

Salmon Allergen Exposure, Occupational Asthma, and Respiratory Symptoms Among Salmon Processing Workers

Anna Dahlman-Höglund, PhD,^{1*} Anne Renström, PhD,² Per H. Larsson, PhD,³
Said Elsayed, PhD,⁴ and Eva Andersson, MD, PhD¹

Background This investigation was triggered by three cases of asthma—about 10% of the workforce—occurring in a salmon processing plant over a short period of time. The aim of the investigation was to characterize the work exposure of inhalable organic particles with personal measurements. Respiratory symptoms at work among workers were also assessed.

Methods Exposures to airborne salmon allergen, airborne mold spores, and endotoxin in water and air were measured during work. To assess the Atlantic salmon (*Salmo salar*) Sal s 1 allergen exposure a polyclonal sandwich enzyme-linked immunosorbent assay (ELISA) was developed. Current workers ($n = 26$) answered questionnaires and underwent allergy and lung function tests.

Results Using the sensitive ELISA method (0.05 ng/ml), we found that workers were exposed to high levels of salmon major allergen at the filleting machine and at the filleting table. Airborne endotoxin levels were low, and mold levels were elevated. Only the three initial asthma cases had IgE to salmon. Of the other workers, 65% reported respiratory symptoms at work. These had lower pulmonary function than workers without such symptoms.

Conclusions We developed a sensitive method to measure salmon antigen in air and found that filleting workers were most exposed. It is important to reduce aerosols by improving the ventilation system, machines and organization of work since respiratory symptoms at work among workers were common. *Am. J. Ind. Med.*

© 2012 Wiley Periodicals, Inc.

KEY WORDS: fish aeroallergen; parvalbumin measurement

INTRODUCTION

Several studies have demonstrated an association between occupational asthma and exposure to allergens during handling and processing of seafood [Cartier et al., 1984; Douglas et al., 1995; Rodriguez et al., 1997; Jeebhay et al., 2001]. However, few studies have performed exposure measurements in salmon industries [Douglas et al., 1995; Bang et al., 2005]. The industrial processing of food is characterized by exposure to high molecular weight proteins and other organic particles that may cause airway symptoms. In seafood industries the molecular weight of allergenic proteins ranges from 10 to 70 kDa [Lehrer et al., 1996; Lindstrom et al., 1996]. A major

¹Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

²Department of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

³Mabtech AB, Stockholm, Sweden

⁴Institute of Medicine, University of Bergen, Bergen, Norway

Disclosure Statement: The authors report no conflicts of interests.

*Correspondence to: Anna Dahlman-Höglund, PhD, Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Box 414 S-405 30 Göteborg, Sweden. E-mail: anna.dahlman-hoglund@amm.gu.se

Accepted 12 April 2012

DOI 10.1002/ajim.22067. Published online in Wiley Online Library (wileyonlinelibrary.com).

allergen is the evolutionarily conserved calcium chelating muscle protein, parvalbumin (Sal s 1), with a molecular weight in salmon of 14 kDa. Antibodies to parvalbumin from one fish species are to varying degrees cross-reactive to others [Lindstrom et al., 1996; Van Do et al., 1999, 2005a].

In one large new salmon plant 8% of the workforce contracted occupational asthma [Douglas et al., 1995]. The reported incidence of occupational asthma among workers handling fish and seafood varies between 7% and 36% [Jeebhay et al., 2001]. However, it is estimated that at most half of the asthma cases are allergic in origin; other causes could be endotoxin, fish enzymes, or irritants [Jeebhay et al., 2001]. Workers in fish processing plants may also develop other work-related respiratory symptoms due to these agents [Sherson et al., 1989; Douglas et al., 1995; Bang et al., 2005; Jeebhay et al., 2008].

A male worker with a severe attack of asthma at work was referred during 1998 to our clinic at the Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital. He worked in a salmon processing plant in our region, and when we contacted the plant they reported two more cases on sick leave due to work-related asthma symptoms. To increase production rate, a gutting machine had recently been introduced in the plant. Many of the workers had symptoms and complained about the work environment. Due to this cluster we performed exposure measurements and clinical investigations of the other plant workers.

The main aim of this study was to characterize work exposure in the salmon plant by measuring airborne salmon allergen and endotoxin in air and water, as well as mold exposure. To measure salmon allergen exposure we developed a polyclonal sandwich enzyme-linked immunosorbent assay (ELISA) against the salmon antigen parvalbumin.

A secondary aim was to measure the frequency of respiratory symptoms among workers present during the investigation, by questionnaire, lung function tests, and blood testing.

MATERIALS AND METHODS

The salmon plant with 38 employees was visited and investigations were performed during one Thursday in November 1998, when fresh salmon was processed. The salmon plant had a main room with the wet processing area (gutting machine, sorting, weighing) consuming large volumes of water, where eight persons worked, a dry processing area (filleting machine, a separate filleting room), where 10 persons worked, and the packing area where eight persons worked. In the separate filleting room,

employees worked around a U-formed table in a room with a low ceiling.

The salmon plant had no mechanical ventilation system. A gutting machine had recently been introduced in the plant increasing the production of slaughtered salmon. During the high season, 25 tons of salmon per day were slaughtered. Previously all salmon were manually degutted. The salmon came from fish farms, were stored 1–2 days and then transported alive in tanks. During the last 24 hr the salmon were starved. They were transported on a conveyor belt from the tank to the gutting machine. After the gutting machine and washing machine one person sorted and weighed the salmon. The salmon were then filleted in the filleting machine and transported on a conveyor belt into the filleting room where bones were manually removed. The salmon fillets were crated up in the filleting room and transported to other companies. There was no separate packing room. About 70% of the salmon were frozen, stored, and then filleted during the low season. The plant produced only salmon.

Exposure Measurements

Exposure to airborne salmon allergen, airborne mold spores, and endotoxin in water and in air were measured during work when handling fresh salmon. Sampling was performed in the wet processing area, in the dry processing area, and in the packing area.

Salmon allergen and endotoxin were extracted from 1 μ m polytetrafluoroethylene (PTFE) filters (Millipore FALP 02500) placed in IOM (Institute of Occupational Medicine, Edinburgh) inhalable dust sampler cassettes (SKC Inc., Valley View, PA) in the breathing zone of the workers, using person-carried pumps (Gilian Gilair), at 2 L/min flow. Sampling time was approximately 4 hr ($n = 9$ samples for salmon antigen and $n = 3$ samples for endotoxin). A stationary pump was placed between the gutting and the washing machine collecting airborne salmon protein. Endotoxin content was measured in the water collected from the incoming tap water, transport tank, gutting machine, wash machine, and ice machine.

For measurement of salmon allergen after sampling, filters were stored at -20°C until extraction. Allergens were extracted from the filter during rotation in 1 ml phosphate-buffered saline (PBS), 0.5% Tween, 0.15% Kathon CGTM at room temperature for 2 hr. Bovine serum albumin (BSA) was added to 1%, and the eluates were aliquoted (Nunc, Intermed, Roskilde, Denmark) and kept at -20°C until analysis.

Levels of endotoxin ($n = 3$) in air and water ($n = 5$) were measured using the endpoint chromogenic Limulus Amebocyte Lysate (LAL) assay (Charles River Laboratories, Charleston, SC).

Mold spores in air were collected by placing one agar plate at 1-m height above the floor in different places in the factory ($n = 4$). The sampling time was 20 min. Sabouraud dextrose medium agar sedimentation plates, pH 5.8–6.0 with a diameter of 9 cm were used. Exposed agar plates were incubated at 25–30°C for 14 days. After incubation the yeast and mold colonies were counted. Two months later when the gutting machine was not in use and frozen salmon processed, new samples were collected for mold spores in air ($n = 4$).

Development of a Salmon ELISA

An ELISA to measure salmon antigen was developed by preparation of allergen, immunization of rabbits, and purification of antibodies. A purified salmon parvalbumin Atlantic salmon (*Salmo salar* Sal s 1) preparation was obtained by anion exchange chromatography, gel filtration chromatography (GFC), and controlled by high-performance liquid chromatography (HPLC) of the muscle extracts as described previously [Van Do et al., 2005b] and stored at -80°C .

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to ascertain the purity of parvalbumin as described previously [Van Do et al., 1999].

Rabbit polyclonal antibodies against the major parvalbumin allergen of salmon (Sal s 1) were prepared by the Routine Immunization Protocol of Vivarium Animal House (University of Bergen). Two rabbits were immunized after 2 weeks acclimatization in the Vivarium, with *Salmo salar* parvalbumin with complete, then incomplete Freund's adjuvant for a total of three immunizations. Immunization was done by an authorized animal technician. Preimmune blood samples and samples prior to each immunization were collected. After a total period of 10 weeks, the animals were sacrificed and the blood was collected. Sera were stored at -80°C until used. The immunoglobulin fraction of the antiserum was salted out with Na_2SO_4 .

Affinity purification of rabbit antisalmon antibodies was performed using a 2 ml AminoLink Plus column (Pierce Biotechnology Inc., Rockford, IL, article 44894) according to the manufacturer's instructions. Briefly, salmon parvalbumin antigen was coupled to the column and rabbit antibodies allowed to bind, and after extensive PBS washing eluted using 0.1 M glycine–HCl, 0.5 M NaCl at pH 2.5. The protein containing fractions (measured as absorbance at 280 nm using a spectrophotometer) from the two rabbits were pooled and neutralized using Tris base. Buffer was changed using prolonged dialysis with PBS.

For use in the salmon parvalbumin ELISA, a fraction of the affinity purified rabbit antibody preparation was biotinylated using aminohexanoyl-biotin-*N*-hydroxysuccinimide

ester (AH-BNHS; Zymed Laboratories Inc, South San Francisco, CA) according to the manufacturer's instructions. In the salmon ELISA, Nunc Maxisorp 96-well plates were coated overnight with 5 $\mu\text{g}/\text{ml}$ affinity purified rabbit polyclonal antibodies against the purified parvalbumin. After three washes, standard dilutions of salmon parvalbumin (0.05–5 ng/ml) and workplace samples diluted from 1/10 to 1/250 were added in duplicate, and the plates incubated at ambient temperature for 60 min. After washing, biotinylated rabbit antibodies against parvalbumin were incubated for 60 min. Bound antibodies were detected after washing using a streptavidin-alkaline phosphatase (Mabtech AB, Stockholm, Sweden), visualized using para-nitrophenyl phosphate (pNPP, Sigma-Aldrich, Stockholm, Sweden S0942) in alkaline phosphatase ALP substrate buffer and measured at 405 nm (absorbance at 650 nm subtracted) using a Spectramax plate reader with Softmax software analysis (Molecular Devices, Sunnyvale, CA).

All use of animals was approved by Ethical Committee. The Ethical Committee of the Faculty of Medicine, Animal House Vivarium, University of Bergen, adheres to international law in animal treatment, in accordance with the Helsinki Declaration.

Salmon Plant Workers

Workers were given questionnaires covering smoking, upper and lower airway symptoms (and for every symptom when it started and if it was present at work), airway symptoms at night, skin symptoms, and abnormal tiredness after work. Respiratory symptoms at work were defined as symptoms present at work, but which may or may not have been present outside of work. This group was compared to workers with no respiratory symptoms at work (who may have had symptoms outside of work). Pulmonary function tests for forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were performed at work on the 4th work day of the week using a dry-wedge spirometer (Vitalograph) with European Respiratory Society (ERS) reference values [Quanjer et al., 1993]. Blood samples were collected for assessment of Phadiatop positivity (Pharmacia Diagnostics, Uppsala, Sweden), total IgE and presence of specific IgE antibodies against salmon. Salmon and cod-specific serum IgE levels were determined by CAP-RAST (f41salmon, f3cod; Pharmacia Diagnostics).

After exclusion of office workers ($n = 3$), persons employed for less than a week ($n = 4$) and workers on long-term sick leave ($n = 5$, the three index cases and two with non-respiratory disease), 26 workers remained and were investigated. Fourteen (54%) had worked at the plant less than 3 months and 73% less than 1 year. No information on workers that had left their work prior to this investigation was available.

As this was an occupational health investigation, prior approval from an Ethics Committee, was not sought. Employee participation was voluntary and the workers were informed about the investigation both before and during the investigation, by the Occupational Health Service and the researchers.

Statistical Analyses

Pulmonary function data are shown as percentage of predicted values; means have been calculated with 95% confidence intervals (CI). The workers were divided into two groups, with and without respiratory symptoms at work, and compared with Wilcoxon rank sum test (two-sided exact test). The statistical analyses were performed with the statistical software package SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Exposure Measurements

Method to measure airborne salmon parvalbumin

The detection limit for the assay was 0.05 ng/ml of parvalbumin. Median (25th–75th percentile) % coefficient of variation between duplicates for standards and samples was 1.6 (0.9–4.0) %. Cross-reactivity to other parvalbumins was studied using the antibodies raised to salmon parvalbumins, which recognized both purified pollack and cod parvalbumin, giving nominal levels of 69% and 84% of the added protein amount, respectively.

Salmon allergens

All samples (except the blank filter control) were positive for airborne Sal s 1. The highest exposure to airborne salmon was around the person handling the filleting machine, 186 ng/m³ (Fig. 1). Working in the packing area gave only one-tenth of the filleting exposure. Working in the filleting room or inserting the salmon into the gutting machine resulted in intermediate parvalbumin exposure levels.

Endotoxin

Personal exposure to airborne endotoxin was 1.6 EU/m³ for the person working with weighing salmon, 2.7 EU/m³ around the person inserting salmon in the gutting machine and 7.1 EU/m³ around the person filleting fish in the filleting room. The endotoxin concentration in water was 39.6 EU/ml from incoming tap water, 779 EU/ml in the water from the transport tank, 100 EU/ml in the water from the gutting

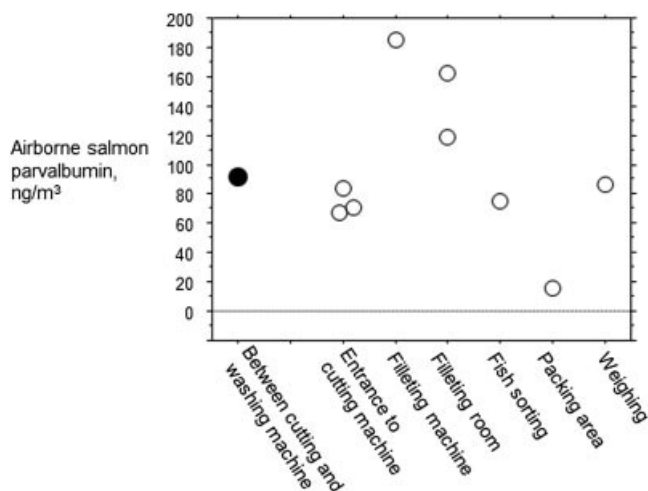


FIGURE 1. Personal exposure to airborne salmon parvalbumin allergen during different work tasks. Note: ● collected using static sampler.

machine, 84.3 EU/ml in the water from the wash machine, and 21.8 EU/ml in water from the ice machine.

Mold spores

High amounts of mold spores in the air were found (*Penicillium notatum*, *Aspergillus aspergillus*, and *Cladosporium herbarum*) in the filleting room where people worked with filleting fresh salmon, about fivefold higher than in normal milieu (the office sample had four mold colonies). The amount of mold spores in air outside the factory buildings was low (two mold colonies). No yeast spores were found. Repeated sampling of mold spores in air 2 months later, when people worked with filleting frozen salmon and the gutting machine was not used, showed low amounts of mold spores (three mold colonies), equal to the amount of mold spores in air outside the factory buildings. Otherwise, no yeast spores were found.

Symptoms and Clinical Findings Among the Workers

Only the three index asthma cases (all smokers) showed specific serum IgE against salmon. None of the 26 investigated workers were IgE positive against salmon. After 8–9 years had passed since the removal from work exposure, the levels of specific IgE antibodies in serum against salmon and cod were retested, and were then reduced to class zero in all three cases.

Of the 26 investigated workers 38% were classified as atopic, having a positive Phadiatop test. Any respiratory symptom at work was reported by 17 (65%) of the workers, but not more frequently among atopics or smokers (Table I). Of these, 13 (50%) reported a time of onset of

TABLE I. Characteristics, Pulmonary Function, and Total IgE by Respiratory Work Symptoms Among Investigated Workers in a Salmon Processing Plant

	All included workers	Respiratory symptoms at work	No respiratory symptoms at work
Number	26	17	9
Women (%)	6 (23)	3 (18)	3 (33)
Mean age ($\pm$ SD)	29.1 ($\pm$ 10.3)	29.2 ($\pm$ 10.8)	29.0 ($\pm$ 9.9)
Employment $\geq$ 3 months (%)	12 (46)	9 (53)	3 (33)
Positive Phadiatop (%)	10 (38)	6 (35)	4 (44)
Smokers	14 (54%)	9 (53%)	5 (56%)
Mean FVC % of predicted, 95% CI	102.5, 98.9–106.1	99.4, 94.9–103.9*	108.4, 103.9–112.9
Mean FEV ₁ % of predicted, 95% CI	100.7, 97.6–103.8	98.2, 95.3–101.1**	105.4, 98.4–112.3
Median total IgE (kU/L), (range) IgE >100 kU/L (%)	35 (2–850), 12%	24 (9–850), 6%	50 (2–390), 22%

*Significant differences between subjects with and without symptoms (Wilcoxon rank sum test) $P = 0.009$.

**Significant differences between subjects with and without symptoms (Wilcoxon rank sum test) $P = 0.045$.

their symptoms that occurred after they were employed at this plant. Upper airway symptoms at work were more often reported (by 58%) than lower airway systems (by 38%; dyspnea by 7, cough by 9, and wheezing by 1 person). Since workers were sometimes moved to the packing area or other work tasks if they had symptoms, it was not possible to assess whether any work task induced more symptoms than another.

Wheeze anytime was reported by nine workers, and 11 declared frequent cough with phlegm. The workers reporting respiratory symptoms at work had significantly lower FVC and FEV₁ than those without symptoms at work (Table I).

DISCUSSION

In the present study, we developed a novel sensitive sandwich ELISA method to measure the exposure to airborne salmon allergen at different work stations in a salmon processing plant. We also measured exposure to endotoxin and mold. High exposure situations were identified at the filleting machine and at the filleting table where bones were removed manually. Of the investigated workers 50% reported respiratory symptoms at work that developed after commencing employment in the plant.

To our knowledge, only one published study has presented levels of airborne salmon allergens [Bang et al., 2005]. It is a study from a salmon factory in Norway, where workers removing entrails after automated gutting were exposed to measurable salmon levels of 1.6 $\mu\text{g}/\text{m}^3$. However, only 5 of 18 air samples were above a non-specified detection limit [Bang et al., 2005]. In our study, those who worked with inserting salmon into the automated gutting machine and nearby were exposed to 75 ng/m^3 salmon Sal s1, which appears to be an order of magnitude lower. However, as the Norwegian study used a patient IgE inhibition ELISA method, it is not possible to

compare their values with those of this study. Indeed, in previous comparisons between inhibition and sandwich set-ups using patient or purified antibodies, similar or even larger differences in nominal allergen levels have been observed [Renstrom et al., 1997; Hollander et al., 1999]. We developed a sensitive (0.05 ng/ml detection limit) polyclonal sandwich ELISA and purified antibodies against the known high-affinity Ca^{2+} -binding protein in white muscle of lower vertebrates, Sal s1 (parvalbumin) from salmon [Van Do et al., 2005a]. The parvalbumin is highly homologous between different species, and our antibodies detected both cod and pollack parvalbumin.

A Danish study from a sea-trout processing plant reported salmon allergen levels between 12 ng/m^3 in the packing area and 1,332 ng/m^3 around the filleting machine [Sommer et al., 2004]. In this study, our ELISA reagents were utilized. In the Danish plant, the fish-processing rate was higher than in our salmon plant. This method was also used in another Danish study, in which the average level of airborne salmon parvalbumin in a salmon plant was 50 ng/m^3 , with the highest levels measured around the filleting machine and at the filleting table [Sommer et al., 2004], as in our study.

When comparing results from different studies it is important to keep in mind that the plants have different ventilation process, production rate of salmon and different ways to handle salmon. An additional benefit of our high sensitivity polyclonal sandwich ELISA is that it makes it possible to compare the same job tasks in different salmon plants.

At the plant the workers handled fresh or frozen salmon, and the asthma index cases were salmon sensitized and also had IgE antibodies against cod. Only these index cases, about 10% of the employees, had IgE antibodies against salmon. One should however bear in mind that only half of the workers had been exposed more than 3 months, and it is possible that symptoms may precede

IgE development. It is known that salmon processing work can induce sensitization [Sherson et al., 1989; Douglas et al., 1995; Tougaard et al., 1997]. A similar prevalence of asthma (8%) as in our study were found by Douglas et al. [1995] in a large salmon processing plant in England ($n = 291$). By contrast, in the Norwegian study described earlier, none of the examined workers ($n = 46$) at the salmon plant had developed IgE antibodies against salmon [Bang et al., 2005].

Many of the non-sensitized workers reported upper (58%) or lower (38%) respiratory symptoms during work. These workers were found to have significantly reduced FEV₁ and FVC compared to workers without respiratory symptoms at work, a finding also noted in the Norwegian study [Bang et al., 2005]. Our cross-sectional investigation does not permit any etiological conclusions. However, the prevalence of airway symptoms and possible pulmonary function decrease is noteworthy considering the mean age of 29 years of the sample and that 73% had worked in the plant less than a year.

Exposure to high levels of endotoxin in air may cause occupational respiratory symptoms. Using a pooled set of data, Castellan et al. [1987] calculated a threshold level of 9 ng endotoxin/m³ for an acute effect on pulmonary function. In one study, the endotoxin content ranged from 1 to 3 ng/ml in drinking water after the ground water has been treated in drinking water treatment plant [Anderson et al., 2002]. Although there was much endotoxin in the plant water (100 EU/ml in the gutting machine), we found relatively low work exposure to airborne endotoxin.

In this investigation mold spores were present at low or moderate levels. Other studies have found that occupational exposure to mold spores was associated with blocked nose, sore throat, cough, and tight chest, while bacteria exposure were associated with nose irritation and unusual tiredness [Melbostad and Eduard, 2001; Haldal and Eduard, 2004].

Other mechanisms that might explain why more than half of the workers experienced work-related respiratory symptoms need to be considered. Other allergens or immune cell-stimulating components may be present in the fish-processing environment: for instance fish parasites, such as *Anisakis simplex*, histamine, gases produced by anaerobic decomposition of fish (hydrogen sulphide), or chemical additives (such as sodium metabisulphite—which was however not used in this plant) [Cartier et al., 1984; Douglas et al., 1995; Rodriguez et al., 1997; Jeebhay et al., 2001]. In this study, we were unable to measure these factors.

Inhalation of biological materials other than fish allergens could also be of importance. In a recent study, Larsen et al. showed that purified salmon trypsin in vitro can activate human pulmonary epithelial cells to induce secretion of the pro-inflammatory cytokine IL-8 [Larsen

et al., 2008; Bang B et al., 2009]. The authors suggest that inhalation of particles containing enzymes like salmon tissue trypsin could produce inflammatory effects in the airways.

In conclusion, we developed a sensitive method to measure salmon antigen in air and found that filleting workers were most exposed. Our salmon parvalbumin ELISA may aid in the detection of generally high allergen exposure situations and the evaluation of exposure reduction measures. Airborne endotoxin levels were low but mold levels were elevated. We also found that 50% of the workers developed respiratory symptoms after commencing employment. Besides sensitization, these symptoms may be induced by non-IgE-dependent mechanisms. Further exposure characterization in fish-processing industries and experimental studies are needed to understand the different mechanisms of these health problems.

In conclusion, it is important to reduce aerosols, including salmon antigen and mold concentrations, in fish-processing plants by improvements in the ventilation system, machines and organization of work. For affected workers who are not sensitized, personal respiratory equipment may be a temporary solution, until a better work environment is achieved. If they are sensitized, a change of job task at the plant with no exposure to salmon allergen or a job outside the seafood industry is recommended. In this case, the salmon plant was closed due to economic reasons 1 year after our investigation. Most of the workers found other jobs, but not in the seafood industry.

REFERENCES

- Anderson WB, Slawson RM, Mayfield CI. 2002. A review of drinking-water-associated endotoxin, including potential routes of human exposure. *Can J Microbiol* 48:567–587.
- Bang B LM, Larsen AK, Aasmoe L. 2009. Proteases from salmon stimulate IL-8 in airway epithelial cells. *J Toxicol Environ Health A* 72:571–573.
- Bang B, Aasmoe L, Aamodt BH, Aardal L, Andorsen GS, Bolle R, Boe R, Van Do T, Evans R, Florvag E, Gram IT, Huser PO, Kramvik E, Lochen ML, Pedersen B, Rasmussen T. 2005. Exposure and airway effects of seafood industry workers in northern Norway. *J Occup Environ Med* 47:482–492.
- Cartier A, Malo JL, Forest F, Lafrance M, Pineau L, St-Aubin JJ, Dubois JY. 1984. Occupational asthma in snow crab-processing workers. *J Allergy Clin Immunol* 74:261–269.
- Castellan RM, Olenchok SA, Kinsley KB, Hankinson JL. 1987. Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Engl J Med* 317:605–610.
- Douglas JD, McSharry C, Blaikie L, Morrow T, Miles S, Franklin D. 1995. Occupational asthma caused by automated salmon processing. *Lancet* 346:737–740.
- Haldal KK, Eduard W. 2004. Associations between acute symptoms and bioaerosol exposure during the collection of household waste. *Am J Ind Med* 46:253–260.

- Hollander A, Gordon S, Renstrom A, Thissen J, Doekes G, Larsson PH, Malmberg P, Venables KM, Heederik D. 1999. Comparison of methods to assess airborne rat and mouse allergen levels. I. Analysis of air samples. *Allergy* 54:142–149.
- Jeebhay MF, Robins TG, Lehrer SB, Lopata AL. 2001. Occupational seafood allergy: A review. *Occup Environ Med* 58:553–562.
- Jeebhay MF, Robins TG, Miller ME, Bateman E, Smuts M, Baatjies R, Lopata AL. 2008. Occupational allergy and asthma among salt water fish processing workers. *Am J Ind Med* 51:899–910.
- Larsen AK, Seternes OM, Larsen M, Aasmoe L, Bang B. 2008. Salmon trypsin stimulates the expression of interleukin-8 via protease-activated receptor-2. *Toxicol Appl Pharmacol* 230:276–282.
- Lehrer SB, Horner WE, Reese G. 1996. Why are some proteins allergenic? Implications for biotechnology. *Crit Rev Food Sci Nutr* 36:553–564.
- Lindstrom CD, van Do T, Hordvik I, Endresen C, Elsayed S. 1996. Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic salmon (*Salmo salar*). *Scand J Immunol* 44:335–344.
- Melbostad E, Eduard W. 2001. Organic dust-related respiratory and eye irritation in Norwegian farmers. *Am J Ind Med* 39:209–217.
- Quanjer P, Tammeling G, Cotes J, Pedersen O, Peslin R, Yernault J. 1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of lung function tests, European Community for steel and coal. Official statement of the European Respiratory Society. *Eur Respir J* 16:5–40.
- Renstrom A, Gordon S, Larsson PH, Tee RD, Newman Taylor AJ, Malmberg P. 1997. Comparison of a radioallergosorbent (RAST) inhibition method and a monoclonal enzyme linked immunosorbent assay (ELISA) for aeroallergen measurement. *Clin Exp Allergy* 27:1314–1321.
- Rodriguez J, Reano M, Vives R, Canto G, Daroca P, Crespo JF, Vila C, Villarreal O, Bensabat Z. 1997. Occupational asthma caused by fish inhalation. *Allergy* 52:866–869.
- Sherson D, Hansen I, Sigsgaard T. 1989. Occupationally related respiratory symptoms in trout-processing workers. *Allergy* 44:336–341.
- Sommer B, Nielsen T, Sabro P. 2004. Work-related asthma at a Danish salmon and sea-trout processing plant (Abstract). EPICOH Conference.
- Tougard AB, Bach B, Taudorf E, Stouby VL. 1997. Occupational respiratory tract allergy in trout processing workers. *Ugeskr Laeger* 159:5800–5804.
- Van Do T, Hordvik I, Endresen C, Elsayed S. 1999. Expression and analysis of recombinant salmon parvalbumin, the major allergen in Atlantic salmon (*Salmon salar*). *Scand J Immunol* 50(6):619–625.
- Van Do T, Elsayed S, Florvaag E, Hordvik I, Endresen C. 2005a. Allergy to fish parvalbumins: Studies on the cross-reactivity of allergens from 9 commonly consumed fish. *J Allergy Clin Immunol* 116:1314–1320.
- Van Do T, Hordvik I, Endresen C, Elsayed S. 2005b. Characterization of parvalbumin, the major allergen in Alaska pollack, and comparison with codfish allergen. *Mol Immunol* 42(3):345–353.