

Environmental Exposure Characterization of Fish Processing Workers

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Received 8 April 2004; in final form 24 December 2004; published online 10 February 2005

Background: Aerosolization of seafood and subsequent inhalation, during processing is a potential high-risk activity for allergic respiratory disease.

Objectives: To quantify total thoracic particulate, protein concentration and specific fish (pilchard, anchovy) antigen concentrations in fish processing plants; to determine the correlation between these exposure metrics; and to identify the major determinants of variability and the optimal grouping strategies for establishing dose–response relationships for fish antigen exposures.

Methods: Exposure assessments were conducted on randomly selected individuals within each of the identified ‘exposure groups’ (EGs) in two fish processing factories. Personal time-integrated sampling was conducted with a thoracic fraction sampler and analysed for particulate mass, total protein and specific fish antigens. Exposure metrics were developed on the basis of individually measured exposures and average levels of these personal samples within EGs. The main components of the exposure variability were determined using ANOVA techniques.

Results: A total of 198 full-shift personal aerosol samples were collected and analysed. Twenty-two percent of the samples were below the limit of detection (LOD) for pilchard and 23% for anchovy assays. Personal sampling revealed wide variations across EGs in arithmetic mean concentrations of thoracic particulate 0.61 mg m^{-3} (range: LOD–11.3), total protein $0.89 \text{ } \mu\text{g m}^{-3}$ (LOD–11.5), pilchard antigen 150 ng m^{-3} (LOD–15 973) and anchovy antigen 552 ng m^{-3} (LOD–75 748) levels. The fishmeal loading and bagging sections of both plants showed consistently high thoracic particulate mass ($0.811\text{--}2.714 \text{ mg m}^{-3}$), total protein ($0.185\text{--}1.855 \text{ } \mu\text{g m}^{-3}$), pilchard antigen ($538\text{--}3288 \text{ ng m}^{-3}$) and anchovy antigen ($1708\text{--}15 431 \text{ ng m}^{-3}$). The a priori strategy that grouped workers according to EGs produced reasonably satisfactory summary exposure metric statistics. An alternative grouping strategy based on department revealed comparable elasticity (exposure contrast). While the correlation between the log-transformed thoracic particulate mass and fish antigen concentrations were generally modest (Pearson’s $r = 0.32\text{--}0.35$, $P < 0.001$), a high correlation was found between pilchard and anchovy antigen concentrations (Pearson’s $r = 0.71$, $P < 0.001$). Models using factory and department grouping strategies accounted for a significant portion of the variability (adjusted $r^2 = 0.18$, $P = 0.043$) in pilchard antigen levels. Grouping strategies using a combination of factory and department yielded the highest degree of elasticity for thoracic particulate (0.38) and pilchard antigen (0.42) levels.

Conclusions: Workers involved in bony fish processing are at risk of inhaling aerosols containing pilchard and anchovy fish antigens. Antigen exposures are highest during fishmeal production and bagging. Grouping strategies based on department and factory

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may provide a more efficient approach than a priori classification of EGs for evaluating fish antigen exposures.

Keywords: bioaerosols; exposure assessment; fish antigens; fishmeal; fish processing; pilchard antigen

INTRODUCTION

Occupational exposure to seafood allergens occurs mainly in the food and fishing industry (Jeebhay *et al.*, 2001). Workers in a number of these industries are exposed to seafood, especially those involved in either manual or automated processing of crabs, prawns, mussels, fish and fishmeal (Malo and Cartier, 1993). Seafood processing plants vary in technology levels, with some of the smaller workplaces relying entirely on manual handling of seafood while larger companies use highly automated modern processes. There is great variation in the processing procedures for the different types of seafood (Moody *et al.*, 1993; Malagie *et al.*, 1998).

Aerosolization of the seafood (meat, exoskeleton, blood, endolymph) during processing has been identified as a potential high-risk activity for sensitization through the respiratory route (Lehrer, 1990; Crespo *et al.*, 1995; Douglas *et al.*, 1995; Smith and Sechena, 1998; Weytjens *et al.*, 1999; Aresery and Lehrer, 2002). Identified processes with high potential for aerosol exposure include: butchering/grinding, degilling, 'cracking' and boiling of crabs; 'tailing' of lobster; 'blowing' of prawns, washing/scrubbing of shellfish; degutting, heading and cooking/boiling of fish; mincing of seafood; cleaning of the processing line and storage tanks with high-pressured water (Jeebhay *et al.*, 2001). Despite high levels of automation in large workplaces, workers employed in these facilities are often found to be at high risk due to inadequate and poorly designed local exhaust ventilation systems (Douglas *et al.*, 1995). Furthermore, processes that generate dry aerosols (prawn blowing using compressed air) appear to generate higher particulate levels than wet processes (prawn blowing using water jets) (Gaddie and Friend, 1980). It has been suggested that because water provides general aerosol suppression, it may also influence the size, lifetime or other dynamics of small protein particles as water is a major feature of this work environment (Ortega and Berardinelli, 1999; Ortega *et al.*, 2001).

A recent review that included evaluation of studies on exposure assessment in the seafood industry found that there is great variability of exposure within and among various jobs involved in seafood processing with reported seafood antigen concentrations ranging from 1 to 5061 ng m⁻³ (Jeebhay *et al.*, 2001). Generally, much higher antigen concentrations have been found using personal sampling as compared with area sampling. The lack of standardized methods for

environmental sample collection, extraction and analysis of high molecular weight sensitizers, such as seafood proteins, makes comparisons between various studies difficult (Heederik *et al.*, 1999). Notably, there have been only two exposure characterization studies on bony fish reported in the literature. Douglas *et al.* found higher respirable particulate (2.71–3.57 mg m⁻³) in area samples of wet processes such as fish gutting and grading than in dry processes such as fish packing in stores (0.04–0.05 mg m⁻³) in a salmon processing plant. These particulate levels of 2.71–3.57 mg m⁻³ corresponded to salmon fish antigen concentrations of 100–1000 ng m⁻³ (Douglas *et al.*, 1995). Taylor *et al.* demonstrated whiff, megrim and hake antigen levels of 2–25 ng m⁻³ obtained from area samples collected in a fish market (Taylor *et al.*, 2000).

The main objectives of the current study were to quantify the total thoracic particulate, total protein and specific fish (pilchard, anchovy) antigen concentrations collected through personal aerosol sampling. The study also aimed to examine correlations among thoracic particulate, protein and specific fish antigen levels. Furthermore, the major determinants of variability of these exposure metrics and optimal grouping strategies for establishing dose–disease response relationships for fish antigen exposures were evaluated.

METHODS

Exposure assessment was conducted using a combination of work history information and personal exposure time-integrated measurements to develop several exposure metrics for analysis.

Sampling strategy

An experienced industrial hygienist (E.R.) first performed a walk-through inspection of each department to identify work groups that were similar with respect to production processes and potential exposures. Job titles and descriptions for each worker in the plant were obtained from production managers and compared with information obtained from questionnaires completed during the health outcome measurement survey. A total of 99 different job titles were reported by workers from the different departments of both factories. The risk of exposure to fish aerosols associated with each job, as reported by the manager and worker, was compared with the estimated risk during the walk-through inspections conducted by the industrial hygienist. Exposure groups (EGs) were then identified to serve as the basis for the sampling strategy to

be used during personal aerosol exposure assessment. Each EG consisted of a number of different job titles within a given department that were assessed as being similarly exposed based on the walk-through inspection together with the worker and supervisor descriptions. Job titles that were difficult to classify into any particular EG due to mobility, were classified as the 'non-stationary exposure group'. Such group average exposure levels can be superior to individual exposure levels that examine exposure health outcome associations when limited data are available on the individual (Heederik *et al.*, 1997).

The walk-through inspections revealed the existence of largely similar departments at the two seafood-processing plants, except for the rock lobster department, which was found only in one plant. Full descriptions of the department-specific processes in these plants have been presented previously (Jeebhay *et al.*, 2004). Some structural processing differences existed between the two factories, which influenced the final compilation of the EGs. Pick-ups, labelling and full can store constituted different departments at Factory B, while they were all in one department in Factory A. The EGs for the respective factories were finally classified from the following departments/sections: jetty, canning, cannery pick-ups, fishmeal manufacturing, fishmeal warehouse (loading, bagging), boiler, rock lobster, labelling, workshop (carpentry, electrical, mechanical), stores, administration and laundry/cleaners. Of the total number of workers that formed part of the study population, 260 workers were employed in Factory A and 383 were employed in Factory B. This constituted 100 and 30% of the respective total workforce populations, with invited Factory B workers being a random sample of all workers stratified on department. Almost 65% of the workers in Factory A and 72% in Factory B were seasonal workers mainly employed in the canning department. The departments employing the largest number of workers were cannery (64% of the total of 643 participants), fishmeal manufacture/warehouse (8%), rock lobster processing (8%) and can labelling (8%). Almost half the workforce (48%) in both factories worked on one of the two night shifts.

Initial observations suggested a total of 36 identifiable EGs (14 EGs in Factory A and 22 in Factory B). In Factory A, this included: five EGs in canning (sorting table, steam exhaust box, retorts/autoclave, pick-ups/labelling, stores), three in fishmeal production (fish pits, manufacturing, loading) and one each in jetty, workshop, boiler room, laundry and administration section. In Factory B, this included seven EGs in canning (sorting table, steam exhaust box, retorts/autoclave, pick-ups, labelling, two stores), three in fishmeal production (maintenance, shovelling, loading/bagging) and one each in the jetty, workshop, boiler room and the administration section. There were six additional EGs in the lobster processing

department (administration, sorting, steaming/washing/scrubbing, packing, weighing, mastering). All non-stationary workers in the cannery and lobster sections were assigned a separate EG in each factory. These EGs formed the basis for the sampling strategy. More detailed descriptions of the tasks in these EGs have been described previously (Jeebhay *et al.*, 2004).

The fishing season for bony fish begins in October and continues until August of the following year. The rock lobster harvesting season starts later in December and ends in August. Seasonal workers work a maximum of 11 months per year and only on production days. In both factories, permanent workers worked mainly on the day shift (0800–1700). Production workers in Factory A worked two shifts (0600–1800, 1800–0600) and in Factory B on three shifts (0700–1500, 1500–2300, 2300–0700). Personal sampling for bony fish processing was done during November and December 2001 and lobster processing in February 2002. Sampling was done on all shifts during the entire work shift in both factories to obtain a full-shift time-weighted average exposure for each job. Ninety-two percent of the samples were collected during the day shift and 8% during the night shift (total number = 198).

All workers on a particular shift were briefed prior to the commencement of sampling by the study's industrial hygienist. Once workers commenced their work shift, they were approached by the industrial hygienist based on the job they performed within a particular EG. At least five personal aerosol exposure samples were obtained from various jobs in each EG in order to account for variability in exposure and to ensure representative exposure assessment. In EGs where there were more than five job titles, jobs entailing similar tasks were grouped together and workers were randomly sampled from each sub group within the EG. In EGs with less than five job titles all jobs were sampled. Moreover, for these EGs with fewer job titles, a random selection of workers in that EG underwent repeat personal sampling on additional days. Most workers (89%) were sampled once, while 7% of workers in the same job were sampled twice and 4% were sampled three or more times. None of the workers refused to undergo personal sampling and all workers provided informed consent before sampling proceeded.

Aerosol sampling instrumentation and procedure

All personal sampling (particulate, protein and specific antigen) was conducted using full-shift-time weighted average samples (range of sampling period: 2.8–7.6 h) collected on each participating worker. A sampling train was set up for each worker by an occupational hygienist, with the flow rates of each pump calibrated according to the specific needs

of that pump. The pumps were calibrated prior to the survey and immediately after sampling using a soap-bubble metre (primary standard) and an electronic stopwatch. A flow rate check was also conducted at the end of each sampling session. Due to the large number of samples being collected over a continuous period (night and day shifts) either an SKC Aircheck Sampler (Model 224-PCCXR) or a Gill Air or Du-Pont Alpha-1 battery operated air-sampling pumps running at an average flow rate of 3 l min^{-1} was used. A two-stage sampler, Personal Environment Monitor (PEM₁₀) Model 200 PEM-2-10 (37 mm filter diameter) (manufactured by MSP Corp., Minneapolis, MN), consisting of a single impaction stage (preselector) and a backup filter was used at the end of the sampling train. The pre-selector has an upper median cut diameter of $10 \mu\text{m}$, resulting in the collection of approximately the 'thoracic' fraction (i.e. reaching the tracheo-bronchial and alveolar regions of the respiratory tract) of particulate on the filter (Lippmann, 1989; Marple, 1989). The PEM₁₀ was fixed to the lapel of the overall/apron, within the breathing zone of the worker. During the sampling period any unusual occurrences were noted and recorded on the sampling data sheet. It must be noted that the PEM₁₀ used in this study is actually designed to operate at a flow rate of 2 l min^{-1} in order to obtain the desired $10 \mu\text{m}$ cut point of the thoracic fraction. However, in an attempt to improve the sampling efficiency for antigen collection the air-sampling pumps were set at 3 l min^{-1} during the collection of all the samples. Increasing the flow rate may have resulted in the actual cut point being reduced from 10 to $8.2 \mu\text{m}$. This value is obtained from particle theory, i.e. the particle diameter collected by an impactor is proportional to the inverse square root of the flow rate (e.g. $10 \mu\text{m} \times 1/\sqrt{(3 \text{ l min}^{-1}/2 \text{ l min}^{-1})} = 8.2 \mu\text{m}$) (Willeke and Baron, 1993). Although the samples do not exactly fulfill the current thoracic fraction definition, since they were all collected in a similar manner, they are all comparable to each other. Furthermore, the cut point is still close enough to represent particles in the thoracic fraction range that penetrate to the bronchial tree.

The aerosols were collected on polytetrafluoroethylene (PTFE) membrane laminated filters ($0.3 \mu\text{m}$ pore size) (Quan-Tec-Air Inc.). These were weighed before and after sampling in a humidity and temperature-controlled environment using a microbalance, to determine particulate mass concentrations. The weighing process was repeated thrice with the average weight being recorded as the filter weight. At least 10% of the filters were submitted together with the field samples as field blanks in order to control for potential contamination during handling, storage, transportation and analysis of the filters. The filters were transported in a cooler box on ice to a freezer at the factory, where it was stored at a temperature

of 4°C until transport to the main laboratory where filters were stored at -20°C until extraction.

Analysis of samples for protein and antigen concentration determinations

Preparation of seafood extracts. Extracts (1:10 w/v) of pilchard (*Sardinops sagax*) and Cape anchovy (*Engraulis capensis*) were prepared from fresh specimens obtained from the factory and extracted into phosphate-buffered saline (PBS) overnight at 4°C . The extracts were sterile filtered using $0.45 \mu\text{m}$ filters (Millipore). The protein concentration of each extract was determined by using bicinchoninic acid assay (BCA protein assay; Pierce) and aliquots were subsequently stored at -80°C . These extracts were used to develop the antisera for the immunoassays and also as internal standards in these assays for detecting and quantifying the protein antigens collected on filters housed in the personal sampling cassettes.

Preparation of filters for personal samples. For elution, the membrane backing was removed, filters were cut into pieces and placed into $0.5 \text{ ml PBS}-0.05\%$ Tween 20. The filter pieces were eluted overnight on a shaker at 4°C , the mixture was centrifuged at 2000 g for 2 min and the supernatant was stored at -80°C until further use. These procedures were conducted according to similar methods described previously for seafood and flour antigen extractions (Lillienberg *et al.*, 2000; Griffin *et al.*, 2001).

Determination of protein concentration of samples. A protein standard was prepared in duplicate using BSA (bovine serum albumin) ranging from 0.05 to 0.25 mg incubated at 60°C for 30 min. After incubation the plate was cooled to room temperature and the absorbance read at 540 nm . A standard curve was used to determine the protein concentration for each unknown protein sample using the BCA protein assay.

ELISA-inhibition assay for antigen determinations of samples. An ELISA-inhibition assay specifically designed for this study was used to identify antigen presence on personal sample filters (Lopata *et al.*, personal communication). These assays were conducted in the Division of Immunology at Groote Schuur Hospital in South Africa. The ELISA method uses the following reagents: sodium carbonate/bicarbonate buffer (100 mM) pH 9.6; Tris-buffered saline (TBS) $10\times$ pH 7.4; 2% blocking reagent (powder skim milk in TBS with 0.05% Tween 20); TBS- 0.05% Tween 20 buffer; TBS-alkaline phosphatase buffer $5\times$ pH 9.5; goat anti-rabbit IgG antibody (Southern Biotechnology Associates, Inc.); substrate (PNPP; Sigma) and polystyrene 96-well microtitre plates (Dynex flat bottom 2HB high binding plates).

The method is explained in detail elsewhere (Lopata *et al.*, personal communication). In brief, polystyrene microtitre plates (Dynex flat bottom 2HB)

were coated with optimized protein concentration for each different seafood extract, followed by blocking and incubation for 60 min at 37°C. One standard inhibition curve was used for each plate assay ranging from 0.2 to 200 $\mu\text{g ml}^{-1}$. The eluant from personal samples was added to each well followed by rabbit antiserum to determine the degree of inhibition. A high degree of inhibition indicates larger amounts of antigen in the samples. Provision was made to demonstrate 'total specific binding' (TSB = 0% inhibition) and 'non-specific binding' (NSB=blank control).

Standard inhibition curves, expressed by a simple linear regression model ($y = mx + a$), were used to determine the limit of detection for each specific plate. As seven plates per antigen were used to analyse the large number of samples, the detection limit was presented as a range. The antigen concentration obtained in micrograms of antigen per millilitre was corrected for the volume of air flowing through the personal air-sampling pump to obtain a final concentration in terms of micrograms of antigen per cubic metre. The detection limit for the pilchard assay was generally lower (range: 0.010–0.235 $\mu\text{g m}^{-3}$) than that of the anchovy assay (range: 0.069–0.225 $\mu\text{g m}^{-3}$). In the statistical analyses, values below the detection limit were considered as being two-thirds of this limit (Helsel, 1990; Hornung and Reed, 1990; Houba *et al.*, 1997).

Quality control of the analytical methods was assured by comparing the standard curves obtained for the pilchard and anchovy antigen assay used by the collaborating laboratories (University of Cape Town, South Africa, and Tulane Medical Centre, USA) using the same protocols. The method recommended by Bland and Altman was used to test the level of agreement of the standard curves ($n = 10$) of the two laboratories (Bland and Altman, 1986). The pilchard and anchovy standard curves both showed good agreement between the laboratories: the confidence interval of the level agreement of the mean $\log_{10}$ difference was 0.008–0.102 for the pilchard standard curves and 0.092–0.172 for the anchovy standard curves. Replicate analyses conducted on a 10% subset of the samples ($n = 20$) also found reasonable agreement between the two laboratories.

The intra-assay (within plate) coefficient of variation (CV = standard deviation/mean) obtained during the analysis of all standards in the main study (by the South African laboratory) varied from 2.2 to 5.1% for the pilchard standard curves and from 1.6 to 3.7% for the anchovy standard curves. The inter-assay variability, reflecting the 'day-to-day' variation between microplates was used as an indicator for the reproducibility of results within the ELISA method. This was assessed by calculating the CV using the mean and standard deviation of all the optical density readings for each standard concentration ranging from 0.20 to 200 $\mu\text{g ml}^{-1}$ for each antigen. The

average inter-assay variation (as assessed by the CV) for the pilchard and anchovy standard curves over 7 days was 12.6 and 8.7%, respectively. These results are considered to be satisfactory since the CV was less than 15% (Sasaki and Mitchell, 2002).

Statistical analysis

All statistical analyses were performed using Stata computer software and graphical illustrations used SPSS software package (SPSS Inc., 2001; Stata Corp, 2001). Descriptive univariate statistics were generated for the total sample distribution. A correlation matrix using Pearson's correlation coefficient (r) was computed on the log-transformed exposure metrics for the overall data and for each department. Correlational analysis using non-parametric tests (Spearman's rank correlation) gave very similar results. Linear regression models were developed to describe the determinants of variability of the various exposure metrics. Since the data showed a lognormal distribution, the natural logarithm ($\ln$) of the measured exposure was used as the dependent variable. Independent variables considered for entry into the models included factory, department, EG, job type and shift sampled. Regression diagnostics were also performed to assess and deal with outlying and influential observations.

The effectiveness of the a priori EG definitions was evaluated using a random effect analysis of variance (ANOVA) and calculation of grouping statistics including a measure of exposure contrast (elasticity) (Kromhout *et al.*, 1995). Groups, workers within these groups and their subsequent measurement periods were assumed to be selected at random during a shift, so random effect models were used (Houba *et al.*, 1997). The structure of the exposure data was regarded as hierarchical (nested) (Samuels *et al.*, 1985) with job type nested in the EG, which in turn was nested in the department of each factory. A two-way nested random effects ANOVA model was applied to estimate a between-group (σ_{bg}^2) and a within-group (σ_{wg}^2) variance component for different grouping schemes. The estimates of the variance components σ_{bg}^2 and σ_{wg}^2 were designated as S_{bg}^2 and S_{wg}^2 , respectively. Using these variance components, the elasticity was calculated as: $\text{elasticity} = S_{\text{bg}}^2 / (S_{\text{bg}}^2 + S_{\text{wg}}^2)$ (Kromhout and Heederik, 1995; Houba *et al.*, 1997). This ratio will reach the value of 1 if each group is entirely distinct. At the other extreme, if all groups are similar then this ratio will approach the value 0. Since most workers were only sampled on one occasion, the 'precision' statistic could not be calculated from the variance components obtained.

RESULTS

A total of 198 full-shift personal aerosol samples were collected from 145 workers for particulate mass,

protein, pilchard and anchovy antigen concentration determinations. There were 20 samples on which total protein determinations could not be done since the amount of eluant obtained was insufficient. Descriptive statistics and results of the one-way ANOVA are presented in Tables 1 and 2. The thoracic particulate mass, protein concentrations as well as pilchard and anchovy antigen concentrations followed a lognormal distribution for most EGs; 23% of the samples were below the detection limits for pilchard and 21% for anchovy.

Summary exposure metrics per EG

This study demonstrated a wide range of mean exposures from least (administration) and most exposed (fishmeal loading/bagging) jobs for thoracic particulate (GM: 0.097 mg m^{-3} versus 0.889 mg m^{-3}), protein (GM: $0.583 \text{ } \mu\text{g m}^{-3}$ versus $1.964 \text{ } \mu\text{g m}^{-3}$), pilchard antigen (GM: 51 ng m^{-3} versus 353 ng m^{-3}) and anchovy antigen (GM: 65 ng m^{-3} versus 1120 ng m^{-3}) (data quoted for Factory A, Tables 1 and 2). The mean exposures for the least and most highly exposed exposure groups in both factories combined varied by about 9-fold for thoracic particulate, 50-fold for protein, and 12- and 18-fold for pilchard and anchovy antigen, respectively. Overall, the mean exposures across EGs varied considerably indicating a reasonably robust exposure gradient for all parameters of interest.

Of interest are the notable differences in exposures between the two factories when comparing groups with similar job titles and departments. For thoracic particulate mass concentrations, higher concentrations were found in the boiler room and fishmeal loading in Factory A as compared with Factory B (Table 1). The wide range of values obtained in the general storeroom and workshop (Factory B) were due to a single outlier observation. High ambient protein concentrations were found in the workshop (Factory B), the cannery exhaust box/saucer area (Factory A) and fishmeal loading (Factory A) (Table 1). Personal exposures to pilchard and anchovy aerosol antigen concentrations were highest in the fish pits, fishmeal manufacture and loading/bagging of both factories, cannery retorts (both factories) and cannery fish sorting table (Factory B) (Table 2). Non-stationary workers in the canning departments also had high mean pilchard antigen exposures in both factories. Surprisingly high ambient anchovy (but not pilchard) antigen levels were found in certain lobster EGs that do not normally handle anchovy, reflecting possible cross-contamination by non-stationary workers.

In each factory, the proportion of thoracic particulate consisting of pilchard or anchovy antigen varied across the EGs, most likely due, in part, to the use of ingredients other than fish in the production process (Table 2). The ratio of the average amount of pilchard

and anchovy antigen to thoracic particulate was the highest in the fishmeal manufacturing EGs of both factories (pilchard: 0.868 parts per thousand by weight; anchovy: 1.839 parts per thousand by weight), reflecting high fish antigen levels in the dust particulate produced in this phase of the production process. Other groups with above average ratios were the jetty and fish pits suggesting bioaerosol generation during handling of large quantities of fish. The isolated unexpectedly high values obtained for the administration EG (Factory B), laundry and certain lobster EGs may point to possible cross-contamination of work environments by non-stationary workers from other departments (Table 2).

Correlation between exposure metrics

There were statistically significant correlations between the log-transformed ambient thoracic particulate mass and protein concentrations ($r = 0.32$, $P < 0.001$, $n = 169$) (Table 3; Fig. 1). Department-specific correlations between these two measures were especially high in labelling ($r = 0.89$) and boiler room ($r = 0.83$). Both these parameters were also modestly correlated with fish (pilchard and anchovy) antigen concentrations, with slightly higher correlations seen between thoracic particulate concentrations and fish antigen concentrations (r : pilchard = 0.32; anchovy = 0.35, $P < 0.001$, $N = 191$) than with total protein (r : pilchard = 0.23; anchovy = 0.17, $P < 0.05$, $n = 171$) (Table 3; Figs 2 and 3). The correlations between particulate and fish antigen were significantly higher in the fishmeal warehouse (r : pilchard = 0.75; anchovy = 0.82). As expected, a significant correlation was found between ambient pilchard and anchovy antigen concentrations ($r = 0.71$, $P < 0.001$, $n = 194$) (Table 3; Fig. 4), again with even significantly higher correlation in the fishmeal warehouse ($r = 0.97$).

Determinants of exposure variability

In the bivariate analyses, the EG explained the greatest variability observed in the thoracic particulate (adjusted $r^2 = 0.31$, $P < 0.001$) and protein (adjusted $r^2 = 0.28$, $P < 0.001$) measurements, followed by job titles (adjusted $r^2 = 0.22$, $P = 0.011$) for thoracic particulate levels (Table 4). The department, however, explained the greatest variability in fish antigen measurements of both pilchard (adjusted $r^2 = 0.15$, $P = 0.013$) and anchovy (adjusted $r^2 = 0.11$, $P = 0.041$) fish species. The work shift did not contribute significantly towards the variability in any of the measurements observed. The addition of other variables such as factory, department and job title to the main explanatory variable did not improve the overall fit of any of the models, except for a model predicting pilchard antigen levels that included factory with department. These variables explained the greatest variability

Table 1. Personal ambient thoracic particulate and protein concentration exposure levels of fish processing workers classified by EG

Factory	EG	k	Particulate mass concentration (mg m ⁻³)				Protein concentration (µg m ⁻³)					
			n	AM	GM	GSD	Range	n	AM	GM	GSD	Range
A	Jetty/holding tank	6	6	0.669	0.387	3.441	LOD-2.954	6	1.853	1.548	2.650	LOD-5.93
	Sorting table	5	5	0.489	0.395	2.125	0.185-0.895	5	1.376	1.658	1.388	LOD-2.16
	Steam exhaust box/saucer	3	6	0.773	0.739	2.105	LOD-2.176	4	1.888	2.170	2.002	LOD-4.13
	Retort/autoclave	4	8	0.938	0.663	2.543	LOD-4.245	4	0.960	1.854	1.458	LOD-2.42
	Pick-ups/labelling	5	5	0.565	0.496	1.755	0.303-1.027	5	0.558	0.269	6.766	0.01-1.14
	General stores	4	4	0.306	0.530	2.173	LOD-0.917	4	1.195	0.990	2.075	0.44-2.17
	Administration	5	5	0.019	0.097	a	LOD-0.097	5	0.322	0.583	3.312	LOD-1.36
	Workshop	5	5	0.431	0.363	2.000	0.149-0.752	5	1.660	1.622	1.260	1.34-2.42
	Boiler room	5	5	2.419	1.994	1.886	1.152-5.926	5	1.590	1.426	1.768	0.56-2.44
	Laundry	1	5	0.275	0.217	2.175	0.096-0.553	4	1.478	1.871	1.449	LOD-2.66
	Non-stationary cannery	5	7	0.285	0.297	1.648	LOD-0.643	6	1.098	1.150	1.827	LOD-2.13
	Fish pits	1	5	0.496	0.453	1.598	0.247-0.898	4	1.058	0.894	2.124	0.3-1.48
	Fishmeal manufacture	5	5	0.653	0.497	2.249	0.206-1.570	5	1.108	1.028	2.681	LOD-2.58
	Fishmeal loading	5	5	2.714	0.889	4.780	0.182-11.293	4	1.855	1.964	2.425	LOD-4.39
B	Jetty	4	6	0.133	0.151	1.484	LOD-0.210	4	0.008	0.030	a	LOD-0.03
	Sorting table	5	7	0.620	0.715	1.887	LOD-2.123	5	0.576	0.264	5.262	LOD-2.37
	Steam exhaust box/saucer	5	6	0.535	0.787	1.258	LOD-1.030	6	0.262	0.227	3.814	LOD-0.81
	Retort/autoclave	5	6	0.521	0.663	2.109	LOD-1.122	6	1.060	0.682	5.319	LOD-2.78
	Pick-ups	5	5	0.343	0.569	1.134	LOD-0.617	5	0.008	0.040	a	LOD-0.04
	Labelling	5	5	0.066	0.157	0.1586	LOD-0.217	5	All <LOD			
	General stores	5	5	2.626	1.335	3.093	0.669-9.846	5	1.218	0.986	3.165	LOD-2.94
	Full can store	4	5	0.439	0.507	1.562	LOD-0.912	5	0.012	0.06	a	LOD-0.06
	Administration	5	5	0.455	0.524	1.599	LOD-0.915	5	0.006	0.03	a	LOD-0.03
	Workshop	5	5	1.805	0.702	6.178	LOD-7.725	5	2.778	5.243	3.037	LOD-11.50
	Boiler room	5	5	0.343	0.366	1.945	LOD-0.762	5	0.382	0.579	1.769	LOD-0.83
	Non-stationary cannery	5	6	0.407	0.401	2.002	LOD-1.031	5	0.858	0.456	4.202	LOD-3.52
	Fish pits	1	5	0.515	0.621	1.378	LOD-0.860	2	0.330	0.660	a	LOD-0.66
	Fishmeal manufacture	5	7	0.126	0.201	1.651	LOD-0.328	6	0.185	1.110	a	LOD-1.11
Overall	Fishmeal loading/bagging	5	5	0.811	0.690	2.063	0.202-1.317	4	1.503	1.442	1.400	0.96-2.08
	Administration (lobster)	4	5	0.173	0.164	2.307	LOD-0.493	5	2.102	0.964	8.723	LOD-4.96
	Lobster sorting	1	5	0.370	0.354	1.395	0.248-0.516	5	0.538	0.271	9.458	LOD-1.23
	Lobster labourers	1	5	0.236	0.194	2.175	0.056-0.414	5	0.188	0.207	1.828	LOD-0.36
	Lobster packers	1	9	0.257	0.296	1.669	LOD-0.602	9	0.309	0.329	5.580	LOD-1.22
	Lobster boxing	5	5	0.282	0.232	1.942	0.117-0.661	5	0.696	0.675	2.496	LOD-1.63
	Lobster mastering	1	5	0.189	0.176	1.567	0.086-0.258	5	0.592	0.953	1.370	LOD-1.37
	Non-stationary (lobster)	4	5	0.130	0.146	1.694	LOD-0.280	5	1.274	1.155	1.678	0.57-1.97
	145	198	0.610	0.420	2.531	LOD-11.293	178	0.894	0.732	3.759	LOD-11.50	

k, number of workers sampled in an EG; n, number of measurements in a group; AM, arithmetic mean; EG, exposure group; GM, geometric mean; GSD, geometric standard deviation; LOD, less than limit of detection.

^aOnly one sample measurement > 0, standard deviation cannot be calculated.

Table 2. Personal ambient thoracic pilchard antigen and anchovy antigen exposure levels of fish processing workers classified by EG

Factory	EG	k	n	Pilchard concentration (ng m ⁻³)				Antigen/particulate ^a (ng mg ⁻¹)				Anchovy concentration (ng m ⁻³)				Antigen/particulate ^a (ng mg ⁻¹)	
				AM	GM	GSD	Range	Mean ratio	AM	GM	GSD	Range	AM	GM	GSD	Range	Mean ratio
A	Jetty/holding tank	6	6	10.2	30.4	1.1	LOD-33	36	22.0	132.0	^b	LOD-132	559				
	Sorting table	5	5	33.8	41.2	1.3	LOD-51	128	115.8	113.4	1.3	88-143	287				
	Steam exhaust box/saucer	3	5	83.2	67.6	2.0	32-204	96	267.2	182.2	2.5	75-795	316				
	Retort/autoclave	4	6	71.7	108.2	2.9	LOD-201	203	221.2	351.4	2.6	LOD-621	503				
	Pick-ups/labelling	5	5	36.8	75.9	2.5	LOD-144	248	85.4	156.2	3.2	LOD-359	511				
	General stores	4	4	90.8	96.1	2.4	LOD-214	292	114.8	219.7	1.5	LOD-296	415				
	Administration	5	5	21.2	50.8	1.5	LOD-68	701	25.8	64.5	1.0	LOD-65	670				
	Workshop	5	5	6.0	30.0	^b	LOD-30	201	24.8	124.0	^b	LOD-124	165				
	Boiler room	5	5	12.6	31.5	1.0	LOD-32	19	59.2	148.0	1.0	LOD-150	88				
	Laundry	1	5	54.0	89.2	1.2	LOD-106	411	90.0	148.1	1.2	LOD-183	682				
	Non-stationary cannery	5	7	68.4	102.9	1.8	LOD-247	315	98.1	151.4	1.8	LOD-276	517				
	Fish pits	1	5	143.4	83.5	4.0	LOD-563	158	317.2	586.3	3.2	LOD -1327	859				
	Fishmeal manufacture	5	5	132.0	110.8	3.0	LOD-347	297	37.8	189.0	^b	LOD-189	632				
B	Fishmeal loading	5	5	3288.2	353.4	15.3	LOD -15973	267	15431.4	1120.5	19.8	LOD -75748	847				
	Jetty	4	6	40.3	78.1	1.4	LOD-94	448	84.0	252.0	1.0	LOD-252	1200				
	Sorting table	5	7	96.3	119.5	1.7	LOD-244	167	269.9	368.0	2.6	LOD-747	459				
	Steam exhaust box/saucer	5	6	53.0	79.6	1.2	LOD-92	98	93.5	111.4	1.1	LOD-129	120				
	Retort/autoclave	5	6	318.7	234.8	2.3	97-898	498	237.3	251.5	1.7	LOD-592	423				
	Pick-ups	5	5	28.2	46.9	1.1	LOD-49	79	39.2	98.0	1.0	LOD-99	160				
	Labelling	5	5	8.6	43.0	^b	LOD-43	198	34.8	86.3	1.2	LOD-98	452				
	General stores	5	5	7.5	38.0	^b	LOD-38	57	33.8	84.1	1.2	LOD-93	114				
	Full can store	4	5	18.2	91.0	^b	LOD-91	100	29.8	149.0	^b	LOD-149	163				
	Administration	5	5	13.6	68.0	^b	LOD-68	74	43.6	109.0	1.0	LOD-109	146				
	Workshop	5	5	14.2	71.0	^b	LOD-71	9	All <LOD								
	Boiler room	5	5	36.0	59.9	1.1	LOD-64	128	All <LOD								
	Non-stationary cannery	5	6	64.2	115.6	1.8	LOD-172	259	145.5	182.7	3.2	LOD-680	315				
	Fish pits	1	5	186.6	1919.3	2.2	LOD-363	308	506.6	428.7	3.0	LOD-1485	690				
	Fishmeal manufacture	5	7	128.9	132.3	1.7	LOD-310	868	294.1	280.1	2.8	LOD-855	1839				
	Fishmeal loading/bagging	5	5	538.4	299.3	3.1	91-1835	109	1708.6	468.0	5.3	81-7458	170				
	Administration (lobster)	4	5	All <LOD					25.6	128.0	^b	LOD-128	260				
	Lobster sorting	1	5	All <LOD					All <LOD								
	Lobster labourers	1	5	All <LOD					All <LOD								
Lobster packers	1	9	All <LOD					173.9	346.7	7.8	LOD-1484	8109					
Lobster boxing	5	5	All <LOD					All <LOD									
Lobster mastering	1	5	All <LOD					317.4	336.7	8.4	LOD-1512	1305					
Non-stationary (lobster)	4	5	29.8	70.7	1.6	LOD-98	604	28.8	72.0	1.0	LOD-73	615					
Overall		145	195	150	99	2.7	LOD-15973	206	552	211	3.1	LOD-75748	462				

k, number of workers sampled in an exposure group; *n*, number of measurements in a group; AM, arithmetic mean; EG, exposure group; GM, geometric mean; GSD, geometric standard deviation; LOD, less than limit of detection.

^aRatio of the antigen to particulate mass (particulate masses are shown in Table 1).

^bOnly one sample measurement > 0, standard deviation cannot be calculated.

(adjusted $r^2 = 0.18$, $P < 0.005$) in the final exposure model (Table 5):

$$\begin{aligned} &\log(\text{pilchard antigen}) \\ &= \beta_0 + \beta_1 \text{ Factory B} + \beta_2 \text{ Canning} \\ &\quad + \beta_3 \text{ Cannery pick-ups} \\ &\quad + \beta_4 \text{ Fishmeal manufacturing} \\ &\quad + \beta_5 \text{ Fishmeal warehouse} + \beta_6 \text{ Boiler room} \\ &\quad + \beta_7 \text{ Rock lobster} + \beta_8 \text{ Labelling} \\ &\quad + \beta_9 \text{ Workshop} + \beta_{10} \text{ General stores} \\ &\quad + \beta_{11} \text{ Jetty} + \beta_{12} \text{ Laundry} \end{aligned}$$

Note: β is regarded as the regression coefficient (parameter estimate) in the multiple linear regression model.

Grouping schemes based on estimation of variance component

Random effect ANOVA and calculation of grouping statistics including a measure of contrast

Table 3. Correlation matrix for various exposure metrics (log-transformed, ln) among fish processing workers

Exposure metric	Pearson's Coefficient (r)		
	Thoracic particulate mass (ln)	Total protein (ln)	Anchovy antigen (ln)
Pilchard antigen (ln)	0.32**	0.23*	0.71**
Thoracic particulate mass (ln)	—	0.32**	0.35**
Total protein (ln)	—	—	0.17*

* $P < 0.05$; ** $P < 0.001$.

(elasticity) between factories, departments and EGs are presented in Table 6. Since the determinants for the variability in thoracic particulate and protein were very similar, the former exposure metric was considered to be representative of both for these comparisons. A similar approach was used for choosing the pilchard antigen levels as being representative for anchovy antigen exposure levels. Among the grouping schemes based on a single factor, the EG yielded the highest elasticity values for thoracic particulate dust (0.34). For thoracic pilchard antigen concentration, grouping workers on the basis of department yielded the highest single factor elasticity value (0.34). Slightly higher levels of elasticity were also obtained for grouping workers by factory and department for both thoracic particulate (0.38) and pilchard antigen levels (0.42).

DISCUSSION

This study used a combination of industrial hygiene, immunological and statistical techniques to characterize the exposure to ambient thoracic particulate, protein and fish (pilchard and anchovy species) antigens among workers in fish processing plants. While exposure variability has been characterized in great detail for crab processing activities (Griffin *et al.*, 1994; Ortega and Berardinelli, 1999; Weytjens *et al.*, 1999; Beaudet *et al.*, 2002), in previous studies, only respirable particulate concentrations for various work activities have been reported for bony fish, i.e. salmon processing workers (Douglas *et al.*, 1995). Notable differences in exposure levels using various

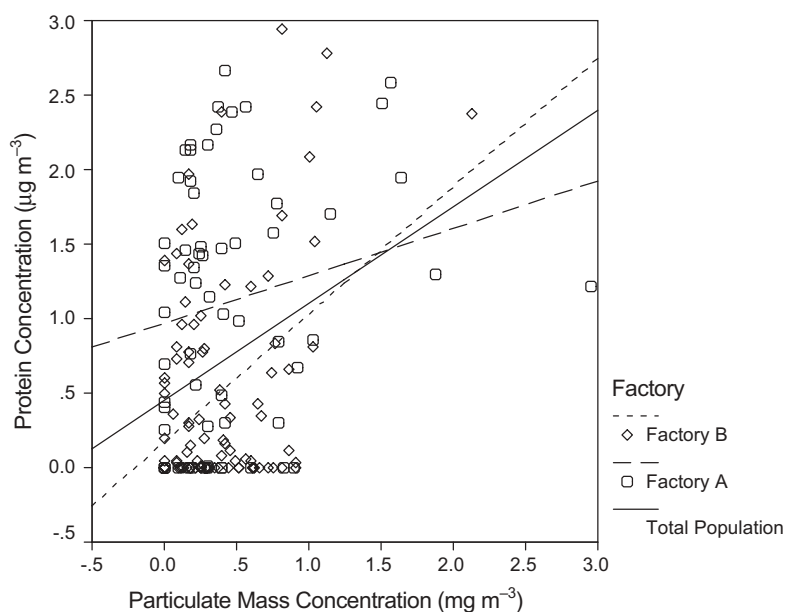


Fig. 1. Relationship between thoracic particulate mass concentration and protein concentration of personal samples among fish processing workers: $\ln \text{ protein } (\mu\text{g m}^{-3}) = 0.07 + 0.52 \ln \text{ particulate mass } (\text{mg m}^{-3})$.

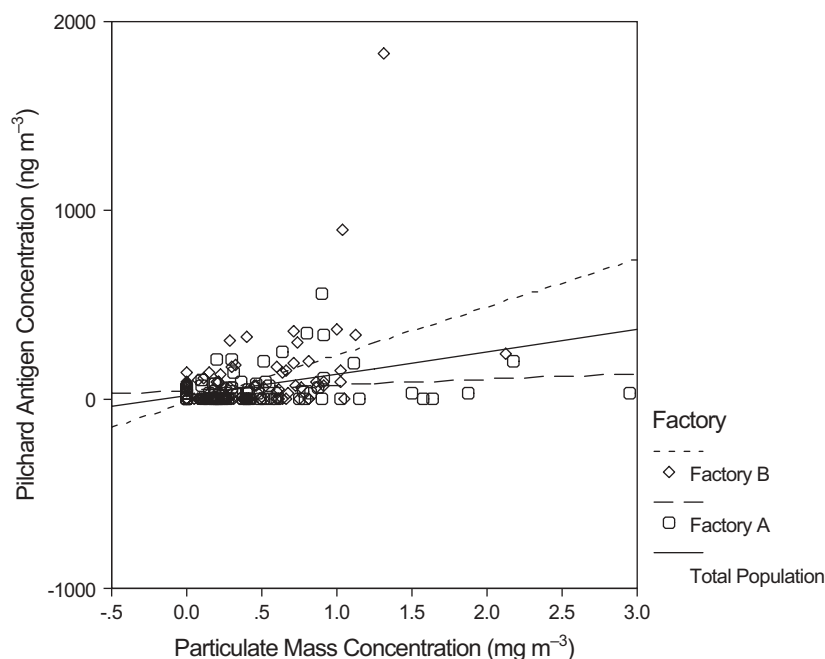


Fig. 2. Relationship between thoracic particulate mass concentration and pilchard antigen concentration of personal samples among fish processing workers: $\ln \text{pilchard antigen (ng m}^{-3}\text{)} = 2.13 + 2.11 \ln \text{particulate mass (mg m}^{-3}\text{)}$.

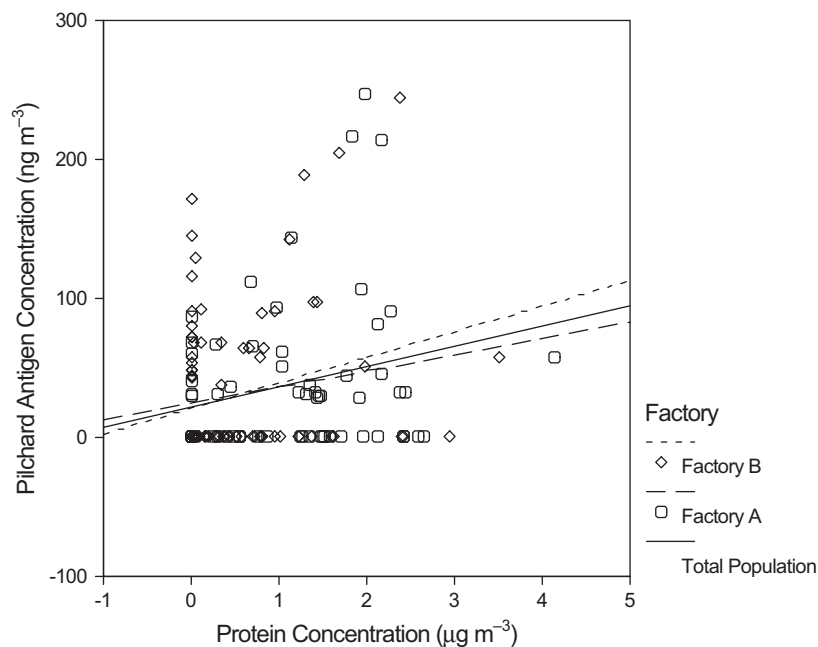


Fig. 3. Relationship between thoracic protein concentration and pilchard antigen concentrations of personal samples among fish processing workers: $\ln \text{pilchard antigen (ng m}^{-3}\text{)} = 1.30 + 0.85 \ln \text{protein (µg m}^{-3}\text{)}$.

metrics were observed when comparing EGs having similar job titles in the two factories in this study. These differences, however, did not demonstrate a consistent pattern in one factory having higher exposures than the other. The observed differences are likely to be attributable to factors such as factory

size (Factory A being much smaller), physical layout and ventilation, handling procedures of fish before and after arrival at factory, actual amount of fish processed during the working day, variations in certain work procedures, activities and equipment used in processing, and a host of other unmeasured covariates

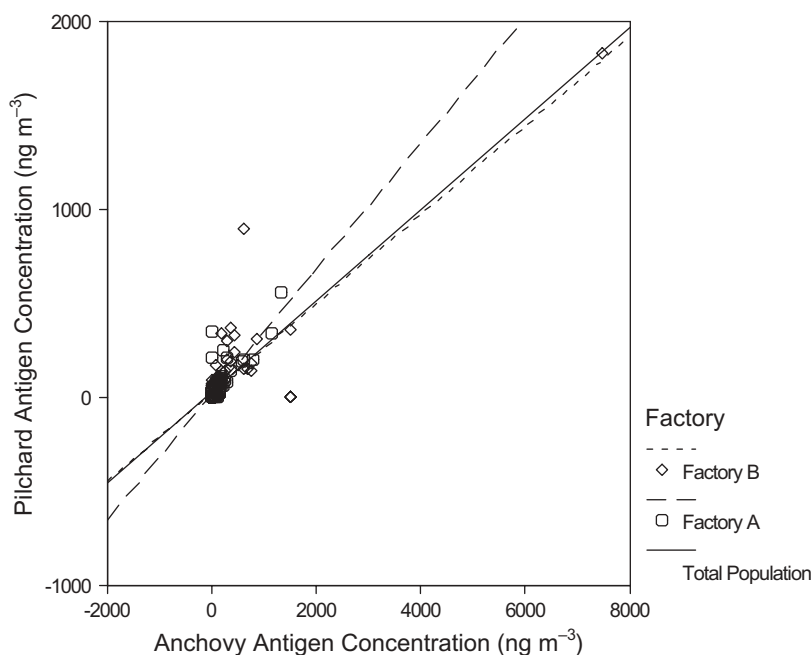


Fig. 4. Relationship between pilchard and anchovy antigen concentrations of personal samples among fish processing workers: $\ln \text{ pilchard antigen (ng m}^{-3}\text{)} = 0.55 + 0.63 \ln \text{ anchovy antigen (ng m}^{-3}\text{)}$.

Table 4. Simple linear regression models^a describing the degree of variability (adjusted r^2) in exposure metrics (log-transformed, $\ln$) explained by specific exposure variables among fish processing workers

Variable	Exposure metric model – adjusted r^2			
	Thoracic particulate $\ln$ (mg m^{-3})	Total protein $\ln$ ($\mu\text{g m}^{-3}$)	Pilchard antigen $\ln$ (ng m^{-3})	Anchovy antigen $\ln$ (ng m^{-3})
Factory	0.02*	0.10**	0.02	0
Department	0.19**	0.10*	0.15*	0.11*
Exposure group	0.31**	0.28**	0.12	0
Job title	0.22*	0.23	0.10	0
Shift	0.01	0.02	0	0

* $P < 0.05$; ** $P < 0.001$.

^aValue for each cell represents a separate regression model.

best summarized as a ‘plant effect’. Despite these variations, consistently high thoracic particulate mass (AM: 0.811–2.714 mg m^{-3}), protein (AM: 1.503–1.855 $\mu\text{g m}^{-3}$), pilchard fish (AM: 538–3288 ng m^{-3}) and anchovy fish antigen (AM: 1708–15 431 ng m^{-3}) concentrations were detected in the fishmeal loading and bagging sections of both plants. These antigen exposures generally appear much higher than those reported by Douglas *et al.* for respirable particulate levels (area samples) of gutting and grading activities (2.71–3.57 mg m^{-3}), which corresponded to salmon fish antigen concentrations of 100–1000 ng m^{-3} (Douglas *et al.*, 1995). Surprisingly, the upper limit in the range of exposures for anchovy fish antigen concentrations in the current

Table 5. Parameter estimates for current pilchard antigen levels – using dependent variable: log (pilchard antigen level) ($\ln[\text{ng m}^{-3}]$)

Variable	Regression coefficient	Standard error	P -value
Intercept	3.48	0.49	<0.001
Factory B ^a	0.41	0.20	0.043
Canning ^b	0.50	0.55	0.359
Cannery pick-ups ^b	−0.09	0.66	0.889
Fishmeal manufacturing ^b	0.71	0.57	0.213
Fishmeal warehouse ^b	1.66	0.60	0.007
Boiler room ^b	−0.30	0.66	0.654
Rock lobster ^b	−0.04	0.83	0.964
Labelling ^b	−0.53	1.05	0.612
Workshop ^b	−0.26	0.83	0.752
Stores ^b	0.32	0.66	0.633
Jetty ^b	−0.01	0.64	0.990
Laundry ^b	0.60	0.74	0.420

^aFactory A as baseline.

^bAdministration department as baseline for all departments.

study was up to seven times higher than for protein (75 748 ng m^{-3} versus 11 500 ng m^{-3}) (Tables 1 and 2). Possible reasons for this could be that not all antigens were of protein origin (e.g. type 1 collagen such as gelatin) (Sakaguchi *et al.*, 2000), thereby interfering with the performance of the protein assay (Luo *et al.*, 2002). In any event, there is considerable evidence that the various exposure metrics examined were robust and valid in that both the pilchard and anchovy standard curves as well as the replicate analysis of a

Table 6. Between- and within-group variability of thoracic particulate and pilchard antigen exposure for grouping schemes among fish processing workers

Grouping variables	<i>g</i>	<i>k</i>	S_{bg}^2	S_{wg}^2	Elasticity
Thoracic particulate exposure (<i>n</i> = 198)					
Factory	2	121	0.03	0.52	0.05
Department	12	121	0.16	0.39	0.29
Exposure group	36	121	0.18	0.35	0.34
Factory and department	22	121	0.21	0.34	0.38
Factory and exposure group	36	121	0.18	0.35	0.34
Thoracic pilchard antigen exposure (<i>n</i> = 195)					
Factory	2	77	0.04	0.30	0.12
Department	12	77	0.11	0.21	0.34
Exposure group	30	77	0.08	0.24	0.25
Factory and department	20	77	0.14	0.19	0.42
Factory and exposure group	30	77	0.08	0.24	0.25

n, number of measurements; *g*, number of groups; *k*, number of workers sampled; S_{bg}^2 , estimated variance component due to groups; S_{wg}^2 , estimated variance component due to workers (between workers within groups); elasticity: $S_{bg}^2 / (S_{bg}^2 + S_{wg}^2)$.

subset of samples showed good agreement between the two participating laboratories.

In this study, marked differences in mean exposures were observed on comparing the lowest (administration) and the highest exposed (fishmeal loading/bagging) jobs for all exposure metrics, with the mean exposures for the least and highly exposed EGs in both factories varying by about 9-fold for thoracic particulate, 50-fold for protein, 12- and 18-fold for pilchard and anchovy antigen, respectively (Tables 1 and 2). The fraction of antigen as a proportion of thoracic particulate was the highest in the fishmeal EGs suggesting that antigens derived from proteinaceous fish material (meat, exoskeleton, blood) are particularly likely to be aerosolized during these processing activities. However, for a number of EGs expected to have low antigen exposures (e.g. administration, laundry, lobster packers and masters) the fraction of thoracic particulate represented by antigen was notably high, perhaps because of the lack of local sources for generating thoracic particulate as well as cross-contamination of these work environments by fish processors transporting fish antigens that may adhere to their shoes and clothing. Unexpectedly, lower correlations were obtained between fish antigens and protein concentrations ($r = 0.17\text{--}0.23$, $P < 0.05$) than fish antigen–thoracic particulate correlations ($r = 0.32\text{--}0.35$, $P < 0.001$). This suggests that the measurement of total protein in ambient personal samples may be a poor surrogate for exposure to a specific fish antigen. This could be due to the inherent inaccuracies involved in wet weight measurement of filters exposed to aqueous aerosols of low protein content as has been demonstrated for crab exposures

(Griffin *et al.*, 1994). The inaccuracies due to wet weight measurement would, however, apply to any correlation between gravimetric analysis and protein, or antigenic, analysis. Since it is more likely that a protein assay has a lower sensitivity than an immunoassay, a protein assay would be more prone to inaccuracy at low concentrations of ambient fish protein levels. Similarly, the generally modest correlations ($r = 0.32\text{--}0.35$) between thoracic particulate mass and fish antigen exposures support the previous reports in the literature concerning the need to directly measure airborne high-molecular weight antigens rather than relying on the particulate mass of total dust as the main surrogate of exposure (Heederik *et al.*, 1999).

A high correlation was found between ambient pilchard and anchovy antigen concentrations ($r = 0.71$, $P < 0.001$), suggesting possible cross-reactivity of antibodies to antigenic determinants of the two fish species. These findings support western blot studies described previously, in which allergens of different fish extracts demonstrated molecular weights below 15 kDa, probably representing the major fish allergen, Gad c 1, previously isolated by Elsayed (Elsayed and Aas, 1970; Lopata *et al.*, 2004). The high levels of ‘anchovy’ antigen (AM: 270 ng m^{-3}) detected in the canning departments (Table 2) that only process pilchard fish can probably be explained on the basis of such cross-reactivity observed.

Some studies have suggested that elevated endotoxin levels may be responsible for symptoms reported by exposed workers. Previous studies by Sherson *et al.* isolated Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas*) and $1 \mu\text{g}$ endotoxin ml^{-1} ($10\,000 \text{ EU ml}^{-1}$) in their investigation of contaminated water from the gutting machine thought to be responsible for the respiratory symptoms among trout processing workers (Sherson *et al.*, 1989). A study among crab processing workers demonstrated relatively low levels of airborne endotoxin levels obtained through personal sampling despite large numbers of Gram-negative Mesophilic bacteria isolated through bulk sampling of plant processing tanks (Ortega and Berardinelli, 1999). The mean levels of endotoxin reported by Ortega *et al.* were 32.6 (total fraction) and 15.6 EU m^{-3} (respirable fraction). Preliminary studies indicate that fishmeal production operations in South Africa produce higher ambient endotoxin levels (mean: 136 EU m^{-3}) than fish canning operations (mean: 49 EU m^{-3}) (Jeebhay *et al.*, 2004). Specific jobs such as autopacker/gutting machine operator (275 EU m^{-3}) and cooker/press operator (195 EU m^{-3}) in the cannery as well as scale operators (258 EU m^{-3}) in fishmeal bagging had very high exposures. Although no health-based occupational exposure limit for airborne endotoxin levels currently exists, these levels are higher than the 50 EU m^{-3} ($\sim 5 \text{ ng m}^{-3}$) based on

inhalable dust exposure that has been proposed (DECOS, 1998). The findings of all these studies suggest a possible role for endotoxin exposures and adverse respiratory effects, including non-allergic asthma, observed among exposed working populations (Douwes *et al.*, 2002). However, more detailed endotoxin exposure characterization and dose-response studies among fish processing workers are needed to assess the relative contribution of this exposure to symptoms experienced by this group.

Exposure characterization grouping techniques are generally based on the underlying assumption that the average exposure of an individual worker should be indistinguishable from the average exposure of the total group (Boleij *et al.*, 1995). The a priori strategy used to group workers in this study produced reasonable summary group exposure metric statistics for most EGs. However, since repeated samples were only taken for a minority of individual workers (10%) the between ($_{bw}R_{0.95}$) and within-worker variability for most EGs could not be fully evaluated as has been possible in other epidemiological studies investigating dose-disease response relationships for high-molecular weight allergens (Rappaport, 1991; Houba *et al.*, 1997). The estimation of the within-worker variance is not crucial in this context since the calculation of elasticity is based on the between-group and within-group variances. However, the within-group variance may be somewhat underestimated since the within-worker variance was not fully incorporated in the calculations.

The environmental exposure assessment strategy sampled workers according to EGs rather than by department. This was done so as to minimize the potential for exposure misclassification if workers were to be sampled at the department level. However, the ANOVA models indicated that factory and department explained a significant proportion (adjusted $r^2 = 0.18$, $P < 0.05$) of the variability in pilchard fish antigen exposure levels, than the a priori EG (adjusted $r^2 = 0.12$, $P = 0.115$) (Table 4). Similarly, using the grouping statistics reported previously in other occupational settings, the highest contrast in exposure to particulates (elasticity = 0.38) and fish antigens (elasticity: pilchard = 0.42) was also obtained when workers were grouped according to a combination of factory and department (Table 6). This provides clear evidence that in this study factory and department characterized exposures more efficiently than the a priori EG classification, because in addition to having higher contrast, there are fewer groups, suggesting a higher precision. However, without information on the within-worker variability, the exact precision cannot be calculated. These data also suggest that should information on EGs or jobs not be available for all workers, especially in resource scarce settings where large number of samples and sophisticated immunological laboratory expertise may not

be easily available (Jeebhay *et al.*, 2000), sampling strategies that group workers per factory and department may yield reasonably valid exposure-response coefficients, although they may lack a certain degree of precision (Tielemans *et al.*, 1998).

Improved understanding of exposure-response relationships in occupational respiratory allergy requires measurement of inhaled particulate aeroallergen exposures using particle-size-specific sampling relevant to their site of biological action (Tarlo and Purdham, 2002). This study has employed a number of approaches for exposure characterization of fish processing workers that have not been previously utilized, which should help to advance understanding of exposure-response relationships. These approaches include random selection of workers for sampling in each EG; use of personal sampling as opposed to area sampling techniques; measurement of the thoracic fraction range (considered to be the more biologically relevant fraction for both airway and alveolar disease processes) versus the total particulate mass of aerosols generated; longer-term measurements (over a full 8 h shift); sampling collection that included day and night shifts in both factories; measuring protein and specific fish antigen concentrations in addition to particulate mass determinations that are conventionally used. Furthermore, it has been demonstrated that pilchard antigen levels can be used as a surrogate measure of exposure to at least some other fish antigens and that sufficient contrast of exposure required for assessing exposure-response relationships could also be obtained by grouping workers by a combination of factory and department. These findings offer the promise of a more viable strategy for personal sampling to be conducted in resource scarce settings such as the large number of small workplaces in South Africa (Jeebhay *et al.*, 2000). This is also the first study demonstrating elevated fish antigen and probably endotoxin exposures in fish processing operations in general and fishmeal operations in particular.

Despite the aforementioned strengths, there were certain logistical and resource limitations to the study. These included the inability to compute variance components for assessing homogeneity of EGs due to insufficient number of repeated samples collected, lack of extensive data collected addressing intra-individual variability that may arise due to daily or seasonal variations in work processes and lack of information obtained on peak exposures since real-time sampling instruments were not used. These factors need to be considered in any future studies of environmental exposure characterization in fish processing plants.

CONCLUSIONS

In conclusion, this study has demonstrated that workers involved in typical bony fish processing

are at risk of inhaling aerosols containing fish antigens. Antigen exposures appear particularly high during fishmeal production and bagging. Grouping strategies based on department and factory may provide a more efficient approach than a priori classification of EGs for evaluating fish antigen exposures. Furthermore, the study demonstrated that successful application of ANOVA enabled the development of a sufficiently robust grouping strategy required for generating valid exposure estimates for investigating exposure-response relationships between fish antigen exposures and adverse allergic respiratory health outcomes among workers in fish processing plants.

Acknowledgements—The contribution of Mr R Beukes, Mr S Mbali and Mr S Hassan from the Department of Health Sciences at Peninsula Technikon in the collection of the personal samples is acknowledged. The assistance of Dr Jeroen Douwes and Dr Inga Wouters from the Institute of Risk Assessment (University of Utrecht, The Netherlands) with the endotoxin analysis is acknowledged as well as Mr J Fernandez from Tulane Medical Centre for his assistance with the validation study of the fish antigen analysis. We would like to thank Ms B Fenemore and Ms A Elliott from the Allergology Unit at the University of Cape Town for the laboratory technical support. The assistance of Prof. H Kromhout (Institute of Risk Assessment, University of Utrecht), Prof. J Myers and Mr R Sayed (Occupational and Environmental Health Research Unit, University of Cape Town) for statistical support is also acknowledged. This study was supported by research grants from the Medical Research Council of South Africa and R01 Grant No. F002304 from NIOSH, CDC, USA. The contents of this paper are solely the responsibility of the authors and do not necessarily reflect the official views of these agencies.

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