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World Health Organization

Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products

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Meeting Report

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Full contact details of the participants are attached as Annex 1

Declarations of Interest

Two of the 14 experts who participated in the meeting declared an interest in the topics under discussion.

Dr Fletcher is an ongoing employee of the New Zealand Institute for Plant & Food Research Limited. This company is a government-owned research organization which also undertakes commercial activities including conducting research and providing scientific advice to the private sector. Dr. Fletcher is engaged in carrying out independent research and providing expert advice on seafood, including the food safety risks of histamine, and received more than US $1 000 but less than US $10 000 per year as remuneration from commercial entities in respect of his activities. Our legal advisors considered that the outcome of this meeting may lead to the development of Codex standards, and that this may have a direct or indirect commercial impact on the New Zealand Institute for Plant & Food Research.

Mr Nolte is an ongoing employee of Connors Clover Leaf Seafoods Ltd, the Canadian subsidiary of Bumble Bee Foods, which is a commercial seafood manufacturer. He is

engaged in quality assurance of seafood and ongoing projects, including hazard analysis and critical control point (HACCP) assessment for tuna processing, which also address histamine. Again it was considered that the outcome of this meeting may lead to the development of Codex standards, which may have a direct or indirect commercial impact on Connors Clover Leaf Seafoods Ltd.

In light of the above, the involvement of Dr Fletcher and Mr Nolte in the meeting was limited in so far as they did not participate in the decision-making process relating to the development of meeting recommendations.

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Executive summary

Scombrotoxin fish poisoning (SFP) (often called "histamine poisoning") is caused by ingestion of certain species of marine fish that contain high levels of histamine and possibly other biogenic amines. Codex Alimentarius through its standards and guidelines aims to provide countries with a basis on which to manage issues such as histamine formation. Several of the existing standards include maximum levels for histamine in different fish and fishery products. The need to harmonize such limits and produce the associated guidance on the relevant sampling plans and other aspects of sampling resulted in the 31st Session of the Codex Committee on Fish and Fishery Products (CCFFP), which agreed to look into the issue of histamine limits in more detail. The Committee established an electronic Working Group in order to facilitate this work and identified the need for scientific advice from FAO and WHO to support this work.

FAO and WHO convened an expert meeting at the FAO headquarters in Rome from 23 to 27 July 2012 to address the public health risks of histamine and other biogenic amines from fish and fishery products. This report summarizes the outcome of that meeting.

Histamine is produced by bacterial actions, e.g. spoilage and fermentation, in fish species which have a naturally high level of the amino acid histidine. Generally, this takes place at a temperature of more than 25 °C over a period of more than 6 hours or for longer at lower temperatures.

A hazard identification process, in which all biogenic amines were considered, concluded that there is compelling evidence that histamine is the most significant causative agent of SFP and that histamine can be used as an indicator of SFP. There are no difficulties in analysing histamine and a number of suitable methods are available. The different species of fish that are reportedly responsible for SFP were identified, including those with a high histidine level which have the potential to cause SFP. Noting that this information should be easily accessible to support risk-based approaches to SFP management, the expert meeting developed the most comprehensive list of fish associated with SFP to date.

The hazard characterization concluded that a dose of 50 mg of histamine, which is the no-observed-adverse-effect level (NOAEL), is the appropriate hazard level. At this level healthy individuals would not be expected to suffer any of the symptoms associated with SFP. In addition, no cumulative effect of consecutive meals containing fish was expected, because histamine usually leaves the body within a few hours.

Using the available fish and fishery products consumption data combined with expert opinion, the meeting agreed that a serving size of 250 g captured the maximum amount eaten in most countries at a single eating event. Based on the hazard level of 50 mg of histamine and the serving size of 250 g, the maximum concentration of histamine in that serving was calculated to be 200 mg/kg. When food business operators apply good hygienic practices (GHP) and hazard analysis critical control point (HACCP), an achievable level of histamine in fish products was reported to be lower than 15 mg/kg, based on data made available by industry (using a test method with a lower detection limit of 15 mg/kg).

Recognizing that the purpose of testing is not to control the problem of SFP, but rather to verify that all the necessary control measures have been implemented effectively, identify failures in the system and remove implicated products from the market, different sampling

approaches and associated plans were presented. In order to provide more explicit guidance on sampling approaches the meeting analysed a range of sampling plans implemented under different scenarios of histamine levels, as defined by the log-transformed mean and standard deviation. Examples of attributes sampling plans appropriate to different levels of tolerance for samples above 200 mg/kg, and for different assumptions about the standard deviation of histamine concentration within lots, were presented. The sampling plans shown were two-class plans and they indicate the number of analytical units required to be tested in order to have 95 percent confidence that the batch as a whole satisfies the desired specified low proportion of samples (such as 1 in 10 000) to exceed 200 mg/kg. The spread of contamination levels in the batch (i.e. the log-transformed standard deviation of contamination levels) has a strong effect on the tolerable average contamination level and, thus, on the number of samples that must be tested to "accept" the batch. Appropriate selection of the criterion against which test units comprising the sample will be assessed for compliance (the *m* value), can considerably improve the time- and cost-effectiveness of sampling: requiring the lowest number of samples to be tested to achieve the same level of confidence about the disposition of the lot being assessed.

The expert meeting concluded that histamine formation and SFP can be easily controlled. The risk from SFP is best mitigated by applying basic GHPs and, where feasible, a HACCP system. Appropriate sampling plans and testing for histamine should be used to validate the HACCP systems, verify the effectiveness of control measures, and detect failures in the system. Sensory evaluation remains a highly useful tool for quality control programmes, but acceptable sensory quality cannot be taken as final assurance of low histamine, nor can low histamine be taken as final assurance that fish is not decomposed. As a result the conclusion of the expert meeting was to focus their advice on histamine limits and related sampling plans to those focused on consumer protection.

Several areas in which further research will be needed have been identified, including the need to clarify the critical role played by histamine and other biogenic amines in the pathogenesis of SFP.

1. Introduction

1.1 Background

Scombrotoxin fish poisoning (SFP) (often called "histamine poisoning") is caused by ingestion of certain species of marine fish that contain high levels of histamine and possibly other biogenic amines. The fish species involved include tuna, which accounts for 8 percent of globally traded fish. Other pelagic species such as mackerel, sardines and anchovy, which account for significant global fish production, can also be involved. These fish species contain high levels of free histidine in their tissues and when conditions are favourable for bacteria to multiply in fish, e.g. when fish are subjected to temperature abuse during and/or after harvest, bacterial decarboxylation of histidine leads to histamine formation. Other biogenic amines produced during bacterial growth in fish may potentiate the effect of histamine. The severity of the symptoms can vary, depending on the amount of histamine and other biogenic amines ingested and the individual's sensitivity to specific biogenic amines. In some parts of the world, SFP accounts for the largest proportion of cases of fish-borne illness.

Fish handling practices are critical with regard to histamine production. For the purposes of consumer protection, fish importing countries have regulations and varying limits for histamine in fish and fishery products. Failure to comply with these regulations and limits leads to import rejection and disruptions in fish trade in major international markets (Ababouch *et al.*, 2005). Thus regulations and limits related to histamine and the fish handling practices that are compatible with these are of great significance for fish producing countries.

Codex Alimentarius, through its standards and guidelines, aims to provide countries with a basis on which to manage issues such as histamine formation. For example, the Codex Code of Practice for Fish and Fishery Products provides guidance on fish handling practices that need to be implemented to minimize food safety problems, including SFP. In addition, the Codex Alimentarius has established several standards that include maximum levels for histamine in different fish and fishery products. Different limits have been established as indicators of decomposition and as indicators of hygiene and handling. However, the associated guidance on the relevant sampling plans and other aspects of sampling is limited or even non-existent. Furthermore, many of these limits (see Annex 2) were established in an era before risk assessment and the scientific basis for the limits is unclear. As food safety management moves towards more risk- and evidence-based approaches, there is a need to review existing limits in the light of the most up-to-date scientific information and to ensure that there is a robust scientific basis for any limits recommended by Codex.

Thus, in April 2011, the 31st Session of the Codex Committee on Fish and Fishery Products (CCFFP) revisited these maximum histamine levels and agreed to look into this issue in more detail. The Committee established an electronic Working Group in order to facilitate this work. The Committee considered that it was important to the decision-making process to have available for their consideration a review of the public health risks and trade implications associated with histamine from fish and fishery products from a more general perspective. This would take into account different maximum levels in various products, existing sampling plans, and risk reductions achieved by various means at the national level. It was also agreed that the Working Group would take into account the work of the Codex

Committee on Food Hygiene (CCFH) on the revision of the Principles for the Establishment and Application of Microbiological Criteria for Foods.

1.2 Objectives

This expert meeting was organized by FAO/WHO to support and facilitate this effort. Its primary objectives were:

- to review the public health impact of histamine and other biogenic amines from fish and fishery products and the trade impacts associated with histamine limits;
- to review the epidemiological and toxicological data and examine whether any risk-based control measures can be recommended for different fishery products;
- to examine the impact of a range of risk-based sampling plans for monitoring histamine levels as a marker for SFP in various fish and fishery products;
- to examine whether fish families mentioned in current Codex standards adequately cover species involved in histamine-associated illness.

1.3 Meeting approach

In order to reach these objectives the meeting decided to take a risk assessment approach and use the available data to estimate a level of histamine at which there is no observed adverse effect, estimate the exposure and characterize the risk. Consideration was also given to risk management options, including a range of sampling approaches. It was also agreed to identify those areas where the scientific knowledge was weak or limited in order to highlight areas where further research is needed.

The aim of this report is to provide the CCFFP and its working group with the scientific basis it needs to make decisions on the management of histamine in fish and fishery products.

The meeting was chaired by Dr Gerard Roessink, and Dr Ronald Benner acted as rapporteur. A group of 14 experts from 12 countries participated in the meeting in their independent capacities and not as representatives of their governments, employers or institutions. They included one expert from the fisheries industry and one expert from a government institution with commercial activities related to the fisheries industry. While these experts participated in the general discussion and exchange of information, they did not participate in the final adoption of the conclusion and recommendations of the meeting.

The deliberations of this meeting were based on three background papers, prepared in advance of the meeting by Dr P. Michael Bolger, Dr Yu (Janet) Zang, Dr Tom Ross and Dr Ronald Allen Benner. The background paper prepared by Dr Bolger and Dr Zang is available in Annex 3 and the relevant information from the papers prepared by Dr Benner and Dr Ross has been incorporated in the report.

2. Hazard Identification

2.1 Biogenic Amines

2.1.1 Histamine

Histamine is a naturally occurring endogenous substance in the human body which is derived from the decarboxylation of the amino acid histidine. Histamine may also be present in certain foods containing free histidine, and is generated by certain bacteria during spoilage and fermentation of fish. Endogenous histamine has important physiological functions related to local immune responses, gastric acid secretion and neuromodulation. Histamine-rich foods may cause food intolerance in sensitive individuals and histamine contamination in fish and fish products may cause food poisoning (Taylor, 1986).

2.1.2 Cadaverine and putrescine

Cadaverine and putrescine are two other biogenic amines found in fish. Like histamine, they are produced from amino acids by bacteria during spoilage and fermentation. The precursors of cadaverine and putrescine are lysine and ornithine, respectively. Cadaverine and putrescine are both found frequently in improperly handled fish, not just those implicated in SFP, and have been studied as spoilage indicators. In some fish spoilage studies, cadaverine appeared to be formed and increased earlier than histamine (Pons-Sanchez-Cascado *et al.*, 2005; Rossi *et al.*, 2002). Although they might act as histamine potentiators (Taylor and Lieber, 1979), the contribution of these biogenic amines to SFP is not clear.

2.1.3 Tyramine

Tyramine is a naturally occurring monoamine compound derived from the amino acid tyrosine. Fresh fish contains little or no tyramine, but a large amount can be found in spoiled or fermented fish (Leuschner and Hammes, 1999; Prester, 2011). Although tyramine might also act as a histamine potentiator (Taylor and Lieber, 1979), the contribution of this biogenic amine to SFP is not clear.

2.1.4 Other biogenic amines

Other biogenic amines detected in fish and fish products include spermine, spermidine, dopamine and agmatine (Park *et al.*, 2010; Visciano *et al.*, 2012). Though some of them might act as histamine potentiators (Taylor and Lieber, 1979), the contribution of these biogenic amines to SFP is not clear.

2.1.5 Micro-organisms involved in biogenic amine production

Biogenic amine production requires available amino acids and amino acid decarboxylases synthesized by bacteria (EFSA, 2011). Histamine is formed in fish by certain micro-organisms capable of producing the enzyme histidine decarboxylase (HDC). The histidine decarboxylases produced by these bacteria catalyse the conversion of free histidine, naturally present at high levels in the muscle of some fish, to histamine. Gram-positive and Gram-negative bacteria can both produce histidine decarboxylase but the forms of the enzymes differ (Bjornsdottir-Butler *et al.*, 2010; EFSA, 2011). In the same way, other biogenic amines (putrescine, cadaverine and tyramine) are synthesized by decarboxylases produced by Gram-positive and Gram-negative bacteria.

In the scientific literature the following species are reported to be those most likely to produce histamine: *Morganella morganii*, *Morganella psychrotolerans*, *Photobacterium damselae*, *Photobacterium phosphoreum*, *Raoultella planticola* and *Hafnia alvei* (Dalgaard *et al.*, 2008; EFSA, 2011). In the case of fermented seafood, *Staphylococcus* spp. and *Tetragenococcus* spp. are reported to be histamine producers (Satomi *et al.*, 2011; Yatsunami and Echigo, 1991). For biogenic amine compounds other than histamine, several families or genera are reported to be involved, such as Enterobacteriaceae, Pseudomonaceae, *Lactobacillus*, *Enterococcus* and *Staphylococcus* (EFSA, 2011). Within different genera or species the ability to generate histamine is very much strain dependent.

In fish, biogenic amine-producing bacteria are most likely to be present on the gills or skin, or in the gastrointestinal tract. Transfer of these bacteria to the flesh of the fish, where free amino acids may be present, leads to development of biogenic amines. Transfer can occur from the gastrointestinal tract after harvest, through migration, or via rupture or spillage of gastric contents during gutting. Micro-organisms may also be transferred from the skin or gills during butchering.

The amount of biogenic amines produced depends on the level of free amino acids present, which is related to the species of fish and the amount and activity of decarboxylase enzymes. The quantity of decarboxylases is related to the number of decarboxylase-producing bacteria transferred to the fish and the extent to which they multiply. Many conditions can affect the growth of biogenic amine producers. Temperature is the main determinant. Biogenic amine concentrations thus depend on the combined influence of time and temperature: longer times and higher temperatures will lead to greater microbial growth and biogenic amine formation. Other important factors can be involved, including pH, salt, oxygen availability and competition with other spoilage micro-organisms.

In summary, the content of biogenic amines in fish products will depend on: (i) the type of fish (i.e. the amount of free amino acids), (ii) the way the fish is handled (i.e. the potential for bacterial growth in the fish products) and (iii) the duration, conditions and temperature of storage of the fish. This combination of factors can lead to highly variable levels of contamination within individual lots of fish, and even within individual fish, and has implications for the efficacy of testing schemes to assess the safety of fish and fish products with respect to histamine contamination.

2.2 Toxicological aspects

2.2.1 Histamine

2.2.1.1 Absorption, distribution, metabolism and excretion

Human subjects can tolerate up to 180 mg of pure histamine orally without having noticeable effects, while intravenous administration of 0.007 mg of histamine produces vasodilatation and an increase in heart rate (Weiss *et al.*, 1932). This difference suggests that histamine is not efficiently absorbed from the gastrointestinal tract. It has been postulated that histamine metabolizing enzymes present in the intestinal tract prevent the absorption of ingested histamine into the circulatory system (Taylor, 1986).

Endogenous histamine is generated in mammals by the enzyme histidine decarboxylase (HDC), which is only synthesized as necessary and is degraded immediately when sufficient histamine has been generated. The HDC exists primarily in mast cells, basophils, enterochromaffin-like cells in gastric mucosa and histaminergic neurons. Generally,

histamine is stored as a histamine–heparin complex in the secretory granules in these cells, and is released upon stimulation to exert its physiological functions. However, recently it has been found that a small amount of histamine is synthesized in some epidermal cells and released immediately (Shahid *et al.*, 2009).

In humans and experimental animals, histamine is primarily metabolized by two enzymes: diamine oxidase (DAO) and histamine-*N*-methyltransferase (HMT) (Maintz and Novak, 2007). DAO converts histamine into imidazole acetic acid, which can be conjugated with ribose before excretion. HMT converts histamine into methylhistamine, which is then converted by monoamine oxidase (MAO) into *N*-imidazole acetic acid. The ultimate end products of histamine metabolism are excreted in the urine.

In humans, DAO is expressed mainly in the intestinal tract, which limits the uptake of exogenous histamine into the circulatory system. HMT, however, is widespread in human tissues, with the order of activity being liver > colon > spleen > lung > small intestine > stomach (Hesterberg *et al.*, 1984). Therefore, DAO is considered to be the major metabolic enzyme for ingested histamine, while histamine injected intravenously or intradermally is primarily metabolized by HMT. HMT is very selective for histamine, while the substrates of DAO include other biogenic amines such as cadaverine and putrescine (Taylor, 1986).

Altered histamine metabolism has been reported in individuals taking isoniazid (Morinaga *et al.*, 1997) and drugs that inhibit DAO or MAO, as well as patients with mastocytosis, tumours or chronic myelocytic leukaemia (Maintz and Novak, 2007). Histamine metabolism may also be influenced by consumption of food-borne DAO inhibitors such as thiamine, cadaverine and tyramine (Taylor, 1986). When ^{14}C-histamine was administered orally to humans, 68–80 percent of the radioactive dose was recovered in the urine. Some histamine does remain in the faeces, and additional amounts are catabolized by intestinal bacteria and exhaled as CO_2 from the lungs (Sjaastad and Sjaastad, 1974).

2.2.1.2 Mechanism of action

Histamine exerts its effects through the activation of four different types of histamine receptor (H_1, H_2, H_3 and H_4) on and/or in the cellular membrane. These histamine receptors are expressed on different cell types and work through different signalling pathways, resulting in multiple biological responses. For example, histamine increases vasopermeability and vasodilatation, causing urticaria, flushing, hypotension and headache. Histamine also induces contraction of intestinal smooth muscle, causing abdominal cramps, diarrhoea and vomiting (Lehane and Olley, 2000).

2.2.1.3 Toxicological responses in animals

The toxicological responses to histamine depend on the method of administration, and the toxicological effects differ among species. Oral administration of histamine, alone or together with spoiled tuna, produced emesis in pigs. An emetic response was also observed in dogs (Blonz and Olcott, 1978). Intraduodenal injection of histamine produced only transient hypotension in dogs and cats, while a histamine-containing yeast extract produced a wider variety of effects in cats, including increased volume and acidity of stomach acid, increased haematocrit and limb volume, and enhanced electromyographic activity (Taylor, 1986). When given intradermally, histamine induced microvascular permeability in the skin of hamsters and rats (Woodward and Ledgard, 1986).

2.2.1.4 Toxicological responses in humans

While endogenous concentrations of histamine are necessary and are required for normal physiological function, histamine is toxic when large doses enter the circulatory system. This often results in symptoms of poisoning, which involve a wide range of organs (Taylor, 1986). The toxicological effects of histamine are related to its normal physiological actions in the body and include the following.

Vascular: Dilatation of the peripheral blood vessels, predominantly arteries, results in hypotension, flushing and headache. Histamine also induces increased capillary permeability, resulting in symptoms such as oedema, urticaria, haemoconcentration and increased blood viscosity. Shock can result from administration of very high doses of histamine. The effect on capillary permeability is mediated by both H_1 and H_2 receptors (Owen and Woodward, 1980).

Heart: Histamine exerts a direct stimulatory action on the heart. Histamine increases heart contractility and increases the rate and strength of the contractions. The effects of histamine on the heart might account for the palpitations noted by some persons experiencing histamine poisoning. Histamine can cause either contraction or relaxation of extravascular smooth muscles. Contraction is mediated by H_1 receptors, while relaxation is associated with H_2 receptors (Shahid *et al.*, 2009).

Gastrointestinal: In humans, the predominant action of histamine on extravascular smooth muscles is contraction. This smooth muscle contraction is most often noted in the bronchi and intestines. In histamine poisoning, the contraction of intestinal smooth muscle is particularly apparent, because histamine enters the gastrointestinal tract initially. Contraction of intestinal smooth muscle leads to the abdominal cramps, diarrhoea and vomiting which are often noted in cases of histamine poisoning (Taylor, 1986).

Neurological: Histamine is also a potent stimulant of both sensory and motor neurons. This stimulation may be important in producing the pain and itching that frequently accompany the urticarial lesions in histamine poisoning. This neural stimulation is mediated by H_1 receptors (Nuutinen and Panula, 2010).

2.2.2 Cadaverine and putrescine

Cadaverine and putrescine are considered to be histamine potentiators, which may explain the lack of toxicity of pure histamine in human oral challenge studies. In guinea pigs, cadaverine and putrescine enhanced histamine-related mortality (Bjeldanes *et al.*, 1978; Vasseur *et al.*, 1968). As evidence of their potentiating effects, cadaverine and putrescine were demonstrated to be functional inhibitors of DAO and HMT in a rat jejunal model (Taylor and Lieber, 1979). Cadaverine is also able to enhance the absorption of histamine in perfused rat intestinal segments (Lyons *et al.*, 1983; Paik and Bjeldanes, 1979). In an *in vivo* study conducted in rats, both cadaverine and putrescine increased the amount of unmetabolized histamine, but decreased the amount of its metabolites in urine (Hui and Taylor, 1985).

The minimum level of cadaverine or putrescine that potentiates histamine toxicity is unknown. The ratio of cadaverine or putrescine to histamine may need to be high to produce an effect, and it is not clear whether the levels present in spoiled fish are sufficient to enhance the toxicity of histamine in humans.

2.2.3 Tyramine

In humans, tyramine acts as a catecholamine (including norepinephrine [noradrenaline], dopamine, epinephrine [adrenaline]) releasing agent, resulting in increased blood pressure. Given that tyramine is metabolized physiologically by MAO, a hypertensive crisis can result when a person who takes MAO inhibitor (MAOI) drugs also consumes foods with high histamine content. This condition, also called the tyramine pressor response, is characterized by an increase in systolic blood pressure of 30 mmHg or more. The displacement of norepinephrine from neuronal storage vesicles by acute tyramine ingestion is thought to cause the vasoconstriction and increased heart rate and blood pressure. In addition to the hypertensive effect, dietary tyramine intake has also been associated with migraine headaches in selected populations, and the mechanism has been linked to tyramine as a neurotransmitter (Jansen *et al.*, 2003).

In animals, tyramine has a low acute oral toxicity of more than 2000 mg/kg body weight (bw). It causes a dose-dependent increase in blood pressure. When using an MAOI, the intake of approximately 10–25 mg of tyramine is required for a severe reaction, compared with 6–10 mg for a mild reaction. For adults, levels of 100–800 mg/kg bw of dietary tyramine have been suggested as acceptable, and levels > 1080 mg/kg bw as toxic (Tenbrink *et al.*, 1990). In individuals using MAOI drugs, ingestion of 60 mg/kg of tyramine can cause migraine headaches, while 100–250 mg/kg bw will produce a hypertensive crisis (Silla Santos, 1996).

There is some evidence that tyramine, like cadaverine and putrescine, potentiates histamine toxicity by inhibitioffing the histamine-metabolizing enzymes DAO and HMT (Bjeldanes *et al.*, 1978; Shalaby, 1996).

2.3 Scombrotoxin fish poisoning (SFP)

SFP is a worldwide food safety problem and is a common cause of fish poisoning that occurs in humans. The food poisoning is caused by heat-stable scombrotoxins, presumably arising from bacterial action in fish. Although detailed components of scombrotoxins have not been identified, it is generally accepted that biogenic amines, especially histamine, play an important role in the pathogenesis of SFP. The incriminated fish usually contain abnormally high levels of histamine due to bacterial activity resulting from inappropriate handling, processing or storage conditions, and histamine has been implicated, at least in part, as an important causative agent. Therefore, SFP is also called histamine fish poisoning (HFP). Although SFP shares some symptoms with histamine intolerance and histamine-induced adverse effects, there are distinctions. Unlike histamine intolerance and histamine induced effects, SFP may involve the presence of other toxic decomposition products or other components unique to fish (Hungerford, 2010). In addition, unlike histamine intolerance, SFP occurs not only in susceptible individuals, but also in those with a normal capacity for histamine degradation.

2.3.1 Symptoms

A variety of symptoms of SFP have been observed among humans (Table 2.1). Poisoned individuals may show one or more of these symptoms, and the severity of the response to the contaminated fish may vary. In several case reports, exacerbation of asthma and more serious cardiac manifestations were reported (Ascione *et al.*, 1997; D'Aloia *et al.*, 2011; Wilson *et al.*, 2012). The symptoms typically develop rapidly (from 5 minutes to 2 hours after ingestion of spoiled fish), with a usual duration of 8–12 hours and with symptoms usually no longer observed after 24 hours. Although symptoms may persist for up to several days,

there are no known long-term sequelae. SFP is considered to be rarely if ever fatal. According to data from the United States Centers for Disease Control and Prevention (CDC) for the period from 1998 to 2002, there were 463 cases reported and no deaths (CDC, 2006). According to the data from the Japanese Ministry of Health, Labour and Welfare for the period from 1998 to 2008, there were 89 incidents, 1577 cases reported and no deaths (Toda *et al.*, 2009).

2.3.2 Diagnosis

The diagnosis of SFP is largely dependent on the symptomology, time of onset, history of food allergy and the consumption of contaminated fish. The diagnosis can be confirmed by detecting high levels of histamine in the implicated food, meal remnants or a similar product obtained from the same source (Ferran and Yebenes, 2006; Predy *et al.*, 2003).

Table 2.1. Common symptoms of scombrotoxin fish poisoning.

Type	Symptoms
Cardiovascular	Flushing, rash (urticaria), hypotension, headache, tachycardia
Gastrointestinal	Abdominal cramps, diarrhoea, vomiting
Neurological	Pain, itching
Other	Oral burning sensation, peppery taste, nausea, swelling of tongue

2.3.3 Treatment

Antihistamine treatment is the optimal mode of therapy for SFP. Symptoms usually subside rapidly after such treatment. Both H_1 antagonists (e.g. diphenhydramine) and H_2 antagonists (e.g. cimetidine) have been used for the treatment of histamine poisoning. Given that the adverse responses are self-limiting and will resolve in a fairly short time, pharmacological intervention may not be necessary in mild cases and these patients require only maintenance support (e.g. fluid replacement) (Taylor, 1986).

2.3.4 Histamine as the causative toxin of SFP

There is compelling evidence that histamine is a significant causative agent of SFP. Examples of the most convincing evidence include high levels of histamine in most incriminated fish, elevated blood or urine histamine in poisoned patients, and the effectiveness of antihistamine drugs in reducing the symptoms. However, oral administration of pure histamine at the same dose as that found in spoiled fish does not elicit the same toxicological effects as those seen in SFP (Taylor, 1986). Some studies suggest that there are histamine potentiators in spoiled fish that contribute to the histamine-related SFP. By competitively inhibiting the histamine detoxification enzymes DAO and HMT, histamine potentiators can decrease the threshold dose of histamine needed to provoke an adverse reaction in humans (Al Bulushi *et al.*, 2009; Bjeldanes *et al.*, 1978; Taylor, 1986; Taylor and Lieber, 1979). Cadaverine and putrescine have been implicated as possible histamine potentiators, on the basis of both *in vivo* and *in vitro* animal studies (Bjeldanes *et al.*, 1978; Lyons *et al.*, 1983; Mongar, 1957).

Another possible mechanism is that potentiators might interfere with the intestinal barrier that prevents the intestinal absorption of histamine. Specifically, intestinal mucin, which is known to bind histamine and prevent its absorption, may be disrupted. This hypothesis is supported by the result of a study involving isolated guinea pig gut sections, which showed that cadaverine was able to increase the histamine transportation rate, yet had a minor effect on histamine metabolism (Paik and Bjeldanes, 1979).

SFP-like symptoms have been reported following consumption of non-scombroid fish, which contain low levels of histidine and histamine (Bartholomew *et al.*, 1987). It has been postulated that unknown toxin(s) in these spoiled fish act as mast cell degranulators to induce histamine release, and that endogenous histamine, rather than ingested histamine, accounts for the adverse reactions (Clifford *et al.*, 1991; Ijomah *et al.*, 1991). In human volunteers who were given marlin with high levels of histamine, researchers failed to detect mast cell secretion when directly measuring mast cell degranulation indicators such as tryptase (Morrow *et al.*, 1991; Sanchez-Guerrero *et al.*, 1997). In a recent case–control study of 10 patients with SFP-like syndrome and 50 non-SFP-like syndrome patients with an established allergic disorder, serum tryptase levels in all 10 patients with SFP-like syndrome were in the normal range, while increased tryptase levels were found in most patients with allergy (Ricci *et al.*, 2010). Therefore, these results do not support the hypothesis that release of endogenous histamine is causative, and the underlying mechanism for SFP-like syndrome caused by eating low-histidine fish is unknown. Given that under-reporting of SFP-like syndrome may be occurring, the public health significance is unclear or unknown.

2.4 Factors influencing sensitivity

2.4.1 Histamine intolerance

Histamine intolerance is a type of food intolerance with allergy-like symptoms. It occurs when histamine-rich foods such as cheese and wine are consumed by susceptible individuals. As a consequence of genetic or acquired dysfunction of DAO or HMT, ingested histamine cannot be degraded efficiently in the gastrointestinal tracts of these individuals. The resulting buildup of histamine in the system causes a series of toxic effects that are similar to a common food allergy, which usually include swelling, rashes, hives and asthma-like symptoms such as difficulty in breathing, wheezing and smooth muscle contractions. Gastrointestinal symptoms, such as bloating and diarrhoea, have also been reported (Maintz and Novak, 2007). The same histamine-rich foods would not cause these reactions in a non-susceptible population. This condition can be used to explain the variations among individuals in their susceptibility to dietary histamine in decomposed fish (Motil and Scrimshaw, 1979). People with histamine intolerance are advised to consume a histamine-free diet (Wantke *et al.*, 1993).

Individual susceptibility to SFP has been observed in multiple epidemiological studies and healthy volunteer challenge tests. It is generally accepted that the ability to tolerate histamine exposure can be compromised when histamine-metabolizing enzymes are impaired. The factors associated with increased sensitivity to histamine have been summarized in a recent report on biogenic amines (EFSA, 2011). Briefly, reduced histamine metabolism can result from genetic polymorphism (Garcia-Martin *et al.*, 2009), certain physiological states/conditions such as menstruation (Jonassen *et al.*, 1976; Kalogeromitros *et al.*, 1995), gastrointestinal diseases (Mainz and Novak, 2007) and the use of certain medications (Hui, 2006; Taylor, 1986). There is suggestive evidence that the incidence and the severity of SFP may depend on age (Ianuzzi *et al.*, 2007). Smoking and drinking alcohol may also increase sensitivity to biogenic amines by reducing the degradation capacity (EFSA, 2011).

2.5   Analytical methods for histamine

A variety of test methods exist for determination of histamine levels in fish (Hungerford, 2010), including the well-accepted Association of Official Analytical Chemists (AOAC) fluorometric method (AOAC 977.13), the spectrofluorometric method (Tine *et al.*, 2008), enzyme-linked immunosorbent assay (ELISA) methods, the colorimetric enzyme test (Sato *et*

al., 2005) and high-performance liquid chromatography (HPLC) methods that can measure multiple biogenic amines (Duflos *et al.*, 1999; Veciana-Nogués *et al.*, 1995). These techniques are discussed and reviewed by Lehane and Olley (2000), Dalgaard *et al.* (2008) and Hungerford (2010). While each method has strengths and limitations, and they vary in terms of related cost, operator expertise, time to obtain a result, portability, etc. (see Table 2.2), most methods provide good agreement and are capable of reliably measuring histamine in seafood at levels of interest. Codex standards propose the use of the fluorometric method (AOAC 977.13) or other scientifically equivalent validated methods. In short, test methods do not appear to limit the detection of histamine in fish.

Table 2.2. Comparison of the most commonly used test methods for determination of histamine levels.

	AOAC method	*HPLC method*	*Spectrofluorometric method*	*ELISA*	*Colorimetric method*
Time needed for one test	1–2 h	1–2 h	1 h	1 h	1 h
Equipment	fluorometer	HPLC	spectrofluorometer	spectrophotometer	spectrophotometer
Limit of quantification	1–5 parts per million (ppm)	1.5–5 ppm	1.5 parts per billion (ppb)	2–5 ppm	20 ppm
Range	1–150 ppm	5–2 500 ppm	1.5 ppb–100 ppm	0–500 ppm	0.8–300 ppm
General advantages	Robust, repeatable, accurate, precise	Quantification of all biogenic amines, accuracy, precision	Accuracy, precision, good recovery, not expensive	Easy (kit), fast, low equipment costs and possibility of multiple tests simultaneously	Easy (kit), fast, low equipment costs, and possibility of multiple tests simultaneously. Simple calibration and possibility of semiquantitative evaluation by visual colorimetry

2.6 Fish species

Table 2.3 lists fish species that have been associated with SFP or elevated levels of free histidine. In addition, mean annual global production of these species in the period 2005–2010 and levels of free histidine were included when data were available. The majority of the fish genera and species listed were sourced from the FDA Fish and Fishery Products Hazards and Controls Guidance (FFPHCG) (FDA, 2011). Others were compiled from the available literature. The fish of concern included representatives of 19 families, 71 genera and more than 111 individual species.

Fish included in Table 2.3 on the basis of elevated free histidine levels had upper-level histidine estimates ranging from 2 600 to 25 070 mg/kg. Fish in the Salmonidae family were included in this table not on the basis of free histidine content, but rather on reported illnesses of SFP-like intoxication. The Salmonidae family had histidine levels ranging from 70 to 2 362 mg/kg. Discussions of SFP-like intoxication are included in Section 2.3. Mean annual production (tonnes of fish) has been included in this table to illustrate which species are of greatest concern for potential formation of histamine. In the European Union (EU) and Codex, fish species of the families Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae and Scomberesocidae are identified as scombrotoxin hazards.

The information provided in Table 2.3 is not ranked in terms of risk for individual fish species because this is challenging from a global perspective. However the meeting recognized that individual countries or regions may need to rank fish species according to their particular situation and needs. Consumption levels and histamine content are important

considerations in such analyses. Further information on such ranking can be found in Guillier *et al.* (2011).

Table 2.3. Scientific names, free histidine levels and mean annual production levels for fish associated with SFP or high free histidine levels.

Market name	Scientific name		Histidine levels (mg/kg)	Mean annual production (tonne, 2006–10)[c]
	Family[a]	Genus and species[b]		
Amberjack or Yellowtail	Carangidae	*Seriola* spp.		158 743
Yellowtail Amberjack		*Seriola lalandi*	7 320[j]	719
Amberjack, Japanese		*Seriola quingueradiata*	2 470–11 600[j,k,l]	152 893
Yellowtail, Longfin		*Seriola rivoliana*		
Greater/Japanese Amberjack or Rudder Fish		*Seriola dumerili*	2 860[j]	2 895
Anchovy	Engraulidae	*Anchoa* spp.		
		Anchoviella spp.		
Anchoveta, Pacific		*Cetengraulis mysticetus*		
		Engraulis spp.		
Peruvian		*Engraulis ringens*		6 630 951
European		*Engraulis encrasicholus*	6 210[m,n]	534 483
South African		*Engraulis capensis*		209 250
Japanese		*Engraulis japonicus*	4 810[o]	1 287 215
		Stolephorus spp.		279 139
Bluefish	Pomatomidae	*Pomatomus saltatrix*		
Bonito	Scombridae			
Leaping		*Cybiosarda elegans*		
Dogtooth Tuna		*Gymnosarda unicolor*		669
Plain		*Orcynopsis unicolor*		759
		Sarda spp.		62 215
Lesser Eel or Small Sandeel	Ammodytidae	*Ammodytes tobianus*		
		Ammodytes spp.		337 923
		Ammodytes personatus		215 806
Escolar or Oilfish or Gemfish	Gempylidae	*Lepidocybium flavobrunneum*	8 000–11 000[q,r]	163
		Ruvettus prestiosus		25 561
Garfish	Belonidae	*Belone belone*[e]	6 084–6 685[s]	
Herring or Sea Herring or Sild	Clupeidae			
		Alosa spp.		
Alewife or River Herring		*Alosa pseudoharengus*		5 286
Herring, Red-eye Round		*Etrumeus teres*		
Tardoore		*Opisthopterus tardoore*		
		Clupea spp.		
Herring, Atlantic		*Clupea harengus*	1 230-2 950[t,u]	2 356 990

Herring, Araucanian		*Clupea bentincki*		624 528
Herring, Pacific		*Clupea pallasii pallasii*		306 839
Herring, Thread		*Opisthonema* spp.		18 717
Herring, Pacific Thread		*Opistonema libertae*		199 899
		Harengula spp.		
Herring, Pacific Flatiron		*Harengula thrissina*		
Herring, Silver-stripe Round		*Spratelloides gracilis*		243
	Pristigasteridae	*Ilisha* spp.		115 921
Indian Pellona		*Pellona ditchela*		16 865
Jack	Carangidae	*Caranx* spp.		
Jack or Blue Runner		*Caranx crysos*		6 473
		Caranx georgianus	1 800–6 300[v]	
		Carangoides bartholomaei		
		Oligoplites saurus		
		Selene spp.		9 163
		Urapsis secunda		
Jack or Crevalle		*Alectis indicus*		
Jack or Rainbow Runner		*Elagatis bipinnulata*	7 090[j]	17 476
Jack or Roosterfish		*Nematistius pectoralis*		
Kahawai/Western Australian Salmon	Arripidae	*Arripis* spp.		5 734
Kahawai		*Arripis trutta*	12 420[v]	
Kohera or Yellowtail	Carangidae	*Decapterus koheru*	2 300–2 700[v]	
Mackerel				
Mackerel, Frigate	Scombridae	*Auxis tapeinocephalus*	14 600[j]	
Butterfly Kingfish		*Gasterochisma melampus*		12
		Grammatorcynus spp.		
		Pneumatophorus diego	5 193–5 999[w]	
Mackerel, Short		*Rastrelliger brachysoma*		311 455
Mackerel, Indian		*Rastrelliger kanagurta*		274 301
		Scomber spp.		
Mackerel, Atlantic		*Scomber scombrus*	2 000–4 500[t,x,y]	664 231
Mackerel, Chub		*Scomber japonicus*	1 063–8 020[k,w,z]	1 767 202
Mackerel, Blue		*Scomber australasicus*	2 600[v]	10 364
Mackerel, Spanish		*Scomberomorus* spp.		
Mackerel, Narrow-Barred Spanish		*Scomberomorus commerson*		228 458
Mackerel, Spanish or King		*Scomberomorus cavalla*		13 508
Mackerel, Japanese Spanish		*Scomberomorus niphonius*	1 990–2 180 Murata *et al.*, 1994	60 950
Mackerel, Jack	Carangidae	*Trachurus* spp.		
Mackerel, Cape Horse		*Trachurus capensis*		251 277
Mackerel, Jack		*Trachurus novaezelandiae*	2 720[v]	
Mackerel, Horse		*Trachurus japonicus*	172–	278 404

			3 680[k,z]	
Mackerel, Atlantic Horse		*Trachurus trachurus*		209 971
Mackerel, Chilean Jack		*Trachurus murphyi*		1 493 793
Mackerel, Atka	Hexagrammidae	*Pleurogrammus monopterygius*		62 282
Mackerel, Okhotsk Atka		*Pleurogrammus azonus*	2 500 Fujii 1954	185 719
Mahi-Mahi (Dolphin Fish)	Coryphaenidae	*Coryphaena hippurus*	1 829–9 370[j,k,aa,ab,ac]	52 642
Marlin	Istiophoridae	*Makaira* spp.		39 285
Marlin, Black		*Makaira mazara*	7 630[j]	
Marlin, Striped		*Makaira mitsukurii*	8 310–13 200[j,k]	
		Tetrapturus spp.		
Menhaden	Clupeidae	*Brevoortia* spp.		
Gulf		*Brevoortia patronus*		435 914
Atlantic		*Brevoortia tyrannus*	1 860–2 790[w]	199 658
Pacific		*Ethmidium maculatum*		26 839
Milkfish	Chanidae	*Chanos chanos*[f]	4 410–5 340, 25 070[ad,ae]	
Mullet, Flathead Grey	Mugilidae	*Mugil cephalus*	2 060–7 600[v,]	
Pilchard or Sardine	Clupeidae			
Sardine, European		*Sardina pilchardus*	2 888[af,ag]	1 101 842
Spotted Sardinella		*Amblygaster sirm*[h] *Sardinella* spp.		
Sardine, Round		*Sardinella aurita*		357 275
Sardine, Indian Oil		*Sardinella longiceps*		408 305
Sardinella, Goldstripe		*Sardinella gibbosa*		178 156
Sardinella, Madeiran		*Sardinella maderensis*		137 814
		Sardinops spp.		
Pilchard, Japanese or South American or Californian		*Sardinops sagax*	1 227–7 626[k,w,z]	837 504
Piper	Hemiramphidae	*Hyporhamphus ihi*	3 200[v]	
Queenfish, Talang	Carangidae	*Scomberoides* spp.[i]		
		Scomberoides commersonnianus[h]		
Sailfish	Istiophoridae	*Istiophorus platypterus*	7 630[ah]	
Salmon	Salmonidae	*Salmo* or *Oncorhynchus* spp.		
Atlantic Salmon		*Salmo salar*	130–300[ai,aj,ak]	
Chincook Salmon		*Oncorhynchus tshawytscha*	70–288[ak,al]	
Chum Salmon		*Oncorhynchus keta*	70–670[ak,am]	
Coho Salmon		*Oncorhynchus kisutch*	219–970[ak,al]	
Amago Salmon		*Oncorhynchus macrostomus*	188–441[ak]	

Cherry Salmon		*Oncorhynchus masou*	387–2362[ak]	
Sockeye Salmon		*Oncorhynchus nerka*	240–590[ak,al]	
Pink Salmon		*Oncorhynchus gorbusvha*	408–1 557[ak,al]	
Saury	Scomberesocidae			
Pacific		*Cololabis saira*	16 100[k]	
Atlantic		*Scomberesox saurus saurus*		
Shad	Clupeidae	*Alosa* spp.		
Bonga		*Ethmalosa fimbriata*		212 076
Shad, Gizzard		*Dorosoma* spp.		
Shad, Western Australian Gizzard		*Nematalosa vlaminghi*		
Shad, Hilsa		*Tenualosa ilisha*		343 058
Spearfish	Istiophoridae	*Tetrapturus* spp.		
Sprat or Bristling	Clupeidae	*Sprattus* spp.		
Blueback		*Sprattus antipodum*	3 900[v]	
Swordfish	Xiphiidae	*Xiphias gladius*[g]		
Trevally	Carangidae	*Caranx* spp.		
		Caranx georgianus	1 800–6 300[v]	
Tuna (Small)	Scombridae			
Slender		*Allothunnus fallai*		
		Auxis spp.		
Bonito		*Auxis thazard*	4 330–10 100[k]	
		Euthynnus spp.		
Little Tuna or Kawakawa		*Euthynnus affinis*	10 900[j]	
Skipjack		*Katsuwonus pelamis*	13 400–20 000[j,w,z,an]	2 529 408
Longtail Tuna		*Thunnus tonggol*	11 540[an]	239 661
Tuna (Large)	Scombridae	*Thunnus* spp.		
Albacore		*Thunnus alalunga*	4 600–6 790[ao]	
Yellowfin		*Thunnus albacares*	2 123–12 200[j,w,ab,ap]	1 113 954
Blackfin		*Thunnus atlanticus*		
Southern Bluefin		*Thunnus maccoyi*	6 670[j]	
Big-eye Tuna		*Thunnus obesus*	7 450[j]	412 616
Pacific Bluefin Tuna		*Thunnus orientalis*	6 850–7 110[ao]	
Atlantic Bluefin		*Thunnus thynnus*		
Wahoo	Scombridae	*Acanthocybium solandri*		
Yellowtail or Amberjack or Kingfish[b]	Carangidae	*Seriola lalandi*	5 500–15 800[v]	

[a] Family names were verified at http://www.fishbase.org/search.php (FishBase, 2012).

[b] All fish genera and species listed were sourced from the FDA Fish and Fishery Products Hazards and Controls Guidance (FFPHCG), 4th Edition (FDA,2011) and other sources as indicated in superscripts below.

[c] (FAO Fisheries and Aquaculture Statistics Service, 2012)

[d] (Fletcher *et al.*, 1998)

[e] (Thaysen and Sloth, 1997; Dalgaard *et al.*, 2006; and Dalgaard *et al.*, 2008)

[f] (Tsai *et al.*, 2007 and Hsu *et al.*, 2009)

[g] (Boutin *et al.*, 1998; Tsai *et al.*, 2007; Chang *et al.*, 2008; and Dalgaard *et al.*, 2008)

[h] (Guillier *et al.*, 2011)

[i] (Sasikala *et al.*, 2005)

[j] (Suyama and Yoshizawa, 1973)

[k] (Hibiki and Simidu, 1959)

[l] (Sakaguchi *et al.*, 1982)

[m] (Özden, 2005)

[n] (Pons-Sánchez-Cascado *et al.*, 2006)

[o] (Arakaki and Suyama, 1966)

[q] (Emborg *et al.*, 2006)

[r] (Kan *et al.*, 2000)

[s] (Dalgaard *et al.*, 2006)

[t] (Mackie *et al.*, 1997)

[u] (Smith, 1980)

[v] (Fletcher *et al.*, 1995)

[w] (Lukton and Olcott, 1958)

[x] (Klausen and Lund, 1986)

[y] (Mackie and Fernández-Salguero, 1977)

[z] (Abe, 1983)

[aa] (Antoine *et al.*, 1999)

[ab] (Antoine *et al.*, 2001)

[ac] (Baranowski *et al.*, 1990)

[ad] (Chiou *et al.*, 1990)

[ae] (Thippeswamy *et al.*, 2002)

[af] (Ababouch *et al.*, 1991)

[ag] (Ababouch *et al.*, 1996)

[ah] (Tsai *et al.*, 2005)

[ai] (Emborg *et al.*, 2002)

[aj] (Espe *et al.*, 1993)

[ak] (Murata *et al.*, 1998)

[al] (Shirai *et al.*, 1983)

[am] (Konso *et al.*, 1983)

[an] (Hiratsuka, 2001)

[ao] (Murata *et al.*, 1994)

[ap] (Emborg *et al.*, 2005)

3. Exposure assessment

3.1 Introduction

Histidine decarboxylating bacteria can be part of the natural microflora in the skin, gills and gut of a freshly caught fish. Given that free histidine is present in the tissues of the fish involved in SFP, the bacterial action could start soon after harvest, and if temperature conditions are suitable these bacteria multiply rapidly and form histamine even before postmortem proteolysis occurs. This could explain the observation that histamine can reach elevated levels before the formation of organoleptic spoilage indicators. Once bacterial multiplication has occurred and histidine decarboxylases are produced, enzyme activity can continue slowly at refrigeration temperatures, even after bacterial growth has ceased (Lehane and Olley, 2000).

Histamine formation in fish is dependent on the time/temperature conditions under which the fish is handled, and therefore time/temperature control needs to be taken into consideration from harvest through consumption. There are many fish harvesting methods used throughout the world, employing hooks, nets and traps. These may involve small vessels, large vessels or be shore based. In all cases, live retrieval of the fish, cooling as quickly as possible to temperatures which do not promote bacterial growth, and maintaining at cool temperatures are critical both to discourage histamine formation and to preserve quality. This translates into a need to supply, wherever possible, small boats with ice in boxes to provide insulation and to protect fish from the elements (Shawyer and Pizzali, 2003) and for larger vessels to be equipped with operational and well maintained refrigeration or freezing equipment. Further along the distribution chain, transport trucks need to be equipped to keep the fish cold and protected from the elements (Johnston *et al.*, 1994). Similarly, fish vendors need to maintain this cold chain by, for example, keeping the fish on ice.

High histamine levels are a result of gross time/temperature abuse during handling and storage. For example, as presented in Table 3.1, skipjack tuna stored at 25 and 31 °C did not accumulate histamine to levels greater than 10 mg/kg during up to 8 hours of storage. In yellowfin tuna stored under the same conditions, histamine levels remained below 10 mg/kg for up to 6 hours of storage but histamine began to accumulate when fish were stored for longer time periods. In fact, after 10.5 hours at 31 °C, histamine levels reached 131 mg/kg (Staruszkiewicz *et al.*, 2004). These data illustrate that the presence of histamine in fish is related to a lack of time/temperature control. Furthermore, histamine formation can be influenced by evisceration. For example, in uneviscerated yellowfin tuna stored at 30 °C for 12 hours, the maximum histamine level reached 2 400 mg/kg but in eviscerated tuna, stored under the same conditions, the levels did not exceed 16 mg/kg (Benner *et al.*, 2009).

3.2 Detection frequency of histamine and levels of contamination

Data describing histamine concentrations and frequencies in seafoods and seafood products were collated to assist in the identification of products representing the greatest risk to public health. These data, together with estimates of the frequency and amount of consumption of specific seafoods and types of fish, are needed to assess human exposure to histamine from seafoods. Data were obtained from existing published literature, or were provided in response to requests for data prior to the meeting.

Table 3.1. Formation of biogenic amines in seawater incubated tuna (Staruszkiewicz *et al.*, 2004).

Incubation (h)	Biogenic amines (mg/kg)[a]			
	Fish no.	Histamine	Cadaverine	Putrescine
Skipjack, 25 °C				
0	58	1.5	0.6	1.7
0	59	1.2	0.5	1.2
6.5	60	0.8	1.1	0.8
8	61	3.3	1.4	1.4
8	62	3.9	3.0	2.3
10	63	8.9	8.5	2.0
Skipjack, 31 °C				
3	64	2.9	0.5	2.0
4.5	65	1.9	2.0	1.4
6	66	2.9	6.5	2.4
7	67	5.3	14	1.6
8	68	5.8	15	2.5
10	69	332	17	4.3
Yellowfin, 25 °C				
0	70	0.7	0	0.7
0	71	0.7	0	0.8
10	72	1.2	1.5	0.9
10	73	1.7	2.7	2.3
12	74	3.7	4.1	2.0
12	75	7.1	7.2	1.9
Yellowfin, 31 °C				
3	76	3.3	0.4	0.8
4	77	0.2	0.3	0.9
6	78	2.6	8.3	2.0
6	79	9.6	8.5	1.5
9.5	80	97	19	4.2
10.5	81	131	19	6.7

[a]Chemical data were acquired from the anterior part of the fish

The data available were from diverse sources and were originally generated for a variety of different reasons. These range from market surveys for routine regulatory surveillance or academic purposes through to "incoming" product testing from a multinational seafood processor/importer/distributor. Similarly, the surveys from which the data were derived varied in study design from generic studies for a wide range of seafood products to those focused on specific products or products believed to be more likely to present a risk of histamine intoxication in consumers. As such, the data do not offer a complete representation of the risk of exposure to histamine but, given the diversity of surveys and number of observations, provide some indication of typical frequencies of different levels.

As far as possible, the survey results were divided into categories that relate to existing regulatory/advisory limits in various jurisdictions and regions, namely:
- < 50 mg/kg
- ≥ 50 mg/kg to < 100 mg/kg
- ≥ 100 mg/kg to < 200 mg/kg
- ≥ 200 mg/kg to < 500 mg/kg
- ≥ 500 mg/kg

The summary data, indicating sources of original data, are shown in Table 3.2. From the data it is apparent that levels above 200 mg/kg in fish and seafood products are not uncommon, often ranging up to 10 percent prevalence in surveys. There is some suggestion from the data that processed products are more frequently contaminated at higher levels, although fresh and frozen fish also sometimes have histamine levels above 200 mg/kg.

The histamine levels for canned tuna in Canada were provided by a major importer and marketer. These data reflect production under extensive good hygienic practices (GHP), good manufacturing practices (GMP) and HACCP from fish sourcing through to processing, and importing. The data suggest that histamine levels can be minimized by appropriate food safety and quality management systems.

From the data presented, a presumptive conclusion is that without well designed GMP and HACCP systems up to 10 percent of product units may develop histamine in excess of 200 mg/kg, whereas well designed GMP and HACCP virtually eliminate product units with more than 200 mg/kg histamine.

As discussed elsewhere, fermented fish sauces are consumed in small quantities (a few grams per serving) and recent Codex decisions (CODEX STAN 302 – 2011; Standard for Fish Sauce) have established that up to 400 mg/kg histamine in such products still provides an acceptable level of consumer protection. Application of the tools provided in this document may be used to reassess safe levels of histamine in fish sauce.

3.3 Consumption

The consumption information available in different databases has been collected using different methodologies, which makes synthesis of the data problematic. Nevertheless an approach to the determination of serving size was made using the data available for a number of food consumption databases. These included:

i. EFSA Comprehensive European Food Consumption Database (EFSA, 2011a);
ii. Database of Food Consumption of Thai People (ACFS, 2006);
iii. Japanese National Household Expenditure Survey (The Management and Coordination Agency of Japan, 2012);
iv. Consumption frequency data published by the JA General Research Institute, results of consumer buying behaviours for meat and seafood products (JA, 2010);
v. Food Safety Commission Investigations (FSCJ, 2006) for food-borne microbiological risk assessments;
vi. UK National Diet and Nutrition Survey (Henderson *et al.*, 2002);
vii. United States Environmental Protection Agency Estimated Per Capita Fish Consumption (EPA, 2002);
viii. New Zealand National Nutrition Survey (Russell *et al.*, 1999).

Serving size/portion size per consumer is defined in different ways in the literature. The United Kingdom Seafish Authority (Seafish, 2012) defines one portion of seafood as 140 g. On the basis of data from the EFSA Concise Food Consumption Database, however, the 95th percentile of consumption among fish consumers ranges between 250 g/day and 422 g/day, with an across-countries median of 300 g/day.

Available data on the 97.5th percentile range in the same European countries varies between 300 g/day and 500 g/day, with an across-countries median of 322 g/day.

Table 3.2. Summary of results of surveys that indicate distributions of histamine levels in seafoods.

description of survey	targetted at scombroid fish?	year	number of samples	histamine levels ≥ 50 mg/kg < 100mg/kg	≥ 100mg/kg < 200mg/kg	≥ 200mg/kg < 500mg/kg	≥ 500mg/kg	Total % ≥ 200 ppm	maximum level (mg/kg)	Reference
Australian Capital Territory, fish at market, various fish, i.e not only scombroid spp	no	1996/1997	64	4.7%	3.1%	0.0%	1.6%	1.6%	653	ACT Health (1997)
Cyprus, imported and market fish	yes	2003-2005	130	0.0%	1.5%	0.0%	0.0%	0.0%	not relevant	Yiannopoulos et al. (2006)
Greece - scombroid fish	yes		55	7.3%	5.5%	0.0%	0.0%	0.0%	220	Vosikis et al (2008)
Victoria (Australia) - scombroid fish at:	yes							0.0%		DHS Vic. (2000)
supermarket		1995	55	0.0%	14.5%	0.0%	0.0%	0.0%	-	"
canned fish		1996	37	0.0%	51.4%	0.0%	0.0%	0.0%	-	"
comment on Import testing of canned fisjh by AQIS		1996	unknown	0.0%	0.0%	16.0%	0.0%	16.0%	-	"
Japan - market fish	yes	2004								
sardines			299	4.5%	4.5%	4.5%	4.5%	9.0%	3400	Kan et al. (2005)
mackerel (5 types)			187	0.5%	2.7%	1.6%	0.5%	2.1%	820	"
tuna			63	0.0%	0.0%	0.0%	0.0%	0.0%	not relevant	"
others			88	0.0%	1.1%	1.1%	0.0%	1.1%	300	"
Morocco - commercially processed samples	yes	1986	248	0.0%	5.2%	5.2%	4.0%	9.3%	6940	Ababouch et al. (1986)
New South Wales (Australia)	yes	2009								NSW FA (2009)
anchovies in oil at reail		2009	45	34.5%	34.5%	2.2%	0.0%	2.2%		"
dried anchovies at retail		2009	34	20.6%	20.6%	2.9%	0%	2.9%		"
New Zealand - various products, inc fish sauces	yes	2011	77	0%	0.0%	12%	1%	13.0%	637	NZFSA (2011)
New Zealand - retail survey fresh whole fish		1995	47	0%	11%	0%	0%	0.0%		Fletcher et al. (1995)
South Australia - whole fresh fish and fillets (42 spp.)	no	2010	51	0%	0%	0%	0%	0.0%	not relevant	SA Health (2010)
Various countries in Europe, Asia, the Pacific, China, Japan, etc..	no	2002 - 2007	159	3%	4%	2%	2%	3.8%	1964	Tao et al. (2011)
South Africa (Cape Town)										
- fresh seafood	no	2004	58	6.9%	0.0%	1.7%	0.0%	1.7%	399	Auerswald et al. (2006).
- processed seafoods		2004	22	9.1%	0.0%	0.0%	4.5%	4.5%	8001	"
Japan - fillet	yes	2010	7	0.0%	0.0%	0.0%	0.0%	0.0%	not relevant	Japan MHLW (2010, unpublished)
- canned		2010	6	0.0%	0.0%	0.0%	0.0%	0.0%	not relevant	"
- fish sauce		2010	5	0.0%	0.0%	0.0%	60.0%	60.0%	2500	"
- salted and dried or seasoned		2010	11	9.0%	9.0%	9.0%	0.0%	9.0%	270	"
- simmered whole in soy sauce and sugar		2010	2	0%	0%	0%	0%	0.0%		"
Japan - summary only of survey of scombroid fish	yes	2010	538	0.7%	1.1%	1.5%	1.5%	3.0%	2515	Japan MAFF (2010, unpublished)
New Zealand - smoked fish	yes	1998	107	4%	2%	1%	1%	1.9%	681.8	Fletcher et al. (1998)
Portugal (fish with high histidine levels)										
- canned seafood samples		2009	135	0.0%	1.5%	0.7%	1.5%	2.2%	not given	Mendes (pers. comm., 2012)
- frozen seafood samples		2009	126	1.6%	0.8%	1.6%	0.0%	1.6%	not given	"
- canned seafood samples		2010	154	5.2%	3.2%	2.6%	0.0%	2.6%	not given	"
- frozen seafood samples		2010	135	3.0%	3.7%	0.7%	0.0%	0.7%	not given	"
- canned seafood samples		2011	356	4.8%	9.8%	5.6%	2.0%	7.6%	not given	"
- frozen seafood samples		2011	100	2.0%	0.0%	0.0%	0.0%	0.0%	not given	"

Table 3.2 (*cont*.). Summary of results of surveys that indicate distributions of histamine levels in seafoods.

description of survey	targetted at scombroid fish?	year	number of samples	histamine levels ≥ 50 mg/kg < 100mg/kg	≥ 100mg/kg < 200mg/kg	≥ 200mg/kg < 500mg/kg	≥ 500mg/kg	Total % ≥ 200 ppm	maximum level (mg/kg)	Reference
Maldives - Rihaakuru (cooked fish paste)	yes	2010	28	3.6%	14.3%	14.3%	21.4%	35.7%	5487.14	Naila *et al.* (2011)
Thailand[§] - (fermented) fish sauce			308	41.6%	32.8%	8.8%	0.0%	0.0%		
Japan - various locations and types of fish		2006 - 2010	822	0.5%	1.6%	0.4%	0.1%	0.5%	999	Japan - call for data
Norway - herring and mackerel, fresh fillets, salted, hot and cold smoked (regulatory data)	yes	2009 - 2011	46	2.2%	2.2%	0.0%	2.2%	2.2%	2114	Norway - call for data
herring, mackerel, salmon and others (regulatory)		1994	209	8.1%	4.8%	0.0%	0.0%	0.0%		"
herring, mackerel, salmon and others (regulatory)		1995	235	8.9%	11.5%	0.0%	0.0%	0.0%		"
heerring, mackerel, salmon and others (regulatory)		1996	50	0.0%	0.0%	0.0%	0.0%	0.0%		"
The Netherlands - fresh/frozen tuna	yes	2006	53	3.8%	0.0%	1.9%	1.9%	3.8%	not recorded	Roesslink (*pers. comm*., 2012)
(regulatory data)		2007	1343	0.0%	0.4%	0.3%	0.1%	0.4%	not recorded	"
		2008	1214	0.0%	0.6%	0.2%	0.8%	1.1%	not recorded	"
		2009	405	0.0%	1.5%	1.5%	1.5%	3.0%	not recorded	"
		2010	224	0.9%	0.0%	0.0%	0.4%	0.4%	not recorded	"
		2011	89	0.0%	0.0%	0.0%	0.0%	0.0%	not recorded	"
Private Company*										
canned tuna imports to Canada	yes	2009	64	10.9%	0.0%	0.0%	0.0%	0.0%	84	Anon., (2012) - call for data
		2009/2010	213	0.0%	0.0%	0.0%	0.0%	0.0%	not relevent	"
		2011	704	0	0.4%	0.0%	0.0%	0.0%	148	"
		2012	416	0.0%	0.0%	0.0%	0.0%	0.0%	not relevent	"
canned fish (various) imports to USA		-	116	0.0%	0.0%	0.0%	0.0%	0.0%	not relevent	"
canned sardines		-	20	0.0%	0.0%	0.0%	0.0%	0.0%	7.7	"
anchovies		2010 - 2012	161	5.0%	0.0%	0.0%	0.0%	0.0%	82.6	"

| unweighted averages | | | | 4% | 5% | 2% | 2% | 4% | | |
| total number of samples | | | 10141 | | | | | | **2.10%** weighted average of samples exceeding the >200mg/kg criterion | |

[§] These data were included for information but were not used in the calculation of "typical" histamine contamination levels because of the known higher levels of histamine typical of fermented Asian fish sauces.

Data for consumption of individual fish species per consumer are very scarce. As presented in Table 3.3, which shows consumption of major fish species in the UK for one day, the median of the 97.5th percentile of fish consumption across species is 258 g/day. At the same percentile the mean consumption for different fish species ranges among fish consumers between 185 g/day and 369 g/day. Among species recognized as frequent histamine affected species, herring, fresh tuna, mackerel, sardines and canned tuna are, in decreasing order, the species with the highest portion size per eater per day.

Table 3.3. Statistical consumption data for fish products in the UK included in the group "Fish and Shellfish" of the UK National Diet and Nutrition Survey (Henderson *et al.*, 2002).

Item	Mean g/day	Median g/day	97.5 Percentile g/day	Maximum g/day
Cod	82	79	207	338
Tuna – canned	75	63	185	265
Tuna – fresh	97	100	258	305
Haddock	82	70	225	340
Salmon	107	96	340	610
Sardine/pilchard	85	80	237	360
Trout	152	154	369	460
Mackerel	101	97	239	486
Herring	125	120	332	370
Plaice	129	106	346	400
Sole	140	145	315	327
minimum	**75**	**63**	**185**	**265**
maximum	**152**	**154**	**369**	**610**
median	**101**	**97**	**258**	**360**

In Thailand, consumption per eater of major fish products linked with histamine production has a mean range from 7.8 to 102.6 g/person/day (Table 3.4). If the 97.5th percentile is considered, consumption will be between 21 g/day for fermented fish and 210 g/day for mackerel. Ranking the most important fish products per portion size gives Spanish mackerel, tuna, sardines, short-bodied mackerel and fermented products in decreasing order of importance.

Data from Japan for consumption of fish species in 2009 is presented in Table 3.5. The range of consumption on a meal basis is calculated as shown in the footnotes to Table 3.5, using the frequency of consumption of a fish meal throughout the year. The estimated mean consumption per meal per person is 8.0–19.6 g for sardine and 32.9–0.3 g for salmon, depending on the frequency data.

For the European Union, Tables 3.6 and 3.7 summarize information from the EFSA Comprehensive European Food Consumption Database. The database reports dietary surveys and food consumption data for each country by food category and covers both regular/"chronic" and high/"acute" consumption. Adult consumers and fish meat consumption were chosen as the criteria for construction of the tables. Looking at the regular consumption per day (Table 3.6) reveals a considerable difference among European countries; Sweden has the lowest 97.5th percentile, 86.4 g/day, and the Czech Republic has the highest, 225.0 g/day. The mean value across EU countries is 162.4 g/day. If, on the other hand, the high/"acute" consumption is considered (Table 3.7), the consumption ranges from 190.5 g/day in Denmark to 600 g/day in Hungary, with a mean value across Europe of 270 g/day.

Data from the United States of America on uncooked marine fish consumption estimate consumption for the year 2002 (Table 3.8). For the purpose of this report, "consumers only" were defined as individuals who ate fish at least once during the 2-day period. In the US data, the 95th percentile of consumption among fish "consumers only" ranges from 250.8 g/day to 283.1 g/day with a mean value of 269.7 g/day.

Data from New Zealand (Table 3.9) indicate that fish portion size ranges between 8 g in the case of anchovy and 200 g in the case of canned mackerel.

Table 3.4. Consumption of fish products in Thailand in g/person/day (ACFS, 2006).

Item name	Per capita (g/person/day)		Eater only (g/person/day)	
	mean g/person/day	percentile 97.5%	mean g/person/day	percentile 97.5%
Tuna, canned in water, liquid excluded	0.57	3.90	63.44	117.0
Sardines, canned in tomato sauce	5.31	39.00	63.30	117.0
Short-bodied Mackerel (Hardtail scad)	9.45	43.00	37.05	86.00
Mackerel, Spanish (Grouper, Giant Sea Perch, steamed, Black-banded Trevally)	5.48	52.50	102.61	210.0
Threadfin/Kurau (Thai), dried Mackerel (Spanish, dried)	0.56	6.40	23.07	32.00
Fermented fish/Pla-ra (Thai), different markets	1.15	12.00	10.33	24.00
Fish sauce	7.67	21.00	9.07	21.00
Fermented fish, liquid part (fermented for 3–12 months)	1.67	14.00	7.80	21.00

Table 3.5. Consumption of fish products in Japan in g/person/meal.

Fish species	Consumption: frequency* by JA (2010) g/person/meal	Consumption: frequency** by FSCJ (2006) g/person/meal
Tuna	25.8	63.1
Horse Mackerel	16.4	40.1
Sardine	8.0	19.6
Bonito	10.7	26.2
Flounder	13.5	33.0
Salmon	32.9	80.3
Mackerel	14.3	35.0
Pacific Saury	25.7	62.8
Sea Bream	25.0	20.4
Yellowtail	62.0	50.5

* Frequency estimated by JA (2010) as: ** Frequency estimated by FSCJ (2006) as:

2.10%	Every day	% Consumers	Frequency of consumption
15.80%	> 3.5/wk	4.40%	> 3 times/week
42.20%	1–3/wk	26.50%	1–2/week
29.80%	< 1/wk	49.10%	1–3/month
3.10%	Seldom/never	15.70%	Few times/year

Table 3.6. Adult "consumers only" chronic[1] consumption of fish meat in g/day (EFSA, 2012[2]).

Country	Survey	Mean	SD	Median	P95	P97.5
Belgium	Diet_National_2004	55.9	47.8	47.5	142.5	183.2
Czech Republic	SISP04	79.1	56.4	75.0	179.0	225.0
Denmark	Danish_Dietary_Survey	18.1	17.0	13.4	51.0	62.0
Finland	FINDIET_2007	62.7	50.4	51.0	139.6	195.3
France	INCA2	28.3	22.9	22.9	74.6	85.9
Germany	National_Nutrition_Survey_II	65.9	49.9	51.0	150.0	190.2
Hungary	National_Repr_Surv	69.2	42.5	50.0	150.0	200.0
Ireland	NSIFCS	31.9	24.6	24.9	78.1	97.1
Italy	INRAN_SCAI_2005_06	50.2	35.3	50.0	118.4	132.3
Latvia	EFSA_TEST	60.7	47.6	50.0	150.0	200.0
Netherlands	DNFCS_2003	41.1	33.6	30.4	110.0	110.7
Spain	AESAN	70.1	53.2	56.0	168.8	192.8
Spain	AESAN_FIAB	70.6	50.9	59.2	166.9	206.7
Sweden	Riksmaten_1997_98	27.6	20.2	21.4	66.1	86.4
United Kingdom	NDNS	33.6	26.6	26.4	81.3	92.9

Table 3.7. Adult "consumers only" acute[3] consumption of fish meat in g/day (EFSA, 2012[4]).

Country	Survey	Mean	SD	Median	P95	P97.5
Austria	ASNS	139.8	88.3	138.0	293.0	376.0
Belgium	Diet_National_2004	100.4	76.9	90.0	275.0	289.0
Bulgaria	NSFIN	198.0	114.5	152.8	413.6	501.0
Czech Republic	SISP04	145.9	104.0	146.3	300.0	406.3
Denmark	Danish_Dietary_Survey	46.3	52.1	25.7	144.9	190.5
Estonia	NDS_1997	138.0	109.7	108.9	329.4	500.0
Finland	FINDIET_2007	102.4	79.7	81.6	245.3	288.9
France	INCA2	95.2	73.4	80.0	220.0	285.0
Germany	National_Nutrition_Survey_II	119.4	90.4	99.0	285.0	302.0
Hungary	National_Repr_Surv	189.6	116.9	150.0	400.0	600.0
Ireland	NSIFCS	130.6	91.6	100.0	319.1	349.8
Italy	INRAN_SCAI_2005_06	112.9	85.7	112.5	240.0	310.0
Latvia	EFSA_TEST	110.6	83.4	100.0	250.0	300.0
Netherlands	DNFCS_2003	78.7	62.1	60.8	215.0	221.1
Poland	IZZ_FAO_2000	159.5	130.0	132.4	404.0	441.2
Slovakia	SK_MON_2008	126.0	182.9	100.0	250.0	300.0
Slovenia	CRP_2008	112.6	75.4	100.0	240.0	420.0
Spain	AESAN	104.7	82.6	84.0	254.0	320.0
Spain	AESAN_FIAB	124.6	85.0	106.3	282.0	336.5
Sweden	Riksmaten_1997_98	108.2	57.9	120.0	210.0	240.0
United Kingdom	NDNS	121.0	77.5	107.1	261.0	307.0

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[1] For chronic consumption, intake statistics have been calculated based on individual average consumption over the total survey period (e.g. 7 days) (EFSA, 2011)
[2] EFSA, 2012. The EFSA Comprehensive European Food Consumption Database. Available from http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm
[3] For acute consumption, statistics have been calculated based on every single reporting day (EFSA, 2011)
[4] EFSA, 2012. The EFSA Comprehensive European Food Consumption Database. Available from http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Table 3.8. Consumption estimates for uncooked marine fish (g/person/day) of consumers only (aged 18 years and older) in the USA (EPA, 2002).

	Estimate	*Lower bound (90% interval)*	*Upper bound (90% interval)*
Mean	107.9	103.0	112.8
95th percentile	269.7	250.8	283.1

Table 3.9. Consumption of fish products in New Zealand in g/meal (Russell *et al.*, 1999).

Fish type	*g/meal*
Tuna, canned, liquid excluded	51–185
Tuna, fresh	72
Sardines, canned	5–120
Mackerel, canned	200
Anchovy	8

The data from various nations and regions indicate that typical serving sizes for fish are in the range of approximately 40 to 100 g and the 97.5th percentiles are typically in the range 250–350 g.

The amount of fish or fishery product consumed during any one eating occasion is rather variable, with clear differences among countries and regions. The meeting considered that because SFP occurs as a result of an acute exposure, it was important to agree on a serving size that would capture this regional variation and still be reflective of the high-volume eating events. As noted earlier, the different approaches to data collection on consumption make it difficult to combine and summarize the available data mathematically. Therefore, the meeting took a more qualitative approach and, using a combination of the information available and expert opinion, agreed that a serving size of 250 g be used in the risk characterization because it captured the maximum amount eaten in most countries on a single eating occasion.

4. Hazard characterization

4.1 Histamine as the exposure marker in SFP

Although other biogenic amines such as cadaverine and putrescine might also play a role in the aetiology of SFP, there are no dose–response data in animals or humans for these biogenic amines. In most epidemiological studies, SFP is associated with abnormally high histamine levels in the incriminated fish. Therefore, histamine is considered the most appropriate marker of dose in this assessment.

4.2 Type of study used in the dose–response assessment

While there have been a number of reports in the scientific literature of human scombrotoxin poisoning, the vast majority of these are case reports. These case reports generally involve only a small number of individuals (e.g. three or four subjects). In a few of the studies multiple subjects are involved, with several case reports involving slightly more than 100 individuals. The difficulty with case reports is that the recapitulation of the dosage/exposure level is almost impossible to determine. Crude measures have been used to estimate what the dose/exposure level was by using levels detected in samples of the suspect fish, and/or detailed recalled by the patients. These estimates of exposure/dosage are highly uncertain and cannot be used to construct a quantitative assessment of dose versus adverse response.

With regard to the few retrospective studies, as is always the case with this type of study, there are important limitations, including reliance on voluntary reporting, limited follow-up, and lack of specific determination of histamine or any other biogenic amine in fish samples consumed by the subjects, or in their biologic fluids. However, the typical histamine-like clinical manifestations together with temporal proximity to consumption of fish known to be involved in scombrotoxin poisoning supports the diagnosis of biogenic amine poisoning.

The other major hurdle in the quantitative use of these studies is the uncertainty associated with a lack of understanding of whether histamine is the sole responsible aetiological toxin(s), whether it is a surrogate of another toxin(s), or whether histamine is working in concert with other biogenic amines or as yet unidentified chemicals in the fish, and what the nature of that relationship is (e.g. additive or synergistic). Histamine levels within fish appear to correlate well with clinical toxicity, but an equivalent oral dose of pure histamine produces few symptoms. Even with several hypotheses attempting to explain the paradox, as previously discussed, the mechanism of toxicity in SFP remains unclear.

To study the health effectsof histamine in humans, a number of volunteer challenge studies have been conducted. Many studies were aimed at investigating the minimal dose of histamine that causes SFP or histamine intolerance symptoms, or the maximal dose of histamine ingested without causing these symptoms. Most of these studies were well-designed randomized trials, in which the doses were well controlled and the symptoms were carefully monitored by medical professionals. Therefore, data from these human trials should reflect the histamine–SFP dose–response relationship better than that from case reports.

4.3 Study selection for dose–response assessment

Human histamine challenge studies are summarized in the EFSA biogenic amine report (EFSA, 2011) and the "Seafood Biogenic Amine Database" (Emborg and Dalgaard, 2007). In these studies histamine was administered with different food matrices and given to healthy

or susceptible adult volunteers, usually in a controlled, blinded study design. The EFSA report covers all human studies regardless of the route of exposure and the food matrices taken with histamine, while the "Seafood Biogenic Amine Database" only includes those oral toxicity studies in which fish was used as the food matrix (Table 4.1).

The critical endpoint in acute histamine intoxication is a spectrum of symptoms including headache, flushing, itching and urticaria. Using data from all 66 healthy and 74 sensitive subjects in trials that included fish, wine and cheese, EFSA (2011) reported that healthy volunteers exhibited no symptoms after consumption of 25–50 mg of histamine; levels from 75 to 300 mg have been reported retrospectively to elicit headache and flushing. The EFSA report identifies a no observed adverse effect Level (NOAEL) of 50 mg histamine for the symptoms of headache and flushing.

Among the five fish-related studies considered by EFSA, three (Clifford *et al.*, 1989; Clifford *et al.*, 1991; Ijomah *et al.*, 1991) failed to establish that histamine was the causative agent of the SFP symptoms, and therefore these were excluded from this dose–response analysis. The two remaining studies (Motil and Scrimshaw, 1979; Van Gelderen *et al.*, 1992) where histamine was administered in the food were used to characterize the dose–response relationship between histamine dose and SFP symptoms.

Table 4.1. Human oral dose–response relationship for histamine in fish.

Histamine dose (mg)	Food ingested	Number of subjects	Number of subjects showing symptoms	Reference
25	Tuna	8	0	Motil and Scrimshaw, 1979
45	Herring	8	0	Van Gelderen *et al.*, 1992
50	Tuna	8	0	Motil and Scrimshaw, 1979
90	Herring	8	2	Van Gelderen *et al.*, 1992
100	Tuna	8	2	Motil and Scrimshaw, 1979
150	Tuna	8	2	Motil and Scrimshaw, 1979
180	Tuna	8	6	Motil and Scrimshaw, 1979

4.4 NOAEL derivation from human challenge studies

On the basis of the data presented in Table 4.1, an oral NOAEL of 50 mg may be identified. The threshold toxic dose for the histamine challenge studies appears to be 90 mg (Table 4.1). However, the precise threshold toxic dose for histamine in SFP is not known with certainty.

It is important to bear in mind that, while the NOAEL is an appropriate hazard threshold value to use for exposures in healthy subjects, this may not be the case for those members of certain segments of the population who may have an increased sensitivity (e.g. metabolic differences, physiological conditions, drug therapies). In these instances a lower hazard level may need to be considered (e.g. the use of an uncertainty factor) or other specific risk management options such as fish consumption advisories should be considered.

4.5 Benchmark dose assessment (BMD)

As an alternative to the NOAEL methodology, the BMD methodology is also commonly used to derive a threshold value in risk assessment. Unlike the NOAEL approach, the BMD approach uses the whole range of available dose–response data by fitting mathematical models to the dataset to derive an estimate of the threshold dose corresponding to a

predetermined level of extra risk, which is normally a 10 percent extra risk. The resulting BMD estimate, BMD_{10}, is the central estimate of the dose that corresponds to the additional risk. The lower 95 percent confidence limit of the BMD ($BMDL_{10}$) is calculated to address and account for uncertainties in the estimate of BMD due to the experimental design (e.g. small sample size).

The United States Environmental Protection Agency's benchmark dose–response modelling software was used to determine benchmark doses. A 10 percent extra risk was selected as the benchmark response value for development of a BMD_{10} (the benchmark dose referring to the 10 percent extra risk) and a $BMDL_{10}$ (the lower limit on the benchmark dose for a 10 percent extra risk). Data from Table 4.1 were analysed using multiple dichotomous models (five). Figure 4.1 shows the Weibull model fitted to the dose–response data.

The calculated BMD_{10} value is 80 mg, and the $BMDL_{10}$ (95 percent lower confidence limit of BMD) is 50 mg. Two measurements of goodness of fit (GOF), the *p*-value and the Akaike information criterion (AIC) value, were calculated and used to compare the models. The Weibull model has the most conservative BMDL, and the best GOF (p = 0.64) and AIC (AIC = 44), among different models tested. In addition the Weibull model was judged to be the most biologically plausible model and was selected as the best model for BMD modelling.

Given that the study of Motil and Scrimshaw (1979) had more dose groups (five doses) than that of Van Gelderen *et al.* (1992) (two doses), a separate BMD modelling procedure was performed using data from Motil and Scrimshaw (1979) only. The BMD_{10} and $BMDL_{10}$ resulting from this assessment were very close to those produced in the assessment of the combined dataset from the two studies. For the Weibull model, the $BMDL_{10}$ was 47.7 mg, compared with 49.7 mg from the assessment of the combined dataset

Both the NOAEL and BMD assessments identified 50 mg of histamine per meal as the dose where either adverse effects were not noted or the estimate of additional risk (lower confidence level) is low. This dosage level will not apply to individuals with a specific sensitivity to histamine and would not apply to children, particularly because they consume more food per unit body weight than adults. It is also important to bear in mind that the 50 mg dosage was derived from data on a small number of subjects, and while the variation of response appears to be reflected in the study results further studies would be most helpful in refining this threshold value.

Further background information on the dose–response evaluation is included in Annex 3.
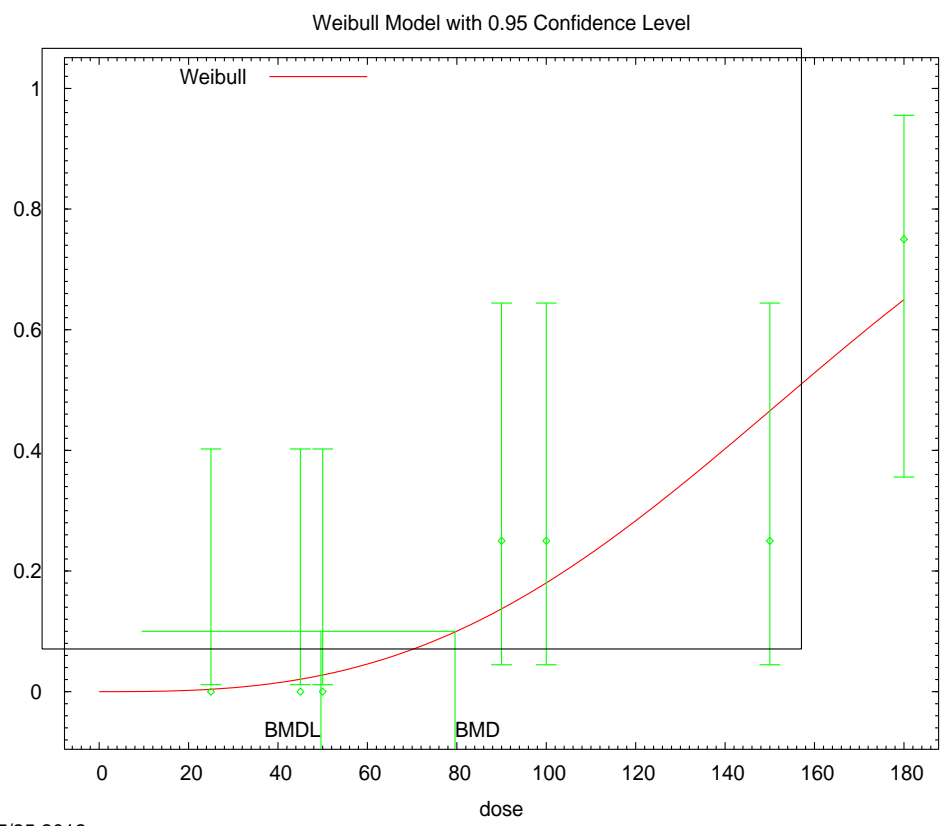
Figure 4.1. Weibull model fitted to the data obtained in fish-related histamine human challenge studies.

5. Risk characterization

5.1 Derivation of a histamine limit based on the NOAEL

The NOAEL (50 mg) for histamine as presented in the previous chapter was used for risk characterization. Based on the consumption data presented in Chapter 3 (Exposure assessment) the meeting agreed to use a serving size of 250 g, and noted that this can be considered as the upper value for a serving size. Based on the hazard level of 50 mg of histamine and a consumption (m) of 250 g, the maximum concentration or level of histamine (L) in that serving that would not cause an adverse effect was calculated consequently as follows:

$$L = \frac{\text{NOAEL}}{m} = \frac{50 \text{ mg}}{250 \text{ g}} = 0.2 \text{ mg/g} = 200 \text{ mg/kg}$$

5.2 Characterization of histamine distribution from censored data

In a given population of fish, the probability of a histamine level equalling or exceeding 200 mg/kg can be estimated by statistical methods, provided that the distribution of histamine is known. To determine what type of distribution the histamine level follows, we first fitted different univariate distributions to some censored data, such as those obtained from the surveys presented in Chapter 3. This was done with the R package fitdistrplus by the maximum likelihood method (Pouillot and Delignette-Muller, 2010).

Based on the Akaike information criterion (AIC), a measure of goodness of fit, it was found that the logarithms of histamine concentrations in different surveys follow a normal distribution, as follows:

$$\log_{10}(C) \sim N(\mu, \sigma)$$

Where C is the histamine concentration, and μ and σ are parameters of the distribution, respectively the mean and standard deviation of the lognormal distribution. An example of the distribution fitted to the survey data is shown in Figure 5.1.

Cumulative distribution

$\log_{10}(C)$ (C in ppm)
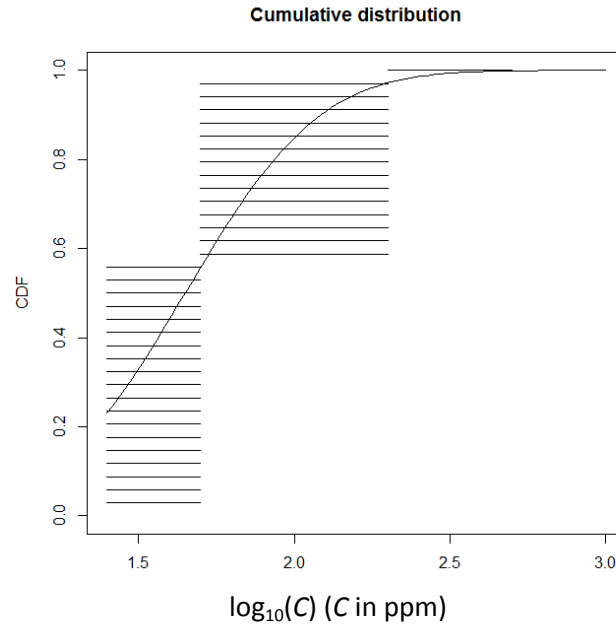
Figure 5.1. Fitting of normal cumulative distribution function (CDF) to data from a survey (dried anchovies at retail, New South Wales, see Table 5.1) using the fitdistrplus R package.

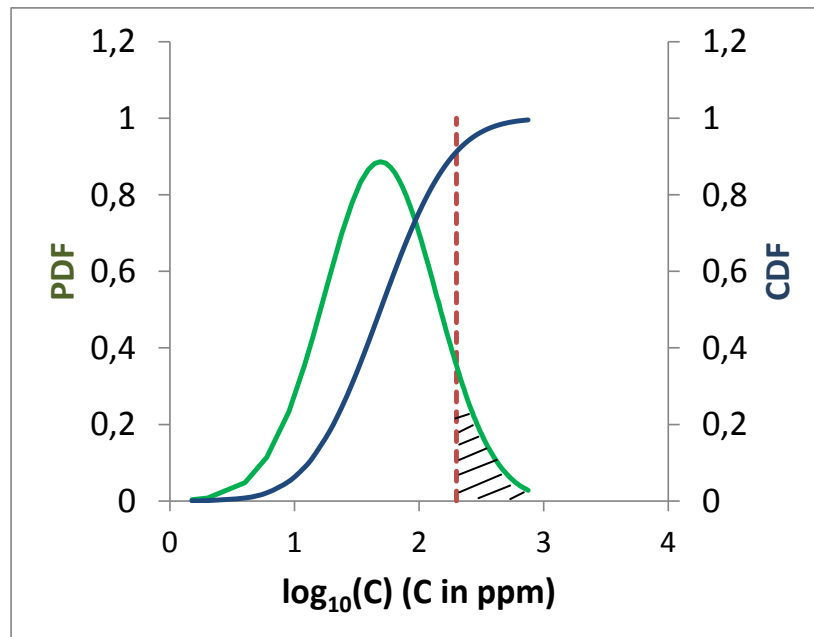## 5.3 Calculating the probability of histamine level exceeding 200 mg/kg

Given the fact that the histamine concentration in seafood (C) follows a lognormal distribution, i.e. $\log_{10}$ (C) follows a normal distribution, the probability of a histamine level higher than the 200 mg/kg limit can be derived using the statistical methods described below.

In a normal distribution, when both the mean and the standard deviation are known, it is straightforward to calculate the probability of being at certain concentration level. Using the same histamine dataset (shown in Figure 5.1) as an example, after a simple logarithmic transformation, Log_{10} (C) can be plotted as a bell-shaped curve of a normal distribution, with μ =1.65 and and σ = 0.34 (Figure 5.2). Thus, after the logarithmic transformation, calculating the probability of C > 200 mg/kg is the same as calculating the probability of $\log_{10}$ (C) > $\log_{10}$ 200, i.e. $\log_{10}$ (C) > 2.3.

In this example, the probability of a sample whose $\log_{10}$ (C) exceeds 2.3 mg/kg can be illustrated as the shaded area in Figure 5.2, and calculated as follows:

$$P(C > L) = 1 - NormalCDF(2.3, \mu, \sigma) = 1 - \text{Normal}(2.3, 1.65, 0.34) = 0.028$$

NormalCDF is the cumulative density function of the Normal distribution (under Excel: NORM.DIST[$\log_{10}$(200),1.65,0.34,cumulative]). It gives the probability that a number falls at or below a given value (here, $\log_{10}(L)$) of a normal distribution.

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Figure 5.2. Probability density function (PDF) and cumulative density function (CDF), describing the $\log_{10}$ of histamine concentration. The dotted red line represents $\log_{10}(L)$, i.e. $\log_{10}(200)$.

Table 5.1 presents the fitted parameters and the probability of reaching or exceeding 200 mg/kg (under the assumption of a 250 g serving size).

The probability of exceeing 200 mg/kg varied from less than 1×10^{-6} to 0.68, according to the survey (Table 5.1). We observed that in surveys in which high contents of histamine were detected the standard deviations of the normal distribution describing $\log_{10}(C)$ were often high (above 1.3), yet the associated means were often low (below 0) (e.g. for mackerel in market fish in Japan or canned tuna imports to Canada). For surveys in which no high concentration of histamine was detected, the standard deviations are comparatively low (about 0.5 or less) but the associated means are comparatively high (greater than 1.0). This is probably due to the limited sample size of these surveys.

These probabilities of exceeding the limit are only representative of the foods analysed. As it is almost impossible from the surveys to know whether sampling was representative of the country consumption profile, the results cannot be compared among countries. In the same way, fish categories cannot be compared directly unless the surveys were conducted in the same country according to a similar sampling plan.

Table 5.1. Parameters of the Normal distribution fitted to the logarithm of the concentration of histamine, and probability of exceeding the limit of 200 mg/kg for each survey referenced in Table 3.2.

Description of survey[a]	$log_{10}(C) \sim N(\mu, \sigma)$		Probability ($C > 200$ ppm)
	μ	σ	
Australian Capital Territory – fish at market, various fish, i.e not only scombroid spp.	1.39	0.44	0.019
Cyprus – imported and market fish	1.85	0.07	< 0.000001
Greece – scombroid fish	1.04	0.64	0.024
Victoria (Australia) – scombroid fish at:			
supermarket	1.93	0.07	< 0.000001
canned fish	2.00	0.05	< 0.000001
Japan – market fish			
sardines	1.35	0.38	0.0061
mackerel (five types)	−0.36	1.29	0.020
tuna	-[b]	-	-
others	−1.03	1.37	0.0075
Morocco – commercially processed samples	0.98	0.97	0.086
New South Wales (Australia)			
anchovies in oil at retail	1.83	0.23	0.020
dried anchovies at retail	1.65	0.34	0.028
New Zealand – various products, inc. fish sauces	1.91	0.35	0.13
New Zealand – retail survey fresh whole fish	1.92	0.06	< 0.000001
South Australia	-	-	-
Various countries in **Europe, Asia, the Pacific, China, Japa**n, etc.	0.31	1.13	0.039
South Africa (Cape Town)			
fresh seafood	0.78	0.67	0.011
processed seafoods	−0.33	1.82	0.074
Japan			
fish sauce	2.57	0.57	0.68
salted and dried or seasoned	1.61	0.14	< 0.000001
Japan – summary only of survey of scombroid fish	1.68	0.45	0.083
New Zealand – smoked fish	0.37	0.92	0.018
Portugal (fish with high histidine levels)			
canned seafood samples 2009	−3.29	2.79	0.023
frozen seafood samples 2009	−0.16	1.06	0.010
canned seafood samples 2010	0.80	0.74	0.021
frozen seafood samples2010	0.66	0.72	0.011
canned seafood samples 2011	1.04	0.88	0.075
frozen seafood samples 2011	1.53	0.08	< 0.000001
Maldives – Rihaakuru (cooked fish paste)	1.86	0.95	0.32
Japan – various locations and types of fish	−0.57	1.16	0.0066

Norway – herring and mackerel, fresh fillets, salted, hot and cold smoked (regulatory data)				
	2009–2011	−0.85	1.69	0.031
	1994	1.19	0.45	0.0067
	1995	1.31	0.48	0.019
The Netherlands – fresh/frozen tuna (regulatory data)				
	2006	−0.44	1.49	0.033
	2007	−2.42	1.86	0.0056
	2008	−1.29	2.42	0.068
	2009	−3.69	2.64	0.011
	2010	−0.55	1.50	0.029
	2011	-	-	-
Private company				-
canned tuna imports to Canada		−2.51	1.32	0.00013
canned fish (various) imports to USA		-	-	-
canned sardines		-	-	-
anchovies		1.62	0.05	< 0.000001
Thailand – fish sauce[c]		1.96	0.24	0.077

[a] See Table 3.2 for more details
[b] Data do not allow estimation
[c] These values were not used in the calculation of summary results

6. Risk management options

6.1 Management of histamine production in fish and fishery products

SFP will only occur in healthy individuals when a dose of at least 50 mg histamine is consumed, and this generally inplies that the fish would have histamine levels exceeding 200 mg/kg. Freshly harvested scombrotoxin-forming fish typically have histamine levels below 2 mg/kg (Frank *et al.*, 1981; Staruszkiewicz *et al.*, 2004). In addition, food business operators that apply GHP and HACCP can achieve a histamine level lower than 15 mg/kg in fish products, based on data made available by industry (using a test method with a lower detection limit of 15 mg/kg). As such, the following conditions must be met for fish to have levels above 200 mg/kg.

- The fish are of a species that have sufficient free histidine to be converted to histamine (only species listed in Table 2.3 or others that may have similar histidine levels can possibly cause outbreaks).
- The presence of histamine-producing bacteria.
- Conditions that support the growth of histamine-producing bacteria and their production of histidine decarboxylase (HDC) enzymes.
- Conditions that allow HDC to convert histidine to histamine (these are normally the same conditions as for bacterial growth, but there are some conditions under which bacterial growth will not occur but preformed HDC may produce histamine and HDC may be suppressed directly).

The risk of SFP is best mitigated by applying GHP and HACCP to interrupt one or more of these conditions, or perhaps to remove histamine that has been formed. Appropriate sampling plans and testing for histamine should be used to validate the HACCP system, verify its effectiveness and detect HACCP failures. Sampling can also be used by regulators and purchasers to identify suppliers that are not applying controls correctly. Controls for histamine and other biogenic amines in susceptible fish and fishery products have been identified (EFSA, 2011; Emborg and Dalgaard, 2007; NZMAF, 2011), and existing and emerging control strategies were specifically reviewed by Naila *et al.* (2010). Risk mitigation strategies discussed in the above citations include:

- post-harvest chilling;
- gutting and gilling of susceptible fish;
- freezing and refrigerated storage;
- heating to destroy histamine-producing bacteria and HDC;
- pH and salt;
- modified atmosphere and vacuum packaging;
- high hydrostatic pressure;
- irradiation;
- food additives;
- using decarboxylase-free starter cultures for fermented fish and fishery products;
- biogenic amine degrading bacteria and enzymes;
- microbiological modelling to select safe storage times under particular conditions;
- sensory assessment for decomposition.

6.1.1 Chilling

Chilling of fish as soon as possible after death is the most important factor in controlling biogenic amine accumulation in scombrotoxin-forming fish (FDA, 2011). Few histamine-producing bacteria will grow at refrigerator temperatures and the growth rates of those that do is much reduced with refrigeration temperatures approaching 0 °C. The following recommendations for chilling of fish after harvest have been provided as procedures that are both readily achievable by industry and will control histamine production (FDA, 2011).

- Fish exposed to air or water temperatures above 28.3 °C should be placed in ice, or in refrigerated seawater, ice slurry or brine at 4.4 °C or lower, as soon as possible after harvest, but not more than 6 hours from the time of death.

- Fish exposed to air or water temperatures of 28.3 °C or lower should be placed in ice, or in refrigerated seawater, ice slurry or brine at 4.4 °C or lower, as soon as possible after harvest, but not more than 9 hours from the time of death.

- Fish that are gilled and gutted before chilling should be placed in ice, or in refrigerated seawater, ice slurry or brine at 4.4 °C or lower, as soon as possible after harvest, but not more than 12 hours from the time of death.

- Fish that are harvested under conditions that expose dead fish to waters of 18.3 °C or lower for 24 hours or less should be placed in ice, or in refrigerated seawater, ice slurry or brine at 4.4 °C or lower, as soon as possible after harvest, but not more than the time limits listed above, with the time period starting when the fish leave the environment of 18.3 °C or lower.

- Further chilling approaching the freezing point is also recommended (FDA, 2011).

6.1.2 Gutting and gilling of susceptible fish

Histamine-producing bacteria appear to be universally present in the gut, gills and skin of fish at the point of capture. Therefore, selecting fish without histamine-producing bacteria does not appear to be an option. However, rapid removal of guts and gills will delay the production of hazardous levels of histamine by such bacteria. For large fish, removing the gut also allows ice or ice slurry to be applied closer to the core of the fish, resulting in more rapid overall chilling. However, during gutting and gilling, care should be taken to minimize the spread of bacteria into the muscle tissue.

6.1.3 Refrigerated storage and freezing

Once they have been chilled, susceptible fish must be kept cold. Refrigerated storage at 4 °C will prevent the growth of mesophilic histamine-producing bacteria and will slow the growth of the less well-known psychrotrophic histamine-producing bacteria. Freezing and frozen storage (–18 °C or below) will stop the growth of all bacteria and, for all practical purposes, will also prevent any preformed HDC from producing histamine. For products whose preparation does not include a heating step or other means to eliminate bacteria, the presence of psychrotrophic organisms may mean that refrigerated storage will not be sufficient to prevent the production of histamine in products with long shelf-lives. If products have a potential refrigerated shelf-life of weeks rather than days, mathematical modelling may be needed to set a refrigerated shelf-life sufficiently short to prevent histamine formation (Emborg and Dalgaard, 2008). Using fresh, high quality materials, chilling and freezing are usually the best methods used to control growth of the bacteria that

produce histamine and other biogenic amines. However, for some types of fishery product, such as fermented and smoked fish, these methods might not be practical. Furthermore, some biogenic amine-producing bacteria can still grow at low temperature and produce enzymes that convert free amino acid precursors to biogenic amines (Emborg and Dalgaard, 2006; Lehane and Olley, 2000; Naila *et al.*, 2010). Other control measures to prevent the formation of biogenic amines have been investigated recently for use in various industries (Naila *et al.*, 2010).

6.1.4 Heating to destroy histamine-producing bacteria and HDC

Most products can be kept under refrigeration until the point of consumption but for some products, such as hot smoked and canned products, processing requires that the frozen products be thawed and chilled, and then warmed. It is important that such products are not held in the temperature zone where histamine-producing bacteria can grow and produce histamine for long enough to become a hazard. Microbiological modelling can be used to determine the effect of various times and temperatures on bacterial growth and histamine production (Emborg and Dalgaard, 2008).

The heating process can be used to eliminate histamine-producing bacteria and their HDC enzymes from the product. However, histamine is heat stable. If histamine is produced in fish, cooking will kill bacteria but will not eliminate the histamine. Therefore, it is important to eliminate or slow the growth of biogenic amine-producing bacteria before they start to produce and release biogenic amine-producing enzymes. Given that histamine-producing bacteria are more heat sensitive than the spore-forming bacteria that are targeted in the canning process, commercially sterile canned products will not contain any histamine-producing bacteria. Furthermore, all HDC enzymes will be denatured by the canning process, meaning that no further histamine can be produced in the product, which can then be stored at ambient temperatures. For products such as hot smoked fish, sufficient heat can also be applied during the process to eliminate the histamine-producing bacteria and inactivate their enzymes. *Morganella morganii* is probably the most heat resistant of the histamine-producing bacteria, and in Australian salmon/kahawai at temperatures between 58 and 62 °C, the *D*-values for eliminating these bacteria and their associated HDC enzymes were between 15 and 1.5 seconds (Osborne and Bremer, 2000). The smoking process might also be designed to eliminate even more resistant vegetative bacteria of concern such as *Listeria monocytogenes* (Fletcher *et al.*, 1998b). However, although heating can destroy the bacteria of concern in food, if recontamination and temperature abuse occur after thermal processing, histamine formation may still occur in the thermally processed product. Thus, for products such as hot smoked fish, care must be taken to avoid recontamination after smoking, and refrigerated storage is still required unless the a_W has been reduced sufficiently to prevent bacterial growth at ambient temperatures.

6.1.5 High hydrostatic pressure and irradiation

High hydrostatic pressure and irradiation are non-thermal treatments that could also be used to eliminate histamine-producing bacteria from susceptible products in a similar manner to heat. The effect on any HDC has been less well studied, and in some cases irradiation was shown to increase histamine production, perhaps as a result of modifying the structure of HDC (Naila *et al.*, 2010).

6.1.6 pH, salt, modified atmosphere and vacuum packaging

Using organic acids to reduce pH, using salt or other means to reduce a_W, and storage under high CO_2 atmospheres can limit or prevent the growth of histamine-producing bacteria for either refrigerated or shelf-stable products such as salted dried fish. The effect of pH, a_W,

temperature and storage atmosphere has been modelled for the fail-safe time to predict when toxic concentrations of histamine might be produced for lightly preserved seafood (Emborg and Dalgaard, 2008).

6.1.6 Food additives

Food additives such as potassium sorbate, sodium nitrites, glucono-delta-lactone and glycine have also been shown to inhibit the growth of histamine-producing bacteria and reduce the production of histamine, and they may be included in formulated products for this purpose (Naila *et al.*, 2010). Various food spices can be applied to similar effect. However, the effectiveness of additives has been little studied and their effects on sensory characteristics, their consumer acceptance in such products and potential negative effects need to be considered. For example, although curcumin can inhibit the growth of histamine-producing bacteria, it also inhibits diamine oxidase, an enzyme that breaks down histamine (Bhutani *et al.*, 2009).

6.1.7 Using suitable starter cultures and/or their enzymes in the preparation of specialist fermented fish and fishery products

Fermented fishery products depend on promoting the growth of certain bacteria to form desirable product characteristics. Their preparation typically requires storage at temperatures that promote rather than inhibit bacterial growth. Bacteria may themselves contain HDC so it is important that decarboxylase-free starter cultures are used for such products. In contrast, some bacteria produce enzymes such as diamine oxidase (DAO) that degrade biogenic amines. These might be included as part of the microflora of starter cultures to provide further protection. In some cases, histamine-degrading bacteria or the enzymes that they produce may also be applied for the removal of preformed amines.

6.1.8 Microbiological modelling

Histamine production in some fish and fishery products stored under different conditions can be estimated using predictive modelling. These models can be helpful to fish processors or buyers when used in conjunction with accurate temperature monitoring. For example, if the temperature history of an incoming fish product is known, an appropriate model can be used to predict the current histamine content and the time remaining until histamine levels in the fish may lead to it being decomposed or toxic. The Seafood Spoilage and Safety Predictor (Dalgaard, 2009) contains models that predict histamine formation for *Morganella morganii* and *M. psychrotolerans*.

6.1.9 Sensory assessment for decomposition

Fish processors have for many decades used sensory assessment of decomposition as a measure of quality, primarily using the odour of the gills and gut cavity (Lassen, 1965). This has proven useful as a screening method for histamine by quickly identifying lots of fish that have been mishandled and, hence, are at risk of elevated histamine content (USTF, 2002). Conversely, histamine is one of the measures of decomposition (Barnett *et al.*, 2006, 2011). However, the correlation between histamine content and odours of decomposition is often inconsistent (Fücker *et al.*, 1974; Kimata, 1965; Veciana-Nogués *et al.*, 1997). Histamine formation without significant odours of decomposition (Özogul *et al.*, 2002), or odours of decomposition without rejectable histamine formation (Du *et al.*, 2002), are both possible. Therefore, sensory evaluation remains a highly useful tool for quality control programmes, but acceptable sensory quality cannot be taken as final assurance of low histamine, nor can low histamine be taken as final assurance that fish is not decomposed.

6.2 Designing a sampling plan to meet an appropriate level of protection (ALOP) for histamine as part of risk management

6.2.1 Understanding attributes sampling plans

"Attributes" sampling plans are defined by several characteristics, namely:

m = the criterion against which test units[5] comprising the sample will be assessed for compliance

n = the number of test units to be tested and evaluated against the criterion (or "attribute"), and

c = the number of test units that are allowed to exceed the criterion m.

The term "*attribute*" arises from the fact that the testing only evaluates whether each test unit meets an absolute criterion, not the actual level of the hazard in the product. That is, a test unit either complies with the criterion (or "attribute") or it does not[6]. The proportion of units that comply is the basis of acceptance or rejection of the lot.

Some sampling plans also specify an additional criterion, M, a level that if exceeded in any test unit will lead to immediate rejection of the entire lot or batch. Where only m is specified, the sampling plan is described as a "two-class" plan, i.e. individual samples either "pass" or "fail" and the proportion passing or failing defines the acceptability of the entire lot. Where M is also specified, the sampling plan is described as "three class": test units with values less than m pass the test; test units with values greater than M fail the test and cause the entire sample to fail; and test units with values between m and M are of "marginal" acceptability. The acceptability of the lot will also be decided on the number of samples with values between m and M.

Attributes sampling plans are designed to determine the proportion of test units in a batch or lot that comply with the criteria. The performance of the sampling plan can be gauged from the number of test units analysed. At the simplest level, the more samples that are tested and shown to be of acceptable quality and/or safety, the greater our confidence that the frequency of unacceptable contamination in the entire batch is low and that the batch as a whole is acceptable.

The number of test units that must be analysed to have confidence about the overall quality and/or safety of the lot can be calculated mathematically and represented by the "*operating characteristic*" curve (see Figure 6.1), often abbreviated as the "OC curve". The mathematics required for the calculations used to generate the OC curve are based on the binomial distribution (or, more correctly, the hypergeometric distribution). The principles of these sampling schemes and the determination of their performance are described in greater detail by Ross *et al.* (2011) and their application in food safety exemplified by van Schothorst *et al.* (2009).

To exemplify the performance of attributes sampling schemes, the EU sampling scheme for assessing histamine levels in a batch of product is characterized by:

n = 9

m = 100 mg/kg

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[5] A sample for testing will typically comprise multiple test units. Thus, for the purpose of this document, a sample is a series of test units all of which are tested and comprise the sample that is used to adjudge the overall acceptability of the batch, or lot, from which it is drawn.

[6] In many cases involving histamine testing, an actual concentration is obtained and this information can be used to assess the overall quality of the lot of product more reliably, rather that regarding the unit as either a "pass" or "fail". Three-class sampling plans begin to use information about distributions of values to assess lot acceptability.

M = 200 mg/kg; c = 2

The FDA (2010) sampling plan is a three-class plan of n = 18, c = 1, m = 50 mg/kg, and M = 500 mg/kg.

The OC curves for the EU sampling scheme and the US FDA scheme are compared in Figure 6.1. Note that, for comparison, the EU three-class scheme is considered as two two-class sampling schemes, one in which n = 9, c = 2, m =100 mg/kg, and another in which n = 9, c = 0, m =200 mg/kg. Similarly, the US FDA sampling scheme is comprised of two two-class sampling plans, in which n = 18, c = 1, m = 50 mg/kg and n = 18, c = 0, m = 500 mg/kg are applied.
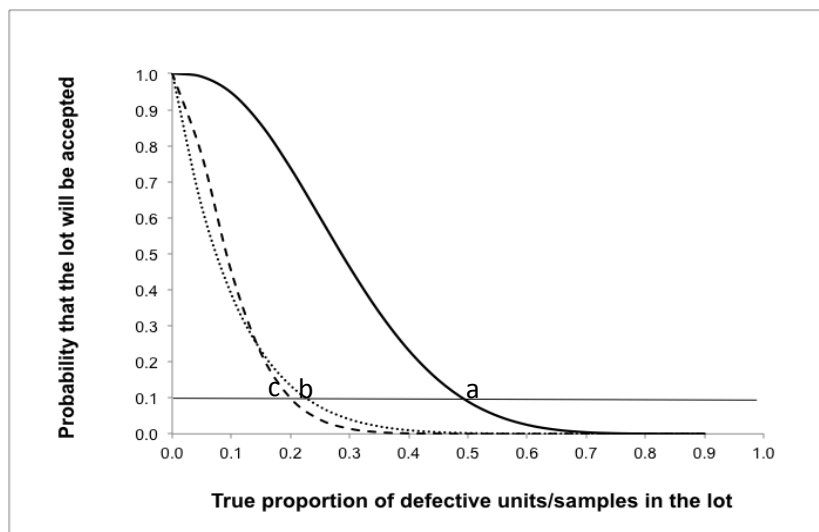
Figure 6.1. Operating characteristic (OC) curves for EU histamine sampling plan (EU 2073/2005, solid line) and US FDA (2010) sampling plan (dashed line). The OC curves describe the probability of accepting a batch as a function of the actual proportion of samples that is defective (histamine level exceeding a given limit, EU: 100 mg/kg; US FDA: 50 mg/kg). See text for detailed explanation. To aid comparison, the dotted line is the OC curve for an n = 9, c = 0 sampling plan, analogous to the EU scheme if m is taken as 200 mg/kg.

Taking nine samples, all of which must comply with the criterion (c = 0), offers 95 percent confidence that a batch that has 28 percent or more defective units (i.e. > 200 mg/kg) will be detected (and hence rejected) by that sampling plan. At 90 percent confidence (Figure 6.1, solid horizontal line), the sampling plan will detect batches with greater than 22 percent defective units (Figure 6.1, solid horizontal line at "b").

For n = 9, c = 2, the plan will, with 95 percent confidence, detect a batch with > 55 percent of defective units or, with 90 percent confidence (Figure 6.1, solid horizontal line), batches with > 49 percent defective units (Figure 6.1, solid horizontal line at "a"). Therefore, this plan is less stringent than the n = 9, c = 0 plan.

The FDA (2010) states that the "number of samples (i.e., scombrotoxin-forming fish) necessary to make a judgment about a lot depends on the anticipated variability, but should not be fewer than 18 samples per lot, unless the lot contains less than 18 fish, in which case a sample should be collected from each fish." Accordingly, that sampling plan (n = 18, c = 1), through requiring more samples, achieves a high level of sensitivity, i.e. it is able reliably to detect lower frequencies of contamination within a lot, or batch, of product. Compared with the above two plans, this plan will, with 95 percent confidence, detect a batch with > 24

percent defective units, or, with 90 percent confidence, batches with > 20 percent defective units (Figure 6.1, solid horizontal line at "c").

Additionally, the criterion for acceptability in the US FDA plan is more rigorous (50 mg/kg). Hence, the US FDA (2010) sampling plan is more stringent than the EU plan and offers more confidence that non-conforming lots will be detected.

6.2.2 Designing a sampling plan

Protection of consumers from food-borne illness involves minimizing their exposure to harmful levels of food-borne hazards. In Section 4 it was concluded that a dose of 50 mg of histamine is unlikely to cause adverse effects in most consumers. For a typical serving of fish, or seafood products, this quantity was translated into a level of 200 mg/kg in fish, or fish products, based on a 250 g serving size. A 250 g serving size represents the typical 97.5th percentile of serving size for fish products in a variety of nations and cultures (see Section 3.3).

Section 3.2 discussed the sources and extent of variability in histamine levels within fish and among fish, and Section 6.1, above, described briefly the statistics of sampling plans. The question arises, then, how a science-based sampling plan can be established that is also practicable. The answer is that this can be developed from an understanding of the distribution of contamination levels within or among batches.

As an example, if we aim to ensure that there is a less than "one in a thousand" probability of any sample in a batch exceeding a specified level, and we know the distribution of contamination levels within the batch, we can use simple statistics based on the Normal (Gaussian) distribution to generate a variety of sampling plans that differ in m and n but all provide the same level of assurance and protection of public health.

6.2.2.1 Using a known standard deviation to derive an acceptable mean

To reveal this variety of sampling plans, we need to have some knowledge of the standard deviation of the contamination levels within the lot to be tested. To be able to estimate this, however, the concentration data must form a Normal distribution, otherwise the statistical inferences will not be valid. In fact, histamine contamination levels do *not* form a Normal distribution, but the logarithm of histamine concentration does[7] (discussed in Chapter 5). As seen in Table 5.1, the standard deviation is not consistent among surveys, but ranges from ~0.05 to 2.7 (in the log-transformed concentration data), depending on the dataset.[8]

Accordingly, the following analyses use $\log_{10}$ (histamine concentration [mg/kg]) for calculations but convert back to histamine concentration (mg/kg) when reporting the results.

In statistics, the "*z*-value" characterizes the probability of any particular value from within a distribution being observed by random sampling. Values closer to the average value in the distribution have small *z*-values and are more likely to be observed, while larger *z*-values are associated with more extreme values within the distribution. Most scientists and technologists are familiar with the idea that ~95 percent of observations from a population are within 2 standard deviations (SD) of the average, or mean, value for that population. The *z*-value is the difference between a specific value and the mean of the distribution, expressed as a number of SDs, i.e. the difference between the two values, divided by the SD.

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[7] This (presumably) arises from the fact that histamine in seafoods is formed by bacteria and that the histamine level has a stoichiometric relationship with bacterial concentration. That bacterial numbers in foods are log-normally distributed in foods is well established and arises as a result of their pattern of exponential increase with time in storage.

[8] It was also noted (Fletcher, 2011; data not shown) that variability in histamine levels was lower when levels were very low (e.g. < 10 mg/kg) or very high (e.g. > 1 000 mg/kg), but that intermediate levels showed greater variability.

To determine the average $\log_{10}$ (histamine concentration) of a population in which fewer than 1 in 1 000 samples contain above 200 mg/kg, the standard deviation of the $\log_{10}$ [histamine] in the population must be known. As an example, 200 mg/kg histamine equals 2.301 $\log_{10}$ ([histamine] mg/kg). In a Normal distribution, values that are likely to occur with less than 1 in 1 000 frequency are 3.09 z-values (i.e. 3.09 SD) away from the average, or mean. If the SD is 0.1, then the mean of the distribution that satisfies our requirement for no more than 1 in 1 000 samples ≥ 200 mg/kg is given by:

$$\log_{10} (\text{mean}) = 2.301 - (3.09 \times 0.1) = 1.992$$

$$\text{mean} = 10^{1.992} = 98.2$$

In other words, our distribution, with 0.1 SD, must have a mean of 98.2 mg/kg or lower to ensure that units containing 200 mg/kg are very unlikely (< 1/1 000) to occur within the lot. Figure 6.2a, below, shows this distribution, as well as the upper tolerable limit of 200 mg/kg.

To illustrate this process further, it might be determined that, to ensure public health, no more than 1 in 10 000 samples above 200 mg/kg histamine can be tolerated. In a Normal distribution, values that are likely to occur with a frequency lower than 1 in 10 000 are 3.72 z-values (i.e. 3.72 SD) away from the mean. Using the same approach as described above, the mean of the histamine concentrations in units in the lot would have to be 84.9 mg/kg (see Figure 6.2b). Similarly, the calculation could be made for a population of samples with an SD of 0.5. In this case, for a lot with fewer than 1 in 10 000 (< 0.01 percent) units likely to exceed 200 mg/kg, the mean histamine concentration would have to be ≤ 2.76 mg/kg, as shown in Figure 6.2c. The information discussed above has been summarized in Table 6.1.

Table 6.1. Comparison of the cumulative distribution function (CDF) plots shown in Figures 6.2a,b,c.

Standard deviation	Level of protection	z-value	Histamine limit (mg/kg)	Maximum mean that meets the criterion (mg/kg)
0.1	1/1000	3.09	200	98.2
0.1	1/10000	3.72	200	84.9
0.5	1/1000	3.09	200	5.71
0.5	1/10000	3.72	200	2.76

Note that, as the SD becomes larger, the mean concentration in the batch has to become lower for the batch to satisfy the criterion of acceptability (e.g. fewer than 1 in 1 000 samples above 200 mg/kg).
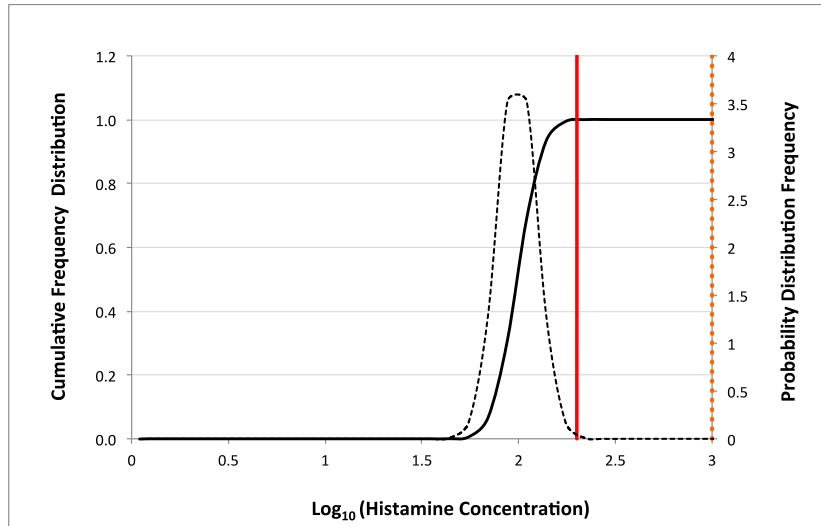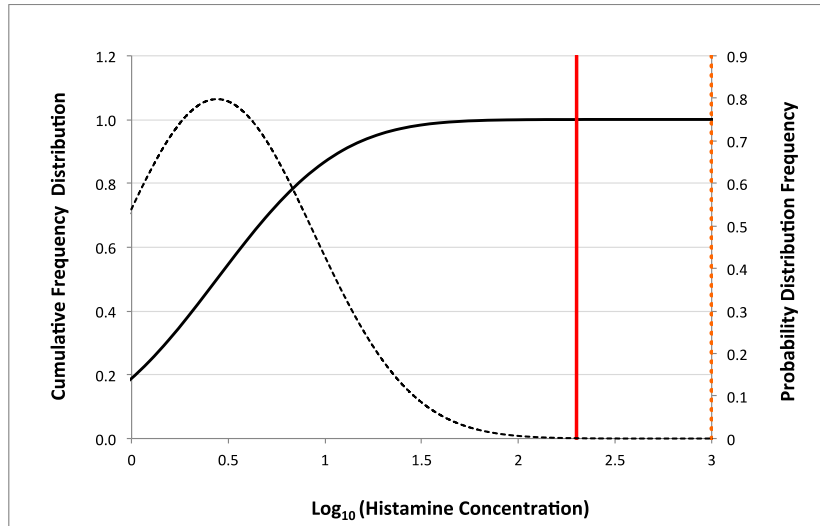
Figure 6.2a. Plot showing a normal distribution (black dotted line) of $\log_{10}$ ([histamine] mg/kg) contamination levels in a lot, or batch, in which ≤ 0.1 percent of samples will be expected to exceed 200 mg/kg (shown as the solid vertical line at 2.301 $\log_{10}$ ([histamine] mg/kg). The standard deviation for this distribution is assumed to be 0.1, meaning that the average contamination in the lot must be 98 mg/kg (or less) so that 99.9 percent of samples in the lot have histamine levels less than 200 mg/kg. The black solid line shows the cumulative distribution function (CDF) for the distribution shown by the dotted line. The CDF can be used to determine the proportion of samples expected to be above, or below, a particular concentration (see Figure 6.2d).
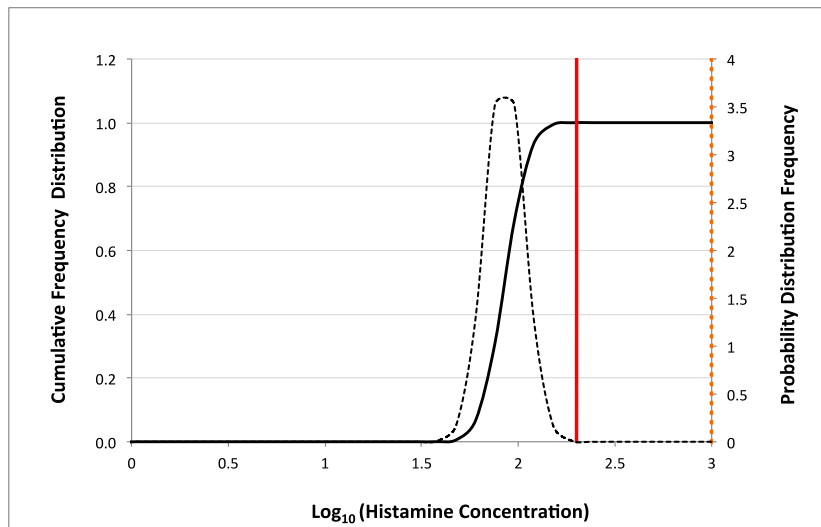

Figure 6.2b. Plot showing a normal distribution (black dotted line) of $\log_{10}$ ([histamine] mg/kg) contamination levels in a lot, or batch, in which ≤ 0.01 percent of units will be expected to exceed 200 mg/kg (shown as the solid vertical line at 2.301 $\log_{10}$ ([histamine] ppm). The standard deviation for this distribution is assumed to be 0.1, meaning that the average contamination in the lot must be 84.9 mg/kg (or less) so that 99.99 percent of samples in the lot have histamine levels less than 200 mg/kg. The black solid line shows the CDF for the normal distribution shown by the dotted line.

Figure 6.2c. Plot showing a normal distribution (black dotted line) of $\log_{10}$ ([histamine] ppm) contamination levels in a lot, or batch, in which ≤ 0.01 percent of samples will be expected to exceed 200 mg/kg (shown as the solid vertical line at 2.301 $\log_{10}$ ([histamine] mg/kg). The standard deviation for this distribution is assumed to be 0.5, meaning that the average contamination in the lot must be 2.76 mg/kg (or less) so that 99.99 pecent of samples in the lot have histamine levels less than 200 mg/kg. The black solid line shows the cumulative distribution function for the distribution shown by the dotted line.

6.2.2.2 Using the known standard deviation and the derived mean to design a sampling plan

Having derived the most extreme distribution that will meet our expectations of acceptably low public health risk, we can derive a sampling plan that provides a specified level of confidence that the batch, as a whole, will satisfy the criterion. To assist in visualizing the process, it is useful to consider cumulative distribution function (CDF) plots. The CDF curve can be interpreted as showing the proportion of samples that fall above, or below, a specified concentration value. From Figure 6.2d, which is based on Figure 6.2c but with several levels of $\log_{10}$ ([histamine] mg/kg) highlighted, by reading from the CDF curve, it can be seen that the distribution which satisfies our objective for tolerable levels and frequencies of histamine has ~56 percent of samples with histamine levels ≤ 0.5 $\log_{10}$ ([histamine] mg/kg) or 3.2 mg/kg, ~87 percent of samples with values ≤ 1.0 $\log_{10}$ ([histamine] mg/kg) or 10 mg/kg, and 98 percent of samples with ≤ 1.5 $\log_{10}$ ([histamine] mg/kg) or 32 mg/kg.

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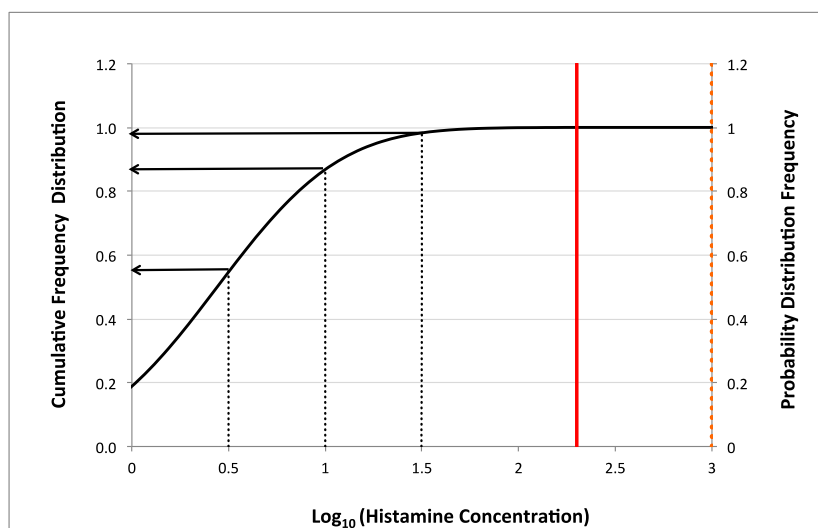
Figure 6.2.d. Plot showing cumulative distribution function (CDF) of $\log_{10}$ histamine contamination in levels in a batch of product in which ≤ 0.01 percent of samples are expected to exceed 200 mg/kg (shown as the solid vertical line at 2.301 $\log_{10}$ ([histamine] mg/kg). The standard deviation for this distribution is *assumed* to be 0.5, meaning that the average contamination in the lot must be 2.76 mg/kg (or less) so that 99.99 percent of samples in the lot have histamine levels less than 200 mg/kg. The vertical black dotted lines are included to show how the proportion of samples below a particular contamination level can be read from the CDF curve. For example, 87 percent of samples from a batch that meets the criterion (not more than 1 in 10 000 samples $\geq$ 200 mg/kg) should have $\log_{10}$ ([histamine] mg/kg) values less than 10 mg/kg.

Table 6.2. Comparison of a family of theoretical sampling plans to ensure the same criterion* for a sample batch**.

m (mg/kg)	% Sample lower than m	No. of samples needed to draw in order to reach 95% confidence level (calculated from OC curves)
200	99.99%	30 102
150	99.97%	11 527
32	98%	178
10	87%	22
3.2	56%	6

*fewer than 1 in 10 000 units exceeding 200 mg/kg
**with mean of 2.76 mg/kg and a log (SD) of 0.5

A preferred value for *m* can now be specified and used to determine how many samples drawn from the derived distribution would be expected to be less than (or above) that value. For example, using the distribution shown in Figure 6.2c (and 6.2d), if 10 mg/kg were chosen for *m*, the required distribution tells us that approximately 87 percent of samples drawn from an acceptable lot will be lower than *m* while 13 percent will not satisfy the attribute. Using the binomial theorem, 22 samples would need to be taken, each containing less than 10 mg/kg, to have 95 percent confidence that the batch as a whole contained fewer than 1 in 10 000 units higher than 200 mg/kg. If the value selected for *m* was 32 mg/kg, however, only ~2 percent of samples would be expected to exceed this level, and to demonstrate (with 95 percent confidence) that $\leq$ 2 percent of samples are "defective" would require 178 samples to be taken, and each shown to contain less than 32 mg/kg histamine. The information discussed above has been summarized in Table 6.2.

Thus, appropriate selection of the *m* value can improve the time- and cost-effectiveness of sampling considerably; *m* values close to the mean of the distribution offer the best discrimination of compliant vs non-compliant lots, i.e. they require the lowest number of

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samples to be tested to achieve the same level of confidence about the disposition of the lot being assessed. A further consideration, however, is the consequence of "false positive" results.

The basis of designing attributes sampling plans is well described elsewhere and this is a well-established approach in technological sciences. While the approach described above is appropriate for consumer protection, there is also the risk that, by chance, a non-compliant unit may be sampled and tested, and lead to inappropriate rejection of the batch, or lot. This false-positive, or "Type I" error, is also termed "the producer's risk".

Ideally the OC curve would be nearly vertical between the contamination frequency accepted with 95 percent confidence and that rejected with 95 percent confidence; in other words the sampling scheme would only infrequently accept unacceptable lots and equally infrequently lead to rejection of acceptable lots. In this way the sampling scheme would protect both the public from exposure to toxic seafood products and also the producers from having to discard product that is actually of acceptable quality and/or safety. The more samples that are tested, the closer one comes to that ideal being achieved.

To illustrate, for the distribution shown in Figure 6.2b, if m were selected to be 84.9 mg/kg, the average of the distribution, then 50 percent of samples tested would be expected to be above m and 50 percent below m. If five test units are shown to be lower than m, there is 95 percent confidence that the batch is of acceptable quality and/or safety overall. However, there is also a high probability of detecting a positive sample with a value higher than m. In this case we might set a sampling scheme with $n = 2$, $c = 1$, $m = 84.9$, but this provides little discrimination. The more samples that are tested, the more closely the performance of the sampling plan approaches the ideal of perfect discrimination between lots that are acceptable and those that are not. This is demonstrated in Figure 6.3.
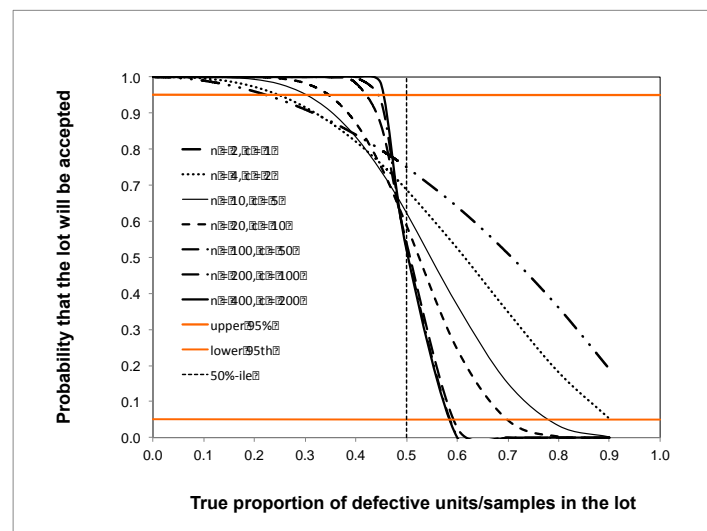
Figure 6.3. Plot comparing OC curves for a sampling plan designed to detect 50 percent of units above the required mean concentrations, for a defined SD of results, and consistent with public health protection (see text for details). The possibility of "false positive" (producer risk) and "false negative" (consumer risk) evaluation decreases with increasing numbers of samples taken, even though the same proportion of units must pass for the lot to be considered acceptable.

6.2.3 Examples and analysis of existing sampling plans

Using the approach described above it is possible to interpret the effectiveness of an existing sampling plan, if an assumption is made about the standard deviation (SD) of the $\log_{10}$ [histamine] within the lot. The EU plan accepts lots in which two of nine samples exceed 100 mg/kg, provided that none exceeds 200 mg/kg. In other words, 78 percent of samples must be lower than 100 mg/kg. Figure 6.4 shows the CDF for a population with SD = 0.5 that satisfies this criterion, and compares it with the example developed above for a population in which SD = 0.5 and fewer than 1 in 10 000 samples exceed 200 mg/kg. Figure 6.4 compares the two distributions directly. This comparison demonstrates that the EU plan is less stringent than that proposed and exemplified above to detect more than 1 in 10 000 units at 200 mg/kg (assuming that the variation in $\log_{10}$ [histamine] has SD = 0.5).
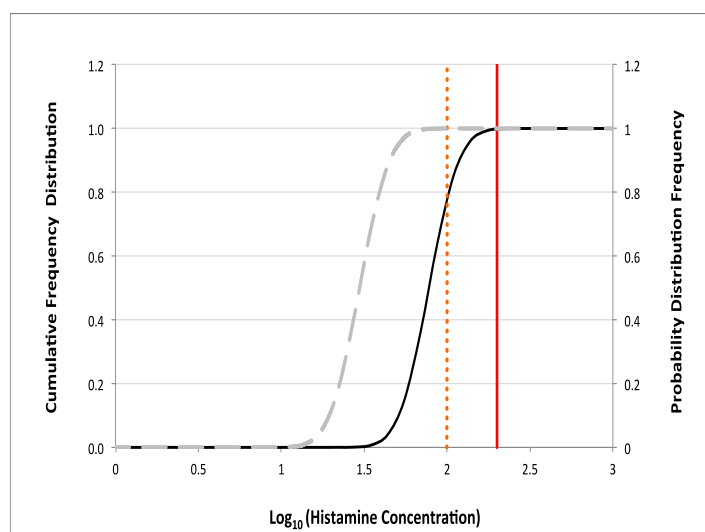
Figure 6.4. Plot comparing distribution curves for the sampling plan designed to detect more than 1 in 10 000 units at 200 mg/kg (dashed line)compared with the EU plan ($n = 9$, $c = 2$; solid line), and assuming that the EU plan (pragmatically) tolerates no more than 1 in 1 000 units that exceed the 200 mg/kg limit. The difference in the mean values of these distributions is apparent (30 mg/kg for the invented sampling plan and 78 mg/kg for the EU-based sampling plan).

It might be argued that the inclusion of M = 200 mg/kg provides an additional level of stringency. If the intent in the EU sampling plan is that fewer than 1 in 10 000 samples exceed 200 mg/kg, and 7 out of 9 (78 percent) samples contain less than 100 mg/kg, the SD of the most extreme distribution that still satisfies these criteria would have to be smaller. Using the concept of z-values described above, a distribution of $\log_{10}$ [histamine] in which ≤ 78 percent of samples contain less than 100 mg/kg, and 99.99 percent of samples contain less than 200 mg/kg implies a (minimum) SD of 0.130 and a mean of no more than 79.4 mg/kg. If the tolerance for exceeding 200 mg/kg is reduced to 99 percent, the distribution has an implied (minimum) SD of 0.193 and a mean no more than 71 mg/kg. If the tolerance for exceeding 200 mg/kg is reduced to 95 percent, the implied (minimum) SD is 0.346 and the mean should be no more than 54 mg/kg. As noted earlier, however, if the actual SD were higher, the mean would also have to be lower, and a smaller proportion of units at 100 mg/kg would be tolerable for the lot overall still to be considered acceptable.

Table 6.3 presents some examples of attributes sampling plans appropriate to different levels of tolerance for samples above 200 mg/kg, and for different assumptions about the SD

of $\log_{10}$ [histamine] within lots. The sampling plans shown are two-class plans and indicate the number of analytical units required to be tested (and to comply with the test criterion, i.e. *m*) in order to have 95 percent confidence that the batch as a whole satisfies our desire for a specified low proportion of samples to exceed 200 mg/kg. In some cases the distributions are so narrow (the SD is very small) that testing samples against a criterion of 100 mg/kg is meaningless because most samples could be above this limit, yet in the lot as a whole it is very unlikely that there is any unit exceeding 200 mg/kg. In this case, it would be more practical to have a higher value for *m*, e.g. 200 mg/kg. Conversely, if the SD is very high, to have confidence that the lot as a whole does not contain an unacceptable proportion of samples above 200 mg/kg, many thousands of samples may be required to attain 95 percent confidence.

Table 6.3 also includes examples that show that appropriate selection of the value of *m* can reduce the number of samples needed to produce the same confidence in the overall quality of the lot from a consumer perspective. However, it also shows that, while fewer samples can protect the consumer, they may also be too protective and result in the disposal of lots that are acceptable. A larger number of samples provides better discrimination of the overall quality and/or safety of the lot and works both to ensure public health and reduce wastage. This principle is evident in the Codex Sampling Plans for Prepackaged Foods (AQL 6.5) in which more samples are taken for larger lots. The consequences of rejecting larger lots that in fact are acceptable justify the additional expense involved with testing more analytical units.

Table 6.3 Examples of attributes sampling plans appropriate to different levels of tolerance for samples above 200 mg/kg, and for different assumptions about the standard deviation of $\log_{10}$ [histamine] within lots.

Log standard deviation (SD, assumed)	Level of protection (allowable probability of any sample in the lot exceeding 200 mg/kg, risk manager's decision)	Mean histamine level (the maximal allowable mean in order to meet the level of protection; back-calculated from SD)	m* (mg/kg)	Percentage of analytical units allowed to have histamine levels > m	n*	c*	Notes
0.05	1 in 20	165	200	5	59	0	[a] In the case of a small SD, a low *m* provides no discrimination – almost all samples (italics) are allowed to exceed this *m*. Therefore, a larger value of *m* is more practical
	1 in 20	165	100	*99*[a]			
	1 in 100	153	100	*99.99*[a]			
	1 in 1000	140	100	*98*[a]			
	1 in 10000	130	100	*99*[a]			
0.1	1 in 20	137	100	*92*[a]	2	0	[b] Increasing the number of analytical units reduces producer's risk due to false positive
	1 in 20	137	150	35	7	0	
	1 in 100	117	100	75	3	0	
	1 in 1 000	98	100	47	10[b]	1	
	1 in 1 000	98	100	47	20[b]	5	
	1 in 1 000	98	100	47	50[b]	17	
	1 in 10 000	85	100	24	11		
0.5	1 in 20	30	100	15	19[c]	0	[c] For a higher level of protection, a larger number of units is needed if *m* doesn't change
	1 in 100	14	100	4	74[c]	0	
	1 in 1 000	6	100	0.6	298[c]	0	
	1 in 10 000	3	100	0.09	3328[c]	0	
1.0	1 in 20	5	100	9	31	0	Using a more stringent *m* can significantly reduce the number of units that need to be tested – yet provide the same level of protection
	1 in 100	0.9	100	2	149	0	
	1 in 1 000	0.2	100	0.26	1151	0	
	1 in 10 000	0.038	100	0.03	9569	0	
	1 in 10 000	0.038	50	0.09	3301	0	
	1 in 10 000	0.038	25	0.24	1239	0	
	1 in 10 000	0.038	1	7.8	37	0	

*See page 45 for an explanation of *m*, *n* and *c*.

6.3 Economic impact of enforcement

This analysis is based on available data for the mean annual production of major species of interest (2005–2010) and histamine distribution in major species of interest, in both risk managed and unmanaged circumstances (Table 6.4). No direct monetary values are used; rather costs are expressed in terms of a percentage of the trade.

Table 6.4. Volumes and histamine distribution in risk managed and risk unmanaged systems.

Species	Mean annual volume (tonnes)	Processed without significant GMP and HACCP			Processed with significant GMP and HACCP		
		> 50– < 100 mg/kg	> 100–< 200 mg/kg	> 200 mg/kg	> 50– < 100 mg/kg	> 100– < 200 mg/kg	> 200 mg/kg
Tunas		4.5%	6.1%	0.1%	0.40%	0.10%	0%
	4 295 639	193 325	263 245	6 287	17183	4 296	0
Clupeidae		4.5%	6.1%	0.1%	0.0%	0.0%	0.0%
	3 656 288	164 551	224 065	5 351	0	0	0
Mackerel		4.5%	6.1%	0.1%	0.0%	0.0%	0.0%
	5 500 460	247 548	337 079	8 050	0	0	0
Anchovy*		4.5%	6.1%	0.1%	5.0%	0.0%	0.0%
	8 941 038	402 392	547 925	13 086	447052	0	0

*Histamine distribution is based on known histamine levels in salted vacuum-packed anchovies – only a minority of anchovy is processed in this manner. The majority is processed as fishmeal or other products which would be expected to demonstrate significantly lower histamine levels.

6.3.1 Estimated cost of rejection

Rejected fish or fish products may be completely rejected or partly rejected. The rejected portion either may be destroyed, resulting in 100 percent direct cost, or may be sold to another customer or jurisdiction at partial or no loss. Thus the direct cost of enforcement when the product is out of compliance ranges from 0–100 percent of the product value. There are frequently additional costs associated with redirecting or destruction of the product. These indirect costs were estimated by a major fish product distributor at 12 percent of product value. Thus the cost of rejection can range widely from 12–112 percent of product value. Hence the cost of enforcement at various histamine limit levels can be expressed as a percentage of the overall value of the trade, as presented in Table 6.5.

Enforcement can be expected to have a number of different impacts on trade. On the positive side the economic cost associated with not meeting requirements will encourage the industry to follow a risk-based system. Unfortunately the effects can also be negative, driving businesses to operate in jurisdictions or with trading partners who are not practising enforcement; in some cases it may lead to a cessation of trade.

Table 6.5. Cost of enforcement by histamine limit level in risk managed and risk unmanaged systems.

Species	Mean annual volume (tonnes)	Processed without significant GMP and HACCP			Processed with significant GMP and HACCP		
		> 50– < 100 mg/kg	> 100–< 200 mg/kg	> 200 mg/kg	> 50– < 100 mg/kg	> 100– < 200 mg/kg	> 200 mg/kg
Tunas	min loss	0.5%	1.3%	1.3%	0.0%	0.1%	0.1%
	max loss	5.0%	11.9%	12.1%	0.4%	0.6%	0.6%
Clupeidae	min loss	0.5%	1.3%	1.3%	0.0%	0.0%	0.0%
	max loss	5.0%	11.9%	12.1%	0.0%	0.0%	0.0%
Mackerel	min loss	0.5%	1.3%	1.3%	0.0%	0.0%	0.0%
	max loss	5.0%	11.9%	12.1%	0.0%	0.0%	0.0%
Anchovy*	min loss	0.5%	1.3%	1.3%	0.6%	0.6%	0.6%
	max loss	5.0%	11.9%	12.1%	5.6%	5.6%	5.6%

*Histamine distribution is based on known histamine levels in salted vacuum-packed anchovies – only a minority of anchovy is processed in this manner. The majority is processed as fishmeal or other products which would be expected to demonstrate significantly lower histamine levels.

6.4 Conclusion

For most products, the risk of SFP can be suitably mitigated by rapid chilling of the raw material and maintaining the cold chain. For such products SFP will only occur when they have been subjected to gross time/temperature abuse. However, for other products, such as smoked and fermented products, other controls may be needed. Sampling and testing are a means to verify that controls have been applied as they should be.

The information and analyses provided in this section enable the development of sampling plans to achieve desired levels of public health protection. The process of development of sampling plans described also demonstrates that public health protection can be achieved using a single criterion (e.g. fewer than 1 in 10 000 units containing more than 200 mg/kg histamine) and an appropriate sampling plan developed to satisfy that criterion. This requires, however, that information about the variation in histamine levels in the lot is known, can be inferred, or can be assumed. Given that this knowledge of the variability of histamine levels within batches underpins the above approach, data that allow better quantification of the distribution of histamine levels in products and batches of products would be useful.

7. Conclusions

- Histamine formation and SFP can be easily controlled. The risk of SFP is best mitigated by applying basic GHP and where feasible a HACCP system. Appropriate sampling plans and testing for histamine should be used to validate the HACCP systems, verify the effectiveness of control measures, and detect failures in the system.

- There is a range of test methods available for the reliable determination of histamine levels in fish and fishery products. While each method has its strengths and limitations, the availability of testing methodology is not a limitation with regard to the detection of histamine in these products at the limits of interest. It was acknowledged that testing may involve the use of several methods, such as in the application of a tiered testing approach where rapid methods (ELISA, colorimetric test, etc.) that allow an initial screening are followed by higher performing and complex methodologies that allow confirmation of results and that may work as reference methods.

- Sensory evaluation remains a highly useful tool in quality control programmes for fish and fishery products, but acceptable sensory quality cannot be taken as final assurance of low histamine, nor can low histamine be taken as final assurance that fish is not decomposed. As a result the conclusion of the expert meeting was to focus their advice on histamine limits and related sampling plans on applications related to consumer protection.

- The process of development of sampling plans demonstrates that public health protection can be achieved using a single criterion (e.g. no more than 1 in 10 000 units containing more than 200 mg/kg histamine), and an appropriate sampling plan was developed to satisfy that criterion. Knowledge of the variability of histamine levels within batches underpins the approach shown here. In order to provide more explicit guidance on sampling approaches, the meeting analysed a range of sampling plans implemented under different scenarios of histamine levels as defined by mean and standard deviation.

- The above histamine limit and related sampling plans are relevant for products at the retail level. However, the meeting recognized that, in order to achieve this limit, more stringent requirements may be applied earlier in the distribution chain.

- While other biogenic amines may play a role in the aetiology of SFP, there is limited evidence of their role and there are no dose–response data in either humans or laboratory animals for these biogenic amines. Given that most epidemiological studies associate abnormally high levels of histamine in the incriminated fish or fish product with SFP, histamine can be considered to be the most appropriate marker of SFP.

- Although a number of hypotheses exist, the mechanism of toxicity in SFP remains unclear.

- SFP will only occur in healthy individuals when a dose of at least 50 mg histamine is consumed in fish and fishery products. Considering a single serving size of 250 g as an estimate of a high consumption level, a limit of 200 mg/kg was calculated. It is important to bear in mind that, while the NOAEL is an appropriate hazard threshold value to use for exposures in healthy subjects, this may not be the case for those members of certain segments of the population who may have an increased sensitivity (e.g. related to metabolic differences, physiological conditions, drug therapies or age). In these instances a lower hazard level may need to be considered (e.g. the use of an uncertainty factor) or other specific risk management options such as fish consumption advisories should be considered.

- A wide range of fish have been associated with SFP and need to be considered in determining exposure and in SFP risk management. Concluding that this information should be easily accessible to risk managers, the expert meeting developed the most comprehensive list of fish associated with SFP to date.

8. Recommendations

- In order to control histamine formation and manage the risk of SFP, fish catchers and handlers need to apply basic GHP and the fishery industry needs to apply GHP/HACCP. It is therefore recommended that regulators and all stakeholders are aware of the basic steps required to control this hazard.

- Fishing methods should be reviewed and adapted, for example by harvesting fish alive, to minimize histamine formation.

- To facilitate implementation of risk-based management plans, it is recommended that the most up-to-date and complete information should be used, including the list of fish species in this report, consumption data, epidemiological data.

- To refine sampling plans, it would be desirable to quantify better the distribution of histamine levels in products and batches of products.

- Recognizing that the lower investment associated with the use of rapid histamine testing methods made them an attractive option for the industry, while also noting the importance of characterizing the performance of these methods under their conditions of use, the expert meeting recommended periodic verification of the level of performance of these methods against the reference methods.

- Epidemiological data can be used to model the dose–response relationship, in addition to the existing volunteer studies model. To do so, in-depth outbreak investigations (e.g. isolating suspected biogenic amine-producing bacteria from implicated fish, testing histamine and other biogenic amine levels in remaining food samples, and estimation of consumption volume) should be encouraged.

- It is recommended that information about SFP outbreaks should be shared internationally. An international SFP alert through an existing emergency network, e.g. INFOSAN[9], is recommended.

- It is recommended that risk-management recommendations should be developed, based on the outcomes of the expert meeting. In particular, consideration should be given to the elaboration of risk-based sampling plans and histamine criteria.

- The experts acknowledged the utility of having access to the mathematical tools used in this meeting to develop different sampling plans. The group therefore recommended that FAO/WHO find ways to make these available in an easy to use format.

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[9] The International Food Safety Authorities Network (INFOSAN) was developed by the World Health Organization (WHO) in cooperation with the Food and Agriculture Organization of the United Nations (FAO), to promote the exchange of food safety information and to improve collaboration among food safety authorities at national and international levels.

8.1 Research Needs and Recommendations for Future Studies

- A reduction in the uncertainty surrounding the critical role played by histamine and other biogenic amines in the pathogenesis of SFP is needed. Studies that will clarify the mechanistic and quantitative roles of histamine and other biogenic amines in the spectrum of adverse effects seen in SFP are deemed to be essential.

- Evidence suggests that potentiators alter the threshold toxic dose for histamine in contaminated fish. Elucidation of the quantitative relationship between the dose of histamine and other biogenic amines and the various adverse effects is needed. Ideally, such work would be derived from human volunteer studies, but the likelihood that such studies will be conducted may be limited. In lieu of human studies, appropriate animal models and studies, such as in pigs, should be investigated. In these studies, the emphasis should be on the dose range between the NOAEL and the low levels associated with the onset of mild symptoms. Beyond the three known potentiators (cadaverine, putrescine and tyramine), studies are needed to characterize and identify currently unknown potentiators, e.g. other biogenic amines. Further studies are also needed to establish the mechanisms of action of all these potentiators.

- Studies are needed to determine the diversity and role of microflora in the production of biogenic amines. This could include the effect of the different microflora populations and the quantitative relationship between their levels and biogenic amine production.

- More studies are needed to determine the quantity of free amino acids other than free histidine in fish normally associated with SFP.

- Other methods to control the growth of biogenic amine-producing bacteria, aside from time/temperature control and using high quality raw materials, have been considered and used. These methods include using starter cultures or enzymes that degrade histamine during fermentation, application of hydrostatic pressure, irradiation, modified atmosphere packaging, adding salt, controlling a_w, and the use of food additives (Naila *et al.*, 2010). Only a few emerging methods have been published and applied to specific fishery products. More studies should be done to help reduce the risk of SFP.

- For consistency of dietary exposure assessments, harmonization of data collection with respect to the identification of typical fishery products consumed in the overall diet and assessment of the dietary intake of histamine and other biogenic amines from these products is recommended. It would be very beneficial if consumption data could be shared internationally.

- Other studies that are deemed to be helpful would be those that investigate the various factors that may enhance the sensitivity of the response to SFP in various populations. This would include investigation of the roles of:
    - genetic polymorphism in histamine toxico-dynamics and -kinetics;

- o certain physiological states/conditions such as menstruation;
- o gastrointestinal tract diseases;
- o certain medications;
- o role of certain lifestyle practices such as smoking and alcohol consumption in altering biogenic amine metabolism;
- o age.

- Studies are needed to investigate and clarify the SFP-like syndrome reported to be associated with consumption of salmonid species. These studies should establish whether the syndrome is SFP and, if it is not, the exact nature of the syndrome should be characterized.

9. References and further reading

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Annex 2 – Histamine limits and sampling plans in current standards for fish and fishery products

Codex Standard	Histamine limit	Sampling plan
Codex Stan 94–1981 Rev 2007. Codex Standard for sardines and sardine-type products. Codex Stan 70–1981 Rev 1995 Codex Standard for canned tuna and bonito Codex Stan 119–1981 Rev 1995. Codex Standard for canned finfish Codex Stan 244–2004 Standard for salted Atlantic herring and salted sprat	3. **Essential composition and quality factors** **3.3. Decomposition** The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested 1. **Hygiene and handling** No sample unit shall contain histamine that exceeds 20 mg per 100 g	Sampling of lots for examination of the final product as prescribed in Section 3.3 shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (AQL-6.5) (CODEX STAN 233-1969)
Codex Stan 36–1981 Rev1–1995. Codex Standard for quick frozen finfish, uneviscerated and eviscerated	3. **Essential composition and quality factors** **3.4. Decomposition** The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested 5. **Hygiene and handling** shall not contain histamine that exceeds 20 mg per 100 g	Sampling of lots for examination of the product shall be in accordance with the FAO/WHO Codex Alimentarius Sampling Plans for Prepackaged Foods (AQL-6.5) CAC/RM 42-1977
Codex Stan 166–1989 Codex Standard for quick frozen fish sticks (fish fingers), fish portions and fish fillets – breaded or in batter Codex Stan 190-1995 Codex Standard for quick frozen fish fillets	3. **Essential composition and quality factors** **3.3. Decomposition** The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested 5. **Hygiene and handling** shall not contain histamine that exceeds 20 mg per 100	Sampling of lots for examination of the product shall be in accordance with an appropriate sampling plan with an AQL of 6.5

Codex Stan 236-2003 Codex Standard for boiled dried salted anchovies	g	
Codex Stan 165–1989 (Rev 1–1995) Codex standard for quick frozen blocks of fish fillet, minced fish flesh and mixtures of fillets and minced fish flesh	3. **Essential composition and quality factors** **3.3. Decomposition** The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested **5. Hygiene and handling** shall not contain histamine that exceeds 20 mg per 100 g	A Table indicating sample size (number of blocks to be tested) and acceptance number in relation to lot size (number of blocks) has been provided
Codex Stan 302–2011 Codex Standard for fish sauce	**6. Hygiene and handling** The product shall not contain more than 40 mg histamine/100g of fish sauce in any sample unit tested	Sampling of lots for examination of the final product shall be in accordance with the *General Guidelines on Sampling* (CAC/GL 50-2004). A sample unit is the individually packed product (bottle) or a 1 l portion from bulk containers

Biogenic amines in fish and fishery products – adverse health effects

Toxicology, epidemiology and dose response

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1. Toxicological aspects

1.1 Histamine

Histamine is a naturally occurring substance in the human body and is derived from the decarboxylation of the amino acid histidine. Histamine is also present in certain foods containing free histidine, and is generated by certain bacteria for example during spoilage and fermentation. Endogenous histamine has important physiological functions related to local immune responses, gastric acid secretion, and neuromodulation (Taylor, 1986). However, histamine-rich foods may cause food intolerance in sensitive individuals. Further, histamine contamination in fish and fish products may cause food poisoning.

1.1.1 Absorption, distribution, metabolism and excretion

Absorption Human subjects can tolerate up to 180 mg histamine orally without having noticeable effects, while intravenous administration of 0.007 mg histamine produces vasodilatation and increased heart rate (Weiss *et al.*, 1932). This suggests that histamine is not efficiently absorbed from the gastrointestinal tract. It has been postulated that histamine-metabolizing enzymes present in the intestinal tract prevent the absorption of ingested histamine into the circulation (Taylor 1986).

Distribution Endogenous histamine is generated by an enzyme called histidine decarboxylase (HDC), which is only synthesized as necessary and is degraded immediately when sufficient histamine has been generated. The HDC exists primarily in mast cells, basophils, enterochromaffin-like cells in the gastric mucosa and histaminergic neurons. Generally, histamine is stored as a histamine–heparin complex in the secretory granules in these cells, and is released upon stimulation to exert its physiological functions. However, recently it has been found that a small amount of histamine is synthesized in some epidermal cells and released immediately (Shahid *et al.*, 2009).

Metabolism In humans and experimental animals, histamine is primarily metabolized by two enzymes, diamine oxidase (DAO) and histamine-*N*-methyltransferase (HMT) (Maintz and Novak, 2007). DAO converts histamine into imidazoleacetic acid, which can be conjugated with ribose before excretion. HMT converts histamine into methylhistamine, which is then converted by monoamine oxidase (MAO) into *N*-imidazoleacetic acid. The ultimate end products of histamine metabolism are excreted in the urine.

In humans, DAO is expressed mainly in the intestinal tract, which limits the uptake of exogenous histamine into the circulation. HMT, however, is widespread in human

tissues with the order of activity being liver >> colon > spleen > lung > small intestine > stomach (Hesterberg *et al.*, 1984). Therefore, DAO is considered the major metabolic enzyme for ingested histamine, while intravenously or intradermally injected histamine is primarily metabolized by HMT. HMT is very selective for histamine, while the substrates of DAO include other biogenic amines such as cadaverine and putrescine (Taylor, 1986).

Altered histamine metabolism has been reported in individuals taking isoniazid (Morinaga *et al.*, 1997) and drugs that inhibit DAO or MAO, as well as patients with mastocytosis, tumor or chronic myelocytic leukemia (Maintz and Novak, 2007). Histamine metabolism may also be influenced by consumption of foodborne DAO inhibitors such as thiamine, cadaverine and tyramine (Taylor, 1986).

Excretion When ^{14}C-histamine was administered orally to humans, 68 to 80 percent of a radioactive dose was recovered in the urine. Some histamine does remain in the feces and additional amounts are catabolized by intestinal bacteria and exhaled as CO_2 from the lungs (Sjaastad and Sjaastad, 1974).

1.1.2 Mechanism of action

Histamine exerts its effects through the activation of four different types of histamine receptors (H_1, H_2, H_3 and H_4) on/in the cellular membrane. These histamine receptors are expressed on different cell types and work through different signaling pathways, resulting in multiple biological responses. For example, histamine increases vasopermeability and vasodilation, causing urticaria, flushing, hypotension and headache. Histamine also induces contraction of intestinal smooth muscle, causing abdominal cramps, diarrhea and vomiting (Lehane and Olley, 2000).

1.1.3 Toxicological responses in animals

The toxicological responses to histamine depend on the method of administration, and the toxicological effects differ among species. Oral administration of histamine, alone or together with spoiled tuna, produced emesis in pigs. An emetic response was also observed in dogs (Blonz and Olcott, 1978). As reviewed by Taylor (1986), intraduodenal injection of histamine produced only transient hypotension in dogs and cats, while a histamine-containing yeast extract produced a wider variety of effects in cats, including increased volume and acidity of stomach acid, increased hematocrit and limb volume, and enhanced electromyographic activity. When given intradermally, histamine induced microvascular permeability in the skin of hamsters and rats (Woodward and Ledgard, 1986).

1.1.4 Toxicological responses in humans

While in physiological concentration a necessary and desirable substance, histamine becomes toxic when large doses enter the circulation. The incidence often results in poisoning symptoms, with a wide range of organs affected (Taylor, 1986). The toxicological effects of histamine are related to its normal physiological actions in the body.

Vascular: Dilatation of the peripheral blood vessels, predominantly arteries, results in hypotension, flushing and headache. Histamine also induces increased capillary permeability, resulting in symptoms such as edema, urticaria, hemoconcentration and increased blood viscosity. Shock can result from administration of very high doses of histamine. The effect on capillary permeability is mediated by both H_1 and H_2 receptors (Owen *et al.*, 1980).

Heart: Histamine exerts a direct stimulatory action on the heart. Histamine increases heart contractility and increases the rate and strength of the contractions. The effects of histamine on the heart might account for the palpitations noted by some persons experiencing histamine poisoning. Histamine can cause either contraction or relaxation of extravascular smooth muscles. Contraction is mediated by H_1 receptors, while relaxation is associated with H_2 (Shahid *et al.*, 2009) receptors.

Gastrointestinal In humans, the predominant action of histamine on extravascular smooth muscles is contraction. This smooth muscle contraction is most often noted in the bronchi and intestines. In histamine poisoning, the contraction of intestinal smooth muscle is particularly apparent, because histamine enters the gastrointestinal tract initially. Contraction of intestinal smooth muscle leads to the abdominal cramps, diarrhea, and vomiting which are often noted in cases of histamine poisoning (Taylor, 1986).

Neurological: Histamine is also a potent stimulant of both sensory and motor neurons. This stimulation may be important in producing the pain and itching that frequently accompany the urticarial lesions in histamine poisoning. This neural stimulation is mediated by H_1 (Nuutinen and Panula, 2010) receptors.

1.1.5. Histamine intolerance

Histamine intolerance is a type of food intolerance with allergic-like symptoms. It occurs when histamine-rich foods, such as cheese and wine, are consumed by susceptible individuals. Owing to genetic or acquired dysfunction of DAO or NMT, ingested histamine cannot be degraded efficiently in the gastrointestinal tracts of these individuals. The resulting build-up of histamine in the system causes a series of toxic

effects that are similar to a common food allergy, which usually include swelling, rashes, hives, and asthma-like symptoms such as difficulty in breathing, wheezing and smooth muscle contractions. Gastrointestinal symptoms, such as bloating and diarrhea, have also been reported (Maintz and Novak, 2007). The same histamine-rich foods would not cause these reactions in a non-susceptible population. This condition can be used to explain the variations between individuals in their susceptibility to dietary histamine in decomposed fish (Motil and Scrimshaw, 1979). People with histamine intolerance are advised to take a histamine-free diet (Wantke *et al.*, 1993).

Individual susceptibility to SFP has been observed in multiple epidemiological studies and healthy volunteer challenge tests. It is generally accepted that the ability to tolerate histamine exposure can be compromised when histamine-metabolizing enzymes are impaired. The factors associated with increased sensitivity to histamine have been summarized in a recent report on biogenic amines (EFSA, 2011). Briefly, reduced histamine metabolism can result from genetic polymorphism (Garcia-Martin *et al.*, 2009), certain physiological states/conditions such as menstruation (Jonassen *et al.*, 1976; Kalogeromitros *et al.*, 1995), gastrointestinal diseases (Mainz and Novak, 2007) or use of certain medications (Y. H. Hui, 2006; Taylor, 1986). There is suggestive evidence that the severity and incidence of SFP may depend on age (Ianuzzi *et al*., 2007). Smoking and alcohol drinking may also increase sensitivity to biogenic amines by reducing the degradation capacity (EFSA, 2011).

1.1.6. Scombrotoxin fish poisoning (SFP)

SFP is a worldwide food safety problem and is a common cause of fish poisoning. The food poisoning is caused by heat-stable scombrotoxins, presumably arising from bacterial action in fish. Though detailed components of scombrotoxins have not been identified, it is generally accepted that biogenic amines, especially histamine, play an important role in the pathogenesis of SFP. The incriminated fish usually contains abnormally high levels of histamine due to bacterial activity resulting from inappropriate handling or storage conditions, and histamine has been implicated as (at least part of) a potential causative agent. Therefore, SFP is also called histamine fish poisoning (HFP). Though HFP shares some symptoms with histamine intolerance, they are two distinct conditions. Unlike histamine intolerance, SFP may involve the presence of other toxic decomposition products or other components unique to fish (Hungerford, 2010). In addition, SFP attacks not only susceptible individuals but also those with normal capacity for histamine degradation.

Symptoms A variety of symptoms of SFP have been observed among humans (Table 1). Poisoned individuals may show one or more of these symptoms, and the severity of the response to the contaminated fish may vary. In several case reports, exacerbation of asthma and more serious cardiac manifestations were reported (Ascione *et al.*, 1997; D'Aloia *et al.*, 2011; Wilson *et al.*, 2012). The symptoms typically develop rapidly (from 5 minutes to 2 hours after ingestion of spoiled fish), with a usual duration of 8–12 hours, and usually resolve themselves within 24 hours. Although symptoms may persist for up to several days, there are no known long-term sequelae. SFP is rarely is considered to be potentially fatal. According to the data from US Centers for Disease Control and Prevention (CDC) for the period from 1998 to 2002, there were 463 cases reported and no deaths (CDC, 2006). According to the data from the Japanese Ministry of Health, Labor and Welfare for the period from 1998 to 2008, there were 89 cases reported and no deaths (Toda *et al.*, 2009) .

Table 1. Common symptoms of scombrotoxin fish poisoning.

Type	*Symptoms*
Cardiovascular	Flushing, rash (urticaria), hypotension, headache, tachycardia
Gastrointestinal	Abdominal cramps, diarrhea, vomiting
Neurological	Pain, itching
Other	Oral burning sensation, peppery taste, nausea, swelling of tongue

Diagnosis The diagnosis of SFP is largely dependent on the symptomology, onset time, history of food allergy, and the consumption of contaminated fish. The diagnosis can be confirmed by detecting high levels of histamine in the implicated food, meal remnants or a similar product obtained from the same source (Ferran and Yebenes, 2006; Predy *et al.*, 2003).

Treatment Antihistamine therapy is the optimal mode of therapy for SFP. Symptoms usually subside rapidly after such treatment. Both H_1 antagonists (e.g. diphenhydramine) and H_2 antagonists (e.g. cimetidine) have been used for the treatment of histamine poisoning. Since the adverse responses are self-limited, pharmacological intervention may not be necessary in mild cases (Taylor, 1986).

Histamine as the causative agent There is compelling evidence that histamine is a significant causative agent of SFP. Examples of the most convincing evidence include high levels of histamine in most incriminated fish, elevated blood or urine histamine

in poisoned patients, and effectiveness of antihistamine drugs in reducing the symptoms.

However, oral administration of pure histamine at the same dose as in spoiled fish is not able to repeat the toxicological effects of SFP (Taylor, 1986). Some studies suggest that there are histamine potentiators in spoiled fish that contribute to the histamine-related SFP. By competitively inhibiting the histamine detoxification enzymes DAO and HMT, histamine potentiators can decrease the threshold dose of histamine needed to provoke an adverse reaction in humans (Al Bulushi *et al.*, 2009; Bjeldanes *et al.*, 1978; Taylor and Lieber, 1979; Taylor, 1986). Cadaverine and putrescine have been implicated as possible histamine potentiators based on both *in vivo* and *in vitro* animal studies (Bjeldanes *et al.*, 1978; Lyons *et al.*, 1983; Mongar, 1957).

Another possible mechanism is that potentiators might interfere with the intestinal barrier that prevents the intestinal absorption of histamine. Specifically, intestinal mucin, which is known to bind histamine and prevent its absorption, may be disrupted. This hypothesis is supported by the result of an isolated guinea pig gut section study showing that cadaverine was able to increase the histamine transportation rate, yet had a minor effect on histamine metabolism (Paik Jung and Bjeldanes, 1979).

SFP has been reported following consumption of non-scombroid fish, such as salmon, that contains low histidine and histamine (Bartholomew *et al.*, 1987). It is postulated that an unknown toxin(s) in these spoiled fish act as a mast cell degranulator(s) to induce histamine release, and that the endogenous histamine, instead of ingested histamine, accounts for the allergy-like reactions (Clifford *et al.*, 1991; Ijomah *et al.*, 1991). However, in human volunteers who were given marlin with high levels of histamine, researchers failed to detect mast cell secretion by directly measuring mast cell degranulation indicators such as tryptase (Morrow *et al.*, 1991; Sanchez-Guerrero *et al.*, 1997). In a recent case–control study of 10 SFP patients and 50 non-SFP patients with an allergic disorder, serum tryptase levels in all 10 SFP patients were in the normal range, while increased tryptase levels were found in most allergy patients (Ricci *et al.*, 2010). Therefore, the underlying mechanism for HFP caused by low-histidine fish is unknown.

1.2. Cadaverine and putrescine

Cadaverine and putrescine are two important biogenic amines found in fish. Like histamine, they are both produced from amino acids by bacteria, for example during spoilage and fermentation. The precursors of cadaverine and putrescine are lysine and ornithine, respectively. Cadaverine and putrescine are both frequently found in all improperly handled fish, not just those implicated in SFP, and have been studied as spoilage indicators. In some fish spoilage studies, cadaverine appeared to be formed and increased earlier than histamine (Pons-Sanchez-Cascado *et al.*, 2005; Rossi *et al.*, 2002).

Cadaverine and putrescine have been considered histamine potentiators, in an attempt to explain the lack of toxicity of pure histamine in human oral challenge studies. In guinea pigs, cadaverine and putrescine enhanced histamine-related mortality (Bjeldanes *et al.*, 1978; Parrot and Nicot, 1965; Vasseur *et al.*, 1968). As evidence of their potentiating effects, cadaverine and putrescine have been demonstrated to be functional inhibitors of DAO and HMT in a rat jejunal model (Taylor and Lieber, 1979). Cadaverine is also able to enhance the absorption of histamine in perfused rat intestinal segments (Lyons *et al.*, 1983; Paik Jung and Bjeldanes, 1979). In an *in vivo* study conducted in rats, both cadaverine and putrescine increased the amount of un-metabolized histamine but decreased the amount of its metabolites in urine (J. Y. Hui and Taylor, 1985).

The minimum level of cadaverine or putrescine that potentiates histamine toxicity is unknown. The ratio of cadaverine or putrescine to histamine may need to be high for the effect, and it is not clear whether the levels present in spoiled fish are sufficient to enhance the toxicity of histamine in humans.

1.3 Tyramine

Tyramine is a naturally occurring monoamine compound derived from the amino acid tyrosine. Fresh fish contains little or no tyramine, but a large amount can be found in spoiled or fermented fish (Leuschner and Hammes, 1999; Prester, 2011).

In humans tyramine acts as a catecholamine (including norepinephrine, dopamine, epinephrine) releasing agent, resulting in increased blood pressure. Since tyramine is physiologically metabolized by monoamine oxidase (MAO), a hypertensive crisis can result when a person who takes an MAO inhibitor (MAOI) drug also consumes foods

with high histamine content. This condition, also called the tyramine pressor response, is characterized by an increase in systolic blood pressure of 30 mmHg or more. The displacement of norepinephrine from neuronal storage vesicles by acute tyramine ingestion is thought to cause the vasoconstriction and increased heart rate and blood pressure. In additional to the hypertensive effects, dietary tyramine intake has also been associated with migraine in select populations, and the mechanism has been linked to tyramine as a neurotransmitter (Jansen *et al.*, 2003).

In animals tyramine has a low acute oral toxicity of more than 2000 mg/kg bw. It causes a dose-dependent increase in blood pressure. When using an MAOI, the intake of approximately 10 to 25 mg of tyramine is required for a severe reaction compared to 6 to 10 mg for a mild reaction. For adults, 100–800 mg/kg of dietary tyramine has been suggested as acceptable, and >1080 mg/kg as toxic (ten Brink *et al.*, 1990). In individuals using MAOI drugs, ingestion of 60 mg/kg of tyramine can cause migraine, while 100–250 mg/kg will produce a hypertensive crisis (Silla Santos, 1996).

There is some evidence that tyramine, like cadaverine and putrescine, potentiates histamine toxicity by inhibition of the histamine-metabolizing enzymes DAO and HMT (Bjeldanes *et al.*, 1978; Shalaby, 1996).

1.4 Other biogenic amines

Other biogenic amines detected in fish and fish products include spermine, spermidine, dopamine and agmatine (Park *et al.*, 2010; Visciano *et al.*, 2012). Though some of them might act as histamine potentiators (Taylor and Lieber, 1979), the contributions of these biogenic amines to SFP are not clear.

2. Epidemiological studies

SFP is generally regarded as one of the common forms of fish-related toxicity in humans. While histamine is usually associated with this clinical syndrome, other biogenic amines, such as tyramine, putrescine and cadaverine, are also thought to be involved. The clinical presentation closely resembles an acute allergic reaction and it is not uncommon to have the condition misdiagnosed as an allergic reaction. Scombroid poisoning is generally associated with the ingestion of spoiled fish, usually of (but not limited to) the scombroid family (tuna, mackerel and related species). The agent of the poisoning is referred to as scombrotoxin. This toxin results from spoilage, and is normally associated with the formation of heat-stable biogenic amines such as histamine. Though freshly caught fish have histamine levels of less than 2 ppm,

histamine levels as high as several thousand ppm were found in the incriminated fish, according to the case reports discussed below. The United States Food and Drug Administration (FDA, 2005) regards histamine levels greater than 50 ppm indicative of decomposition, and levels greater than 500 ppm are associated with illness (FDA, 2011), although some individuals may become ill after consuming fish with histamine levels below 500 ppm. Based on very limited human data, the European Food Safety Authority (EFSA, 2011) indicates that no adverse health effects have been observed in healthy volunteers exposed to a level of 25 to 50 mg of histamine per person per eating occasion, though some individuals may demonstrate particular sensitivity to histamine. For tyramine, there is currently insufficient information related to establishing a threshold toxicological dosage in humans. Based on very limited information, no adverse health effects have been observed in healthy individuals exposed to a level of 600 mg of tyramine per person per eating occasion. For those with reduced MAO activity (e.g. those on MAOI drugs), this threshold value will be reduced. The toxicity of putrescine and cadaverine appears to be less potent than that of histamine and tyramine, and available information is insufficient to identify concentrations that directly cause acute adverse health effects and/or potentiate the toxic effects of histamine and other biogenic amines.

SFP occurs throughout the world and is perhaps the most common form of toxicity caused by the ingestion of fish (Huss *et al.*, 2004). However, good statistical information about its incidence does not exist. This may be because of underreporting due to the mild nature of the illness, to lack of adequate systems for reporting food-borne diseases, or ignorance by medical personnel who misdiagnose histamine poisoning as a food allergy (Lehane and Olley, 2000; Taylor, 1986). In this section SFP case reports and related cohort/case–control studies are discussed. Only those published in the international journals and those which provide dose information are covered in this document.

2.1 Case reports

Two separate outbreaks of scombroid poisoning occurred in South Australia in 1992, involving seven patients who consumed Western Australian salmon (*Arripis truttaceus*). The onset of symptoms occurred within half an hour of consumption. The clinical syndrome included erythema and urticaria of the skin, facial flushing and sweating, palpitations, hot flushes of the body, headache, nausea, vomiting and dizziness. The fish implicated in one outbreak was noted by the subjects to have a

peppery taste. The presence of high histamine levels in the cooked fish was noted. Levels of 80 mg/100 g and 254 mg/100 g were determined in the two outbreaks. Two patients had minor symptoms which resolved fairly quickly. Another two patients had mild symptoms which disappeared after two hours. Four patients demonstrated major toxicity and were treated with parenterally administered promethazine. No patient had symptoms for longer than 12 hours. The patients had minimal gastrointestinal symptoms; swelling of the tongue, mouth blistering and bronchospasm (Smart, 1992).

A report of two cases originated from Australia in 1993. The first case involved a 35-year-old woman who presented with a florid flush of her skin, a pounding heart rate and tightness of the chest. Her symptoms occurred about 75 minutes after a meal of tuna. The patient's entire skin was reddish purple in color, her heart rate was 90 per minute with regular blood pressure 120/70. A diagnosis of scombroid poisoning was made and she was given the appropriate treatment to relieve her symptoms. A second patient, a 31-year-old male, had eaten a tuna dish and experienced a hot sensation, nausea, diarrhea, shaking and headache that lasted several hours (Brown, 1993).

In 2003 six cases of scombroid poisoning after ingestion of fish from the same Canberra restaurant were reported in Australia. The clinical presentation had features typical of histamine toxicity, typically with urticaria, flushing, headache, abdominal cramps, diarrhea and vomiting. One case resulted in significant hypotension necessitating a prolonged hospital stay. All of the patients reported eating yellow fin tuna (*Thunnus albacares*) with wasabi and Japanese spices. The restaurant stated that eight portions of the dish were served that night, giving a case attack rate of at least 75 percent. Histamine levels of 470 mg/kg and 490 mg/kg were reported (Hall, 2003).

Three outbreaks of gastroenteritis believed to be associated with consumption of "butterfish" occurred in Australia in the late 1990s and early 2010s. Escolar and rudderfish are commonly marketed under the name "butterfish". The first outbreak was reported in a group of approximately 80 people who consumed their meals at a restaurant. A cohort study was conducted and 63 percent (50/80) of the guests who attended the function were interviewed. Eleven subjects developed symptoms, predominantly of diarrhea (92 percent), abdominal pain (92 percent) and nausea (50 percent). Vomiting was not a major outcome of this outbreak, with only 8 percent reporting this symptom. The median incubation period after consumption of the meal was 2.5 hours with most cases recovering within 24 hours. Only one food item, crumbed and deep-fried fillets of butterfish served as a main course, had a statistically significant relative risk (RR = 9.37; 95 percent confidence interval [CI] 1.31–67.20). A sample of "butterfish", taken from the wholesale suppliers to the restaurant, was

analysed and found to be either escolar (*Ruvettus pretiosus*) or rudderfish (*Centrolophus* sp.). A second outbreak was reported in a group of 15 people who also eat their meals at a restaurant. While complete interviews were not conducted, 10 subjects reported diarrhea after consumption of grilled "butterfish", which was the common food consumed by all of the reported cases. A sample of left-over butterfish from the restaurant was obtained and was found to be either escolar or rudderfish. A third outbreak was reported in 2001, involving five individuals out of a group of 15 who ate at a restaurant. Four of these individuals consumed 'butterfish' and experienced symptoms of diarrhea and nausea within 2 hours of consumption. Leftover fish sampled from the restaurant was analyzed and found to be escolar (Gregory, 2002).

In 1978 several cases of scombroid food poisoning were reported in Britain. Several hours after consuming smoked mackerel, a male patient developed severe headache, nausea, slight diarrhea and redness of the trunk and arms. Two hours after the onset the redness faded and all the symptoms rapidly disappeared. In two other cases one patient began to feel unwell after eating smoked mackerel and became conscious of his elevated heart beat, as well as feeling hot and looking red and flushed in the face and hands. A little later he had one episode of diarrhea. He had neither headache nor nausea. The second patient became ill about half an hour after her husband, with a moderately severe headache; a feeling of hotness, especially in the face, which flushed bright red; and, a little later, a brief attack of diarrhea. Both complained of feeling slightly itchy. Within 3 hours their symptoms began to subside and shortly thereafter they felt completely normal. The fish was found to contain 1480 ppm histamine (Cruickshank and Williams, 1978).

A case report from Canada involved a 51-year-old female with a history of migraine headaches but no known food allergies who ate a tuna salad and drank bottled orange juice. Within 30 minutes she developed a severe headache, as well as nausea and palpitations. No vomiting, diarrhea or abdominal pain occurred. Over the next 30 minutes her symptoms intensified and her face became flushed and erythematous. Her pulse was 90 (usually 50) beats/min and her blood pressure 190/105 (usually 125/85) mmHg. She was transferred to a local emergency department, where her pulse rate was 100 beats/min and her blood pressure 200/120 mm Hg. The patient advised the attending emergency physician that scombroid poisoning was a possibility. This was discounted by the attending physician because the patient was hypertensive and the facial flushing had subsided. The results of neurologic examination and computed tomography (CT) of the head were normal. A CT scan of the abdomen for an adrenal

mass was also normal. A complete blood count and serum electrolyte levels were within normal limits, as were serum creatinine and calcium levels. The serum creatine kinase level was minimally elevated. Plasma histamine level was not measured for evidence of scombroid poisoning. The salad contained 35 mg of histamine per 100 g; the leftover canned tuna contained less than 1 mg/100 g, the concentration typical of canned tuna (Predy *et al.*, 2003).

A 60-year-old British man was admitted with a 4-day history of palpitations that had started after a large meal which included smoked mackerel. Within 2 hours after consumption of the meal he had developed flatulence, upper abdominal pain and nausea, followed by weakness, pulsating headache, a sensation of fear, itching of the scalp, increased frequency of micturition, and rapid regular palpitations. He was in atrial flutter with a ventricular rate of 150/minute; blood pressure was 160/90 mmHg, with no evidence of cardiac failure. Heart sounds were normal. Routine clinical blood work and chemistries, including blood count, erythrocyte sedimentation rate, cardiac enzymes, urea, electrolytes and thyroid function tests, were all normal. Chest radiography showed normal heart size and no pulmonary congestion. He reverted spontaneously to sinus rhythm the next day. Arrhythmia did not recur during follow-up of 18 months, which included four 24-hour ambulatory electrocardiograms taken in relation to similar meals. Of 10 people who had also eaten the mackerel at the dinner, three had developed headache and itching of the scalp and six had felt generally unwell for a few hours (Borysiewicz and Krikler, 1981).

An outbreak of scombroid poisoning occurred in San Francisco, United States, in the fall of 1977. The vehicle was sashimi prepared from spoiled tuna fish. Laboratory studies showed the presence in the tuna of bacterial species capable of producing large amounts of histamine, a substance strongly implicated in scombroid poisoning. Chemical analysis showed that histamine is very unevenly distributed in the flesh of spoiling tuna, therefore accounting for the sometimes random occurrence of disease among people eating the same food at the same table. On investigation 15 cases of scombroid poisoning were found. All of the persons affected had eaten sashimi (raw tuna fish) and became ill 15 to 45 minutes later. In one party of nine people who ate together, only the seven who ate sashimi became ill. In nearly all cases typical symptoms of facial flushing and headache were reported, but there were reports also of rash, swollen tongue, abdominal cramps, nausea, diarrhea, tachycardia and dizziness. At least 11 of the people felt ill enough to seek medical attention, nine of them at hospital emergency rooms. For most, the illness lasted only a few hours (Lerke *et al.*, 1978).

From 1994 to 1997, North Carolina, United States, averaged two cases annually; however, from July 1998 to February 1999, a total of 22 cases of histamine fish poisoning were reported. A study was instigated to examine the increase in histamine case reports, identify risk factors for poisoning, and develop recommendations for prevention. Reported case-patients had two of the following symptoms within 2 hours of eating tuna: rash, facial flushing, vomiting, diarrhea, dyspnea, a tight feeling in the throat, headache, or a metallic or peppery taste in the mouth. Twenty cases occurred during five outbreaks, and there were two single occurrences. Of the 22 persons affected, 19 (86 percent) sought emergency medical care. All case-patients ate tuna: 18 ate tuna burgers, 2 ate salad containing tuna, and 2 ate fillets. Tuna samples (available from three outbreaks) had histamine levels above 50 ppm (levels were between 213 and 3245 ppm). In 19 cases, the tuna used to prepare burgers or salads was frozen and thawed more than once before serving. Violations of recommended temperature controls were identified in two of the five restaurants, accounting for 14 (64 percent) cases. Tuna burgers, a relatively new menu item in restaurants, were associated with an increase in histamine poisoning cases in North Carolina. Tuna ground for burgers can be susceptible to both temperature fluctuations and bacterial contamination (Becker *et al.*, 2001).

Russell and Maretic (1986) reported two cases of SFP: one was caused by marlin (*Markair audax*), the other by mackerel (*Scomber japonicas*). In 1980, 35 of 1100 people attending a fish fry on Catalina Island, California, United States, were poisoned when they ate portions of 11 marlin, *Markair audax*. The fishes had been caught 1 to 3 days previously, gutted and cleaned the same day and then either kept in the cold or at 0–10 °C in brine until the time of the fry. It is not known which of the fish were responsible for the poisoning or how many were involved, for the 11 marlin had been cut and divided into 27 buckets on the morning of the fish fry. No muscle pain or cramps, other than abdominal, were reported by these patients, nor was there any hypotension, disorientation or changes in body temperature. Unfortunately, no samples of the offending fish were obtained for laboratory study.

In the other fish poisoning case, which occurred in August 1981, tourists from three families purchased several fresh mackerel, *Scomber japonicus*, in the fish market at Pula, Istria, Yugoslavia, at approximately 07.00 hours. The fish had not been gutted. In the absence of facilities for refrigeration, the fish were placed in a net and submerged in the sea at a temperature of 23–24 °C, where they remained until 20.00 hours. They were then gutted, grilled and eaten, each person consuming

approximately 250 g of fish. Eight of the subjects reported that the fish tasted good, but two complained that they noted a "strange peppery taste" to the fish. Within 10 minutes of eating the fish, six of the patients complained of numbness about the mouth and over the tongue, described by some as a burning sensation. These individuals also complained of dryness in the mouth. However, two of the patients did not develop significant symptoms until 1 to 2 hours later. Four of the cases complained of headache, weakness, dizziness and epigastric cramps, while three complained of nausea. On admission, swelling of the lips and tongue were observed in most patients and were most marked in a boy aged 8 years, whose lower lip was markedly edematous and his cheeks flushed and swollen. He also had hemorrhages on the palatal arches. This child had a previous history of bronchial asthma. He was somnolent, voided frequently and without control and had reportedly suffered respiratory collapse on the way to the hospital, requiring mouth-to-mouth resuscitation. Vomiting occurred in four patients and watery stools in two. The epigastrium was sensitive to pressure in four patients. In one patient there was gastric distension, hyperemia was observed and the patient complained of a burning sensation. Conjunctivitis was observed in all patients. In four patients mydriasis was present and the pupillary reaction was decreased. Mild hypertension was found in four patients. One patient complained of palpitations. Four patients showed mild diffuse dysrhythmias in their electroencephalograms, while in the other four there were no changes. All eight patients received gastric lavage and the stomach washings and the remains of the fish were subjected to bacteriological and biochemical studies. Analysis by thin-layer chromatography revealed excessive amounts of histamine (500 mg/100 g meat). Bacterial examination revealed *Enterobacter aeogenes*, *Escherichia coli*, *Klebsiella* sp. and others (Russell and Maretic, 1986).

Two incidents of food-borne poisoning, causing illness in 59 and 43 subjects, due to ingestion of billfish, occurred in May 2004, in Pingtung, southern Taiwan, and in December 2004, Taichung, central Taiwan, respectively. One fried billfish fillet and five frozen billfish fillet samples collected, respectively, from the suspected restaurants in Pingtung and Taichung, were tested to determine the histamine levels and identify fish species. Analyses of histamine showed that the suspected billfish samples in the two food poisoning incidents contained more than 150 mg/100 g of histamine, which is higher than the hazard action level of 50 mg/100 g. Judging from the allergy-like symptoms of the victims and the high histamine levels in the suspected billfish samples, both food-borne poisoning episodes were strongly suspected to be caused by histamine intoxication. A polymerase chain reaction–restriction fragment

length polymorphism (PCR–RFLP) method was used to identify the species of the suspected billfish samples in both food poisonings. The species of the Pingtung and Taichung billfish samples implicated in food poisoning were identified as *Makaira nigricans* and *Xiphias gladius*, respectively (Tsai *et al.*, 2007).

In August, 1973, 30 of 298 children attending a daycare center in Mississippi, United States had the sudden onset of a pruritic maculopapular rash 15 minutes after beginning lunch. Three children had urticarial lesions on the head and neck, and another developed periorbital edema. Symptoms lasted from 15 minutes to 2.5 hours. The children ate lunch in small groups divided by age. At approximately 10:30 hours, three groups (aged 11–13 months, 14 months to 2 years, and 2–3 years) began to eat. Fifteen minutes later, a pruritic erythematous maculopapular rash began to appear which subsequently affected 10 of 11 in the youngest age group, 13 of 17 in the middle group, and 7 of 20 in the oldest group. Lunch consisted of tuna casserole, string beans, banana pudding, bread, milk and grape juice. Investigation revealed that all 30 children who became ill had eaten the tuna casserole. The one child in the youngest age group who did not eat the casserole did not become ill. Suspecting that the tuna casserole was responsible for the illness, the staff did not serve it to the older children who ate later. These children experienced no symptoms. Seven adults who ate the casserole remained well and reported that the casserole looked and tasted normal. The casserole was prepared on the morning of August 2 in the school kitchen from cans of commercially packaged tuna fish, cream of mushroom and cream of celery soup and spices, and was served hot within minutes after preparation. Cultures of specimens performed by the Mississippi Public Health Laboratory from an opened can of tuna used to prepare the casserole and from an unopened can of tuna incubated at 37 °C were sterile. Cultures of the remaining tuna casserole grew only a few colonies of diphtheroids. Analysis of the tuna casserole prepared from tuna from an open can revealed no detectable histamine (MMWR, 1973).

An 80-year-old British woman presented to hospital after collapsing in a restaurant. She had consumed mackerel for lunch. She felt generally unwell with dizziness and complained of severe nausea and vomiting after finishing her meal. She subsequently collapsed and lost consciousness while having tea. There was no history of chest pain, palpitations, breathlessness, headache, diarrhea or abdominal pain. Apart from hypertension, which was well controlled by medication, she was relatively fit and healthy. She had no known drug or food allergies. On examination, she was found to have significant hypotension with a blood pressure of 60/40 mmHg. Her pulse was

regular at 90 beats/minute with a normal character. A localized erythematous rash was found on the anterior aspect of her neck. She did not complain of any itching or pain from the rash. Cardiovascular examination revealed normal heart sounds with no murmur, rub or gallop heard on auscultation. Neurological examination was normal. Examination of the respiratory and abdominal systems was unremarkable. Blood biochemistry and hematology were within normal limits. Electrocardiography was unremarkable and there were no significant abnormalities noted on the chest X-ray (Borade *et al.*, 2007).

In July 2000 a 42-year-old woman in Italy developed hives and hypotension 90 minutes after eating anchovies. Initially, the patient showed marked hypotension (80/60 mmHg), a heart rate of 92 beats/minute, a respiratory rate of 16 breaths/minute, and a body temperature of 37 $^{\circ}$C. The hematological parameters were normal. After 48 hours of hospitalization the patient showed no symptoms and was discharged (Tursi *et al.*, 2001).

Seven cases of scombroid poisoning occurred over a period of several weeks in 1995 in Spain. A healthy 45-year-old man was admitted to an emergency department for treatment of dyspnea, vomiting, diarrhea, generalized erythema, and pruritus, which started 20 minutes after consumption of tuna. Hypotension, tachycardia, bronchospasm, cyanosis, hypoxia and metabolic acidosis were found. The patient was thought to be experiencing acute anaphylaxis caused by fish allergy, and he was treated with epinephrine, fluids, oxygen, steroids and antihistamines. Symptoms disappeared in 15 to 17 hours. A 30-year-old man was first seen 30 minutes after tuna ingestion with erythema, cutaneous pruritus, facial flushing, palpebral angioedema, sweating, palpitations, dizziness, hypotension, headache, nausea, vomiting and dyspnea; he was diagnosed with acute anaphylaxis and was treated with epinephrine, fluids, oxygen, steroids and antihistamines. Eradication of the symptoms occurred in hours. A 54-year-old man was first seen a few minutes after tuna ingestion with urticaria, facial flushing, headache and glottis angioedema; epinephrine was required for resolution of symptoms. Four persons were first seen with headache and urticaria after tuna ingestion; their symptoms disappeared with administration of antihistamine. Subsequently six more cases were also reported. The epidemiologic investigation showed that in all cases the individuals had eaten tuna (*Thunnus thynnus*) from the same grocer's shop. The incubation period ranged from 10 minutes to 4 hours with a median of 40 minutes. All cases recovered within 24 hours. In two of the cases,

increased levels of serum histamine (115.4 and 89.6 μg/dl), urine histamine (74 and 57 μg/dl), and 24-hour urine methylhistamine (250 and 213 μg/24 h, respectively) were observed, with undetectable tryptase serum levels (< 2 UI/l). All patients were studied to rule out fish allergy and all were negative. Quantification of the histamine content of the fish showed elevated levels (2000 mg/100 g of fish) (Sanchez-Guerrero *et al.*, 1997).

In a study of an outbreak of food poisoning in Taiwan, 340 questionnaires (68 percent) were returned; 115 subjects (34 percent) reported signs and symptoms. The time of onset of illness suggested a common source of exposure. The lunch menu consisted of fried white-tipped mackerel, and fish was the only food associated with illness: 115 (56 percent) out of a total of 204 subjects who ate fish were ill, compared with none of 136 who did not eat fish. An analysis of attack rates revealed a clear trend toward higher attack rates in those who ate during later lunch shifts. Fish was not served after 13:00 hours because several employees were already ill and complained the fish had a peculiar taste. None of 112 employees who ate lunch after 13.00 hours compared with 115 of 228 who ate lunch before 13.00 hours became ill. The fish were found to contain histamine at 10 mg/100 g (Tong and Malison, 1987).

In a single event in Japan in 2006 a total of 32 cases were reported, with 13 of them hospitalized. About half of the patients said they had experienced an unusual taste (sour or peppery) when they ate teriyaki of marlin. The incubation period was 5–90 minutes after eating and most patients showed edematous erythema with heat sensation distributed throughout their bodies. Blood cell count and blood biochemical examination were conducted for all patients, as well as toxicology testing of urine. Gastric contents from severe patients were also taken and kept. After histamine poisoning was indicated, patients were administrated *d*-chlorpheniramine maleate intravenously. Patients with mild symptoms showed improvement of symptoms but severe cases did not. All patients were hospitalized because of the possibility of other causes and uncertainty regarding the duration of symptoms. However they all checked out of hospital the next day after their symptoms had ceased. Later, a local health center confirmed the food-borne outbreak of histamine in marlin (Otani and Ishimatsu, 2006).

In a 1999 report, 12 cases of scombroid poisoning that occurred in Italy were described. The patients ranged in age from 15 to 52 years. Two-thirds of the patients presented with rapid worsening of their clinical condition and hypotension severe

enough to require hospitalization. Symptoms occurred 15 minutes to 2 hours after eating cooked fresh fish (*Euthynnus pelamis*). The patients started feeling malaise, nausea and itching; in three cases vomiting, abdominal pain and diarrhea occurred, and in one case bronchial spasms. Inflammation was evident in the face, the neck and above the torso, and the skin was hot to the touch. None of the patients had a history of allergies or other pathology of any significance and in no case had any patient shown similar symptoms in the past when consuming the same type of fish. Initial blood pressure averaged 128 ± 8 mmHg systolic and 85 ± 5 mmHg diastolic; the electrocardiology examintaion results were normal, with a heart rate of 75 ± 4 beats/minute. Overall the patients responded to initial therapeutic support with almost full disappearance of symptoms within 1 hour in four of the 12 patients, with no further treatment needed. In contrast, the symptoms in seven patients showed no change, particularly in the dermatologic areas which, amongst other things, showed signs of eritemato-pomfoid: one had a consistent blood pressure in the average range of 30 mmHg. The youngest patient (15 years old) showed signs of rapid deterioration with severe skin manifestations, bronchial spasms, low blood pressure (80/60 mmHg) and profuse sweating necessitating the administration of massive doses of corticosteroid, plasma-expanders and, in the end, the use of epinephrine intravenously. The patient, after relative clinical improvement and stabilization of the vital signs, was admitted to an intensive care unit. After an average of 2 days, all patients were released in good health and with no side effects (Di Grande *et al.*, 1999) .

In June 2000 in Italy, a 31-year-old man experienced approximately 2 hours of nausea, extreme weakness and confusion. The patient had consumed an "oily fish" in a restaurant. Tests showed that his blood pressure was 90/60 mmHg, heart rate 120 beats/minute, respiratory rate 18 breaths/minute and a body temperature of 38 $^{\circ}$C. The patient was conscious but disoriented. His other vital signs were normal. A CT scan ruled out any cerebral pathology, and the hematochemical parameters were in the normal range. The patient was given 40 mg of prednisone intravenously, and plasma expanders. The patient's sense of orientation improved after 2 hours, but his hypotension worsened. After appropriate treatment the patient's vital signs returned to normal in 12 hours. All other vital signs had remained normal, no other symptoms were present, no other treatments were necessary and the patient was released after 2 days.

In 2007, 28 people were admitted to a hospital in SamutPrakarn province, Thailand. The patients had various symptoms including headache, nausea, vomiting, numbness

in the hands and feet, and diarrhea. After an investigation by the Research and Training Section Bureau of Epidemiology, Ministry of Public Health, it was found that all 28 patients worked at the frozen seafood factory nearby and had started to develop illness an average of 2 hours after the consumption of fried fermented tuna at the factory's cafeteria. The team then interviewed all the workers who ate fried fermented tuna on the day of the incident and found additional 64 individuals who developed mild symptoms but were not hospitalized, making the total number of cases 92. It was also found that a total of 193 workers ate the implicated fried fermented tuna that day and 91 workers became ill (47 percent), including mild and severe cases. Chemical analysis confirmed a high level of histamine, 446.18 mg/kg, in the implicated fried fermented tuna. Further investigation suggested that temperature abuse and improper handling were the cause of the histamine reaching a high level (Hongchumpon *et al.*, 2007).

On 26 November 2010, an outbreak of scombroid fish poisoning occurred in the French Armed Forces in Dakar, Senegal. This chemical intoxication, due to high histamine concentration in fish, is often mistaken for an allergic reaction. A case–control study was undertaken, including the 71 cases and 78 randomly selected controls among lunch attendees. The usual symptoms of scombroid fish poisoning were observed in the cases, i.e. flushing (85.9 percent), headache (83.1 percent), rapid/weak pulse (59.1 percent) and diarrhoea (47.9 percent). Symptoms occurred from within a few minutes to up to 3 hours following the meal. Most patients quickly recovered with antihistamine and/or symptomatic treatment. Tuna was the only food item positively associated with illness (odds ratio 36.3, 95 percent CI 6.3–210.0), with the risk of illness increasing with the quantity of fish consumed. No bacterial contamination was found in leftover food, but the histamine concentration in the tuna was found to be 4900 mg/kg, almost 50-fold higher than the concentration allowed by European regulations. This report is unique because of the large size of the case series – to our knowledge, the largest event of scombroid fish poisoning ever reported – and the results of chemical and bacteriological analyses obtained on leftover food (Demoncheaux *et al.*, 2012).

In November 2007, a healthy 28-year-old female ate seared tuna in a Baltimore restaurant. As she ate the tuna, she experienced a burning, peppery sensation on her lips. The tuna was pink in color, was not malodorous and did not taste spoiled. She assumed this sensation was due to spices coating the tuna and completed her meal. Minutes after leaving the restaurant, she developed a headache accompanied by a

flushing feeling in her face and shoulders. A little later, while sitting in a movie theater, she developed a rapid heart rate and a feeling that she was going to pass out. She also experienced crampy abdominal pain accompanied by two episodes of diarrhea. She noted facial flushing but no facial swelling, lip swelling or tongue swelling. She noted no rashes. She had no facial tingling, muscle weakness or ataxia. She noticed no shortness of breath, pruritus, or eyelid swelling. She took two antihistamine tablets and went to bed. In the morning she felt fine. She sought medical attention 48 hours after the fish ingestion. She denied any history of fish or shellfish allergies or other food allergies. She denied a history of asthma or eczema. She had eaten in this restaurant in the past and had eaten this menu item as well. She was taking no new medications. Samples of the suspect tuna were collected and sent to an FDA laboratory for histamine testing. Eighteen pieces of the suspect tuna were evaluated for the presence of histamine, and histamine levels ranged from 0 ppm to 24.4 ppm.

Table 2. Histamine levels related to scombroid fish poisoning case reports.

Country	Year	Number affected	Causative food	Level of histamine or biogenic amines (mg/100 g)	Reference	Note
Australia	1990	3	Western Australian Salmon	80	Smart, 1992	
	1991	4		254		
Australia		6	Yellow-fin tuna	470–490	Hall, 2003	
USA	1973	232	Canned tuna	68–280	Merson *et al.*, 1974	
USA	1977	15	Raw tuna (sashimi)	160–919 (mg/l)	Lerke *et al.* 1978	
USA	1998	11	Tuna burgers	274–325	Becker *et al.*, 2001	
USA		1	Smoked salmon	0.19	Gessner *et al.*, 1996	High toxicity in mouse bioassay was detected despite the low histamine level
USA	2003	42	Escolar fish	200–380	Feldman *et al,.* 2005	
Canada	1991	12	Marlin	360 (cooked) 331 (raw)	Todd *et al.*, 1992	
Canada		1	Canned tuna	35 (in salad)	Predy *et al.*, 2003	Histamine level was < 1 mg/100 g in canned tuna
UK		4	Smoked mackerel	250	Cruickshank and Williams, 1978	
Spain	1995	7	Tuna	2000	Sánchez-Guerrero *et al.*, 1996	
Taiwan, China	1986	41	Fried mackerel	10	Chen *et al.*, 1987	The implicated food was displayed at room temperature 2 hours longer than

|						the tested samples
Taiwan, China	2004	59 43	Fried billfish	257 157–270	Tsai *et al.*, 2007	
Taiwan, China	2007	347	Fried fish cubes	40 and 52	Chen *et al.*, 2009	Only two samples tested
Taiwan, China	2006	7	Tuna dumpling	161	Chen *et al.*, 2008	
Thailand	2007	91	Fried fermented tuna	446	Hongchumpon *et al.*, 2007	

From 1998 to 2008, 89 histamine fish poisoning incidents affecting 1 577 individuals (with no deaths) were reported in Japan, giving an average of around eight incidents with 150 patients every year. Among these 89 incidents, 72 incidents had remaining food samples for histamine testing. Histamine concentrations varied from below the detection limit to 1 267 mg/100g in these samples, with 8 percent containing < 10 mg/100mg and 12 percent containing < 20 mg/100mg.
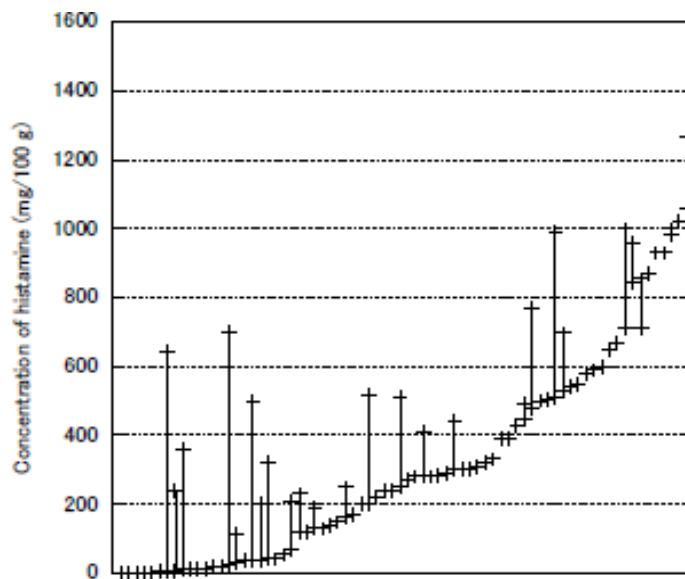
Figure 1. Histamine levels in fish samples associated with the 72 histamine fish poisoning reported in Japan from 1998 to 2008. Each data point refers to one incident.

Regarding the species of implicated fish, in the 89 histamine food poisoning incidents, 29 (33 percent) involved tuna, followed by billfish in 16 (18 percent) and mackerel (13 percent), yellowtail in 9 (10 percent), pacific saury in 8 (9 percent), sardine in 7 (8 percent), horse mackerel and dolphin fish in 3 (3 percent). Histamine concentrations in different fish species involved in 74 histamine food poisoning incidents, for which remaining samples were available, are shown in Figure 2. The highest concentration (1 267 mg/100g) was found in a sample of mackerel seasoned with mirin and then grilled. Generally tuna, billfish and horse mackerel contained relatively high concentrations.

Regarding the preparation/cooking methods of the implicated food, backed and grilled fish comprised 62 percent, followed by fried (21 percent); only 7 percent involved raw fish.
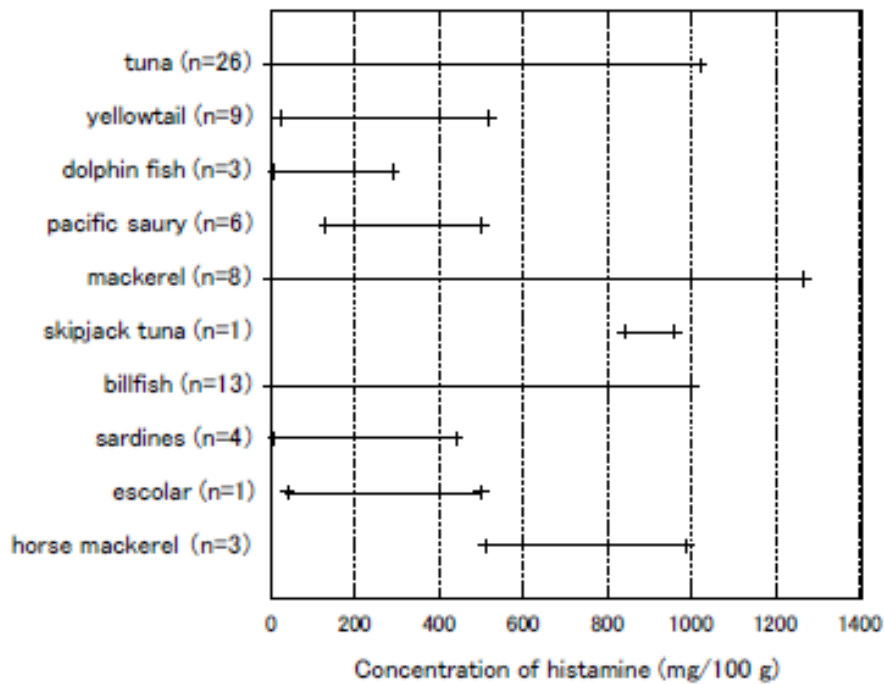
Figure 2. Histamine concentration in different fish species associated with the 74 histamine food poisoning incidents reported in Japan 1998–2008.

2.2 Cohort studies

In 2003, an outbreak of SFP occurred in California, USA at a retreat center. The retreat center provided a list of all workshop attendees. In a retrospective cohort study, 42 (75 percent) of the 56 dinner attendees who ate escolar fish (*Lepidocybium flavobrunneum*) met the case definition. Case-patients ranged in age from 19 to 64 years (median 47 years). There were no significant differences between the 42 case-patients and the 14 well individuals who consumed fish with respect to gender, race, allergy history, chronic medical conditions, or taking medicines other than medication for SFP on 11 August. Case-patients experienced from 1 to 18 symptoms that started immediately after eating the fish to as long as 2 hours afterwards. Acute symptoms lasted from 15 minutes to 24 hours after onset (median, 3 hours). The most common symptoms reported were headache (67 percent), facial flushing (62 percent), palpitations or a rapid pulse rate (57 percent), nausea (48 percent), dizziness (48 percent), and diarrhea (41 percent). Attack rates and relative risks for each food item served at the dinner (Table 3) show that only fish was positively associated with

illness, and all case-patients ate some fish. No other food item was significantly associated with illness.

Table 3. Food-specific attack rates and relative risks in a 2003 outbreak in the USA.

Exposure	Ill among exposed (%)	Ill among unexposed (%)	Relative risk	Confidence interval	Mantel–Haenszel p-value
Mixed greens	34/52 (65)	2/4 (50)	1.3	0.5–3.6	0.54
Poppy seed roll	19/25 (76)	16/24	1.1	0.8–1.6	0.47
Butter	17/20 (85)	19/29	1.3	0.9–1.8	0.13
Corn on the cob	18/26 (69)	17/25	1.0	0.7–1.5	0.93
Escolar fish	42/56 (75)	0/8 (0)	Undefined		< 0.0001
Baby carrots and	29/42 (69)	8/11 (73)	1.0	0.6–1.4	0.81
Brown rice	23/33 (70)	15/22	1.0	0.7–1.5	0.91
Nectarine and blueberry	12/26 (46)	26/31	0.6	0.4–0.9	0.003
Vanilla bean ice cream	11/23 (48)	28/35 (80)	0.6	0.4–0.9	0.01

Individuals who ate at least 2 oz of fish were 1.5 times more likely to develop symptoms than those who ate less (relative risk 1.5, 95 percent CI 0.9–2.6), and to develop more symptoms (median 7 vs 3 symptoms, $p = 0.03$). Patients who took medicine had a longer duration of symptoms than those who did not (median 4 vs 1.5 h, $p = 0.05$), and experienced a greater number of symptoms (median 8 vs 3 symptoms, $p = 0.0002$). Samples of fish contained markedly elevated histamine levels (from 2000 to 3800 ppm). The incubation period and duration of symptoms were not significantly associated with the amount of fish consumed; however, patients who ate less than half a piece of fish experienced fewer symptoms than those who ate half a piece or more (median 3 vs 7 symptoms, $p = 0.03$). The histamine levels in the fish subsamples were markedly elevated at 2 000, 2 700, 2 800 and 3 000 ppm (Feldman *et al.*, 2005).

To ascertain the prevalence and characteristics of SFP in Israel, a retrospective poison center chart review was conducted from January 2005 to December 2007. All consultations of the Israel Poison Information Center (IPIC) were provided by specialized physicians. Data were recorded in a comprehensive structured form that included caller and patient demographic details, route, site and circumstances of exposure, time elapsed until consultation, clinical manifestations via a system-oriented approach, evaluation, management and follow-up recommendations. Exposure and causative agents were classified according to a list of categories, classifications and subclassifications available at the IPIC. Mild SFP was defined as mainly mild dermatological manifestations. Patients with moderate poisoning were symptomatic with mainly gastrointestinal complaints. In severe cases at least one life-threatening

sign was present (hypotension, bronchospasm, angioedema, etc.). During the study period, 21 events of scombroid poisoning involving 46 patients were recorded. Tuna was the commonest fish consumed (84.7 percent). Clinical manifestations developed within 20 minutes in 65.2 percent of the patients. The main clinical manifestations included rash (41 percent), flushing (37 percent), gastrointestinal complaints (37 percent) and headache (30.4 percent). About 25 percent had abnormal vital signs; two patients developed hypotension (Lavon *et al.*, 2008).

2.3 Factors influencing sensitivity to histamine

Individualized susceptibility to SFP has been observed in multiple epidemiological studies and healthy volunteer challenge tests. It is generally accepted that the ability to tolerate histamine exposure can be compromised when histamine metabolizing enzymes are impaired. The factors associated with increased sensitivity to histamine have been summarized in a recent report on biogenic amines (EFSA, 2011). These include the following.

- Food allergy, defined as an immune-mediated hypersensitivity to ingested allergens (Raithel *et al.*, 1999).

- Histamine intolerance, which results from reduced histamine degradation due to low DAO activity or quantity; both could be associated with genetic polymorphism of the enzyme (Garcia-Martin *et al.*, 2009). About 1 percent of the general human population has histamine intolerance; 80 percent of these are middle-aged female patients (Maintz and Novak, 2007).

- Certain physiological states can also modify the sensitivity to biogenic amines.
  - In women, a premenstrual decrease in the activity of B-type MAO may cause hypersensibility to both histamine and tyramine (Bardocz, 1995). Clinical observations indicate that women are more sensitive to histamine on days 12 to 16 of the menstrual cycle (Kalogeromitros *et al.*, 1995).
  - In contrast a physiological increase of DAO production (up to 500-fold) has been reported in pregnant woman, which would explain remissions of food intolerance frequently observed during pregnancy (Mainz and Novak, 2007).

- Individuals with chronic urticaria, atopic eczema, respiratory and coronary problems or those suffering from hypertension or vitamin B6 deficiency are particularly sensitive because of their sensitivity to lower doses of biogenic amines (Mainz and Novak, 2007).

- Gastrointestinal conditions with altered enterocytes as well as inflammatory and neoplastic diseases (gastritis, irritable bowel syndrome, Crohn's disease, colorectal

neoplasia, stomach and colonic ulcers) may elevate sensitivity because of the lower activity of oxidases in the intestine compared withg healthy individuals (Jarisch, 2004; Mainz and Novak, 2007).

- Tobacco smoke reduces MAO levels by up to 40 percent and several cigarette smoke compounds have been shown to inhibit MAO enzyme activities (Broadley, 2010).

- Certain food components may also compromise the ability of detoxifying enzymes (MAO, DAO, HMT) to degrade ingested amines, such as other amines, alcohol and its metabolite acetaldehyde, and phenols (J. Y. Hui and Taylor, 1985; Zimatkin and Anichtchik, 1999).

There is suggestive evidence that the severity of the symptoms and the incidence of SFP may be age-related (Iannuzzi, 2007; FDA, 2011).

3. Dose–response assessment

3.1. Histamine as the exposure marker in SFP

Though other biogenic amines such as cadaverine and putrescine might also play a role in the etiology of SFP, there are no dose–response data for the co-administration of either biogenic amine with histamine in laboratory animals or humans. In most epidemiological studies, SFP is associated with abnormally high histamine levels in the incriminated fish. Therefore, histamine is considered the most appropriate marker of dose in this assessment.

3.2. Study selection for dose–response assessment

While there have been a number of reports in the scientific literature of human scombroid poisonings, the vast majority of these are case reports of generally a few cases and in a few instances of multiple cases of more than 100 subjects. The difficulty with the use of case reports in a dose–response assessment is that the recapitulation of the dosage/exposure level in these studies is almost impossible to determine. Crude measures have been used to estimate the dose/exposure level by using levels detected in samples of the suspect fish and/or the recall of the patients of the amounts of fish consumed. These estimates of exposure/dosage are highly uncertain and cannot be used to construct a quantitative assessment of dose versus adverse response.

In regards to the few published retrospective studies, there are important limitations including reliance on voluntary reporting, limited follow-up, and the lack of data on histamine levels or those of any other biogenic amines in fish samples consumed by the subjects, or in their biologic fluids. However, the typical histamine-like clinical

manifestations seen in SFP together with temporal proximity to consumption of fish known to be involved in scombroid poisoning supports the diagnosis of biogenic amine poisoning. The critical endpoint in acute histamine intoxication is a spectrum of symptoms including headache, flushing, itching and urticaria.

The other major hurdle in the quantitative use of these studies is the uncertainty associated with the lack of understanding of whether histamine is the sole responsible etiological toxin(s), whether it is a surrogate of another toxin(s), or whether histamine is working in concert with other biogenic amines or as yet unidentified chemicals in the fish, and what the nature of that relationship is (e.g. additive, synergistic). Histamine levels within fish appear to correlate well with the clinical toxicity of SFP, but an equivalent dose of orally administered pure histamine does not produce the same spectrum of symptoms as seen in SPF. Even though there are several plausible hypotheses which attempt to explain this paradox, the mechanism of toxicity in SFP remains unclear.

To study health effects of histamine in humans, a number of volunteer challenge studies have been conducted. Many studies were designed to investigate the minimal dose of histamine that causes SFP or histamine intolerance symptoms, or the maximum dose of histamine that can be taken without causing these symptoms. Most of these studies are well-designed randomized trials, in which the doses were well controlled and the symptoms were carefully monitored by medical professionals. Therefore, data from these human trials should reflect the histamine–SFP dose–response relationship better than data from case reports.

Histamine challenge studies in humans are summarized in the EFSA biogenic amine report (EFSA, 2011) and the "Seafood Biogenic Amine Database" (Emborg and Dalgaard, 2007). In these studies histamine was administrated with different food matrices and given to healthy or susceptible volunteers, usually in a controlled, blinded study design. The EFSA report includes all human studies regardless of the route of exposure and the food matrices in which histamine was administered, while the "Seafood Biogenic Amine Database" only includes those oral toxicity studies in which fish was used as the food matrix (Table 4).

Among the five studies listed in Table 4, three studies (Clifford *et al.*, 1989; Clifford *et al.*, 1991; Ijomah *et al.*, 1991) failed to establish that histamine was the causative

agent of the SFP symptoms, and therefore these were excluded from the dose–response assessment.

Table 4. Published histamine oral challenge studies (from Emborg and Dalgaard, 2007).

Food or medium	Histamine (mg)	Potentiator (mg)	Symptoms	References
Grapefruit juice	100	None[a]	Mild headache (1 of 4 volunteers)	(Motil and Scrimshaw 1979)
High quality tuna fish	100	None[a]	Mild headache and flushing (2 of 8 volunteers)	(Motil and Scrimshaw 1979)
Grapefruit juice	180	None[a]	Severe headache and flushing (1 of 4 volunteers)	(Motil and Scrimshaw 1979)
High quality tuna fish	180	None[a]	Severe headache and flushing (2 of 8 volunteers)	(Motil and Scrimshaw 1979)
Hot smoked mackerel from HFP outbreak	68	Not reported	Mild flushing (1-2 of 9 volunteers)	(Clifford et al. 1989)
Hot smoked mackerel (fresh)	300	None[a]	Headache (4 of 7 volunteers), flushing (2 of 7 volunteers), oral tingling (5 of 7 volunteers)	(Clifford et al. 1989)
Hot smoked mackerel (spoiled)	300	Not reported	Headache (1 of 7 volunteers), flushing (1 of 7 volunteers), oral tingling (5 of 7 volunteers)	(Clifford et al. 1989)
Mackerel spoiled and hot smoked	20	Not reported	Vomiting and/or diarrhoea (1 of 9 volunteers)	(Ijomah et al. 1991)
Mackerel spoiled and hot smoked	150	Not reported	Vomiting and/or diarrhoea (2 of 9 volunteers)	(Ijomah et al. 1991)
Hot smoked mackerel from HFP outbreak	28-66	Not reported	Diarrhoea, headache, flatulence, stomach ache, nausea or oral tingling	(Ijomah et al. 1991)
Hot smoked mackerel from HFP outbreak	62±29	Cad (6 ± 4) Tyr (3 ± 3)	Nausea/vomiting and/or diarrhoea. Other symptoms were not reported	(Clifford et al. 1991)
Herring, fresh with added histamine	90	< 1 mg cad and put	Warm face, flushing or headache (2 of 8 subjects)	(van Gelderen et al. 1992)
Herring spoiled by photobacteria	88	22 mg cad, 18 mg put	Warm face and flushing or headache (1 of 8 subjects)	(van Gelderen et al. 1992)

a) Assumed

3.3. Derivation of a no-observed-adverse-effect limit (NOAEL)

Data used to characterize the dose–response relationship between histamine in fish and SFP-like symptoms are presented in Table 5. Based on these data, a NOAEL of 50 mg histamine is identified, which is consistent with that established by EFSA. The threshold toxic dose for the histamine challenge studies appears to be about 90 mg (Table 5). However, the threshold toxic dose for histamine in SFP is not known.

Table 5. Human oral dose–response relationship for histamine in fish.

Histamine dose (mg)	Food ingested	Number of subjects	Number of subjects showing symptoms	Reference
25	Tuna	8	0	Motil and Scrimshaw, 1979
45	Herring	8	0	Van Gelderne *et al.*, 1992
50	Tuna	8	0	Motil and Scrimshaw, 1979
90	Herring	8	2	Van Gelderne *et al.*, 1992
100	Tuna	8	2	Motil and Scrimshaw, 1979
150	Tuna	8	2	Motil and Scrimshaw, 1979
180	tuna	8	6	Motil and Scrimshaw, 1979

It is important to bear in mind that while the NOAEL is an appropriate hazard threshold value to use for exposures in healthy subjects, this may not be the case for those members of certain segments of the population who may have an increased sensitivity (e.g. metabolic differences, physiological conditions, drug therapies). In these instances a lower hazard level (e.g. the use of an uncertainty factor of 10) or other specific risk management options such as fish consumption advisories should be considered.

3.4. Derivation of a benchmark dose (BMD)

As an alternative to the NOAEL methodology, the BMD methodology is also commonly used to derive a threshold value in risk assessment. Unlike the NOAEL approach, the BMD approach uses the whole range of available dose–response data by fitting mathematical models to the dataset to derive an estimate of the threshold dose corresponding to a predetermined level of extra which is normally a 10 percent extra risk. The resulting BMD estimate, BMD_{10}, is the central estimate of the dose that corresponds to the additional risk. The lower 95 percent confidence limit of the BMD ($BMDL_{10}$) is calculated to address and account for uncertainties in the estimate of BMD due to the experimental design (e.g. small sample size).

The US Environmental Protection Agency's BMD modelling software (BMDS 2.2) was used to determine benchmark doses for histamine. Using a 10 percent extra risk, data from Table 5 were analyzed using multiple dichotomous models (Logistic, LogLogistic, Weibull, Probit, LogProbit). The calculated BMD_{10} and $BMDL_{10}$ are presented in Table 6. Two measurements of goodness of fit (GOF), the *p*-value and the Akaike information criterion (AIC) value are also presented in Table 6.

Table 6. Results from the BMD dose–response modelling.

Model	BMD_{10}	$BMDL_{10}$	p	AIC
Logistic	85.7	61.1	0.53	44.63
LogLogistic	78.7	50.5	0.64	43.82
Weibull	79.6	49.6	0.64	43.74
Probit	83.1	58.4	0.58	44.20
LogProbit	78.1	51.9	0.67	43.43

As shown in Table 6, all models attained an acceptable goodness of fit ($p > 0.1$), and produced similar BMD_{10} and $BMDL_{10}$. The Weibull model gave the most conservative $BMDL_{10}$ (49.6 mg, rounded to 50 mg) of the five models, and the best overall goodness of fit ($p = 0.64$, AIC = 43.74). In addition, the Weibull model is biologically relevant for use in the dose–response modelling of histamine, which is a product of micro-organism activity. Figure 3 shows the Weibull model fit to the dose–response data in Table 5.

Figure 3. Weibull model of combined data from fish–histamine human challenge studies.
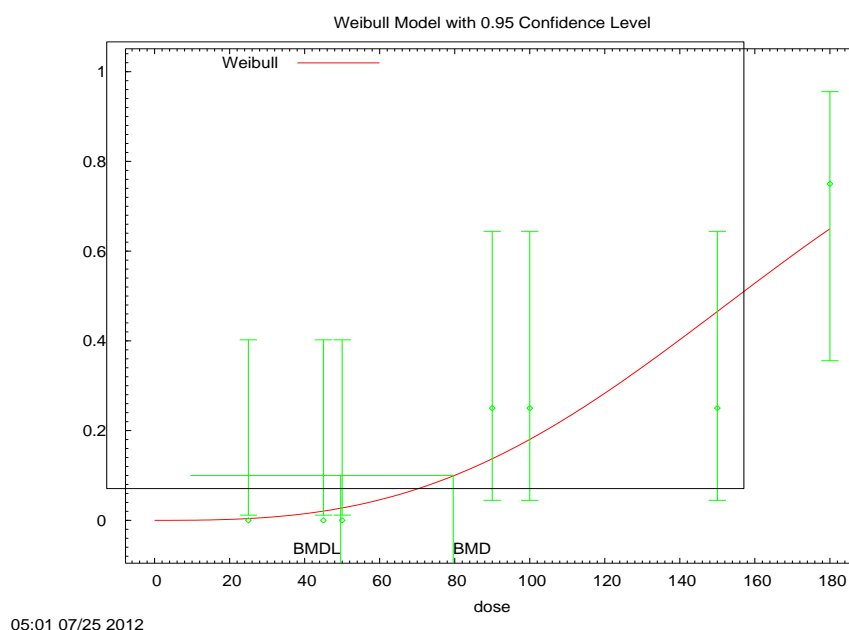
Since the study of Motil and Scrimshaw (1979) had more dose groups (five doses) than the Van Gelderen (1992) study (two doses), separate BMD modelling was performed using data from Motil and Scrimshaw (1979) only. The BMD_{10} and $BMDL_{10}$ resulting from this assessment were very close to those produced in the assessment of the combined dataset of these two studies. For the Weibull model, the $BMDL_{10}$ was 47.7 mg, as compared to 49.7 mg from the assessment of the combined dataset.

4. Recommended studies

The reduction of the uncertainty surrounding the critical role played by histamine in the pathogenesis of SFP is needed. Studies that will clarify the mechanistic role and the quantitative relationship of histamine to the spectrum of adverse effects seen in SFP are deemed to be essential.

Evidence suggests that potentiators alter the threshold toxic dose for histamine in contaminated fish. The elucidation of the quantitative relationship between the dose of histamine and other biogenic amines and the various adverse effects is needed. Ideally such work would be derived from studies of human volunteers, but the likelihood that they can be conducted may be limited. In lieu of human studies, appropriate animal models and studies, such as in the swine, should be investigated. In these studies, the emphasis should be on a dose range between the NOAEL and the low levels associated with the onset of mild symptoms.

Beyond the three known potentiators (cadaverine, putrescine and tyramine), studies are needed to characterize and identify currently unknown potentiators, e.g. other biogenic amines.

Further studies are needed on the identity and mechanisms of action of potential histamine potentiators. The types and levels of the potentiators may vary depending on a variety of factors, including the types of microflora, the metabolic capabilities of the microflora, the natural constituents of the fish, and the conditions of spoilage.

Other studies that are deemed to be helpful would be those that consider and investigate the various factors that may enhance sensitivity of the response to SFP in various populations and would include study of the role of:

- genetic polymorphism in histamine metabolism;
- certain physiological states/conditions such as menstruation;
- gastrointestinal tract diseases;
- certain medications;
- certain lifestyle practices such as smoking and alcohol consumption in altering biogenic amine metabolism.

Acknowledgement

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