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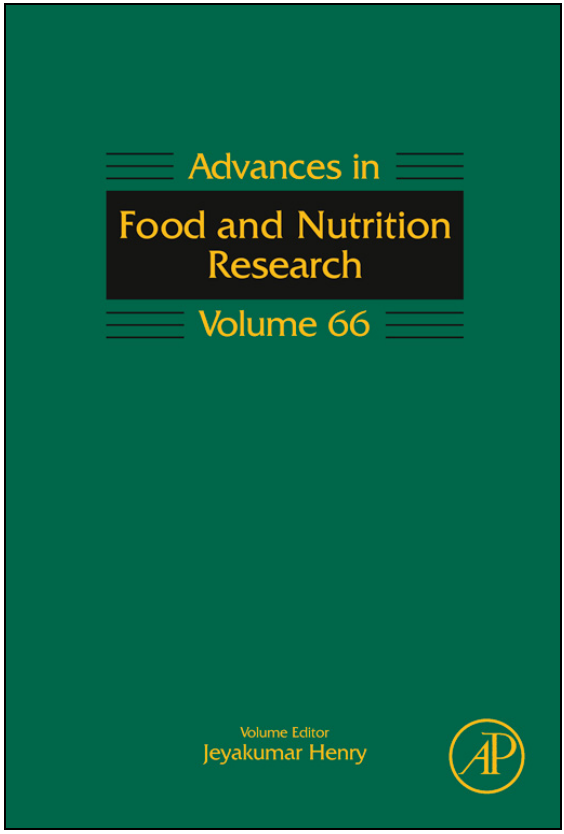

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CHAPTER 2

Occupational Allergies in Seafood-Processing Workers

Mohamed F. Jeebhay[*,1] and **Andreas L. Lopata**[†]

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Abstract Global increased demand for seafood and its products has been associated with a concomitant rise in fishing, aquaculture, and processing activities. This increased harvesting of seafood is associated with more frequent reporting of allergic health problems among seafood processors. This review outlines the high-risk working populations, work processes, as well as host and environmental exposure risk factors for occupational respiratory and skin allergies. It also provides insights into the major and minor allergens as well as the pathophysiological mechanisms implicated. Diagnostic and preventive approaches are outlined in managing work-related allergy associated with seafood processing.

I. INTRODUCTION

This chapter provides an overview and update of previous reviews (Jeebhay and Cartier, 2010; Jeebhay *et al.*, 2001) on this subject with a greater focus on the contribution of the seafood-processing environment in causing occupational allergies. Workers in this industrial setting are increasingly at risk of developing occupational allergic reactions of the lung and skin due to high-risk seafood handling and processing activities that characterize this working environment.

II. SEAFOOD INDUSTRY, PROCESSING ACTIVITIES, AND POPULATIONS AT RISK

A. The seafood industry

Occupational exposure to seafood allergens occurs mainly in the food and fishing industry. The Food and Agriculture Organization (FAO) estimated that in 2006, fishery and aquaculture production activities provided direct employment and revenue for 43 million people worldwide, largely from developing countries, harvesting 110 million tons of food fish (FAO, 2009). The number of fishers and fish farmers has been growing at an

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average rate of 3.5% per year since 1990. It is estimated that approximately 52% of this population work aboard fishing trawlers, 32% in aquaculture production (marine and freshwater), and 16% work inland as capture fishers or in other land-based activities such as processing (International Labour Organization, 1999). More than 30% of fish for human consumption derives from aquaculture (Hastein *et al.*, 2006). In many countries, labor in the fishing industry is divided along gender lines with men almost exclusively involved in harvesting the seafood at sea and women doing most of the processing ashore (Jeebhay, 2004). Most of the workforces are seasonal and often migrant working populations and are vulnerable to various workplace organizational factors which, in addition to the gendered distribution of work, may mediate hazardous occupational exposures (Howse et al., 2006).

B. Populations at risk

Workers in several of these industries are exposed to seafood, especially those involved in either manual or automated processing of crabs, prawns, mussels, fish, and fishmeal in factories. Other occupations associated with exposure to seafood include harvesting activities (fishermen, aquaculture, oyster shuckers, fishmongers, truck drivers, maintenance), food preparation activities (restaurant chefs and waiters), laboratory technicians and researchers, pet food production and value added (shell grinders, jewelry polishers) (Jeebhay and Cartier, 2010, Jeebhay *et al.*, 2001). Recently, a worker developed fatal asthma following inhalation of dust produced from grinding shark cartilage intended for use as a natural remedy (Ortega *et al.*, 2002).

Seafood-processing plants vary in technology levels, with some of the smaller workplaces relying entirely on manual handling of the seafood and larger companies using modern highly automated processes. There is a great variation in processing procedures for the different types of seafood (Malagie *et al.*, 1998). Common processing, preservation, and storage techniques employed for the major seafood groupings and sources of potential exposure to seafood product/s are outlined in Table 2.1.

C. High-risk work processes

Work processes causing aerosol production include butchering or grinding; degilling, ''cracking,'' and boiling of crabs; cleaning and brushing of crab; ''tailing'' of lobster; ''blowing'' of prawns; washing or scrubbing of shellfish; degutting, heading, and cooking/boiling of fish; mincing of seafood; and cleaning of the processing line or storage tanks with

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TABLE 2.1 Common processing, preservation, and storage techniques employed for seafood groups that are sources of potential occupational exposure to seafood products

Seafood category	Processing techniques	Preservation techniques	Packaging of final products	Sources of occupational exposure to seafood product/s
Crustaceans				
Crabs, lobsters, crawfish	Cooking (boiling or steaming), ''tailing'' lobsters, ''cracking,'' butchering and degilling crabs, manual picking of meat, cutting, grinding, mincing, scrubbing and washing, cooling	Deep freezing, pasteurizing, sterilization, liquid freezing	In refrigerated containers, polyethylene bags or in cans	– Inhalation of wet aerosols from lobster ''tailing,'' crab ''cracking,'' butchering and degilling, boiling, scrubbing and washing, spraying, cutting, grinding, mincing, prawn ''blowing,'' cleaning processing lines/tanks with pressurized water
Prawns	Heading, peeling, deveining, prawn ''blowing'' (water jets or compressed air)	Deep freezing, drying	In refrigerated containers or in cans	– Dermal contact from unprotected handling of prawn; hand immersion in water containing extruded gut material
Molluscs				
Oysters, mussels clams, scallops, abalone	Washing, oyster ''shucking,'' shellfish depuration, chopping, dicing, slicing	Deep freezing, freezing, sterilization, smoking, cooking	In refrigerated containers, or in cans	– Inhalation of wet aerosols from oyster ''shucking,'' washing – Dermal contact from unprotected handling
Finfish				
Various species	Heading, degutting, skinning, mincing, filleting, trimming, cooking (boiling or steaming), spice/batter application, frying, milling, bagging	Deep freezing, drying, smoking, sterilization, liquid freezing	Loose in refrigerated containers, cans or in bags	– Inhalation of wet aerosols from fish heading, degutting, boiling – Inhalation of dry aerosols from fishmeal bagging – Dermal contact from unprotected handling

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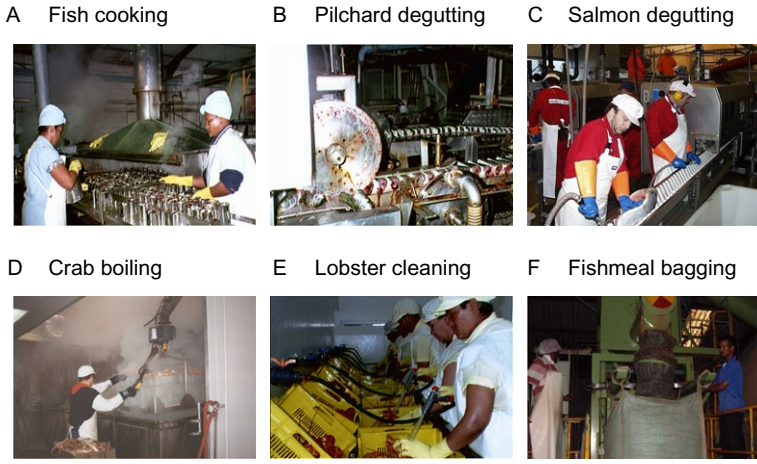

FIGURE 2.1 (A–F) Inhalation of dust, steam, vapors, and seafood proteins generated during cutting, scrubbing or cleaning, degutting, cooking or boiling, and drying (fishmeal) activities during fishmeal production.

high-pressured water hoses (Fig. 2.1A–F) (Jeebhay *et al.*, 2001). Since water is a major feature of this work environment, it may also influence the size, lifetime, or other dynamics of small protein particles. Processes that generate dry aerosols such as prawn blowing operations using compressed air and fishmeal loading/bagging appear to generate higher concentrations of particulate than wet processes (prawn blowing with water jets).

Dermatological symptoms experienced by workers are the result of direct contact with the actual seafood or a systemic response to inhalational exposures. Occupational dermal exposure can occur as a result of unprotected handling of various seafood and their products (fish juice, meat, skin, skin slime/mucin, entrails) at various stages in the production process (Halkier-Sorensen, 1996; Jeebhay *et al.*, 2001). This generally occurs under wet and low-temperature conditions. Manual cutting and generation of fish juice in various work processes can expose the skin to mechanical damage (inoculation by spinous appendages), protein fragments, and fish enzymes capable of causing dermatological symptoms.

III. HEALTH EFFECTS ASSOCIATED WITH SEAFOOD PROCESSING AND THEIR EPIDEMIOLOGY

A. Lung

Reactions to seafood commonly present with adverse respiratory health effects, the most common being occupational asthma. Occupational asthma is a ''disease characterized by variable airflow limitation and/or

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hyperresponsiveness and/or inflammation due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace'' (Bernstein *et al.*, 2006). Two types of occupational asthma are distinguishable viz. occupational allergic asthma, which appears after a latency period of respiratory sensitization, and irritant-induced asthma or reactive airways dysfunction syndrome (RADS), which may occur after single or multiple exposures to high concentrations of nonspecific irritants without a latency period.

Occupational allergic asthma is the most frequent work-related respiratory disease reported in the seafood industry, with the prevalence varying from 2% to 36% (Jeebhay and Cartier, 2010). Differences in prevalence are partly due to varying definitions of occupational asthma, most studies relying on questionnaires and assessment of specific sensitization; the allergenic potential of the seafood proteins involved; and the type of work process resulting in excessive exposure (e.g., steam, organic dust, air blowing, water jets). A higher prevalence of occupational asthma is associated with exposure to aerosols arising from arthropods (crab and shrimp) than to molluscs and bony fish. Symptoms of asthma may develop after only a few weeks or months or after several years. Symptoms are worse at work, improving on weekends or holidays and often awaken affected subjects at night. Rhinitis and conjunctivitis (and less frequently skin rashes on exposed parts of the body) are often associated and may precede the development of chest symptoms. It is estimated that 7% of workers with ingestion-related seafood allergy also have asthma symptoms associated with inhalational exposure to seafood (Jeebhay *et al.*, 2008). There have also been isolated case reports of workers (fishmonger handling shrimp and lobster, fish smoking factory worker handling trout, anchovy, salmon, and sardines) with work-related asthma symptoms who subsequently developed ingestion-related allergic symptoms (Jeebhay *et al.*, 2001).

Irritant-induced asthma or RADS can be induced by exposure to high concentrations of irritants such as sulfite preservatives (Steiner *et al.*, 2008) or ammonia used as a refrigerant, which could be encountered in seafood industries. Furthermore, asthma triggered by exposure to sulfites in prawn- and lobster-processing workers has been described simulating occupational asthma (Madsen *et al.*, 2004), although the exact mechanism of sulfite asthma is unknown (Taylor and Nordlee, 1993).

Although no cases of *hypersensitivity pneumonitis* have been described in the seafood industry, there are reports of hypersensitivity pneumonitis in workers exposed to the dust of molluscs (oyster, sea snail) used in the manufacture of various ornaments such as buttons (Jeebhay and Cartier, 2010).

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B. Skin

By contrast with respiratory allergy, dermatologic allergy to seafood has been to a lesser degree studied and documented in the medical literature. The major skin manifestations associated with seafood are contact *urticaria and eczematous contact dermatitis* of various types (Jeebhay *et al.*, 2001). Contact urticaria is associated with direct contact with raw seafood proteins. At least 75% of eczematous dermatitis in the fish-processing industry is of an irritant nature commonly due to contact with water and fish products (fish juice, slime, skin, and fillet). Contact with the proteinacious material such as seafood also causes a chronic recurrent dermatitis commonly known as protein contact dermatitis (PCD). However, biochemical sensitizers (e.g., garlic, onion, spices) added to seafood can also cause a delayed allergic contact dermatitis. Various reports have indicated that these dermatological outcomes can also coexist in affected individuals.

Contact urticaria and PCD initially manifest as itchy, erythematous, and vesicular lesions. PCD usually goes on to present as a chronic eczema with episodic acute exacerbations a few minutes after repeated contact with the offending allergen. The development of immediate contact reactions usually requires repeated skin contact, although prior sensitization through ingestion or inhalation and subsequent dermal contact can also result in PCD. Predominantly affected areas are the volar aspect of the forearm and dorsum of the hands. In the more severe form, local skin contact with seafood may result in generalized urticaria and/or systemic symptoms (angioedema, wheezing). The diagnosis can only be made by means of skin prick tests since patch tests with the responsible allergen are usually negative. Sometimes, specific IgE antibodies can be detected in the sera (Jeebhay *et al.*, 2001).

In the seafood industry, the reported prevalence of occupational PCD is between 3% and 11% (Jeebhay *et al.*, 2001).

IV. SEAFOOD MATRIX, ALLERGEN STRUCTURE, AND DISEASE MECHANISMS

A. Components of seafood

Adverse reactions to seafood result from exposure to the seafood itself or to various non-seafood components present in the product. The phylogeny of the commercially important seafood species causing occupational allergies is presented in Table 2.2. Among the arthropods, the crustacean class includes some of the most allergenic species of seafood such as prawns,

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lobsters, and crabs. Allergens in the various seafood species are primarily high-molecular-weight proteins ranging in molecular weight from 10 to 70 kDa (Lopata and Lehrer, 2009a,b). It is these proteins present in aerosols that have been associated with the allergic respiratory symptoms. The composition of aerosols generated by snow crab and king crab processing has been found to contain crab exoskeleton (chitin), meat (mainly muscle protein), gills, kanimiso (internal organs), and background material such as sodium chloride crystals, cellulose, synthetic fibers, silicate, pigment constituent particles, and inorganic particles (silicon, aluminum, iron) (Jeebhay *et al.*, 2001). Most of the particles are irregular, and at least 30% of airborne particulate are within the respirable range (<5 μm). Investigation of contaminated water during processing has isolated gram negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas*) and endotoxin (1 μg endotoxin/ml) in the water from a gutting machine thought to be responsible for the respiratory symptoms among trout-processing workers (Jeebhay *et al.*, 2001). Constituents of fish juice associated with skin symptoms have been shown to comprise traces of biogenic amines, histamine, and cadaverine; degradation compounds associated with postmortem changes; digestive enzymes (pepsin and trypsin); and high-molecular-weight proteins (>10 kDa). No well-known human pathogenic bacteria were present. It has been suggested that fish muscle proteases lead to hydrolysis of large muscle proteins which accumulate in fish juice. These denatured proteins are thought to be responsible for inducing skin symptoms. Furthermore, it has been shown that storage conditions may also influence the allergenicity of seafood extracts by influencing the relative distribution of various IgE-reactive proteins. Fish kept on ice for several days showed additional high-molecular-weight allergens and higher IgE-binding capacity.

While the composition of aerosol can contain a variety of environmental contaminants, also seafood can comprise additional non-seafood contaminants which can generate allergic/inflammatory reactions. These include parasites (e.g., *Anisakis simplex*), protochordates (*Hoya*), algae (dinoflagellates *Hematodinium*), bacteria (*Vibrio*), viruses (hepatitis A), marine or bacterial toxins (saxitoxins, scombroid toxin, histamine), gases produced by anaerobic decomposition of fish (hydrogen sulfide), chemical additives (sodium metabisulfite), spices (mustard, paprika, flour additives, garlic), and hidden ingredients (casein) in canned or processed fish products (Jeebhay *et al.*, 2001).

B. Seafood allergen families

The three most important seafood groupings of marine species most often consumed and processed are Arthropoda, Mollusca, and Pisces (subphylum Chordata) (Table 2.2). It is important to note that most of the allergens

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TABLE 2.2 Seafood species causing occupational allergy and other possible associated allergens

Phylum	Class	Family species (common name)	Allergens	
			Major	Minor
Arthropoda	Crustacea	Crabs, lobsters, prawns, shrimp, scampi, shrimp meal	Tropomyosin	Arginine kinase Myosin light chain Sarcoplasmic calcium-binding protein
Mollusca	Gastropoda	Abalone	Tropomyosin	
	Bivalvia	Clams, oysters, mussels, scallops	Tropomyosin	
	Cephalopoda	Cuttlefish (and bone), octopus, squid	Tropomyosin	
Pisces (subphylum Chordata)	Osteichthyes (bony fish)	Salmon, plaice, tuna, hake, cod, herring, pilchard, anchovy, trout, swordfish, sole, pomfret, yellowfin, fishmeal (flour)	Parvalbumin	Collagen Vitellogenin Glyceraldehyde-3-phosphate dehydrogenase
	Chondrichthyes (Cartilaginous fish)	Shark	Parvalbumin	

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listed for the different seafood species have been identified in ingestion-sensitized patients. Only very few studies have actually identified the allergens aerosolized and causing allergic sensitization to seafood primarily encountered in the workplace. Crustaceans, perhaps surprisingly, are classified as arthropods together with spiders and insects (Lopata and Lehrer, 2009a,b). Over 30,000 living crustacean species are found worldwide, and a large number of varieties are consumed raw or cooked. The second group of molluscs, a large and diverse group, is subdivided into the classes Bivalve, Gastropods, and Cephalopods and comprises over 100,000 different species, including several economically important seafood groups such as mussels, oysters, abalone, snails, and squid (calamari). Lastly, the fish subgroup can be divided into the bony fish and cartilaginous fish. Most edible fish belong to the bony fish, whereas sharks and rays are cartilaginous and belong to a different order. Most studies of fish allergens have focused on cod and carp. Although there are more than 20,000 different species of fish, consumption and processing depends on regional availability.

1. Shellfish allergens

The major allergen in shellfish is the muscle protein, tropomyosin, in crustaceans and mollusks (Lopata and Lehrer, 2009a,b). Shrimp tropomyosin has a slightly acidic isoelectric point, seems to have minor glycan modifications, and is water soluble and heat stable. While tropomyosin migrates on SDS–PAGE as a single band of between 34 and 39 kDa, the protein in its native state is a coiled-coil homodimer with much higher molecular weight. Tropomyosin has a highly conserved amino acid sequence among the different crustaceans and to a lower extent the molluscs and is present in muscle and non-muscle cells. Cross-reactivity occurs frequently to ingested seafoods within a certain group or family such as crab, lobster, shrimp among the crustaceans (Lopata and Lehrer, 2009a,b, Motoyama *et al.*, 2008), suggesting that cross-reactivity frequently occurs between phylogenetically related organisms.

Exposure to crustacean and other invertebrate tropomyosins via the inhalation route that mainly occurs in the seafood-processing industry may also generate additional sensitization profiles resulting in different IgE responses than that commonly reported for ingestion-related allergy (Jeebhay and Cartier, 2010, Binder *et al.*, 2001, Garcia-Orozco *et al.*, 2007). A recent study on allergic sensitization among snow crab processors identified several IgE-binding proteins among snow crab-processing workers (Gill *et al.*, 2009). While several allergens of 14.4–50 kDa were identified, the nature of these proteins was not characterized. Nevertheless, a potential association of protein reactivity with disease was suggested for the 34 and 14.4 kDa allergens. Subsequently, Anas *et al.* (Abdel Rahman *et al.*, 2010a, 2011) quantified aerosolized snow crab tropomyosin using mass

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spectroscopy, demonstrating that this allergen of 33 kDa could be the occupational allergy-eliciting agent. In addition, arginine kinase (40 kDa) was identified in the later study, which could represent a 43-kDa IgE-binding protein in the air samples analyzed by Gill *et al*. It is important to note that tropomyosin is not only a crustacean allergen, but it has also been confirmed in a number of mollusk species (e.g., mussels, oysters, squid), although mollusk allergens are not as well characterized as those of fish or crustaceans (Taylor, 2008, Desjardins *et al*., 1995). Even though IgE cross-reactivity between different crustacean tropomyosins are commonly reported, the amino acid sequence identity of mollusk tropomyosin with the one from shrimp is fairly low, with 57% and 61% homology (similar to nonallergenic vertebrate tropomyosin). It is therefore likely that workers sensitized to crustacean tropomyosin will not react to mollusk tropomyosin.

While currently no other aerosolized allergenic proteins have been characterized on a molecular level, other potential allergens could include myosin light chain (Ayuso *et al*., 2008) and sarcoplasmatic calcium-binding protein (Shiomi *et al*., 2008).

It is noteworthy that crustacean and mollusc allergens do not cross-react with fish allergens and no reactivity between known allergens or homologous proteins has been demonstrated (Lopata and Lehrer, 2009a, b) which is of importance when identifying the offending occupational seafood allergens.

2. Fish allergens

The major allergen in fish is the muscle protein parvalbumin, which belongs to a group of Ca-transporting muscle proteins (Beale *et al*., 2009; Thien Van Do *et al*., 2005). The molecular weight of parvalbumin, which can range from 10 to 15 kDa, can be subdivided into two distinct isoforms, alpha and beta. In fish, the allergenic beta-form is considered a cross-reactive pan-allergen. Although most heat-resistant food allergens contain linear epitopes, parvalbumin has conformational epitopes (Untersmayr *et al*., 2006), stabilized by the interaction of metal-binding domains. Comparative studies of natural and recombinant allergens from cod and carp indicate that these proteins are equally good in the detection of parvalbumin-specific IgE antibodies (Swoboda *et al*., 2002). High-molecular-weight polymers of parvalbumin have previously been documented in snapper (Rosmilah *et al*., 2005), but the clinical relevance is not known. Parvalbumin shares various degrees of amino acid homologies ranging from 60% to 80%, which probably explains variable degrees of clinical cross-reactivity in fish-allergic patients (Van Do *et al*., 2005).

A recent study on occupational exposure to fish allergens in a pilchard-processing factory characterized a highly cross-reactive allergenic isoform of pilchard parvalbumin (Andreas Lopata and Mohamed Jeebhay, unpublished data). The cDNA sequence of the β-form of pilchard parvalbumin was

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subsequently determined and designated as Sar sa 1.0101 (Beale *et al*., 2009). In contrast to ingestion-sensitized patients, who recognized mainly the monomeric isoform, sensitized workers appear to recognize higher molecular weight proteins, which are most likely dimers of parvalbumin and collagen (Lopata and Jeebhay, unpublished data). A very recent study demonstrated that, during processing of pilchard, the major IgE reactivity seems to be directed against a novel 36-kDa allergen, which was also recognized by fish-processing workers (van der Ventel *et al*., 2011). This allergen was identified as fish glyceraldehyde-3-phosphate dehydrogenase, which interestingly is the most frequently recognized allergen in *Aspergillus versicolor*, a mold species that is also responsible for inhalant allergy (Benndorf *et al*., 2008). Glyceraldehyde-3-phosphate dehydrogenase may therefore be a novel fish allergen that is important in the occupational setting.

In addition to parvalbumin, other allergens have been identified in fish such as collagen (Sakaguchi *et al*., 2000), from the skin and muscle tissue, as well as the hormone, vitellogenin (Hickey 2007; Perez-Gordo *et al*. 2008), found particularly in fish roe (caviar).

C. The immune system and inflammatory responses to seafood components

The term hypersensitivity is defined as a reaction that induces reproducible symptoms and signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects. Hypersensitivity can be differentiated into IgE and non-IgE allergic and nonallergic hypersensitivity; the latter one does not involve the immune system. Allergy-causing IgE antibodies are produced by B-cells against a specific allergenic protein. These antibodies bind onto receptors on cells such as mast cells and basophils, and bind the allergen on subsequent exposure, causing the release of preformed mediators such as histamine, enzymes, and cytokines (see detailed mechanism in Fig. 2.2). These mediators trigger an allergic reaction by acting on various direct target organs. Importantly, not only does the IgE–allergen interaction cause activation of cells, but other components can also do this such as histamine (binding on histamine receptors) and specific proteases. The latter mechanism has recently been characterized for salmon trypsin, which is able to activate cells via binding to protease activation receptors (PAR) (Bang *et al*., 2009; Larsen *et al*., 2007, 2008). Furthermore, trypsin activity has recently been demonstrated in several crustacean species implicated in occupational allergies (Sun and Lopata, 2010). While PAR activation enzymes have been characterized for few seafood species, their relevance in causing clinical symptoms associated with exposure at the workplace has still to be evaluated.

In addition, other components can be found in seafood which can affect the immune system or cause toxic reactions (Table 2.3). In

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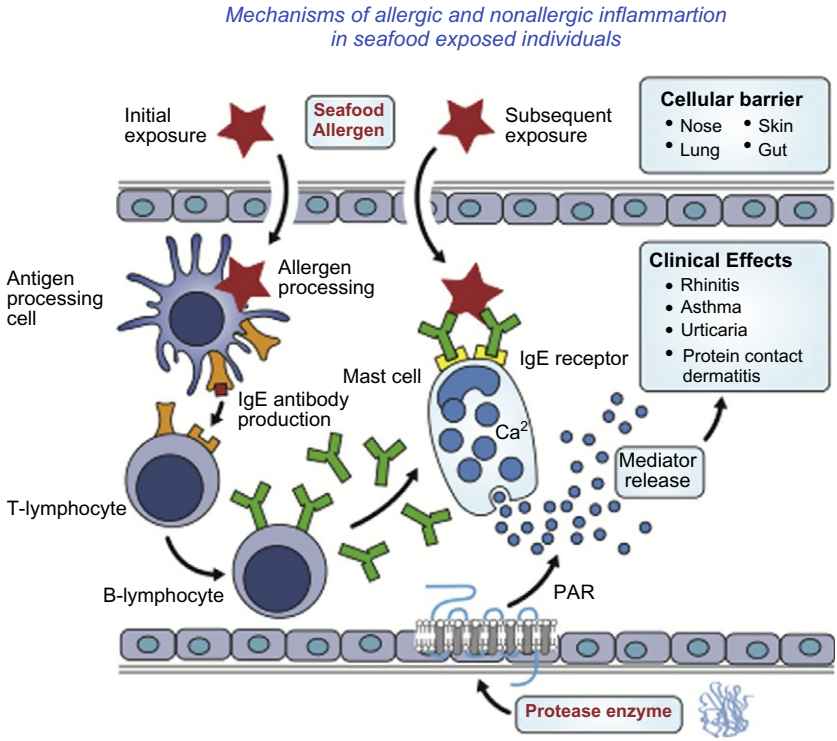

FIGURE 2.2 Mechanisms involved in cellular activation of cells via allergen recognition by IgE antibody and direct activation via protease activation receptors (PAR) and proteases. Modified and adapted from Lehrer *et al.* (1996).

TABLE 2.3 Seafood-associated agents causing adverse reactions in seafood processors

Seafood type	Agents
Fish	*Anisakis* (parasite), sulfur dioxide, histamine, trypsin (enzymes), lipopolysaccharide (endotoxin), Daphnia (fish feed)
Crustaceans	Chitin, kanimiso (internal organs), algae
Mollusks	Hoya (sea squirt), algae

particular, chitin, found in all crustacean and some mollusks, is known to stimulate the innate immune system (Reese *et al.*, 2007; Strong *et al.*, 2002). The chitin of shellfish is covalently linked to proteins and tanned by quinones. Chitin and modified chitins (e.g., chitosan) are known to stimulate particularly macrophages, possibly via receptor interaction

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(e.g., Toll-like, Dectin-1), and generate a strong Th1 immune response (Sigsgaard *et al*., 2008). Murine studies support these finding by demonstrating a downregulation of allergic Th2 responses. However, other studies have demonstrated a Th2-enhancing effect, and therefore, the effect on the immune system in the occupational setting is currently unclear.

Bacteria on seafood products or in the working environment can generate endotoxins, also called lipopolysaccharides (LPSs), which are found in the outer membrane of various Gram-negative bacteria. These LPSs are strong inducers of the innate immune response and can have direct effects on the respiratory system and cause airway hyperresponsiveness in an occupational setting (Jeebhay and Cartier, 2010). Endotoxins have not only been implicated in organic toxic dust syndrome but may also act as an adjuvant, facilitating sensitization to other allergens, or alternatively, they may increase the severity of allergic disease (Huntington *et al*., 2007; Sigsgaard *et al*., 2008).

D. Cross-reactivity with other allergen sources

Patients with allergy to ingested seafood are frequently reported to also have allergic reactions to mites and insects (Lopata and Lehrer, 2009a,b). This cross-reactivity is probably due to the high amino acid homology of these invertebrate tropomyosins and may have significant clinical implications. Molecular studies suggest similar IgE-binding epitopes in these very different arthropods. A multivariate statistical analysis of IgE antibody serum concentrations of 1011 individuals to 89 allergen extracts identified 12 stable clusters (Soeria-Atmadja *et al*., 2007). Interestingly, reactivity to shrimp and mussels was clustered with cockroach but unexpectedly not with HDM. This IgE cluster analysis suggests that mite tropomyosin (Der p 10) is not the major allergen and is probably based on the much lower amounts of this muscle protein in mites. A recent review on crustacean allergy highlights that in the group of arthropods, the crustaceans are phylogenetically closer related to the ''insects'' than to the group of ''mites'' (arachnids) (Lopata and Lehrer, 2009a,b). Other possible IgE reactivities to tropomyosin-containing allergen sources have been documented, such as the cross-reactivity with the nematode parasite, *Anisakis* (Audicana and Kennedy, 2008; Lopata and Lehrer, 2009a,b), found in many seafood species. A recent study among pilchard-processing workers identified a high prevalence of *Anisakis* sensitization which seems to be related to fish exposure (Nieuwenhuizen *et al*., 2006). A subsequently developed mouse model demonstrated clearly that the exposure to *Anisakis* allergens can cause severe allergic reactions upon ingestion but also skin contact or inhalation (Kirstein *et al*., 2010; Nieuwenhuizen *et al*., 2009).

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V. IMPACT OF SEAFOOD-PROCESSING ACTIVITIES ON ALLERGENICITY AND AIRBORNE EXPOSURE LEVELS

A. Seafood processing and changes in allergenicity

Food is subjected to a large variety of processing conditions to prolong storage time or improve sensory qualities. Many different processes are used, often in combination, but can be generally categorized into thermal and nonthermal procedures. A recent workshop evaluated the effects of food processing on the allergenicity of food allergens (Thomas *et al.*, 2007). Various food processes have been used to reduce allergenicity of certain foods, but very few studies focused on seafood. In fact, even enhanced allergenicity has been demonstrated through heat treatment of food allergens. Since tropomyosin of invertebrates is typically a lysine-rich protein (up to 12% in scallops), it reacts easily with reducing sugars through the Maillard reaction during food processing such as grilling, steaming, and roasting. Particularly, the brown color of dried seafood is caused by the Maillard reaction. Studies on the effect of sugar residues on two different mollusk species showed opposite effects on IgE reactivity. Studies have shown that heating of scallops (a bivalve) in the presence of sugar residues increased IgE binding, as demonstrated by competitive enzyme-linked immunosorbent assay (ELISA), while a decrease of allergenicity has been observed for squid (calamari) in the presence of the reducing sugar ribose (Nakamura *et al.*, 2006). These contradictory results need therefore to be interpreted with caution in the context of their ability to cause allergic sensitization among exposed workers.

In addition, nonthermal processes have been investigated such as gamma radiation of crustaceans and mollusks as well as high-intensity ultrasound treatment of shrimp that resulted in reduced IgE-binding capacity of the allergens. Meyer-Pittroff *et al.* (2007) suggest that pressure above 600 MPa causes reversible and irreversible changes to the secondary, quaternary, and tertiary structure, particularly in helical proteins, and demonstrated reduced allergenicity. Nevertheless, complete loss of allergenicity or allergen concentrations has not been demonstrated which is probably due to the fact that even small protein fragments of about 3.5 kDa can still cross-link mast cell IgE and elicit an allergic reaction (Thomas *et al.*, 2007). Furthermore, the solubility and extractability of treated tropomyosin might be affected and result in underdetection as has been demonstrated for radioactive-treated crustacean and mollusks. In addition, it cannot be ruled out that processing has different affects on the less well-characterized seafood allergens. Most of the investigated processes to reduce allergenicity are purely experimental, but it is an important area of research for seafood allergy which has to be further explored.

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The allergenicity of seafood allergens seems also to vary with storage procedures. Codfish stored for several days (at 4 °C) displays a much higher IgE reactivity than very fresh fish. These biochemical changes of allergens, even during longer freezing periods, may be attributed to the natural development of components such as formaldehyde in fish tissue, which might affect the allergenicity of some proteins (Lopata, 2010).

More recently, work among crab workers provided strong evidence that airborne proteins derived from snow crabs, released during boiling and cracking of crab legs, are responsible for the immunological reactivity in sensitized or symptomatic workers (Gill *et al*., 2009). Other studies of the allergenicity of emissions from boiling fish indicate that the ''steam aerosols'' from salmon share IgE-binding components with raw and boiled salmon but did not identify the actual protein (Crespo *et al*., 1995; Pascual *et al*., 1996). Finally, there is also some evidence emerging that processing raw versus cooked crustaceans and fish may be less sensitizing to exposed workers (Carnes *et al*., 2007, Gautrin *et al*., 2010; Lopata and Jeebhay, unpublished data).

B. Environmental airborne exposures in seafood-processing environments

Studies of environmental exposure assessments in seafood-processing environments are summarized in Table 2.4. These studies demonstrate a wide range of total inhalable airborne particulate (0.001–11.293 mg/m^3), protein (0.001–6.4 mg/m^3), and allergen (0.001–75.748 mg/m^3) levels (Jeebhay and Cartier, 2010). Particulate and allergen levels reach very high levels in the processing operations aboard vessels at sea in the case of crab processing and during fishmeal operations. Work processes causing aerosol production include butchering or grinding; degilling, ''cracking,'' and boiling of crabs; cleaning and brushing of crab; ''tailing'' of lobster; ''blowing'' of prawns; washing or scrubbing of shellfish; degutting, heading, and cooking/boiling of fish; mincing of seafood; and cleaning of the processing line or storage tanks with high-pressured water hoses (Jeebhay *et al*., 2001). Since water is a major feature of this work environment, it may also influence the size, lifetime, or other dynamics of small protein particles. Processes that generate dry aerosols such as prawn blowing operations using compressed air and fishmeal loading/bagging appear to generate higher concentrations of particulate than wet processes (prawn blowing with water jets).

Aside from allergen exposures, endotoxin levels during seafood operations have also been investigated. Whereas surveys among crab-processing workers reported mean concentrations of airborne endotoxin to be 32.6 EU/m^3 (total fraction) and 15.6 EU/m^3 (respirable fraction), recent studies of shrimp processors have demonstrated

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TABLE 2.4 Exposure assessment studies of seafood-processing workers on land and aboard vessels

Seafood category	Particle fraction measured	Particulate conc. (mg/m^3) range	Protein conc. (mg/m^3) range	Allergen conc. (μg/m^3) range
Crustaceans				
Crabs (snow, Tanner, common, King)	Total inhalable	0.001–0.680	0.001–6.400	0.001–5.061
Crabs (snow)[a]	Total inhalable	ND	ND	0.079–21.093
Prawns	Total inhalable	0.100–3.300	ND	ND
Shrimp	Total inhalable	ND	ND	1.500–6.260
Rock lobster	Thoracic	LOD—0.661	LOD—0.002	ND
Scampi	Total inhalable	ND	ND	0.047–1.042
Finfish				
Salmon	Respirable	0.040–3.570	ND	0.100–1.00
Pollock	Total inhalable	0.004	ND	ND
Whiff megrim/hake	Total inhalable	ND	ND	0.002–0.025
Pilchard	Thoracic	LOD—2.954	LOD—0.006	0.010–0.898
Cod	Total inhalable	ND	ND	3.800–5.100
Salmon	Total inhalable	ND	ND	LOD—1.600
Herring	Total inhalable	ND	ND	0.300–1.900
Fishmeal (anchovy)	Thoracic	LOD—11.293	LOD—0.004	0.069—75.748
Shark cartilage[b]	Respirable	0.920–5.140	ND	ND
	Total inhalable	26.400–44.700	ND	ND

ND, not done; LOD, limit of detection.
Reproduced with permission from Jeebhay and Cartier (2010).
[a] Processing aboard vessels.
[b] Nonfood-handling environment.

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mean levels between 5.8 and 29.9 EU/m^3 (total fraction) (Bang *et al.*, 2005). Fish-processing operations have reported highly variable mean airborne endotoxin levels for pilchard-canning operations (49 EU/m^3); filleting/slaughtering of cod (8.6–23.9 EU/m^3), salmon (6.8–7.9 EU/m^3), and herring (103.7 EU/m^3); and anchovy fishmeal (136 EU/m^3) (Jeebhay *et al.*, 2004, 2005). It would appear that gutting and fishmeal operations demonstrate consistently elevated endotoxin exposures.

Almost all studies evaluating total protein exposure and quantifying specific allergenic proteins have until recently relied on immunological reactivity techniques. These include ELISA (Lopata *et al.*, 2005), radioallergosorbent test, and immunoblotting. These techniques measure the allergenic protein based on the recognition by specific antibodies, which in turn are labeled and react with colorimetric substrates, enabling the indirect quantification of these proteins. Consequently, the sensitivity, specificity, and selectivity of these indirect techniques are limited by the recognition of the proteins in air samples by these antibodies. Therefore, modifications of allergenic proteins during processing could result in an underestimation of actual allergen concentrations in air samples. In addition, cross-reactivity of antibodies with similar proteins could result on the other hand in overestimation of allergen concentrations. Recent studies have therefore investigated alternative techniques in quantifying the exact amount of allergen present as demonstrated for snow crab tropomyosin and arginine kinase (Abdel Rahman *et al.*, 2010a,b, 2011). The lower limit of detection for sampling airborne tropomyosin in a simulated processing plant was with 33 ng/m^3 extremely sensitive compared to conventional assays and could be achieved in a very short sampling time of <1 h.

VI. RISK FACTORS FOR OCCUPATIONAL ALLERGIES ASSOCIATED WITH SEAFOOD

A. Host-associated factors

Individuals with preexisting asthma are not at increased risk of developing occupational asthma unless they are atopic. Indeed, atopy is the most important host factor associated with the development of sensitization to high molecular allergens such as crabs, prawns, cuttlefish, pilchard, and anchovy (Jeebhay and Cartier, 2010, Jeebhay *et al.*, 2001) as well as for the development of occupational asthma although the risk associated with atopy varies between studies. Smoking has been associated with an increased risk of developing sensitization to high molecular agents and in the seafood industry particularly to prawns, crab, and fish (pilchard,

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anchovy, and salmon) (Jeebhay and Cartier, 2010, Jeebhay *et al*., 2001). It is also a risk factor for developing occupational asthma in salmon- and crab-processing workers. The presence of rhino-conjunctivitis has been associated with an increased risk of developing occupational asthma to a number of high molecular agents including seafood. Analysis of fish consumption patterns among seafood processors has shown that high seafood consumption (as measured by serum levels of omega-3 fatty acids) is positively associated with neither occupational sensitization nor occupational asthma (Jeebhay *et al*., 2008).

Atopy and skin integrity constitute important host-associated risk factors for the development of urticaria and PCD (Jeebhay *et al*., 2001). Various studies among food handlers and caterers have shown an association between atopy and these skin outcomes induced by crustaceans. Skin integrity and physiological factors (such as temperature below 19 °C) also seem to be important in determining the location of skin symptoms (urticaria) and recovery of skin barrier function among workers handling fish (Halkier-Sorensen, 1996).

B. Environmental factors

Regarding environmental factors, exposure to the causative agent/substance in the food matrix, its physical state, and the exposure dose (duration and level of exposure) all appear to play a role. Several studies have shown dose–response relationships between the level of exposure to occupational sensitizers and the development of either symptoms or sensitization. As reported previously, Gaddie *et al.* reported that 15 of 18 workers in a prawn-processing plant experienced relief of allergy symptoms including asthma when compressed-air jets used to extrude prawns from their carapace were replaced by cold-water jets, the wet weight of material filtered in the air decreasing from 1.8–3.3 to 0.1–0.3 mg/m^3 (Jeebhay *et al*., 2001). Similarly, Douglas *et al*. reported that adding an exhaust ventilation over gutting machines in a salmon-processing facility reduced respirable aerosol levels from a mean of 3.14 to <0.01 mg/m^3. Since then, no new cases of occupational asthma occurred over 24 months versus an initial prevalence of 8% over an 18-month period (Jeebhay *et al*., 2001). Recently, Gautrin *et al*. showed that cumulative exposure to snow crab allergens is positively associated with occupational asthma and allergy in a dose–response manner (Gautrin *et al*., 2010). Exposure response relationships in fish-processing workers have also demonstrated that workers with work-related asthma symptoms have a twofold increased risk of being exposed to pilchard-antigen concentrations >30 ng/m^3 at the time of onset of new symptoms (Jeebhay, 2003).

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VII. DIAGNOSING SEAFOOD ALLERGY

Hypersensitivity to seafood can be differentiated into IgE and non-IgE allergic hypersensitivity, implicating the immune system, and nonallergic hypersensitivity, which does not involve the immune system. Therefore, different tests must be used to distinguish between these conditions.

The term hypersensitivity is defined as a reaction that induces reproducible symptoms and may involve antibodies of the IgE, IgG, and IgA type as well as other immune cells. Allergic type 1 hypersensitivity is usually mediated by IgE antibodies. Most affected individuals are sensitized to more than one allergen, which might trigger clinical symptoms, and often, it is difficult to distinguish the major offender. In addition, the symptoms are dependent not only on IgE antibodies but also on a number of other confounding factors. These can include inflammation, presence of infection, physical and psychological stress, and hormonal influences. With these issues in mind, *in vitro* and *in vivo* assays are used to determine the mechanisms behind the clinical symptoms.

In vitro diagnostic methods rely on the detection and/or quantification of specific IgE antibodies using assays such as the ImmunoCAP (Phadia), ELISA, immunoblotting, and allergen microarray (Table 2.5). All these various techniques rely on the presence of relevant allergen components in the test system. Natural allergen extracts are routinely used for *in vitro* and *in vivo* diagnostic procedures. However, these allergen preparations can vary significantly in their composition and allergenicity. In addition, allergic workers commonly generate antibodies to a specific allergen rather than multiple allergens. A recent study by Gill *et al*. (2009) among snow crab-processing workers indicated that reactivity to certain allergens was associated with an increased risk of disease. To address these problems, recombinant allergens have recently been introduced into conventional *in vitro* testing, a strategy termed component-resolved diagnosis that potentially also has applications for evaluating sensitized seafood-processing workers (De Knop *et al*., 2010; Mothes *et al*., 2006; Swoboda *et al*., 2007).

During the allergic reaction, new and preformed mediators are released from human cells, such as mast cells, basophils, and eosinophils, into the surrounding tissue or blood system. These mediators include histamine, tryptase, leukotrienes, eosinophil cationic protein, and cytokines and can directly be quantified using various assays (Table 2.5).

During an immune reaction to foreign antigens at the workplace, antibodies of the IgG type are also generated as part of the body's complex defense mechanism. It is well known that specific IgG antibodies to aerosolized organic dust can cause allergic alveolitis in the workplace as demonstrated for farmers keeping birds or growing mushrooms

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TABLE 2.5 Tests for the presence of sensitization and identification of offending allergens

Aim of the test is to identify	Principle of the test	Basic technology	Major test system
Presence of sensitization to specific allergen	IgE/IgA/IgG antibodies tests to antigens from one allergen source or one single allergen component	• Different assays using a solid phase to bind allergen-specific antibodies and detect with anti-IgE/IgA/IgG reagents	• UniCAP • ELISA • Immunoblot • Allergen microarray
Presence of inflammation mediators released from different cells	• Histamine from basophils/mast cells • Tryptase from mast cells • Leukotrienes and prostaglandins • Eosinophil mediators such as ECP • Lymphocyte mediators such as cytokines	• Solid phase with attached antibodies and labeled anti-mediator reagents	• UniCAP • UniCAP • ELISA • UniCAP • ELISA
Cellular immune response	• T-cell proliferation • Basophil activation	• Analysis of cell proliferation • Cell stimulation with specific allergen/antigen	• Tissue culture • CAST • Flow cytometry

(Di Stefano *et al.*, 2007; Lopata *et al.*, 2004). In addition, specific IgG antibodies can be a good marker of increased exposure to particular antigens and is frequently used in the evaluation of inhaled fungal proteins, cow's milk allergy, or celiac disease. Although various other isotypes of seafood specific immunoglobulins such as the IgG and IgG subclasses, IgM and IgA have also been detected in patients sensitized to seafood, their potential role in providing protection from or eliciting

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adverse reactions to various shellfish or bony fish has not been fully evaluated.

Aside for these immunodiagnostic tests, specific bronchial inhalation challenge with the implicated seafood still remains the gold standard in diagnosing occupational asthma associated with airborne exposure to seafood.

VIII. PREVENTION

Although a number of studies suggest that atopic individuals are at higher risk of developing occupational asthma, there is no way of predicting which individuals will become symptomatic. Furthermore, even preexisting sensitization to a workplace allergen is not sufficient to predict which individual will develop allergic symptoms. As a result, there is general consensus that there is no place for prescreening and exclusion from employment of atopic individuals (Tarlo *et al.*, 2008). Primary prevention measures to minimize exposure to offending agents by eliminating exposure or worker relocation are therefore key to reducing the risk of occupational allergy and asthma among workers in the seafood industry. However, regular follow-up of exposed workers employed particularly in high-risk industries such as crab and prawn processing is warranted. Although regular assessment using skin prick tests to specific occupational allergens has been used successfully in the enzyme industry to detect those at risk of developing symptoms, this may not be as useful in some seafood industries such as snow crab processing in which skin prick tests appear to be less sensitive (Jeebhay and Cartier, 2010). Short questionnaires administered on a regular basis may allow early detection of allergic symptoms. Prior to work withdrawal, proper evaluation should be conducted to confirm the diagnosis of occupational allergy or asthma. Affected individuals should be transferred to a low allergen exposure environment, while measures are undertaken to reduce allergen exposures in high-risk work processes.

Monitoring of allergen exposure in seafood-processing plants would be ideal but is not readily available. Furthermore, there are currently no regulatory exposure standards for seafood allergens, and these should be developed once standardized assays for quantifying these allergens become available (Jeebhay and Lopata, 2006). An alternative strategy may be to monitor total particulate or proteins in the air as a proxy for allergen exposure, provided that these exposure metrics are highly correlated. Identifying departments and activities with high aerosol exposure such as fishmeal bagging, gutting machines, cleaning and brushing crabs, and compressed-air jets should lead to the introduction of improved local exhaust ventilation systems and change in work processes to reduce aerosol exposures (Jeebhay and Cartier, 2010). Preliminary studies suggest that

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processing (cleaning and brushing) raw crab before it is cooked may help reduce exposures; however, further studies are needed to confirm this.

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