Occupational Allergy and Asthma Among Salt Water Fish Processing Workers

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Background *Fish processing is a common economic activity in Southern Africa. The aim of this study was to determine the prevalence and host determinants of allergic symptoms, allergic sensitization, bronchial hyper-responsiveness and asthma among workers processing saltwater fish.*

Methods *A cross-sectional study was conducted on 594 currently employed workers in two processing plants involved in pilchard canning and fishmeal processing. A modified European Community Respiratory Health Survey (ECRHS) questionnaire was used. Skin prick tests (SPT) used extracts of common airborne allergens, fresh fish (pilchard, anchovy, maasbanker, mackerel, red eye) and fishmeal. Spirometry and methacholine challenge tests (MCTs; tidal breathing method) used ATS guidelines.*

Results *Work-related ocular-nasal symptoms (26%) were more common than asthma symptoms (16%). The prevalence of atopy was 36%, while 7% were sensitized to fish species and 26% had NSBH ($PC_{20} \leq 8$ mg/ml or $\geq$12% increase in FEV_1 post-bronchodilator). The prevalence of probable occupational asthma was 1.8% and fish allergic rhino-conjunctivitis 2.6%. Women were more likely to report work-related asthma symptoms (OR = 1.94) and have NSBH (OR = 3.09), while men were more likely to be sensitized to fish (OR = 2.06) and have airway obstruction (OR = 4.17). Atopy (OR = 3.16) and current smoking (OR = 2.37), but not habitual seafood consumption were associated with sensitization to fish.*

Conclusions *Based on comparison with previous published studies, the prevalence of occupational asthma to salt water fish is lower than due to shellfish. The gendered distribution of work and exposures in fish processing operations together with atopy and cigarette smoking are important determinants of occupational allergy and asthma.* Am. J. Ind. Med. 51:899–910, 2008. *© 2008 Wiley-Liss, Inc.*

KEY WORDS: fish processing; occupational allergy; work-related asthma; atopy; smoking; gender

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INTRODUCTION

Occupational allergic reactions to seafood was first reported by De Beshce [1937], when he described a fisherman who developed asthma, angioedema and conjunctivitis when handling codfish. Various studies subsequently confirmed that an occupational allergic reaction to seafood can manifest as rhinitis, conjunctivitis, asthma, urticaria, protein contact dermatitis, and occasionally systemic anaphylactic reactions [Jeebhay et al., 2001]. These reactions are predominantly IgE-mediated due to high molecular weight agents such as proteins present in seafood.

Occupational asthma has been associated with occupational exposure to all the major seafood groupings in various epidemiological studies viz. arthropods (crabs, prawns), mollusks (cuttlefish), pisces (salmon), as well as other seafood derived agents (sea-squirt, *Anisakis* and red soft coral) [Jeebhay et al., 2001]. A higher prevalence is associated with arthropods (crustaceans) than with Pisces (bony-fish) and mollusks. Rhino-conjunctivitis and skin symptoms commonly occur in association and usually precede asthmatic symptoms. Upper airway symptoms can be an early risk marker for occupational asthma due to high molecular weight agents such as seafood [Malo et al., 1997]. Various cross-sectional studies reported the prevalence of occupational asthma due to seafood to be between 7% and 36% and due to fish in particular to be between 2% and 8% [Jeebhay et al., 2001]. Differences in prevalence data observed across these studies may be due to varying definitions of occupational asthma; varying exposure to seafood constituents; and the allergenic potential of seafood proteins involved. The most important host-associated risk factors reported for sensitization, IgE-mediated immunologic reactivity and the development of asthma are atopy and cigarette smoking. Atopy has been more consistently associated with sensitization to shellfish (clam, shrimp, crab, prawn, and cuttlefish) in particular [Gaddie and Friend, 1980; Cartier et al., 1984; Desjardins et al., 1995; Olszanski and Katlowski, 1997]. Smoking has been demonstrated in one study among prawn processors as an independent risk factor for increased specific IgE production [OR = 2.4; Mc Sharry et al., 1994]. A detailed study of these host factors in fish processors has not been conducted.

The seafood industry in South Africa employs over 30,000 mainly seasonal women workers in over 100 workplaces involved predominantly in bony fish (anchovy, pilchard and hake) processing, with 50% of workplaces reporting at least one worker with work-related allergic health problems annually [Jeebhay et al., 2000]. The spectrum of occupational allergy associated with processing of bony fish species (pilchard and anchovy) has not been previously investigated in epidemiological studies of seafood working populations. The aim of this study was to (1) determine the prevalence of allergic sensitization and work-related symptoms (ocular-nasal and asthma symptoms), non-specific bronchial hyperresponsiveness (NSBH) and asthma in relation to commonly processed fish species (pilchard, anchovy); (2) determine the host risk factors (age, gender, atopy, smoking, habitual seafood ingestion estimated by serum omega-3 fatty acids levels) for allergic sensitization, work-related symptoms and asthma due to fish. The environmental exposure risk factors and dose–response relationships are the subject of another article.

MATERIALS AND METHODS

Study Design, Population and Sampling

A cross-sectional study was conducted on 594 currently employed workers in two fish processing plants working in fish canning (pilchard) and fishmeal processing (mainly anchovy, red-eye and pilchard offal) along the West Coast of South Africa. All 260 workers in Factory A were investigated. For efficiency reasons, 334 workers from Factory B of a total workforce of 1,275 were chosen by stratified random sampling according to departments. Based on power calculations using $\alpha = 0.05$, a background prevalence of seafood allergy in the adult population as 0.1% (Nordic estimates) and conservative estimates for asthma (7%) for working populations exposed to seafood, a sample size of 400 was estimated to be appropriate to investigate the parameters of interest [Aas, 1987]. Ethical clearance of the protocol was obtained from the University of Cape Town, University of Michigan and the NIH (USA) prior to the study being conducted. Each participant signed informed consent prior to being tested.

Health Outcome Measurements

Questionnaire

Each worker answered a standard questionnaire of the European Community Respiratory Health Survey [ECRHS, Burney et al., 1994]. The questionnaire was modified to include questions relating to current and previous employment, exposure to seafood aerosols, tobacco smoke and patterns of seafood consumption. The questionnaire was also adapted for local conditions and translated into Afrikaans and Xhosa, and back translated to assess validity and reproducibility. It was administered by trained interviewers in whichever language the worker was most fluent.

Smoking status was classified into three categories viz. non-smoker as lifelong abstinence from smoking; ex-smoker if ceased smoking completely more than 1-month before the survey; and current smoker. Symptom variables included: respiratory (wheeze and/or tight chest); ocular (itchy eyes, red eyes); and nasal (runny nose, blocked nose, stuffy nose) symptoms. Symptoms were considered to be work-related if they worsened at work and improved on weekends or vacations.

In-house preparation of specific seafood extracts

Specific seafood extracts were prepared from fresh (raw) West Coast rock lobster (*Jasus lalandii*); pilchard (*Sardinops sagax*) in various processing stages (gut, raw, cooked, canned); and fresh (raw) anchovy (*Engraulis capensis*), redeye (*Etrumeus whiteheadi*), mackerel (*Scomber japonicus*) and maasbanker (*Trachurus trachurus capensis*); and fishmeal dust (containing mainly anchovy and pilchard offal) obtained from the factory. Extracts were diluted (1:1) with glycerol and standardized to a protein concentration of 3 mg/ml for skin prick tests (SPT). Extracts and controls were cross-validated in a second laboratory (Prof. Samuel Lehrer, Tulane University) by SDS gel electrophoresis and immunoblotting using sera of patients with confirmed sensitization to the major allergens parvalbumin and tropomyosin in fish and crustaceans, respectively [Lopata et al., 2005].

Skin prick tests (SPT)

The SPT were performed on each worker using standard common local aeroallergens (ALK-Abelló, A/S, Horsholm, Denmark) that included house dust mite (*Dermatophagoides pteronyssinus*), bermuda grass (*Cynodon dactylon*), rye grass (*Lolium perenne*), cockroach (*Blatella germanica*), cat (*Felis domesticus*), dog (*Canis familiaris*), mouldmix (*Cladosporium herbarum, Alternaria alternata, Fusarium*), Aspergillus (*Aspergillus fumigatus*) and mussel (*Mytilus edulis*; LETI alergia, Barcelona, Spain). The specific seafood extracts were prepared in-house [for details see Lopata et al., 2005]. Histamine dihydrochloride was used as positive control and diluent of glycerol/sodium chloride as a negative control.

Workers were instructed to not take any anti-histamines for 3 days prior to the test. SPT for atopy were done on 579 subjects only (six subjects had active eczema, four subjects were pregnant, four subjects admitted to recent use of anti-histamines, one subject had skin scar tissue). One subject was excluded from the data analysis since the subject displayed skin dermographism. A further three subjects did not undergo SPT with seafood extracts since they reported severe reactions on exposure to seafood in the past. A positive SPT was regarded as a wheal read 15 min after testing with a diameter (mean of two perpendicular measures) of $\geq$3 mm more than the negative control. Areas of wheal were traced on clear tape and stored for later verification. Atopic status was considered to be present if the SPT to one or more common aeroallergens was positive [Pepys, 1973]. Fish sensitization was defined as a positive SPT to any one or more of the specific fish allergens tested. For the analysis of correlation between various allergens, SPT reactivity was expressed as the allergen histamine wheal ratio (AHWR), that is the mean wheal diameter at the allergen site divided by the mean wheal diameter at the histamine site [Aas and Belin, 1973].

Serum analysis for specific IgE antibody determination

Blood samples for serum analysis for determining specific IgE levels to common inhalants and certain fish species was obtained from workers (n = 15) who did not undergo SPT. A 10-ml venous blood sample was taken from each worker (BD Vacutainer® Blood Collection Tube SST, Franklin Lakes) and stored at -80°C. The presence of atopy was ascertained by Phadiotop® testing using the UniCAP system (PHADIA, Sweden). For quantification of specific IgE levels ImmunoCAP tests were performed according to manufacturers instructions using pilchard—*Sardinops melanostica* (f-61) and anchovy—*Engraulis encrasicolus* (Rf-313) with values >0.35 ku/L regarded as positive. From the 15 workers who provided sera for serological analysis, there were two workers on whom specific IgE levels could not be determined as the sera were not suitable for analysis.

Serum determination of omega 3-fatty acids

For serum determinations of omega 3-fatty acids, serum was thawed and extracted with chloroform/methanol (2:1; v/v) according to a modified method of Folch et al. [1957] containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The total phospholipid band was scraped off and analyzed for fatty acid composition as described previously [van Jaarsveld et al., 2000]. The fatty acid methyl-esters (FAME) were identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, MN). The weight % μg/ml (relative composition) of the major marine n3-polyunsaturated fatty acid, eicosapentaenoic acid (EPA; 20:5n-3) was used as an index of habitual seafood consumption.

Spirometry

American Thoracic Society (ATS) guidelines were followed for spirometry tests [American Thoracic Society, 1995]. Vitallograph S model bellows volume-time spirometers, calibrated at least twice a day with a three-liter syringe, were used [Vitallograph Limited, 1982]. Lung volumes obtained by spirometry were adjusted for body temperature and atmospheric pressure levels. For logistical reasons spirometry tests were conducted during the working day and throughout the working week. Special instructions were given to workers not to smoke tobacco (at least 1 hr before) and to stop anti-asthmatic inhalers (4 hr before) or oral asthma medications (8 hr before) prior to the test. Pulmonary function reference values of the European Community for Coal and Steel (ECCS) with lower limits corresponding to the 95th percentile were used where appropriate [Quanjer et al., 1993]. Among the 584 workers (98%) that presented for spirometry, 32 workers (5%) were not eligible due to contra-indications. A further nine workers (1%) were unable to perform spirometry due to poor coordinative efforts despite eight attempts, resulting in 543 subjects with acceptable traces on spirometry.

Methacholine challenge testing (MCT)

Non-specific inhalation challenge testing using methacholine chloride powder mixed with normal saline was performed during the working day and scheduled throughout the working week using an abbreviated protocol. The 2 min tidal breathing method was used [American Thoracic Society, 2000]. The diluent was administered using the Salter 8900 Series nebulizer set (reference 8900; Salter Labs, 100 W Sycamore Road, Arvin, CA), with a nebulizer output volume 0.13 ml per minute $\pm$ 10% and particle size <5 microns (85%).

In all subjects eligible for methacholine challenge test (MCT), saline diluent was first administered before inhalations of methacholine were done every 5 min. Subjects underwent either a short, medium or full protocol depending on the presence of asthma symptoms and baseline lung function. If the FEV_1 was 70–80% of predicted or symptoms were present, concentrations commenced at 0.03 mg/ml and doubled until 16 mg/ml (long protocol). In subjects with an asthma history or symptoms controlled and $FEV_1 \geq 80\%$ of predicted, concentrations commenced at 0.125 mg/ml and doubled until 16 mg/ml (medium protocol). Those with no symptoms or history of asthma and FEV_1 was $\geq$80% of predicted, concentrations of 2, 4, 8, and 16 mg/ml were used (short protocol). This short protocol procedure was completed in 35 min. A positive MCT with a $PC_{20} \leq 8$ mg/ml was considered highly suggestive of asthma [Cockroft et al., 1985]. In subjects in whom MCT was contraindicated, such as those with acute asthma symptoms or a baseline FEV_1 $<$1.5 L or $FEV_1 < 70\%$ predicted, a bronchodilator (400 μg salbutamol) was administered instead [Sterk et al., 1993]. A change in FEV_1 of $\geq$12% and 180 ml increase after 10 min of bronchodilator administration was considered to confirm NSBH.

Among the 543 subjects who underwent spirometry, 15 subjects were unable to generate reproducible curves. There were 83 subjects (16%) who underwent bronchodilator challenge since MCT was contraindicated. Among the remaining 445 subjects, 21 subjects had $\geq$10% decrease in FEV_1 after administration of saline diluent, and did not proceed with MCT. From the 424 subjects remaining, 259 subjects followed the short protocol, 102 subjects the medium protocol and 63 subjects the long protocol. The MCT was discontinued in 18 subjects as they were either unable to perform the procedure satisfactorily. An audit of positive MCT records by an experienced panel of pulmonary function technologists and pulmonologists scored 56% as excellent, 36% satisfactory and only 7% scored poorly according to ATS standards [ATS, 1995, 2000].

Statistical Analysis

Key associations of interest involved investigating the relationships between host factor attributes and occupational disease outcomes. Dependent variables of interest included work-related asthma symptoms (wheeze and/or chest tightness); allergic sensitization; the pattern of baseline spirometry (primarily FEV_1/FVC ratio, FEV_1% of predicted); and the presence of NSBH as defined by a positive MCT on its own or together with positive post-bronchodilator test. The key disease *outcome variables* are presented below:

(a) allergic sensitization to fish (measured by positive immediate skin reactivity or antigen-specific circulating IgE antibodies in human serum to fish).
(b) occupational fish allergic rhinoconjunctivitis (work-related specific symptoms and presence of allergic sensitization).
(c) occupational fish allergic asthma symptoms (work-related specific symptoms and presence of allergic sensitization).
(d) probable occupational asthma to fish (as defined by presence of allergic sensitization to fish and a positive MCT) [Beach et al., 2007].

Statistical analyses were performed using STATA version 6 computer software [StataCorp., 2001]. The general approach involved univariate, bivariate and multivariate analyses of the outcomes of interest in relation to the predictors of interest. Spearmans Correlation Coefficient was used for analysis of continuous health outcomes (SPT antigen/histamine wheal ratio) since the data were skewed. Generalized linear models were used for logistic regression analyses with individual dichotomous outcomes. Key associations of interest involved investigating the relationships between host factor attributes (age, gender, smoking, atopy, and seafood intake), in relation to work-related symptoms, airway obstruction, NSBH and allergic disease outcomes using bivariate unadjusted models. Multivariate logistic regression models adjusting for age, gender, smoking, atopy, and plant (factory) was used to examine the role of certain predictors (“seasonal work,” smoking) in relation to allergic disease outcomes.

RESULTS

Demographic Characteristics

The demographic characteristics of the entire study population are outlined in Table I. The overall proportion of females to males in this study population was 3:1, although a slightly greater proportion of males (54%) were employed in Factory A ($n = 260$) and females (76%) in Factory B ($n = 334$). A significant correlation (Spearman $r = 0.50$, $P < 0.001$) existed between employment duration and age. Almost 70% of workers were employed as seasonal workers. A slightly higher proportion of workers worked on the day shift (52%) compared to the night shift (48%). Almost half of the workforce were current smokers, having, on average, a nine pack year smoking history. A family history of atopy was reported in 39% of subjects. Asthma was twice as common (22%) as hayfever and eczema. Almost all workers reported habitual seafood consumption on a regular basis mainly of fish (99%) and crustaceans (71%).

TABLE I. Demographic Characteristics ($n = 594$) of Salt Water Fish (Pilchard and Anchovy) Processing Workers

Demographic characteristics (n=594)	
Age (years)	36 ± 11
Gender no. (%)	
Female	374 (63%)
Male	220 (37%)
Height (cm)	164 ± 9
Female	159 ± 6
Male	171 ± 7
Employment history	
Employment duration in current factory (years)	10 ± 9
Employment duration in current job (years)	7 ± 7
Employment duration in seafood industry prior to current employment (years)	3 ± 4
Current employment status	
Seasonal	409 (69%)
Permanent	176 (30%)
Casual	9 (1%)
Smoking history	
Tobacco smoking status: no. (%)	
Current smokers	305 (51%)
Ex-smokers	67 (11%)
Non-smokers	222 (37%)
Packyears smoking	
Current smokers	9 ± 9
Ex-smokers	11 ± 13
Allergy history	
Family history of any allergy	230 (39%)
Asthma	132 (22%)
Hayfever	85 (14%)
Eczema	67 (11%)
Seafood dietary history	
Consumption of any seafood type	589 (99%)
Fish	589 (99%)
Rocklobster/prawns	421 (71%)
Oyster/mussels	377 (64%)
Squid (calamari)	354 (60%)
Abalone (perlemoen)	164 (28%)
Eicosapentaenoic acid—EPA (% wt/wt; 20:5n-3)	2.13 ± 1.43

Continuous variables, mean ± SD; categorical variables, number (%).

Occupational History Characteristics

Most currently employed workers who participated in the study were from the canning-related (69%) department, followed by fishmeal manufacturing/warehouse (10%), labeling (9%), jetty (3%), boiler room (3%), workshop (2%), administration (2%), and laundry/cleaners (2%). A large proportion (70%) reported their current jobs producing aerosols (sprays/mist/dust), with 41% reporting excessive levels. Despite 71% working close to the aerosol source, respiratory protective equipment (mainly polypropylene masks) was only worn by 12% of the workforce. Workers who wore goggles and masks on a regular basis, did so for an average of 11 and 10 years, respectively, while gloves were worn on average for 7 years.

Respiratory Symptoms

Chest symptoms (any one of wheeze, tight chest wakening, shortness of breath, shortness of breath wakening, cough wakening) were reported by 4–19% of workers (Table II). A relatively low proportion of workers (3%) had symptoms suggestive of chronic bronchitis. Among the 7% with doctor-diagnosed asthma and 23% reporting hayfever, a large proportion developed these conditions in adult life.

At least 20% of the entire workforce reported inhaling an excessive amount of aerosols in the factory and 16% of workers admitted to having work-related asthma symptoms. The most common suspected putative agents reported by subjects ($n = 79$) included steam vapors from cooking fish in the cannery (62%), dust in the boiler room (11%), fishmeal dust (9%) and fish handling in the cannery (5%). There were 10 workers (2%) who reported job changes following work-related chest symptoms. Among these, five worked in the jetty (pipe switch operators), three in the cannery (sorting table and sealer operator), one each in the ice plant and fishmeal department (scale operator).

A much higher proportion of workers reported work-related ocular-nasal symptoms (26%) than seasonal hayfever symptoms (12%). The most common agents suspected by subjects ($n = 118$) to be responsible for ocular-nasal symptoms were the steam vapors produced during cooking

TABLE II. Respiratory Symptoms Among Salt Water Fish (Pilchard and Anchovy) Processing Workers

Symptom history	Prevalence (%; n = 594)
Chest symptoms	
Wheezing in the past year	86 (14%)
Woken up by tight chest in the past year	49 (9%)
Shortness of breath in the past year	26 (4%)
Woken up by shortness of breath in the past year	23 (4%)
Woken up by cough in the past year	110 (19%)
Asthma history	
Doctor diagnosed asthma	42 (7%)
Current use of asthma medication	24 (4%)
Work-related asthma symptom experience	
Ever inhaled an excessive amount of dust/vapors/mist	124 (21%)
Work-related asthma symptoms (tight chest or wheezing)	93 (16%)
Job change due to work-related chest symptoms	10 (2%)
Ocular-nasal symptoms	
Hayfever current	134 (23%)
Work-related ocular-nasal symptoms	157 (26%)

of fish in the cannery (29%), dust in the boiler room (21%), spices (19%), fishmeal dust (11%) and fish handling in the cannery (10%).

Seafood-Related Allergic Symptoms

The overall prevalence of self-reported seafood-related allergic symptoms (domestic and/or work-related) reported in this working population was 5% (n = 30; data not shown). While most of these workers reported symptoms after eating (87%) seafood, 40% also reported symptoms after skin contact and 17% after smelling seafood vapors. The most common symptoms experienced by subjects were hives/itchy wheals (63%) and gastrointestinal symptoms (57%), while 7% admitted to specific asthma symptoms. Rock lobster (33%), mussels (30%) and pilchard (23%) were the common seafood cited as being associated with these allergic symptoms. A large proportion (43%) reported an immediate (within 1 hr) allergic, mainly skin reactions while working or handling rock lobster and pilchard. These reactions were encountered in the domestic home environment (69%), in the occupational context (53%) and very rarely in the recreational setting (15%).

Patterns of Allergic Sensitization

The overall prevalence of atopy was 36% (Table III). The prevalence of sensitization to any of the fish extracts was 6–7%, with half of these sensitized to either pilchard or anchovy. A larger number of workers were sensitized to pilchard gut than to the other forms of pilchard. Positive results for specific IgE among the 15 workers who did not undergo SPT were obtained for Phadiotop® (9/15), anchovy—*Engraulis encrasicolus* (5/15) and pilchard—*Sardinops melanostica* (4/15). Statistically significant but modest correlations were obtained for subjects sensitized to the different fish extracts (Spearmans r = 0.27–0.38, $P < 0.001$). Much lower, but significant associations were found between sensitization to lobster and mussel (Spearmans r = 0.21, $P < 0.001$), between any fish extract and mussel (Spearmans r = 0.17, $P < 0.001$), between lobster and any fish extract (Spearmans r = 0.14, $P < 0.001$).

Pulmonary Function and Non-Specific Bronchial Challenge Tests

The results of pulmonary function and non-specific bronchial challenge tests completed are presented in Table IV. While 28% of workers had FEV_1 less than 80%

TABLE III. Patterns of Allergic Sensitization on Skin Prick Testing of Salt Water Fish (Pilchard and Anchovy) Processing Workers

Allergen	Prevalence (%)
Common inhalant allergens (n = 578)	
House dust mite (*Dermatophgoides pteronyssinus*)	142 (25%)
Cockroach (*Blatella germanica*)	86 (15%)
Rye grass (*Lolium perenne*)	78 (13%)
Bermuda grass (*Cynodon dactylon*)	46 (8%)
Dog (*Canis familiaris*)	30 (5%)
Cat (*Felis domesticus*)	18 (3%)
Mouldmix (*Cladosporium herbarum, Alternaria alternata, Fusarium*)	18 (3%)
Aspergillus (*Aspergillus fumigatus*)	12 (2%)
Atopy	210 (36%)[a]
Seafood and associated allergens (n = 575)	
Positive to any fish	36 (6%)[b]
Positive to Pilchard in any form (*Sardinops sagax*)	15 (3%)
Pilchard gut (*Sardinops sagax*)	8 (1%)
Pilchard cooked (*Sardinops sagax*)	5 (1%)
Pilchard raw (*Sardinops sagax*)	4 (1%)
Pilchard canned (*Sardinops sagax*)	4 (1%)
Anchovy (*Engraulis capensis*)	15 (3%)
Maasbanker (*Trachurus trachurus capensis*)	8 (1%)
Redeye (*Etrumeus whitehead*)	5 (1%)
Mackerel (*Scomber japonicus*)	2
Fishmeal	5 (1%)
Rock lobster (*Jasus lalandii*)	11 (2%)
Mussel (*Mytilus edulis*)	4 (1%)

[a]Atopy prevalence, 37% (including phadiotop results).
[b]Fish sensitivity, 7% (including specific IgE results).

TABLE IV. Pulmonary Function and Non-Specific Bronchial Challenge Tests Among Salt Water Fish (Pilchard and Anchovy) Processing Workers

Pulmonary function indices	
Males (n = 200)	
FEV_1 (liters)	3.35 ± 0.84
FVC (liters)	4.05 ± 0.79
FEV_1 % predicted	86 ± 16
FVC % predicted	89 ± 13
FEV_1/FVC %	83 ± 22
Females (n = 343)	
FEV_1 (liters)	2.46 ± 0.50
FVC (liters)	2.86 ± 0.55
FEV_1 % predicted	87 ± 13
FVC % predicted	87 ± 13
FEV_1/FVC %	86 ± 7
Entire group (n = 543)	
FEV_1 (liters)	2.79 ± 0.78
FVC (liters)	3.30 ± 0.87
FEV_1 % predicted	86 ± 14
FVC % predicted	88 ± 13
FEV1/FVC %	85 ± 14
No. with FEV_1/FVC < 70% (absolute)	29 (5%)
No. with FEV_1 < 80% predicted	153 (28%)
No. with evidence of bronchial hyperresponsiveness (n = 510)	
No. ≥12% FEV_1 increase post-bronchodilator (n = 83)	11 (2%)
No. ≥10% FEV_1 decrease post-saline diluent (n_c = 445)	21 (4%)
No. ≥20% FEV_1 decrease to methacholine at ≤8 mg/ml (n_c = 424)	123 (24%)
Short protocol (n_c = 259)	53 (10%)
Medium protocol (n_c = 102)	39 (8%)
Long protocol (n_c = 63)	31 (6%)
No. ≥20% FEV_1 decrease to methacholine at >8 mg/ml but < 16 mg/ml (n_c = 424)	17 (3%)

Continuous variables, mean ± SD; categorical variables, number (%); n_c, number completed test; reference values are from the European Community for Coal and Steel (ECCS), 1993.

of predicted values, a much lower proportion (5%) of workers had evidence of airway obstruction on baseline spirometry (FEV_1/FVC <70%). This prevalence of airway obstruction did not change even after computing the proportion of workers having FEV_1/FVC % predicted less than the 5th centile. A total of 26% of workers demonstrated evidence of non-specific bronchial responsiveness (24% on MCT and 2% post-bronchodilator), with a further 3% having "borderline" results (as defined by a ≥20% decrease in FEV_1 in response to methacholine concentrations >8 mg/ml but <16 mg/ml). A positive trend was found between the proportion of workers with a positive MCT and the length of the protocol used: short (42%), medium (33%) and long (25%) protocol.

Prevalence of Work-Related Symptoms, Lung Function and Allergic Disease Outcomes

As discussed previously, the prevalence of work-related ocular-nasal symptoms (26%) was much more common than work-related asthma symptoms (16%; Table II). While the prevalence of sensitization to any fish species was 7% (Table III), the prevalence of occupational allergic rhinoconjunctivitis (ORC) due to fish was 2.6% and occupational asthma due to fish was 1.8% (data not shown).

Host Factors Associated With Work-Related Symptoms, Lung Function and Allergic Disease Outcomes

Logistic regression analysis revealed that female gender was significantly associated with work-related asthma symptoms (OR: 1.94, CI: 1.17–3.21) and the presence of NSBH (OR: 3.09, CI: 1.91–5.01; Table V). Male gender on the other hand, was significantly associated with the presence of airway obstruction (FEV_1/FVC < 0.7; OR: 4.17, CI: 1.85–9.09). Males were also twice more likely to have allergic sensitization to fish (OR: 2.06, CI: 1.09–3.91).

Analysis for associations with atopic status demonstrated that atopy was statistically significantly ($P < 0.01$) associated with work-related asthma symptoms (OR: 2.17, CI: 1.39–3.40); NSBH (OR: 1.59, CI: 1.04–2.43); airway obstruction (OR: 2.16, CI: 1.01–4.58); and allergic sensitization to fish (OR: 3.16, CI: 1.64–6.12; Table V). These associations with atopy were borderline ($P = 0.076$) for ORC due to fish but surprisingly absent for occupational asthma due to fish.

A statistically significant trend ($P = 0.023$) of increasing prevalence of sensitization to fish was observed across smoker status categories, with elevated odds ratios for current smokers compared to non-smokers (OR: 2.37, CI: 1.09–5.13; Table V). This association persisted in the multivariate models that adjusted for known potential confounders (OR: 2.19; CI: 1.01–4.79). A significant trend was also observable between smoking and probable occupational asthma due to fish ($P < 0.001$), but the numbers were too small to generate stable and meaningful odds ratios.

Analysis for associations with seafood consumption as measured by serum levels of an omega-3 fatty acid [EPA % (wt/wt); 20:5n-3] did not reveal any significant association with sensitization to fish ($P = 0.451$; Table V). Furthermore, none of the allergic asthma outcomes in general or specifically due to fish were related to habitual fish consumption as measured by serum EPA levels.

In the bivariate models work-related asthma symptoms was significantly associated with seasonal as opposed to permanent employment status (OR: 1.76, CI: 1.04–2.99). However, after adjusting for potential confounders in the

TABLE V. Host-Associated Predictors of Work-Related Symptoms, Lung Function and Allergic Disease Outcomes Among Salt Water Fish (Pilchard and Anchovy) Processing Workers in Bivariate (Unadjusted) Models

	Host predictor variable (odds ratio, confidence interval)				
Outcome	**Age**	**Female**	**Current smoker**	**Atopy**	**Seafood intake (EPA)**
Work-related symptoms					
Work-related asthma symptoms	1.02 (0.99–1.04)	1.94 (1.17–3.21)*	0.86 (0.54–1.39)	2.17 (1.39–3.40)**	0.94 (0.80–1.11)
Work-related ocular-nasal symptoms	0.99 (0.97–1.01)	1.12 (0.76–1.65)	0.83 (0.55–1.24)	1.12 (0.77–1.64)	0.91 (0.79–1.05)
Baseline pulmonary function indices					
$FEV_1/FVC < 0.7$	1.09 (1.05–1.12)***	0.24 (0.11–0.54)**	1.55 (0.65–3.66)	2.16 (1.01–4.58)*	0.96 (0.73–1.27)
FEV_1 <80% predicted	1.05 (1.03–1.07)***	1.24 (0.83–1.83)	1.40 (0.92–2.12)	1.12 (0.76–1.65)	0.99 (0.87–1.13)
Non-specific bronchial hyperresponsiveness					
Positive methacholine test	1.03 (1.01–1.05)*	3.09 (1.91–5.01)***	1.53 (0.96–2.42)	1.59 (1.04–2.43)*	0.99 (0.86–1.15)
Positive methacholine or post-bronchodilator	1.01 (0.99–1.03)	2.66 (1.69–4.19)***	1.42 (0.92–2.21)	1.50 (1.00–2.24)*	0.99 (0.86–1.13)
Allergic sensitization to fish	1.03 (1.00–1.06)*	0.48 (0.26–0.92)*	2.37 (1.09–5.13)*	3.16 (1.64–6.12)**	1.08 (0.88–1.33)
Occupational rhinoconjunctivitis due to fish	1.02 (0.98–1.07)	1.19 (0.40–3.52)	3.69 (0.80–17.03)	2.58 (0.91–7.36)	1.03 (0.73–1.46)
Occupational asthma symptoms due to fish	1.07 (1.01–1.14)*	0.99 (0.23–4.16)	3.65 (0.42–31.46)	2.84 (0.67–11.99)	0.90 (0.52–1.55)
Probable occupational asthma due to fish	1.03 (0.97–1.09)	0.76 (0.20–2.86)	—	2.10 (0.56–7.91)	0.91 (0.55–1.51)

Each OR is derived from a separate unadjusted model.
EPA, % (w/w) of 20:5n-3 omega fatty acid.
*<0.05.
**<0.01.
***<0.001.

multivariate models, these associations did not reach statistical significance for either work-related asthma symptoms (OR: 1.86, CI: 0.95–3.65) or airway obstruction (OR: 1.65, CI: 0.56–4.86).

DISCUSSION

Workers in this study processing saltwater bony fish (pilchard, anchovy) appear to be at increased risk for developing work-related upper and lower allergic respiratory outcomes. While allergic sensitization to fish was present in 7% of workers, 2.6% had ORC and 1.8% had occupational asthma due to fish. Due to the low prevalence of fish-related occupational asthma (1.8%), it is probable that the healthy worker effect could be operational since only current but not former workers were studied. This is also suggested by the significant association observed between work-related asthma symptoms and seasonal as opposed to permanent employment status. It is however generally accepted that the prevalence of occupational asthma due to high molecular weight agents is estimated to be between 2% and 5% [Chan-Yeung and Malo, 1995]. The prevalence of occupational allergy and asthma due to bony fish, predominantly pilchard and anchovy species, reported in this study is therefore consistent with these figures. There have been very few epidemiological studies of occupational asthma among fish processors reported in the literature. These studies have been conducted among processors of fishmeal (pickling, cod, plaice, tunny, salmon, herring, sardine), cod and salmon, reporting prevalences of 2%, <7%, and 8%, respectively [Dorszscz et al., 1981; Douglas et al., 1995; Bang et al., 2005b]. Occupational asthma due to bony fish is less commonly encountered than to crustacean species where prevalences of up to 16% and 36% have been documented in cross-sectional studies of workers exposed to crab and prawn processors, respectively [Gaddie and Friend, 1980; Cartier et al., 1984].

Among the 6–7% of workers sensitized to any fish species, sensititization to pilchard and anchovy species appeared to be the most common, with a larger number sensitized to pilchard gut compared to pilchard (mainly muscle) in its other prepared forms. Aerosolisation of seafood (meat, internal organs, blood) during processing has been previously identified as a potential high-risk activity for sensitization through the respiratory route [Crespo et al., 1995; Douglas et al., 1995; Ortega et al., 2001; Lopata and

Jeebhay, 2001]. While no specific allergens were identified in these occupational studies, sensitization to a number of allergens in fish muscle tissue proteins (parvalbumins) and gelatin (collagen) have been demonstrated among patients with food allergies due to ingestion [Elsayed and Aas, 1970; Sakaguchi et al., 2000]. Furthermore, bony fish species such as the pilchard and anchovy, belonging to the Class Osteichthyes, have been shown to have high IgE binding activity that correlates with the expression of symptoms in affected individuals [Koyama et al., 2006]. The higher prevalence of sensitization to fish among male workers mainly employed in the fishmeal (containing mainly fish offal) loading and bagging departments, which have been previously shown to have the highest concentrations of these fish antigens (pilchard = antigen 538–3,288 ng/m^3; anchovy = 1,708–15,431 ng/m^3) lend further support to the increased risk of sensitization via inhalation [Jeebhay et al., 2005].

The prevalence of work-related ocular-nasal (26%) and asthma (16%) symptoms was much higher than ORC (2.6%) and asthma (1.8%) due to fish. This suggests that not all symptoms experienced by workers can be attributable to fish allergens aerosolized in the working environment, and that other allergens such as the *Anisakis* fish parasite (aerosolized during degutting/cutting fish or fishmeal production) could possibly be important in causing occupational allergic IgE-mediated sensitization and respiratory allergy as has been demonstrated in previous studies on this group of workers as well as in other studies [Armentia et al., 1998; Purello-D'Ambrosio et al., 2000; Nieuwenhuizen et al., 2006].

Recent studies estimate that, at most, only 50% of asthma cases are attributable to eosinophilic allergic airway inflammation, suggesting a possible role of neutrophil-mediated asthma triggered by endotoxins and other non-specific irritant factors causing asthma symptoms [Douwes et al., 2002]. In this study, 20% of workers reported an episode of inhaling excessive vapor, gas, dust or fumes in their job resulting in work-related asthma symptoms, with 62% of them attributing this to steam vapors produced by cooking fish in the cannery. Concomitant exposures to toxins such as histamine, endotoxin (as our preliminary studies in fishmeal operations have shown) and mycotoxins in organic dust and bioaerosols have also been known to cause mucous membrane irritation and/or asthma on an inflammatory basis [Sherson et al., 1989; Jeebhay et al., 2001; Bonlokke et al., 2004]. Workplace exposure factors other than those of biological origin such physical factors (e.g., hypertonic saline aerosols, cold air, steam vapors), chemicals (e.g., formaldehyde used in fishmeal production; sulphite preservatives, amines and other anti-microbial agents used to soak gloves, forklift exhaust emissions) and other biological contaminants in organic dust have also been suggested as triggers for non-allergic respiratory symptoms of asthma [Ortega et al., 2001; Madsen et al., 2004; Bang et al., 2005a,b].

In this study, the most important host-associated risk factors associated with allergic sensitization to fish were atopy (OR: 3.16, CI: 1.64–6.12) and current cigarette smoking (OR: 2.37, CI: 1.09–5.13). While atopy was not significantly associated with any of the fish-related occupational allergic respiratory outcomes, a significant trend was observable between smoking and probable occupational asthma due to fish ($P < 0.001$). Atopy and cigarette smoking have been the most frequently reported host-associated risk factors for IgE-mediated immunologic reactivity and the development of asthma among seafood processing workers. Atopy has been more consistently associated with sensitization to mainly shellfish [clam, shrimp, crab, prawn, and cuttlefish; Gaddie and Friend, 1980; Cartier et al., 1984; Desjardins et al., 1995; Olszanski and Kotlowski, 1997]. However, Douglas et al. [1995] were unable to demonstrate atopy as a risk factor for occupational asthma among fish (salmon) processing workers. Smoking, on the other hand has been demonstrated in a study among prawn processors as an independent risk factor for increased specific IgE production [OR = 2.4; Mc Sharry et al., 1994]. A significant association between serum antibodies and smoking was also demonstrated among salmon processors with smokers having higher IgE and non-smokers higher IgG levels [Douglas et al., 1995]. In studies focusing on occupational asthma as the outcome, a significant but weak relationship between smoking habits and occupational asthma has been described among snow crab workers [Cartier et al., 1984].

The significance of the different patterns of gender status in relation to work-related symptoms, allergic sensitization and pulmonary function outcomes in this study is of interest. While female gender was a significant predictor of work-related asthma symptoms (OR: 1.94) and non-specific bronchial hyperresponsivess (OR: 3.09), male gender was significantly associated with fish sensitization (OR: 2.06) and airway obstruction (OR: 4.16). Similar associations between female gender and work-related asthma symptoms (OR: 1.73) have been reported among New Zealand mussel processing workers and Canadian crab processors [Glass et al., 1998; Howse et al., 2006]. A high prevalence of NSBH (29%) was also observed in this current study in which women comprised 63% of the overall study population. This is consistent with the findings of previous studies in which women comprised the major proportion of study subjects [Britton et al., 1994; Gautrin et al., 1997]. This current study also demonstrated similar trends for airway hyperresponsiveness viz. gender (OR: 3.09), current smokers (OR: 1.53), atopy (OR: 1.59) and age (OR: 2.27 for group 50–59 vs.18–29 years) to the study by Britton et al. among the general population in the United Kingdom viz. gender (OR: 2.05), current smokers (OR: 1.89), atopy (OR: 1.39) and age (OR: 2.15 for group 50–59 vs. 18–29 years). A possible reason for a higher prevalence of work-related symptoms among women without airway obstruction may be due to the fact that a larger proportion of women are seasonal workers, who are unlikely to return to work the following season should their asthma symptoms persist. On the other hand, male workers being permanently employed may be more likely to move jobs but remain in employment, thereby presenting with progressive deterioration in pulmonary function. The gendered distribution of work, women doing repetitive canning activities in humid environments while men are engaged in more manual and dusty fishmeal production operations, could also explain the elevated risk of fish sensitization observed in men, but more airway symptoms found among women [Jeebhay et al., 2005]. Overall, these findings demonstrate that the expression and experience of health and illness may be moderated by the interplay of a number of factors such as biological vulnerability, exposure to health risks, perception of symptoms, evaluation of risk, information processing and societal role expectations [DunnGalvin et al., 2006].

The current study also investigated the relationship between seafood ingestion and the risk of occupational allergic respiratory disease. The prevalence of reported general seafood allergy obtained from questionnaire interviews was 5%, which is much higher than the 2.8% reported by studies among adults in the USA [Sicherer et al., 2004]. Among the 30 subjects with symptoms of seafood allergy (mainly due to lobster, mussel, pilchard, and mackerel), a large proportion (87%) experienced symptoms as a result of ingestion. Only 17% of cases also reported symptoms after inhaling seafood vapors with 7% admitting to specific asthma symptoms. This is much higher than a recent study reporting 6% of individuals with food allergy who reacted to inhalational exposure to the putative agent [Eigenmann and Zamora, 2002]. Furthermore, almost half (43%) of the respondents in our study reported symptoms while working/handling seafood and only 13% (3/23) of workers sensitized to fish reported an allergic reaction after ingesting seafood. This suggests that seafood ingestion-related allergy alone cannot explain the work-related symptoms and allergic diseases observed in this study. This was confirmed by logistic regression models in which increasing seafood consumption, as measured by the relative composition of serum marine omega 3-fatty acids, did not significantly predict ocular-nasal WRS, asthma WRS, fish sensitization nor fish-allergic occupational rhinoconjunctivitis and asthma. These results corroborate the findings of the Norwegian study in which no association between high-level fish consumption and self-reported asthma symptoms was observed [Fluge et al., 1998].

In conclusion, this study has demonstrated that workers involved in bony fish processing (pilchard, anchovy) are at increased risk of becoming sensitized to fish and developing work-related asthma symptoms. In addition to atopy and cigarette smoking patterns, the gendered distribution of work and related occupational exposures appear to play an important role in the manifestation of allergic respiratory disease outcomes. Future studies could better document the true incidence of occupational allergic sensitization and asthma due to fish processing as well as the exposure-response relationships associated with upper and lower respiratory disease outcomes. Furthermore, studies need to focus on identifying and characterizing the specific protein allergens and other agents present in aerosols generated during fish processing that are responsible for these allergic respiratory disease outcomes.

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