

INAUGURAL LECTURE

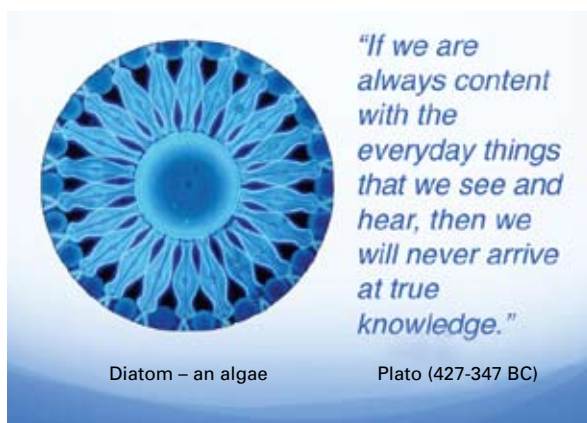
FROM FARM TO FORK – A FISHY STORY OF ALLERGY AND ASTHMA



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This statement by Plato, the Greek philosopher, forms the basis of many a scholarly research endeavour trying to understand the current human experience. Join me on a journey of discovery through the recollection of some aspects of my research over the past decade in unravelling the first case report of seafood allergy in 1937 reported by the Norwegian bacteriologist Arent de Besche of a fisherman who developed allergic symptoms and asthma when handling codfish.

De Besche reported: *'for this reason patients who suffer from fish asthma will rarely discover for themselves the connection between fish and their malady, and as it seldom occurs to their medical advisors, the patients continue to ... suffer from asthma throughout their lives, whereas an investigation which revealed the real cause of the disease would have relieved them of it...'* (Arent de Besche, On Asthma bronchiale in Man provoked by various Kind of Animals, *Acta Medica Scandinavica*, Vol XCII, Fasc I-III, 1937).

WHY A FOCUS ON SEAFOOD?

Worldwide, food allergies and anaphylaxis are increasing at a faster rate than any other allergic disorder, affecting up to 13% of adults and children. The World Allergy Organisation estimates that allergic diseases affect at least 30-40% of the world's population, increasingly affecting the young and those in the developing world as these diseases plateau in the industrialised world. Most allergic reactions to foods can be attributed to a few food groups including seafood, which

form important sources of protein for populations from developing countries.

In industrialised countries, there has also been a move to healthier eating habits that promote consumption of functional foods such as vitamin-enriched and fermented foods. Seafood comprises one of these food options since it is rich in omega-3 fatty acids, bioactive peptides, chitin and carotenoids when compared to other food sources. Various studies show that fatty acids have potent anti-inflammatory properties and other beneficial effects on brain development and function in infants as well as preventing coronary artery disease in adults.

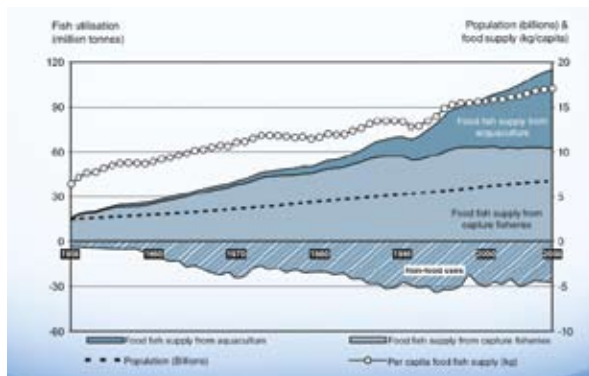
The increased demand and consumption for seafood and its by-products has been associated with a concomitant rise in fishing and aquaculture activities. Aside from the potential depletion of marine stocks if not managed in a sustainable manner, recent studies suggest more frequent reporting of allergic health problems among consumers as well as processors of seafood.

On a more local level, fishing has been a strong economic thread that has woven itself into the fabric of the Western Cape economy from the early 19th century, providing a means of livelihood for many a coastal inhabitant.

THE FARM: SEAFOOD HARVESTING AND EMPLOYMENT TRENDS

Recent data from the Food and Agriculture Organisation indicates that while fish capture has stabilised in the past decade, aquaculture has increased at a rate of 6% per annum to just under 50% of the total global fish supply. In 2008, this sector provided direct employment and revenue for 45 million people worldwide, most of whom were from developing countries, especially Asia. The International Labour Organisation estimates that approximately 50% of the fishing population work aboard fishing trawlers, 30% in aquaculture production and 20% work inland as capture fishers and seafood processors. A characteristic feature of employment in the fishing industry is the seasonal nature of the work due to weather variations and the migratory nature of marine species. As a result, the number of full-time fishers has declined while the number of part-time fishers has grown quite rapidly. In many countries, labour in the fishing industry is divided along gender lines with men almost exclusively involved in harvesting and some processing at sea and women doing most of the seafood processing ashore and some inland capture.

The South African fisheries sector plays a small part in direct economic terms in the economy of the country, contributing about 1% to GDP. However, fishing contributes primarily to the economies of the coastal provinces with the majority of the contribution to the national fishing industry derived from the Western Cape. Over the last decade the industry, which at one time employed approximately 30 000 workers and a further 100 000 workers in secondary industries, has decreased to 17 000 people directly employed in the fishing industry and 11 000 in associated industries.



World fish utilisation and supply (Source: Food and Agriculture Organisation, 2010).

THE SEAFOOD MATRIX

The three most important groups of marine species frequently consumed and processed are Arthropods, Molluscs, and Pisces (subphylum Chordata).

Crustaceans, surprisingly, belong to the arthropod family together with spiders, insects and mites such the house-dust mite. Over 30 000 living crustacean species are found worldwide. This class includes some of the most allergenic species of seafood such as prawns, lobsters and crabs. The second group of molluscs constitute a large and diverse group, of over 100 000 different species including mussels, oysters, abalone, snails, and squid. Lastly the Pisces fish family comprises two subgroups, namely bony fish and cartilaginous fish. Most edible fish belong to the bony fish group, whereas sharks and rays are cartilaginous and belong to a different order. Although there are more than 20 000 different species of fish, consumption and processing depends on regional availability.

It is estimated that in most Asian countries at least 70% of seafood is eaten fresh. However, this proportion is on the decline, mimicking trends seen in industrialised countries as the marine seafood stocks decline and aquaculture and processing activities take hold. The processed products are mainly filleted frozen seafood, as well as dried, salted, canned, surimi products, seafood additives and fishmeal.

Experimental studies in factories have shown that the aerosols generated when crab is processed contain mainly the exoskeleton containing chitin, muscle, gills and internal organs. Fish juice produced in fish filleting and canning plants contain biogenic amines, digestive enzymes, skin mucin, collagen and fish muscle.

Aside from the actual seafood, various non-seafood contaminants and additives have been detected in the seafood matrix. These include parasites such as the Anisakis worm, sea-squirt, red soft-coral, algae, bacteria, viruses, marine toxins such as saxitoxins, scrobroid and histamine, bacterial endotoxin, hydrogen sulphide gas produced by anaerobic decomposition of fish, residues of antibiotics or hormones used in aquaculture, chemical preservatives such as sulphites, and other spice additives.

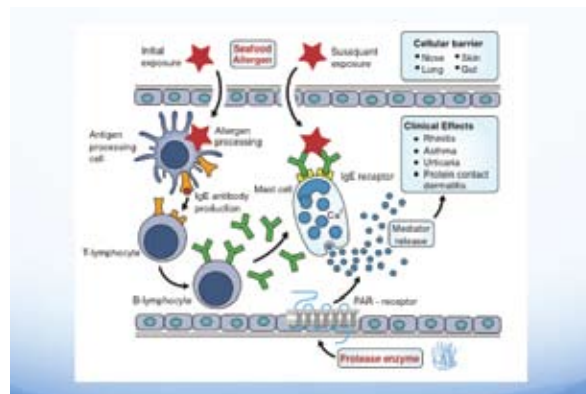
Finally, in heavily polluted seas, rivers, and estuaries abnormally high concentrations of industrial chemicals such as heavy metals and organic compounds such as polychlorinated biphenyls (PCBs), dioxins and chlorinated pesticides have been detected.

AT THE FORK: HEALTH EFFECTS ASSOCIATED WITH SEAFOOD

Aside from the commonly encountered oral route in domestic settings, seafood also finds its way into the

human body through inhalation and skin contact, in domestic, recreational and work settings. Adverse reactions have been reported in individuals consuming, handling and processing seafood in these settings. The reaction could be due to a toxin, as commonly encountered with food poisoning in a seafood restaurant. Alternatively the reaction could be of a non-toxic nature that may be due to a hypersensitivity or intolerance to a component in the seafood matrix.

In seafood-exposed individuals, both allergic hypersensitivity and pro-inflammatory reactions have been observed. The allergy is commonly mediated by IgE antibodies produced in response to a specific allergen, commonly a protein, present in the seafood matrix. There is also evidence from our Norwegian colleagues that certain seafood digestive enzymes such as trypsin can activate receptors on epithelial cells of airways and cause airway inflammation. Individuals with allergic reactions to seafood may have a single symptom, but often more than one organ is involved.



Mechanisms of allergic and non-allergic inflammation in seafood exposed individuals (modified and adapted from Lehrer et al. *Critical Reviews in Food Science and Nutrition*, 1996).

While the vast majority of published reports have focused on allergic symptoms following ingestion or skin contact, I would like to focus on the less apparent route – that of inhalation. This, in an attempt to address the problem described by de Besche in the 1930s and the subsequent reports of highly allergic individuals having an asthmatic reaction without eating seafood in a restaurant or when seafood is cooked in the home.

Fifteen years ago, a Spanish study by Crespo reported 11% of children from a group of 200 children with fish allergy placed on a strict fish avoidance diet that experienced repeated reactions upon incidental inhalation of fish odours or vapours. In most cases, these episodes occurred at home when other people were eating fish. This was followed by a similar observation in a South African study of over 100 individuals with self-reported seafood allergy, among whom 30% reported allergic symptoms after handling or inhaling seafood at home.

Yet, 1-2 decades prior to this, occupational asthma was being reported among fish and shellfish fishers and processors exposed to mother of pearl dust, abalone, clam liver extract, prawns, sea squirt, fishmeal, crab and cuttlefish. Since then various other seafood has been associated with occupational allergy and asthma including other crustaceans such as shrimp and lobsters, molluscs such as mussels, octopus and scallops and bony fish including trout, salmon, pilchard, anchovy, hake and tuna. Various epidemiological studies indicate that the prevalence of occupational asthma among these processors is between 2% and 36%, with occupational

Table 1. Seafood species causing occupational allergy and asthma

Phylum	Class	Family species (common name)
Arthropoda	Gastropoda	Crabs, lobsters, prawns, shrimp, scampi, shrimpmeal
Mollusca	Gastropoda	Abalone, clams, oysters, mussels, scallops, cuttlefish (and bone), octopus, squid
	Cephalopoda	
Pisces (sub-phylum Chordata)	Osteichthyes (bony fish)	Salmon, plaice, tuna, hake, cod, herring, pilchard, anchovy, trout, swordfish, sole, pomfret, yellowfin, fishmeal (flour)
	Chondrichthyes (Cartilaginous fish)	Shark (cartilage)
Other non-seafood associated biological agents		Hoya (sea-squirt), Anisakis, red coral, daphnia, marine sponge, algae

asthma more commonly associated with shellfish than with bony fish.

Our epidemiological studies of over 600 workers along the west coast at St Helena Bay, the epicentre of fish harvesting and processing in South Africa, show that symptoms of work-related asthma may develop after only a few months or after several years of commencing work. Symptoms are generally worse at work, improving on week-ends or holidays and often awaken affected subjects at night. Rhinitis, conjunctivitis and less frequently urticaria are often associated with and may precede chest symptoms.

RISK FACTORS FOR OCCUPATIONAL/INHALANT ALLERGY AND ASTHMA ASSOCIATED WITH SEAFOOD

The aetiology and development of asthma is due to an interaction between various genetic, environmental and host factors giving rise to different expressions or phenotypes of the disease. The seafood industry provides an ideal context to analyse both upstream and downstream risk factors for occupational allergy and asthma associated with seafood.

Environmental factors

Working populations with seafood contact

Occupational exposure to seafood allergens occurs mainly in the food and fishing industry. Seafood processing plants vary in technology levels, with some of the smaller workplaces relying entirely on manual handling of seafood and larger companies using modern highly automated processes. In these plants, there is great variation in processing procedures and preservation techniques including filleting, freezing, drying, cooking, smoking and high pressure techniques. Other occupations associated with exposure to seafood include those involved in food preparation activities such as restaurant chefs and waiters, pharmaceutical technicians, laboratory researchers, pet food production workers, jewellery and souvenir makers.

Work processes generating seafood aerosols

Our studies among fish processors on the west coast and those of our North American and Scandinavian colleagues show that various work processes cause bio-aerosol production. In shellfish processing plants these include butchering and degilling of crabs, washing and brushing of shellfish, 'cracking' of legs, boiling of crabs, 'blowing' of prawn meat through shells. In fish process-

ing, activities include degutting, heading, cooking/boiling of fish and mincing of seafood. Processes that generate dry aerosol particulates such as prawn blowing using compressed air and fishmeal bagging appear to generate higher levels of particulate than wet processes. It is these aerosolised wet or dry particulates produced from seafood during processing operations that are ultimately inhaled by workers.

Seafood allergens present in aerosols

In order to demonstrate that seafood allergens are present in the aerosols produced by work processes, we developed the first antibody assay to measure allergens of pilchard and anchovy

produced in factories. Our environmental exposure assessments and those of other colleagues have demonstrated a wide range of concentrations reaching as high as 11 mg/m³ for total inhalable particulates, 6 mg/m³ for protein and 75 µg/m³ for allergen levels. Particulates and allergens reach very high levels during degutting and dusty fishmeal operations. Recent studies also suggest that particle concentrations are higher in very old processing machines. Studies of crab processors show that activities aboard vessels at sea produce relatively higher levels than similar land based processes due to very confined spaces and poor ventilation systems.

Aerodynamic studies of particles in crab processing environments indicate that at least 30% of airborne particulates are <5 µm and almost 100% <1 µm in herring fish filleting environments. This indicates that a large proportion of particles are less than 10 µm and are therefore able to enter the nose and penetrate to reach the small airways of the lung. The size of these seafood particles is similar in size to other domestic allergens that are known to cause allergic disease when inhaled.

Food processing changes the nature, dose and allergenicity of seafood

As alluded to previously seafood aerosols and particulates are not inert but are in fact biologically active. There is increasing evidence that food processing techniques such as heating, freezing and high pressures have the ability to change the nature, dose and allergenicity of food. During processing, the seafood is concentrated into major allergen source compartments such as muscle, visceral contents, skin mucin or collagen that more readily enters the body. In addition, other by-products such as protease enzymes from the gut, chitin from shell fish, and endotoxin from Gram-negative bacteria are also produced in high concentrations and promote inflammation in the airways. Furthermore, it has been shown that storage conditions may also influence the allergenicity of seafood. Fish kept on ice or completely frozen displays a much higher IgE reactivity than very fresh fish. Our studies and those of our Canadian colleagues also show that processing raw crustaceans or fish appears to be less sensitising than cooked seafood.

Identifying the seafood allergen

The molecular characterisation studies using immunoassays and mass spectroscopy to detect aerosolised seafood allergens with Andreas Lopata in Australia and



Food processing changes the nature, dose and allergenicity of seafood.

Canadian colleagues, confirm that these allergens are primarily high-molecular-weight proteins.

Immunological studies of serum obtained from crab processing workers with occupational asthma identified a major allergen in shellfish responsible for inhalant-related seafood allergy to be tropomyosin, a 34 kDa muscle protein, which is important for muscle contraction. This is the same allergen that has been identified in patients with ingestion-related allergy. Another minor inhaled seafood allergen, arginine kinase (40 kDa) has also been recently identified using mass spectroscopic techniques.

Our studies among fish processing workers suggest that a highly cross-reactive allergenic isoform of pilchard parvalbumin, the major allergen causing ingestion-related seafood allergy, is also one of the allergens responsible for symptoms in workers. Parvalbumin is a 12 kDa calcium binding protein also involved in muscle contraction. In contrast to patients with ingestion-related seafood allergy, who recognise mainly the single molecule form, occupationally sensitised workers appear to recognise proteins of higher molecular weight, including parvalbumin molecules that exist in pairs. There are however, other potential allergens that have yet to be identified. Recently, we characterised a novel allergen (36 kDa), which has been identified as fish glyceraldehyde-3-phosphate dehydrogenase.

One of the fascinating aspects of molecular studies is to explore cross-reactivity patterns in allergic patients using component resolved methods such as protein microarrays to study pan allergens – these are allergens widely distributed in nature. Tropomyosin is one such pan-allergen as it is a highly conserved protein among species. Individuals, allergic to one seafood type such as crab, who have cross-reactive allergies can experience similar symptoms as a result of a common allergen found in a range of other sources such as crustaceans, as well as molluscs, insects, roundworms and house-dust mites. This is also the reason why some individuals sensitised to house-dust mites could have an allergic reaction after eating seafood. While there appears to be very little or no cross-reactivity between shellfish and bony fish, there exists a high degree of cross-reactivity between fish species, because of the presence of parvalbumin.

More recent studies also suggest that chitin, a polysaccharide found abundantly in nature, may be another source of a pan allergen causing cross-reactivity. The reason for this is that chitin forms the basis of the hard shells of crustaceans, such as the crab, lobster, and shrimp. The exoskeleton of insects is also chitinous, and the cell walls of certain fungi also contain this substance.

Exposure-response relationships to seafood aerosols

There is increasing evidence that the risks of sensitisation and occupational asthma are increased with higher exposures of seafood aerosols. This has been concluded

from British studies after introducing interventions demonstrated a complete reduction in the incidence of new cases of asthma. Our studies among fish processing workers found positive relationships between incident work-related asthma symptoms and increasing concentrations of pilchard fish antigens, as well as for cumulative exposures across the working life of processors in the factory. The risk for work-related asthma symptoms doubles with pilchard-antigen concentrations $>30 \text{ ng/m}^3$ at the time of onset of their symptoms. Furthermore, this risk is modified in atopic individuals, who also demonstrate a two-fold higher risk than non-atopic individuals. Recently, Canadian colleagues also showed that cumulative exposure to snow crab allergens was positively associated with occupational asthma and allergy in a dose-dependent pattern.

Workplace organisation factors mediating exposure to seafood

An analysis of other upstream social factors has also assisted in moving beyond the scientific biological understanding of the disease. Our studies have shown that industrial change linked to over-fishing and globalisation interacts with work organisational factors in fish harvesting and processing including the lack of adequate preventive measures in factories to mediate hazardous exposures and worker vulnerability.

Our studies along the west coast showed that almost 70% of workers, mostly women, had a seasonal employment status and that these seasonal workers were twice as likely to report work-related asthma symptoms as permanent workers. We also found that women, employed predominantly in canning operations, were more likely to develop asthma symptoms and airway hyper-responsiveness (twitchy airways) as a result of the gender distribution of work.

In many parts of the world, a large proportion of the fishermen are migrant Asian workers employed by large fishing companies of the North to work on vessels for long periods away from home. This is reminiscent of the way in which South Africa's gold mining industry was built on the sweat and labour of migrant workers from remote rural areas and neighbouring countries.

Both Canadian and South African seafood processing communities are typically rural and remote, single-industry coastal towns that offer few alternatives to working in the seafood processing plants. A large proportion of symptomatic workers refuse to leave their jobs at the plant and choose to risk further exposure.

Access to health care and occupational health services is characterised by uneven and fragmented service provision. Workers are only able to access workplace-based health services during the season and, for the remaining half of the year, they are reliant on under-resourced public sector health care services. Our earlier surveys revealed that only around 50% of these workplaces provided on-site occupational health services and medical surveillance.

Host factors

Atopy

In our studies we observed a high prevalence of atopy, close to 37% among seafood workers, which is similar to our other studies of urban adult populations. This rising prevalence has been attributed to global warming and climate changes causing increased counts of airborne pollen that is more likely to be inhaled. Atopy is by far the strongest identifiable predisposing factor for the development of asthma, especially in children. Among seafood processors, it is also the most important host factor associated with the development of al-

lergic sensitisation and occupational asthma to crabs, prawns, cuttlefish, pilchard and anchovy.

Smoking

In our study along the west coast, 51% of workers, many of whom were female, were current smokers. Smoking has been associated with an increased risk of developing sensitisation and asthma to proteins in the seafood industry particularly to prawns, crab and certain fish such as pilchard, anchovy and salmon. Various reasons have been postulated for this association, including disruption of the natural epithelial barrier of the respiratory tract thereby facilitating allergen entry or smoking acting as an adjuvant for other allergens, thereby enhancing the allergenicity of the inhaled allergen.

Upper airway disease – occupational rhinitis

Many studies show that occupational rhinitis and occupational asthma frequently occur as co-morbid conditions. Rhinitis may precede or coincide with the onset of occupational asthma. The presence of rhinitis has been associated with an increased risk of developing occupational asthma to a number of proteins including seafood. Studies also show that the risk of developing occupational asthma is highest in the year after the onset of occupational rhinitis.

Seafood consumption

Our studies of fish consumption patterns among seafood processors in the Western Cape have shown that high seafood consumption (as measured by serum levels of omega-3 fatty acids) was not associated with occupational sensitisation nor with occupational asthma. However, what we have shown is that while there was no relationship between atopy and serum omega-3 fatty acids, there was a 50% decreased risk of asthma symptoms or current asthma diagnosis in those who consumed rock lobster or mussels regularly. We concluded that this decreased risk may be mediated by omega-3 fatty acids reducing non-specific airway hyperresponsiveness or twitchy airways since we found an inverse relationship between these two variables.

Genetic factors associated with asthma and atopy

Various studies have been conducted to understand the genetic basis for general asthma, with a number of candidate genes identified on various chromosomes. This is however a complex area for which the methodological issues are still being refined. What is known however is that genetic factors that influence the expression of atopy are different from those that influence asthma or its severity. One of the main areas of research today is to determine how environmental factors, in the womb environment to the general and occupational environment, interact with genes and how this influences disease susceptibility.

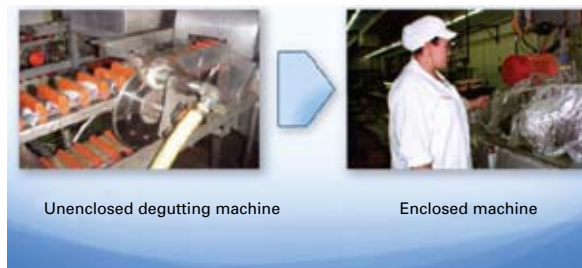
TOWARDS INTERVENTION AND PREVENTION STRATEGIES

Reducing exposures can decrease the risk of occupational/inhalant allergy and asthma

At an individual level, in addition to the conventional treatment of rhinitis and asthma to relieve symptoms, avoidance of exposure to seafood during preparation and cooking activities is an important strategy to reduce airborne exposures to seafood in the domestic environment.

In the workplace, exposure control measures including eliminating exposure or worker relocation are key to re-

ducing risk. This can be done by identifying high-risk activities for aerosol exposure such as fishmeal bagging and gutting machines, and the introduction of improved local exhaust ventilation systems. Our work in some of the factories along the west coast has also sought to address this issue. Preliminary studies suggest that processing raw seafood before it is cooked may help reduce the risk of sensitisation. In the long term legal exposure standards for seafood allergens in the workplace need to be developed. In the interim, the recent ILO 'Work in Fishing Convention (2007)' will no doubt contribute towards improving occupational health and safety conditions at sea to ensure social protection for these vulnerable groups of workers.



Reducing exposures can decrease the risk for inhalant seafood allergy and asthma in the domestic and work environment

Mandatory product labelling for seafood allergens and increased consumer and worker awareness

Recent developments in this country have made it compulsory for product manufacturers to label food products containing commonly recognised food allergens such as seafood as well as egg, cow's milk, peanut, soybeans, tree nuts and grain cereals. This is contained in the recent Regulations related to labelling and advertising of foodstuffs under the Foodstuffs, Cosmetics and Disinfectants Act (1972), which became effective this year. The major relevance of the Regulations in relation to seafood allergy is to prevent accidental exposure to seafood constituents present in these products. Both consumers who are allergic to seafood and workers in high-risk working environments with exposure to seafood need to be educated and made aware of the health risks associated with handling seafood containing products.

Surveillance of workers can reduce morbidity and long-term disability

Regular medical surveillance of exposed workers employed particularly in high-risk seafood industries such as crab and prawn processing is another strategy that can be used to reduce the incidence of occupational allergy and asthma and long-term disability. Symptomatic individuals should be transferred to a low-allergen exposure environment, while measures are undertaken to reduce allergen exposures in high-risk work processes. Surveillance of workers is also important as it may point to early presentation of allergies to previously unknown allergens that may be newly introduced into the work process before the product is released for broader domestic consumption.

Allergen identification can assist with developing diagnostic and immunotherapy modalities

Our molecular studies have characterised a highly cross-reactive isoform of parvalbumin from indigenous pilchard species. We have subsequently developed a model of fish allergy that could be used to test recom-

binant allergens. Generation of a recombinant form of parvalbumin could be used for improved diagnostic use of skin-prick tests and allergen-specific IgE for identifying an allergy in an individual. It also has the potential for developing immunotherapy, which is currently not available for seafood allergic individuals. Immunotherapy involves administering increasing doses of the purified allergen to the allergic individual in a controlled setting over a period of time. With progressively increasing dosages over time, the body adjusts to the allergen exposure such that the individual becomes desensitized and the allergic response suppressed. Immunotherapy against fish allergy could be used for those who are at risk of accidental exposure to aerosolised fish proteins where allergen avoidance measures are ineffective. It also has the potential to reduce allergic reactions among symptomatic seafood workers where exposure reduction measures may not be possible or prove ineffective.

CONCLUSION

In conclusion, as I reflect on the original report by de Besche of the fisherman with seafood allergy, I can say that it has taken me along a journey of discovery engaging with colleagues from all over the world. I have engaged with colleagues from various disciplines, from marine biology to molecular biology, from environmental health and conservation to food security and nutritional health, from allergy and immunology to respiratory medicine, from occupational medicine to occupational hygiene, from quantitative epidemiology to the more qualitative sociology. We have answered some questions and at the same time opened the door to others. In this process, I have come to learn and appreciate that in the vast universe of knowledge, I have just skimmed the surface, a truly humbling experience. So too, in the quest for answers to the major challenges for better health – these lie in multidisciplinary collaboration using laboratory, clinical, public health and social science approaches as I have endeavoured to illustrate in my lecture.

Acknowledgements

Sincere appreciation to my family, mentors, collaborators, students, funders, administrative and research support staff in the university for all your valuable support. Special thanks to my colleague, immunologist Dr Andreas Lopata, who has walked most of this journey of discovery together with me.

Dedication

To all the workers and my patients, I have come to learn, know and appreciate your struggle and toil through working with you. Whether you

work thousands of metres underground unearthing its mineral wealth to build our country, till the soil or fish the seas to bring forth food to still our hunger, or work in the factories night and day sewing the cloth that adorns our bodies, I remember you all. My intellectual efforts pale in comparison to your work. Your struggle and toil has and always will leave an indelible mark in my consciousness.

Chronology of Scientific Communications: 2000-2011

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