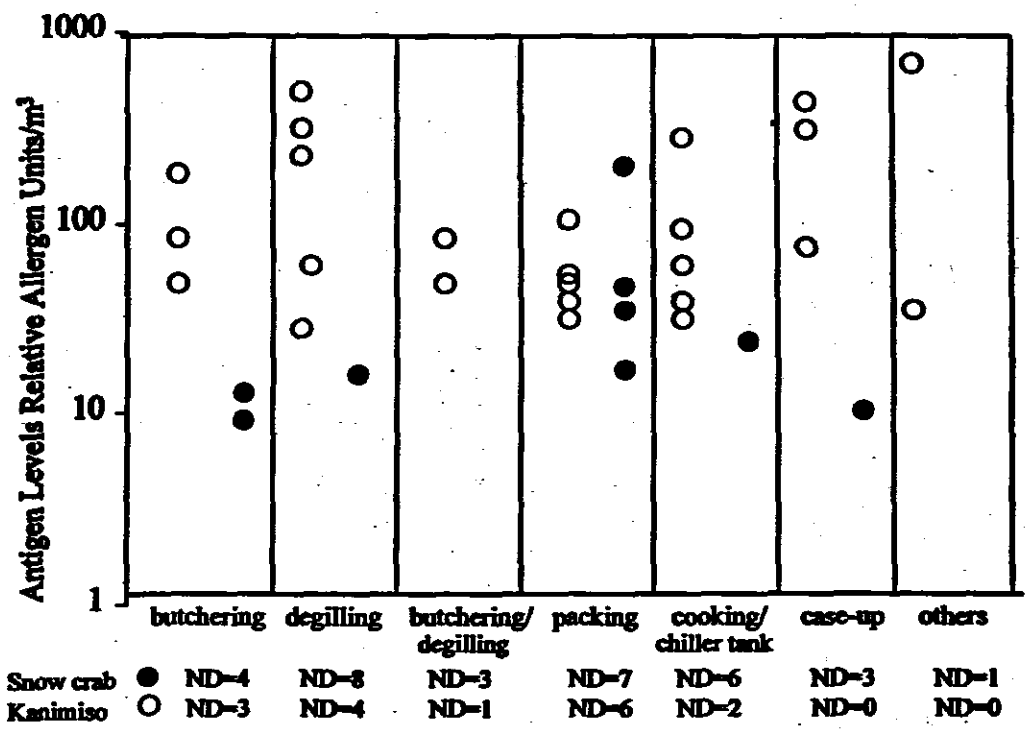


Figure 4. Personal Sampling Results with Detectable Levels



ND: Not detected

Table 1. Summary of the Environmental Evaluations Conducted during Crab Processing

Author	Analyte	Number of samples (N)	Range of Exposure	Potential Exposure Sites
Beaudet,[6] 1994	Total Particulate	$N_{split\ line}$=2 $N_{score\ line}$=3	0.11, 0.16 mg/m^3 3-hr TWA ≤0.03 mg/m^3 3-hr TWA	Splits & Scoring Lines
Edelman,[7] 1994	Total Particulate Sodium Bisulfite Organic Matter Inorganic Matter	N=4 N=6	0.14-0.68 mg/m^3 Not Detectable protein, cellulose, synthetic fibers, silicate & paint Si, Al, Fe	Butchering & Packing Areas
Malo,[8] 1997	Snow Crab Allergen	N=4	1.7 µg allergen 2-hr TWA	Cooking Area
Chretien,[9] 1997	Snow Crab Allergen	N_{area} =5 $N_{personal}$ =7	53-547 ng/m^3 179-5061 ng/m^3	Crab cracking & Cooling sections

Table 2. Summary of the Environmental Methods

Analytes	Media/Sampler	Flowrate (L/min)	Analytical Methods
Aerosolized Protein	37mm Glass Fiber Filter, Closed Face Filter Cassette for personal sampling Open Face Filter Cassette for area sampling	Total @ 2.0 Respirable @ 1.7	micro-Kjeldahl
Aerosolized Protein Crab Allergen Kanimiso Allergen Endotoxin	37mm Glass Fiber Filter, Closed Face Filter Cassette for personal sampling Open Face Filter Cassette for area sampling	*Jan '98 Survey:* Total @ 2.0 Respirable @ 1.7 *March '98 Survey:* Total @ 4.0 & 10.0	Protein determined by Lowry Assay Allergen determinations by RAST inhibition Endotoxin determinations by Chromogenic Limulus Amebocyte Lysate Test Assays performed by the NIOSH-DRDS Immunological Lab (all analytes were determined from one filter)
Microbiological	Bulk process water		Commercial Microbiological Laboratories
Volatile Organic Compounds	Thermal Desorption Tubes	50 cc/min	Gas Chromatography / Mass Spectrometry
Elemental Metals Analysis	Bulk process water		Inductively Coupled Plasma Emission Spectroscopy by NIOSH Method 7300.
Ammonia in air	gas monitoring instrument	diffusion	Direct reading instrument
Carbon Monoxide in air	gas monitoring instrument	diffusion	Direct reading instrument

Table 3. Summary of Serological Studies

Analysis	Collected	Method	Interpretation
Total IgE Antibodies	January and March '98	CAP®	≥100 kU/l was considered abnormally elevated
Eosinophil Cationic Protein (ECP)	January and March '98	CAP®	≥23 µg/l was considered suggestive of an active inflammatory process
Specific IgE Antibodies†			
House dust mite mix	January '98	CAP®	Positive ≥ class II
Mold mix	January '98	CAP®	Positive ≥ class II
Weed mix	January '98	CAP®	Positive ≥ class II
Epidermal mix	January '98	CAP®	Positive ≥ class II
Crab-Specific IgE Antibodies			
Pagurus crab	January and March '98	CAP®	Positive ≥class II
Snow crab - cooking water	January and March '98	RAST	Positive ≥90th percentile for naive workers, early season survey
Snow crab- kanimiso	January and March '98	RAST	Positive ≥90th percentile for naive workers, early season survey
Snow crab - meat uncooked*	January and March '98	RAST	Positive ≥90th percentile for naive workers, early season survey
Snow crab - meat cooked*	January and March '98	RAST	Positive ≥90th percentile for naive workers, early season survey

† Used to define atopy (one or more positive tests)
* data not shown

Table 4. Potential Employee Exposures during Snow Crab Processing

Employee Task	Potential Exposures	Method of Aerosol Generation
Butchering	Live crab, seawater Uncooked crab meat Kanimiso	Cracking of shell against metal blade, pulling of legs from body, throwing legs to conveyor against back splash over conveyer
Degilling	Uncooked crab meat Crab gills Kanimiso	Contact of crab legs and attached gills with rotating blade, throwing leg clusters onto conveyor
Packing	Uncooked crab meat	Material handling of crab leg clusters
Cooking	Uncooked crab meat at tank entrance Cooked crab meat at tank exit Cooking water with crab proteins	Steam and condensation generated from cooking tank Splashing of cooking water from exit of tank
Case-up	Cooked and frozen crab meat Frozen kanimiso	Material handling of frozen crab leg clusters Material handling of frozen kanimiso Cleaning of crab cooking pans and kanimiso trays

Table 5. Summary of Aerosolized Protein Sampling Results

Job or Area Location	Number of Samples	Range ($\mu g/m^3$)	Mean Protein Concentration ($\mu g/m^3$)
Personal Total micro-Kjeldahl Aerosolized Protein			
Butcher	1	81	81
Degiller	4	34 - 1500	461
Area Total micro-Kjeldahl Aerosolized Protein			
Butchering	7	ND - 32	4.5
Degilling	2	ND	-
Packing Line	8	9.6 - 20	3.7
Cooking Tank	4	10 - 6400	1605
Shipping	2	ND - 39	19.5
Ambient Water Tank	1	ND	-
Loading Dock	3	ND - 11	3.6
Bunk House	1	ND	-
Area Respirable micro-Kjeldahl Aerosolized Protein			
Butchering	3	ND	-
Degilling	2	ND	-
Packing Line	3	ND	-
Cooking Tank	2	ND	-
Loading Dock	2	ND	-

Table 6. Summary of Environmental Allergen Sampling Results

Job Title	Number of Samples	Snow Crab Antigen (RAU/m³)		Kanimiso Antigen (RAU/m³)		Endotoxin (EU/m³)	
Total Personal Sampling		Range	Mean	Range	Mean	Range	Mean
Unloading	1	ND	-	54	-	1.6	-
Butcher	6	ND-11	3.4	ND-140	50.2	7.6-82.4	25.8
Butcher-Degiller	3	ND	-	ND-86	46.3	2.5-28.4	11.7
Butcher-Degiller-Packer	2	ND	-	ND-47	23.5	2.5-9.8	6.2
Degiller	8	ND	-	ND-570	118	3.0-9.1	6.0
Post Gilling Inspector	1	18	-	130	-	3.3	-
Packer-Sorter	11	ND-150	22.6	ND-82	24.5	2.0-11.0	5.5
Cooker (entrance)	2	ND	-	ND -56	28.0	4.9-17.3	11.1
Cooker (exit)	3	ND	-	ND-66	37.3	1.1-6.6	4.4
Chiller Tank	2	ND-21	10.5	55-170	113	3.9-59.6	31.8
Shipping-Caseup	2	ND	-	61-220	141	23.8-13	18.6
Shipping-Caseup-Pan Cleaning	1	ND	-	330	-	949	-
Freezer Forklift	1	ND	-	830	-	2.4	-
Respirable Personal Sampling							
Butcher	3	ND-38	12.7	844-2270	1511	ND-9.2	3.1
Degiller	3	ND	-	ND	-	4.4-22.7	14.4
Packing Line	2	ND-15	7.5	ND	-	14.5-32.6	23.6
Cooking Tank	3	ND-58	19.3	ND	-	12.6-18	15.8
Shipping	1	ND	-	ND	-	39	-

Table 7. Demographic characteristics of crab processing workers

	Completed early and late surveys n=107	Completed early and late surveys but excluded because not crab processor n=15	Completed only early survey n=30
Age, yr ± SE	36 ± 1	40 ± 3	35 ± 2
Gender, in (%)			
Males	84 (78)	7 (46)	25 (83)
Females	23 (22)	8 (54)	5 (17)
Race (%)	¶		
Whites	44 (41)	8 (53)	11 (37)
Asian/ Pacific Islander	39 (37)	1 (6)	3 (10)
Blacks	16 (15)	6 (40)	12 (40)
American Indians	5 (5)	.	.
Other	2 (2)	.	4 (13)
Place of longest residence (%)			¶
US	41 (39)	6 (40)	11 (38)
South East Asia	32 (30)	5 (33)	9 (31)
Latin America	19 (18)	3 (20)	6 (20)
Africa	11 (10)	.	3 (10)
Other	4 (4)	1 (6)	.
Tenure status			
Experienced* (%)	55 (51)	6 (40)	11 (37)
Median number of months of experience (25^{th} - 75^{th} percentiles)	5 (2-8)	3 (1-6)	3 (0-5)
Smoking status (%)		¶	¶
Never smoker	51 (48)	9 (64)	17 (60)
Current smoker	38 (35)	4 (29)	3 (11)
Former smoker	18 (17)	1 (7)	8 (29)
Family history of allergies (%)	22 (21)	5 (33)	9 (30)
Total IgE >100kU/ml (%)	45 (42)	4 (27)	9 (36)
Atopy (%)**	48 (45)	6 (40)	11 (42)

* experienced workers were those with more than four weeks of work in the crab industry at the early season

** atopy was defined as a positive CAP to one or more common allergen at the early season

¶ missing data

Table 8. Percent Prevalence and Incidence of Symptoms among Crab Processing Workers

Symptoms	Prevalence Early season (n=107)	Prevalence Late season (n=107)	Incidence ¶ (New onset)
Upper respiratory			
usually stuffy nose or drainage back of nose	22 %	43 %†	37%
two or more episodes of blocked, itchy or runny nose	26 %*	53 %†**	45%
Asthma-like			
chest ever sound wheezing or whistling apart from colds or on most days each week	10 %	17 %	11%
attacks of SOB with wheezing with normal breathing between attacks	7 %	14 %	13%
tightness in chest	8 %*	22 %†**	19%
awaken by an attack of cough, wheezing or SOB	12 %	35 %†	32%
attack of SOB or cough after exercise	15 %	21 %†	16%
Bronchitic			
usual morning cough	3 %	28 %†	26%
usual morning phlegm	6 %	34 %†	30%
Functional impairment			
SOB hurrying or walking up hill	13 %	16 %	9%
SOB walking with other own age	4 %	2 %	2%
stop for breath when walking alone	2 %	3 %	2%

* in the last 12 months, ** in the last 6 weeks (during crab season)

¶ workers without symptoms in the early-season survey were followed during 6 weeks for the development of incident symptoms.

† p value <0.05 (comparison to early-season survey by McNemar's test)

Table 9. Percent Health Outcome Prevalence and Incidence among Crab Processing Workers

Health Outcome	Prevalence Early season (n=107)	Prevalence Late season (n=107)	Incidence¶ (New Onset)
Upper Respiratory	31 %	57 %†	56%
Asthma-like	14 %	32 %†	26%
Bronchitic	2 %	21 %†	19%

¶ workers without health outcomes in the early-season survey were followed over 6 weeks for the development of health outcomes.

† p value <0.05 (comparison to early-season survey by McNemar's test).

Table 10. Summary of Pulmonary Function Results among 91 Crab Processing Workers

Spirometry Test	Early season	Late season
Percent predicted FEV_1 ± SE	109.6 ±1.9	107.9 ± 1.9
Percent predicted FVC ± SE	109.3 ±2.0	109.5 ±1.9
Mean FEV1/FVC ±SE	84.6 ±0.6	83.1 ±0.7
Obstruction (% workers)	2%	4%
Restriction (% workers)	3%	3%
Bronchodilator improvement >8% (%workers)	7%	10%

Table 11. Summary of Immunology Results, Stratified by Tenure Status among 96 Crab Processing Workers

Tenure Status	Snow crab RAST-IgE kanimiso		Snow crab RAST-IgE crab-water		Pagurus crab CAP-IgE		Medians for Total IgE kU/l (25th, 75th percentiles)	
	early	late	early	late	early	late	early	late
Naive workers with IgE (n=45)	12%	10%	12%	12%	12%	10%	91 (37, 419)	94 (38, 392)
Experienced workers with IgE (n=51)	5%	2%	2%	0%	14%	11%	64 (18, 224)	61 (20, 223)
All Workers (n=96)	8%	6%	7%	6%	13%	10%	84 (25, 273)	74 (30, 279)

Table 12. Risk Ratio (RR) for Predicting Development of Upper Respiratory Outcomes among 74 Crab Processing Workers Without the Condition at the Early-season Survey

Risk Factors (n)		Percent Incidence	Relative Risk (95% CI ‡)	p value
Age	≥ 35 years old (43) < 35 years old (31)	37 58	1.0 1.6 (1.0-2.5)	0.08
Gender	female (16) male (58)	38 48	1.0 1.3 (0.7-2.6)	0.45
Race	non-white (43) white (31)	47 45	1.0 1.0 (0.6-1.6)	0.91
Smoking *	no (47) yes (27)	51 37	1.0 0.7 (0.4-1.3)	0.25
Atopy	no (42) yes (32)	48 44	1.0 0.9 (0.5-1.5)	0.74
Family History of Allergies	no (65) yes (9)	42 78	**1.0** **1.9 (1.2-3.0)**	**0.04†**
Kanimiso RAST- IgE *	negative (70) positive (2)	46 50	1.0 1.1 (0.3-4.5)	0.71†
Crab-water RAST- IgE *	negative (68) positive (4)	46 50	1.0 1.1 (0.4-2.9)	0.63 †
Pargus-crab CAP- IgE *	negative (67) positive (5)	45 60	1.0 1.3 (0.3-0.6)	0.42†
Eosinophil Cationic Protein*	negative (73) positive (1)	45 100	1.0 ND	0.46†
Housing	off-site (45) on-site (29)	47 45	1.0 1.0 (0.6-1.6)	0.88
Tenure in Crab Industry	experienced (34) naive (40)	41 50	1.0 1.2 (0.7-2.0)	0.45
Potential high exposures**	no (33) yes (20)	48 60	1.0 1.2 (0.8-2.0)	0.42
Task Categories	unloading (7) butchering (6) degilling (12) packing (20) case-up (13) mixed/dirty (8) mixed/clean (8)	14 83 50 50 46 38 38	1.0 **5.8 (0.9-37.1)** 3.5 (0.5-23.4) 3.5 (0.5-22.6) 3.2 (0.5-21.7) 2.6 (0.3-19.8) 2.6 (0.3-19.8)	referent **0.02 †** 0.14 † 0.11 † 0.18 † 0.34 † 0.34 †

* status at the end of the season

** yes (butchering, degilling, cooking), no (packing, case-up)

† Fisher's exact test

‡ denotes confidence interval

ND not determined; one cell contained a zero value

Statistical significant values are shown in bold

Table 13. Risk Ratio (RR) for Predicting Development of Asthma-like Outcomes among 92 Crab Processing Workers Without the Condition at the Early-season Survey

Risk Factors (n)		Percent Incidence	Relative Risk (95% CI ‡)	p value
Age	≥ 35 years old (50) <35 years old (42)	20 33	1.0 1.7 (0.8-3.4)	0.15
Gender	female (18) male (74)	6 31	1.0 5.6 (0.8-38.7)	**0.02 †**
Race	non-white (54) white (38)	28 24	1.0 0.9 (0.4-1.7)	0.66
Smoking *	no (57) yes (35)	21 34	1.0 1.6 (0.8-3.2)	0.16
Atopy	no (51) yes (41)	27 24	1.0 0.9 (0.4-1.8)	0.74
Family History of Allergies	no (75) yes (17)	21 47	1.0 2.2 (1.1-4.3)	**0.03**
Kanimiso RAST- IgE *	negative (85) positive (4)	25 50	1.0 2.0 (0.7-5.8)	0.26 †
Crab-water RAST- IgE *	negative (85) positive (4)	27 0	1.0 ND	0.29 †
Pagurus crab CAP- IgE *	negative (81) positive (8)	23 50	1.0 2.1 (0.9-4.7)	0.11 †
Eosinophil Cationic Protein *	negative (88) positive (4)	23 100	1.0 ND	**0.003 †**
Housing	off-site (64) on-site (28)	30 18	1.0 0.6 (0.3-1.5)	0.24
Tenure in Crab Industry	experienced (44) naive (48)	20 31	1.0 1.5 (0.8-3.1)	0.24
Potential high exposures**	no (40) yes (26)	30 38	1.0 1.3 (0.7-2.5)	0.48
Task Categories	unloading (10) butchering (10) degilling (14) packing (25) case-up (15) mixed/dirty (8) mixed/clean (10)	0 40 36 28 33 25 10	1.0 ND ND ND ND ND ND	referent **0.04 †** **0.05 †** 0.07 † 0.06 † 0.18 † 0.50 †

* status at the end of the season
** yes (butchering, degilling, cooking), no (packing, case-up)
† Fisher's exact test
‡ denotes confidence interval
ND not determined; one cell contained a zero value
Statistical significant values are shown in bold

Table 14. Risk Ratio (RR) for Predicting Development of Bronchitic Outcomes among 105 Crab Processing Workers Without the Condition at the Early-season Survey

Risk Factors (n)		Percent Incidence	Relative Risk (95% CI ‡)	p value
Age	≥ 35 years old (55) < 35 years old (50)	7 32	1.0 **4.4 (1.6-12.3)**	**0.001**
Gender	female (22) male (83)	5 23	1.0 **5.0 (0.7-35.8)**	**0.04 †**
Race	non-white (61) white (44)	16 23	1.0 1.4 (0.6-3.0)	0.42
Smoking *	no (64) yes (41)	14 27	1.0 1.9 (0.9-4.2)	0.10
Atopy	no (57) yes (48)	40 60	1.0 1.8 (0.8-4.0)	0.16
Family History of Allergies	no (83) yes (22)	16 32	1.0 2.0 (0.9-4.5)	0.09
Kanimiso RAST- IgE *	negative (97) positive (5)	15 80	1.0 **5.2 (2.7-9.8)**	**0.004 †**
Crab-water RAST- IgE *	negative (97) positive (5)	18 40	1.0 2.3 (0.7-7.3)	0.23 †
Pagurus crab CAP- IgE *	negative (93) positive (9)	16 44	1.0 **2.8 (1.2-6.5)**	**0.04 †**
Eosinophil Cationic Protein *	negative (100) positive (5)	17 60	1.0 **3.5 (1.5-8.1)**	**0.05 †**
Housing	off-site (69) on-site (36)	17 22	1.0 1.3 (0.6-2.8)	0.55
Tenure in Crab Industry	experienced (51) naive (54)	12 26	1.0 2.2 (0.9-5.3)	0.07
Potential high exposures**	no (45) yes (30)	16 30	1.0 1.9 (0.8-4.6)	0.14
Task Categories	unloading (11) butchering (12) degilling (16) packing (29) case-up (16) mixed/dirty (10) mixed/clean (11)	0 25 38 10 24 20 18	1.0 ND ND ND ND ND ND	referent 0.12 † **0.03 †** 0.37 † 0.10 † 0.21 † 0.24 †

* status at the end of the season
** yes (butchering, degilling, cooking), no (packing, case-up)
† Fisher's exact test
‡ denotes confidence interval
ND not determined; one cell contained a zero value
Statistical significant values are shown in bold

Appendix 1
Allergen Extract Preparation

Cooking Water

Samples of water used to cook crab legs were collected at the end of a work shift just before the cooking tank water was to be replaced. The cooking water sample was frozen, shipped to the laboratory, and stored at minus 80°C until analyzed. The thawed cooking water samples were clarified by centrifugation followed by filtration through a 0.45μm filter. The filtrate was dialyzed against a sodium carbonate buffer, concentrated approximately four-fold using an Amicon ultrafiltration system with a YM-2 membrane, and stored at minus 20°C until used.

Crab Meat

Extracts of both cooked and uncooked crab meat were prepared from crab legs shipped frozen to the laboratory. The crab meat was dissected out of the shell, minced into fine pieces, suspended in 0.05M ammonium carbonate (10% w/v), and extracted overnight at 4°C with constant stirring. The samples were then centrifuged, filtered, dialyzed and stored as above.

Kanimiso

A frozen sample of kanimiso was thawed, subjected to a low speed centrifugation to remove particulate matter followed by a high speed centrifugation (10,000 g) for two hours to separate the lipids (oils) from the mixture. The aqueous material was processed by filtration and dialyzed as above, and stored at minus 20°C until analyzed.

Preparation of solid-phase for RAST studies

The allergen extracts were coupled to CNBr activated Sephrose® 4B (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's recommended procedure. Coupling efficiency was determined by monitoring the absorbance at 280 nm of the wash fluids and >90% of the protein in each sample was bound to the beads. Unreacted sites on the beads were blocked by incubation in TRIS buffer overnight at 4°C. The allergen coated beads were stored at 4°C until used for RAST assays.

Appendix 2
Environmental Samples for Allergen Content

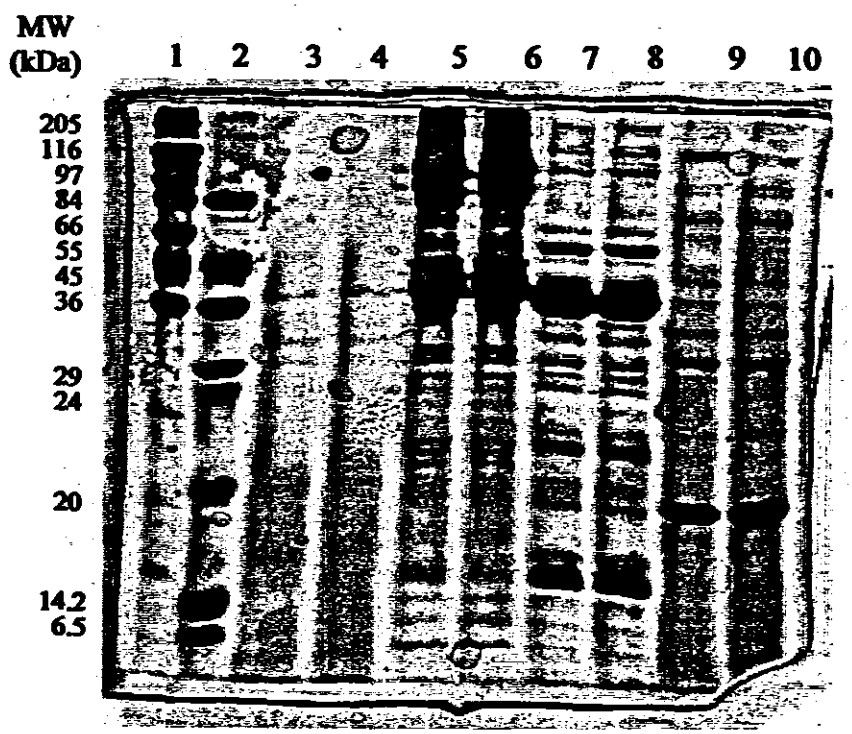
The glass fiber filters used to collect air samples were stored at 4° in their cassettes until extraction. The filters were aseptically transferred to sterile 50 ml centrifuge tubes, and extracted with 10 ml of pyrogen free water for 60 minutes at room temperature on a rocker platform. The samples were then centrifuged for 10 minutes at 2500 rpm, and the supernatant fluid was recovered and stored in three aliquots at minus 85°C until assayed for either total protein, allergen, or endotoxin content.

Allergen content in extracts of air samples was determined by a RAST inhibition assay. Because the results of the four allergens RAST indicated that cooking water, cooked meat, and uncooked meat gave nearly identical results, the analysis of the air samples included only two allergens, crab meat and kanimiso. For the crab meat allergen levels, the cooking water was selected as the allergen source material because sodium dodecylsulphate (SDS) gel electrophoresis analysis revealed that this preparation contained significantly fewer proteins than either the cooked or uncooked meat extracts, (see Figure A2-1) but had similar binding activity in the RAST assay. The staining of gels for protein by Commassie blue revealed that uncooked crab meat, cooked crab meat, and cooking water extracts showed similar protein bands. The kanimiso extract shared many proteins with the meat extracts, but contained some bands not seen in the meat extracts. Analysis of pooled sera using Western blot (picture not shown) showed IgE binding to several bands with both extracts, including from 34-36 kd (kilodaltons) range.

The stock allergen extracts (cooking water and kanimiso) were defined as containing 1000 relative allergen units (RAU) per milliliter, and serial dilutions were prepared from these stocks each day to construct a standard curve for each inhibition assay. The sera used for the inhibition assay were selected on the basis of the RAST results. The anti-crab meat pool contained sera that reacted strongly with the meat allergens (>10% counts bound) but weakly or not at all with the kanimiso extract, and vice versa for the anti-kanimiso serum pool. The limits of detection were found to be approximately 1 RAU/ml or 10 RAU/filter for the crab meat assay and 5 RAU/ml or 50 RAU/filter for the kanimiso assay. The minimum detectable concentration (MDC) for the crab assay was 5.2 RAU/m^3 and 26.0 RAU/m^3 for the kanimiso assay, based on an air sampling volume of 1920 liters (4.0 L/min for 8 hours).

Figure A2-1

SDS Gel of Crab Extracts



1 = High molecular weight markers
2 = Low molecular weight markers
3 and 4 = Crab Cooking Water
5 and 6 = Uncooked Crab Meat Extract
7 and 8 = Cooked Crab Meat Extract
9 and 10 = Kanimiso Extract

Note: Crab cooking water contained three bands indicating proteins with molecular weights of 40, 33, and 17 kd. The uncooked crab meat extract had the most protein per gram of sample, and the most individual bands. A total of 19 bands could be detected, with major bands around 75 to 84 kd, 50 kd and 42 kd. The cooked crab meat extract had 13 bands, with major bands at 50 kd, 38 kd, 22 kd, and 17 kd. The kanimiso had eight distinct bands with major bands at 31 kd and 20 kd.

Appendix 3
Environmental 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 this 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),[1] (2) the American Conference of Governmental Industrial Hygienists' (ACGIH®) Threshold Limit Values (TLVs®),[2] and (3) the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs).[3] 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.[4] Thus, employers should understand that not all hazardous chemicals have specific OSHA exposure limits such as PELs and 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 short-term exposure limits (STEL) or ceiling values which are intended to supplement the TWA where there are recognized toxic effects from higher exposures over the short-term.

Ammonia

Ammonia is a severe irritant of the eyes, respiratory tract and skin. It may cause coughing burning and tearing of the eyes, runny nose, chest pain, cessation of respiration, and death. Symptoms may be delayed in onset. Exposure of the eyes to high gas concentrations may produce temporary blindness and severe eye damage. Exposure of the skin to high concentrations of the gas may cause burning and blistering. Repeated exposure to ammonia gas may cause chronic irritation of the eyes and upper respiratory tract.

Carbon Monoxide

Carbon monoxide (CO) is a colorless, odorless, tasteless gas which can be a product of the incomplete

combustion of organic compounds. CO combines with hemoglobin and interferes with the oxygen carrying capacity of blood. Symptoms include headache, drowsiness, dizziness, nausea, vomiting, collapse, myocardial ischemia, and death.

Microorganisms

Microorganisms (including fungi and bacteria) are normal inhabitants of the environment. The saprophytic varieties (those utilizing non-living organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide an ample supply of a nutrient substrate. Under the appropriate conditions (optimum temperature, pH, and with sufficient moisture and available nutrients) saprophytic microorganism populations can be amplified. Through various mechanisms, these organisms can then be disseminated as individual cells or in association with soil/dust or water particles. In the outdoor environment, the levels of microbial aerosols will vary according to the geographic location, climatic conditions, and surrounding activity. Generally, indoor workplace levels are expected to be below the outdoor levels, with similar microbial species and relative frequencies.

Acceptable limits on worker exposure to airborne microorganisms have not been established, primarily because allergic reactions can occur even with relatively low air concentrations of allergens, and individuals differ with respect to allergic susceptibilities. The current strategy for on-site evaluation of environmental microbial contamination involves an inspection to identify sources (reservoirs) of microbial growth and potential routes of dissemination. In those locations where contamination is visibly evident or suspected, bulk samples may be collected to identify the predominant (fungi and bacteria) species.

Algae and Dinoflagellates

Dinoflagellate contamination of crab endolymph has been suggested as an etiological agent for respiratory, dermatological and systemic symptoms. Dinoflagellates are unicellular, free-floating algae. Certain marine dinoflagellates can produce neurotoxins that cause paralytic shellfish poisoning. Blooms of dinoflagellates are also responsible for seasonal red tides.

Dinoflagellates resembling *Hematodinium* sp. can cause fatal infection in crabs resulting in the bitter crab syndrome. Infected crabs have drooping limbs and mouthparts, milky-white endolymph and when cooked, the meat has a chalky texture and an astringent after-taste. Prevalence and intensity of infection are highest between July and October, with peak crab mortality in August and September. Prevalence of infection is usually zero by mid-winter during the harvesting season.

Endotoxins

Endotoxin is a component of the outer cell wall of Gram-negative bacteria. It is often a contaminant of organic materials and, when inhaled, can cause symptoms of upper and lower airway irritation, as well as systemic flu-like symptoms. Endotoxins have a wide range of biological activities including inflammatory and immunological responses. The pulmonary macrophage is extremely sensitive to the effects of endotoxins and a primary target cell for endotoxin-induced pulmonary injury following respiratory exposure. Exposures to endotoxins have been reported to cause acute fever, dyspnea, chest tightness, coughing, and decreases in pulmonary function. Asthmatics are more sensitive to the airways effects of endotoxin than nonasthmatics.

There are no OSHA, ACGIH, or NIOSH standards or criteria for occupational exposures to endotoxin. The scientific literature contains research describing human threshold exposure limits for endotoxins. The lowest endotoxin exposure reported to cause adverse pulmonary response was measured in exposure studies among subjects sensitive to cotton dusts, [9 nanograms of elutriated endotoxin per cubic meter of air (9 ng/m^3)]; this concentration is equivalent to approximately 90 endotoxin units per cubic meter of air (EU/m^3). Threshold endotoxin exposures among healthy human subjects exposed to cotton dusts are reported by Rylander as approximately 1000 to 2000 EU/m^3 for an across shift acute pulmonary response (decline in FEV_1) and 5000 to 10,000 EU/m^3 for fever. However, a recommended endotoxin exposure limit of 50 EU/m^3 based on inhalable dust sampling has recently been adopted in the Netherlands. This limit was established as about half of the 90 EU/m^3 level that induces measurable airways obstruction.

Volatile Organic Compounds

Volatile organic compounds describe a large class of chemicals which are organic (i.e., containing carbon) and have a sufficiently high vapor pressure to allow some of the compound to exist in the gaseous state at room temperature. These compounds are emitted in varying concentrations from numerous indoor sources including, but not limited to, adhesives, solvents, paints, cleaners, cigarettes, and combustion sources. They can also be of microbial origin

Occupational exposure criteria and standards for air contaminants measured

SUBSTANCE	NIOSH REL	ACGIH TLV	OSHA PEL
Aerosolized protein, Crab allergens, Endotoxin, and Microorganisms	No RELs	No TLVs	No PELs
Ammonia	25 ppm TWA 35 ppm STEL	25 ppm TWA 35 ppm STEL	50 ppm TWA
Carbon Monoxide	35 ppm TWA 200 ppm Ceiling	25 ppm TWA	50 ppm TWA

1. NIOSH [1992]. Recommendations for occupational safety and health: compendium of policy documents and statements. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 92-100.

2. ACGIH [1999]. 1998 TLVs® and RELs®: threshold limit values for chemical substances and physical agents. Cincinnati, OH: American Conference of Governmental industrial Hygienists.

3. Code of Federal Regulations [1997]. 29 CFR 1910.1000. Washington, DC: U.S. Government Printing Office, Federal Register.

4. Public Law 91 – 596 Occupational safety and Health Act of 1970, Sec. 5.(a)(1).

Table A-1. Personal Sampling Results - Total micro-Kjeldahl Aerosolized Protein
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
January 23, 1998
HETA 98-0069

Job Title	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Protein Concentration ($\mu g/m^3$)
Butcher	1/23/98	8898-33	537	1074	81
Degiller	1/23/98	8898-06	553	1106	1500
Degiller	1/23/98	8898-12	553	1106	140
Degiller	1/23/98	8898-32	548	1096	170
Degiller	1/23/98	8898-30	589	1178	34

- Samples were collected on glass fiber filters mounted in 3-piece 37mm cassettes at 2.0 L/min.
- Samples were collected with an open-face cassette.
- Examination of the filters after the work-shift indicated that 'crab bits' were splashing onto the sampling media. All subsequent personal sampling was done closed faced to minimize collection of such material.
- These data act as a positive control indicating that the micro-Kjeldahl method was effectively detecting crab protein.

Table A-2. Area Sampling Results - Total micro-Kjeldahl Aerosolized Protein
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
January 23-28, 1998
HETA 98-0069

Area Location	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Protein Concentration ($\mu g/m^3$)
Butchering	1/23/98	8898-09	495	990	ND
Butchering	1/24/98	8898-17	517	1034	ND
Butchering	1/25/98	8898-39	495	990	ND
Butchering	1/26/98	000025	468	936	32
Butchering	1/27/98	8898-R-09-CF	482	819	ND
Butchering	1/27/98	8898-R-16-CF	480	960	ND
Butchering	1/28/98	8898-R-38-CF	492	836	ND
Degilling	1/26/98	000018	454	908	ND
Degilling	1/28/98	8898-R-40-CF	465	791	ND
Packing Line	1/23/98	8898-27	567	1134	ND
Packing Line	1/24/98	8898-07	518	1036	9.6
Packing Line	1/25/98	8898-35	494	988	20
Packing Line	1/26/98	000012	454	908	ND

Table A-2. Area Sampling Results - Total micro-Kjeldahl Aerosolized Protein (continued)
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
January 23-28, 1998
HETA 98-0069

Area Location	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Protein Concentration ($\mu g/m^3$)
Packing Line	1/23/98	8898-22	571	1142	ND
Packing Line	1/24/98	8898-26	518	1036	ND
Packing Line	1/25/98	8898-16	494	988	ND
Packing Line	1/26/98	000007	453	906	ND
Cooking Tank	1/23/98	8898-31	174	348	ND
Cooking Tank	1/24/98	8898-40	117	234	6400
Cooking Tank	1/25/98	000006	494	988	10
Cooking Tank	1/26/98	000011	452	904	11
Ambient Water Tank	1/23/98	8898-11	566	1132	ND
Shipping	1/24/98	8898-24	518	1036	39
Shipping	1/25/98	8898-36	495	990	ND
Loading Dock	1/24/98	8898-05	493	986	ND
Loading Dock	1/25/98	8898-34	482	964	ND
Loading Dock	1/26/98	000019	453	906	11
Bunk House	1/23/98	8898-15	567	1134	ND

Table A-3. Area Sampling Results - Respirable micro-Kjeldahl Aerosolized Protein
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
January 27-28, 1998
HETA 98-0069

Job Title	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Respirable Protein Concentration ($\mu g/m^3$)
Butchering	1/27/98	8898-R-17	482	819	ND
Butchering	1/27/98	8898-R-21	483	821	ND
Butchering	1/28/98	8898-R-28	492	836	ND
Degilling	1/28/98	8898-R-18	466	792	ND
Degilling	1/27/98	8898-R-23	467	794	ND
Packing Line	1/27/98	8898-R-25	479	814	ND
Packing Line	1/27/98	8898-R-13	480	816	ND
Packing Line	1/28/98	8898-R-36	286	486	ND
Cooking Tank	1/27/98	8898-R-24 8898-R-22	267 179	454 304	ND
Cooking Tank	1/28/98	8898-R-35	304	517	ND
Loading Dock	1/27/98	8898-R-30	473	804	ND
Loading Dock	1/28/98	8898-R-33	474	806	ND

NOTE: Samples were collected on 37-mm glass fiber filters mounted cyclones at 1.7 L/min.

Table A-4. Personal Sampling Results
Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen and Endotoxin (n=43)
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
HETA 98-0069

Job Title	Date	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Aerosolized Protein ($\mu g/m^3$)	Snow Crab Antigen (RAU/m^3)	Kanimiso Antigen (RAU/m^3)	Endotoxin (EU/m^3)
Offloader	3/8/98	000056	259	1036	ND	ND	54	1.6
Butcher	1/27/98	8898-R-10	481	962	ND	ND	ND	7.6
Butcher	1/27/98	8898-R-14	546	1092	ND	9.2	ND	21.3
Butcher	1/27/98	8898-R-20	517	1034	ND	11	ND	11.1
Butcher	3/6/98	000019	441	1764	ND	ND	66	24.7
Butcher	3/8/98	000039	319	1276	ND	ND	95	7.6
Butcher	3/8/98	000051	144	576	ND	ND	140	82.4
Butcher-Degiller	3/6/98	000006	455	1820	ND	ND	ND	2.5
Butcher-Degiller	3/8/98	000072	283	1132	ND	ND	53	28.4
Butcher-Degiller	3/6/98	000015	481	1924	ND	ND	86	4.1

Table A-4. Personal Sampling Results (continued)
Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen and Endotoxin
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
HETA 98-0069

Job Title	Date	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Aerosolized Protein ($\mu g/m^3$)	Snow Crab Antigen (RAU/m^3)	Kanimiso Antigen (RAU/m^3)	Endotoxin (EU/m^3)
Butcher-Degiller-Packer	3/8/98	000049	322	1288	ND	ND	47	9.8
Butcher-Degiller-Packer	3/8/98	000055	290	1160	ND	ND	ND	2.5
Degiller	1/26/98	000009	516	1032	ND	ND	ND	6.2
Degiller	1/26/98	000016	511	1022	ND	ND	ND	4.8
Degiller	1/26/98	000017	512	1024	ND	ND	ND	6.8
Degiller	1/26/98	000023	507	1014	ND	ND	ND	3.0
Degiller	3/6/98	000007	510	2040	ND	ND	30	9.1
Degiller	3/6/98	000016	506	2024	ND	ND	570	5.3
Degiller	3/8/98	000034	259	1036	ND	ND	270	5.0
Degiller	3/8/98	000090	346	1384	ND	ND	70	7.9
Post Gilling Inspector	3/8/98	000060	289	1156	ND	18	130	3.3

Table A-4. Personal Sampling Results (continued)
Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen and Endotoxin
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
HETA 98-0069

Job Title	Date	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Aerosolized Protein ($\mu g/m^3$)	Snow Crab Antigen (RAU/m^3)	Kanimiso Antigen (RAU/m^3)	Endotoxin (EU/m^3)
Packer/Sorter	1/26/98	000020	370	740	ND	ND	ND	9.8
Packer/Sorter	1/26/98	000022	467	934	ND	ND	ND	5.5
Packer/Sorter	1/26/98	000024	504	1008	ND	150	ND	4.7
Packer/Sorter	3/6/98	000001	501	2004	ND	15	ND	3.2
Packer/Sorter	3/6/98	000013	480	1920	ND	46	ND	2.7
Packer/Sorter	3/6/98	000027	484	1936	ND	ND	37	6.5
Packer/Sorter	3/6/98	000005	489	1956	ND	ND	ND	2.0
Packer/Sorter	3/8/98	000097	350	1400	ND	ND	53	8.1
Packer/Sorter	3/8/98	000057	354	1416	ND	ND	82	3.5
Packer/Sorter	3/8/98	000032	351	1404	ND	ND	44	3.9
Packer/Sorter	3/8/98	000024	348	1392	ND	38	53	11.0
Cooker Entrance	1/26/98	000008	511	1022	ND	ND	ND	4.9
Cooker Entrance	3/8/98	000023	331	1324	ND	ND	56	17.3

Table A-4. Personal Sampling Results (continued)
Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen and Endotoxin
UniSea, Inc. Snow Crab Processing
Dutch Harbor, Alaska
HETA 98-0069

Job Title	Date	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Aerosolized Protein ($\mu g/m^3$)	Snow Crab Antigen (RAU/m^3)	Kanimiso Antigen (RAU/m^3)	Endotoxin (EU/m^3)
Cooker Exit	1/27/98	8898-R-15	536	1072	ND	ND	66	6.6
Cooker Exit	3/6/98	000009	465	1860	ND	ND	46	1.1
Cooker Exit	3/8/98	000068	333	1332	ND	ND	ND	5.5
Chiller Tank	1/26/98	000010	501	1002	69	ND	170	59.6
Chiller Tank	3/8/98	000040	283	1132	ND	21	55	3.9
Shipping-Caseup	1/26/98	000015	478	956	63	ND	61	23.8
Shipping Caseup	3/8/98	000031	146	584	ND	ND	220	13.3
Shipping Caseup Pan Cleaning	3/8/98	000092	199	796	ND	ND	330	949
Freezer Fork Lift Driver	3/8/98	000079	246	984	ND	ND	830	2.4

Table A-5. Personal Sampling Results
Respirable Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen, and Endotoxin (n=12)
UniSea, Inc. Snow Crab Processing, Dutch Harbor, Alaska
January 27-28, 1998
HETA 98-0069

Job Title	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Respirable Aerosolized Protein ($\mu g/m^3$)	Respirable Snow Crab Antigen (RAU/m^3)	Respirable Kanimiso Antigen (RAU/m^3)	Respirable Endotoxin (EU/m^3)
Butcher	1/28/98	8898-R-26 8898-R-34	340 172	578 292	ND	ND	1420	ND
Butcher	1/28/98	8898-R-29	498	847	ND	38	844	9.2
Butcher	1/28/98	8898-R-32	186	316	ND	ND	2270	ND
Degiller	1/27/98	8898-R-11	501	852	ND	ND	ND	22.7
Degiller	1/28/98	8898-R-31	527	896	ND	ND	ND	16.2
Degiller	1/28/98	8898-R-37	524	891	ND	ND	ND	4.4
Packing Line	1/27/98	8898-R-12	513	872	ND	ND	ND	14.5
Packing Line	1/28/98	8898-R-39	520	884	ND	15	ND	32.6
Cooking Tank	1/27/98	8898-R-08	524	891	ND	58	ND	12.6
Cooking Tank	1/28/98	8898-R-27	515	876	56	ND	ND	16.2
Cooking Tank	1/26/98	8898-R-19	529	899	ND	ND	ND	18.5
Shipping	1/27/98	8898-R-07	501	852	ND	ND	ND	39.0

Table A-6. Area Sampling Results - Low Exposure Comparison Work/Production Areas
Aerosolized Protein, Snow Crab Antigen, Kanimiso Antigen and Endotoxin (n=14)
UniSea, Inc. Dutch Harbor, Alaska
HETA 98-0069

Area Sample Location	Date Sample Collected	Sample Number	Sampling Time (min)	Total Volume Collected (l)	Aerosolized Protein ($\mu g/m^3$)	Snow Crab Antigen (RAU/m^3)	Kanimiso Antigen (RAU/m^3)	Endotoxin (EU/m^3)
Loading Dock	1/24/98	8898-19	493	986	ND	ND	ND	2.0
Loading Dock	3/6/98	000018	493	1972	ND	180	ND	2.7
Bunkhouse	1/23/98	8898-23	567	1134	ND	ND	ND	29.0
G1 Break Room	3/6/98	000029	553	2212	25	ND	ND	11.2
G1 Break Room	3/8/98	000083	262	1048	ND	ND	ND	8.5
Glove Wash Room	3/6/98	000025	568	2272	37	ND	50	8.7
Glove Wash Room	3/8/98	000080	265	1060	ND	ND	ND	12.6
G1 Production Office	3/6/98	000017	458	1832	ND	ND	ND	2.8
Screening Plant Deck	3/6/98	000022	487	1948	ND	7.9	ND	2.5
Meal Plant Hopper	3/9/98	000077	629	2516	ND	ND	ND	25.3
Meal Plant Storage	3/6/98	000014	482	1928	ND	ND	ND	20.2
G2 Surimi Plant Fillet Area	3/9/98	000020	559	2236	33	ND	ND	13.6
G2 Surimi Plant Fillet Area	3/9/98	000095	559	2236	38	ND	ND	10.0
G2 Surimi Plant Screening Deck	3/9/98	000058	556	2224	31	ND	ND	13.5

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