Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research report)

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Abstract

The Joint FAO/WHO/IOC ad hoc Expert Consultation on Biotoxins in Molluscan Bivalves performed risk assessments for a number of biotoxins present in bivalve molluscs. For performing risk assessments, the Expert Consultation categorized the biotoxins into eight distinct groups based on chemical structure. The Expert Consultation established LOAELs for the azaspiracid (AZA), okadaic acid (OA), saxitoxin (STX), and domoic acid (DA) toxin groups. The derived provisional acute RfDs for the AZA, OA, STX, and DA toxin groups were 0.04 µg/kg bw, 0.33 µg/kg bw, 0.7 µg/kg bw, and 100 µg/kg bw, respectively. For the yessotoxin (YTX) group, a NOAEL was established, based on animal studies. Applying a safety factor of 100, a provisional acute RfD of 50 µg/kg bw was suggested for the YTX group. The Expert Consultation considered that the database for cyclic imines, brevetoxins, and pectenotoxins was insufficient to establish provisional acute RfDs for these three toxin groups.

Keywords: Marine biotoxin; Provisional acute reference dose (RfD); Azaspiracid (AZA); Domoic acid (DA); Okadaic acid (OA); Saxitoxin (STX)

1. Introduction

Bivalve shellfish are a major internationally traded seafood commodity. Specific control measures have been implemented to prevent food-borne illness associated with marine biotoxins in bivalves. One control measure is the establishment of maximum levels of marine toxin in bivalves, and several countries have set national and regional regulatory levels. However, no international maximum standard level for marine biotoxin has been established.

Codex is the international standard-setting intergovernmental body, and the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) benchmarked Codex standards as the international standards for food safety. Food safety and health aspects of Codex standards and related texts are based on risk analysis. Within the framework of the Codex Alimentarius Commission and its procedures, the responsibility for providing recommendations on risk management lies with the Commission and its subsidiary bodies (risk managers), e.g., for fish and fishery products, the Codex Committee on Fish and Fishery Product (CCFFP) is the risk manager, while the responsibility for risk assessment lies primarily with the joint FAO/WHO expert bodies and consultations (risk assessors) (Codex, 2005a).

During its 25th session, the CCFFP requested FAO and WHO to provide scientific advice on biotoxins in conjunction with its work on Proposed Draft Standards for Live and Processed Bivalve Molluscs. The CCFFP, during its 26th session, elaborated the following specific questions:

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels of shellfish toxins in shellfish;
- Provide guidance on methods of analysis for each toxin group;
• Provide guidance on monitoring of biotoxin-forming phytoplankton and bivalve molluscs (including sampling methodology);
• Provide information on geographic distribution of biotoxin-forming marine phytoplankton.

To address this request, FAO/WHO/IOC agreed to organize a joint FAO/IOC/WHO workshop on biotoxins in bivalve molluscs in Dublin in March 2004 to identify the scope, content of the work, candidates for the electronic drafting groups, and information needed to compile scientific advice to be discussed during the Expert Consultation session. In May 2004, three working groups were established to develop drafts on (1) analytical methods, (2) toxicological aspects, and (3) marine biotoxin management programmes. The working groups examined available relevant information and prepared technical documents. From September 26th to 30th, 2004, the Expert Consultation met in the National Veterinary Institute (Oslo, Norway) to review the technical reports drafted by the working groups, and prepare the scientific advice and recommendations for the CCFFP.

Historically, marine biotoxins were classified based on the clinical symptoms they caused. However, at the Dublin meeting, the workshop agreed to classify the toxins into eight groups based on chemical structure [i.e., azaspiracid (AZA) group, brevetoxin group, cyclic imines group, DA (DA) group, okadaic acid (OA) group, pectenotoxin (PTX) group, saxitoxin (STX) group, and yessotoxin (YTX) group] because chemical classification is more appropriate for the analytical purposes of the Codex standards.

Although the Expert Consultation covered risk assessments, analytical methods, and monitoring of biotoxin-forming phytoplankton and bivalve molluscs, this report focuses on the risk assessment.

2. Approach taken

2.1. Risk assessment

The Expert Consultation was asked to perform risk assessments for a number of biotoxins present in bivalve molluscs. Since exposure generally involves only occasional consumption, and because most of the available toxicological data involve only acute and short-term studies, priority was given to the establishment of an acute reference dose1 (acute RfD) based on the standard JMPR procedure. Although more frequent exposure also may occur, the Expert Consultation could not establish tolerable daily intake2 (TDI) values, due to a lack of relevant toxicological data. The size of the safety factor is a crucial issue when deriving either acute RfD or TDI from the relevant toxicological information. Generally, default values of 10 and 100 are used, based on human and animal data, respectively. A reduced safety factor between 1 and 10 for human data may be used depending upon the magnitude and severity of the effect, the amount of information, and the variety of the populations included in the human data.

The risk assessments for the individual toxin groups were performed in a stepwise fashion, including hazard identification, hazard characterization, exposure assessment, and risk characterization.

An adverse health effect is more likely in susceptible individuals who consume large amounts of contaminated shellfish. Occurrence data were not available to allow the consultation to conduct a probabilistic risk assessment. The Expert Consultation recognized that regulatory limits already established within existing monitoring plans minimize the probability of adverse health effects.

2.2. Intake and exposure

Because of large seasonal variations, the frequency of consumption and the number of consumers should be determined on a one-year basis. Within the entire population, 35% consume bivalve molluscs in Norway (Fish and Game study 1999) and in France (SECODIP3 1999). With shorter surveys, this percentage is 11% in France (7 days), 8% in Italy (7 days), 4% in the US (2 days), 3% in New Zealand (1 day), and 2% in Australia (1 day). In France the frequency of bivalve mollusc consumption is 4.2 eating occasions per year. In the US, the frequency of consumption is 8.6 eating occasion per year (USFDA – Market Research Corporation of America). In Norway, 33% of consumers eat bivalve molluscs between 1 and 11 times a year and 2% of consumers eat these molluscs between 1 and 8 times per month.

Short-term dietary intake assessment is conducted to obtain the estimated toxin intake over a single day or for a single eating occasion. The procedure used by JMPR for acute toxicity of pesticide residues employs the WHO/GEMS Food Database, which has compiled the highest reported 97.5th percentile consumption figures for “eaters only” for each single food category. For bivalve molluscs, a large portion corresponds to 380 g for adults (Netherlands). The conservatism of this figure is confirmed by additional information received from Member States about the 97.5th percentile consumption figures for edible shellfish portions by adults, which are, respectively, 133 g in Japan, 181 g in Australia, 225 g in the US, and 263 g in New Zealand. A maximum consumption level of 182 g has been reported in Norway. For children, the highest reported 97.5th percentile consumption figures were 70 g (Australia) and 27 g (Japan).

1 The acute RfD is an estimate of the amount of a substance in food, normally expressed on a body-weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer, on the basis of all known facts at the time of evaluation (JMPR, 2002).
2 An estimate of the amount of substance in food, normally expressed on a body-weight basis (mg/kg or μg/kg of body weight), that can be ingested daily over a lifetime without appreciable health risk.
3 SECODIP = Société d’Études de la Consommation, de la DIstribution et de la Publicité.
3. Toxin group specific section

3.1. Azaspiracid (AZA) group

3.1.1. Background information
The syndrome later named azaspiracid poisoning (AZP) was reported for the first time in 1995 among consumers in The Netherlands after eating blue mussels from Ireland. The symptoms were similar to those of diarrhoeic shellfish poisoning (DSP), but the concentration of the DSP toxins was low. Subsequently, the azaspiracid toxin group was discovered. AZAs have thus far been detected only in Europe. The EU has set a regulatory level of 0.16 mg/kg using the mouse bioassay (MBA) as the reference method. However, an MBA protocol with adequate specificity or detectability has not been validated.

3.1.2. Toxicity in animals
Preliminary experiments indicate that AZA 1, administered once or twice by gavage at dose levels of 250–450 µg/kg bw, caused death in some mice and serious gastrointestinal, pulmonary, and hepatic effects that persisted for a prolonged period in those that survived (Ito et al., 2002). In a preliminary long-term experiment, repeated administration of 20 µg/kg bw once or twice a week by gavage for 10–20 weeks caused death in some mice, and doses of 5–20 µg/kg bw caused a statistically insignificant increased incidence of lung tumours at one year in survivors (Ito et al., 2002). Because the strain of mouse used in this experiment normally has a high background incidence of pulmonary as well as hepatic tumours, these results may indicate that AZA is carcinogenic, or more probably, that it is a tumour promoter. No genotoxicity data are available and no definitive conclusions regarding relevance to humans can be drawn.

No oral toxicity data are available on AZA analogues, but intraperitoneal administration: i.p.) studies in mice suggest that AZA 2 and 3 are somewhat more toxic than AZA 1, and that AZA 4 and 5 are less toxic (Ofuji et al., 1999a, 2001).

3.1.3. Human epidemiological data
In November 1995, at least eight people in The Netherlands became ill after eating mussels (Mytilus edulis) cultivated at Killary Harbour, Ireland. Although human symptoms such as nausea, vomiting, severe diarrhoea, and stomach cramps were similar to those of diarrhoeic shellfish poisoning (DSP), contamination by the major DSP toxins okadaic acid (OA) and dinophysistoxins (DTXs) were very low. These observations prompted the investigators to identify the causative toxin in the mussels by structural studies. After chemical analytical research, the investigators identified and quantified AZA (Satake et al., 1998a,b). Based on these results, the toxicity of the mussels was estimated to be 0.15 mouse units (MU)/g (equivalent to 0.6 µg AZA/g) (EU/SANCO, 2001). A higher toxin content of 1.4 µg AZAs/g of meat (0.4 MU/g of meat) was reported by Ofuji et al. (1999b). Human toxicity was observed between 6.7 (5%) and 24.8 (95%) µg/person with a mean value of 15 µg/person. However, new data on the heat stability of AZA suggest that a reduction in AZAs concentration due to heating is not appropriate. Therefore, the recalculated range of the LOAEL is 23–86 µg per person with a mean value of 51.7 µg/person (EU/SANCO, 2001).

3.1.4. Evaluation
The Expert Consultation established a provisional acute reference dose (RfD) of 0.4 µg/kg bw, based on the LOAEL of 23 µg per person in humans and a body weight of 60 kg, using a 10-fold safety factor because of the small number of people involved.

The Expert Consultation insufficient data on the chronic effects of AZA prevented the establishment of a TDI.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would result in a derived guidance level of 0.0096 or 0.0063 mg/kg, respectively.

3.2. Brevetoxin group

3.2.1. Background information
The dinoflagellate Karenia brevis, which causes red tides annually along the Gulf of Mexico off of the Florida coast, produces neurotoxins named brevetoxins. In humans, brevetoxins induce acute gastrointestinal and neurologic symptoms after ingestion of contaminated shellfish (oysters, clams). The syndrome was named neurotoxic shellfish poisoning (NSP). Recent history has shown that brevetoxin-producing algae, and consequently NSP, have expanded well beyond the Gulf of Mexico. In 1987, a major Florida K. brevis bloom was dispersed by the Gulf Stream northward into waters of North Carolina, US, where 48 people became ill with NSP. Karenia brevis has continued to be observed in North Carolina waters. In 1992/1993, more than 180 people became ill in the first recorded outbreak of NSP in New Zealand. Neither the algal species responsible for this outbreak nor the circumstances surrounding its presence was determined. An extensive review concluded that K. mikimotoi was the most likely causative agent, but at least three other suspect species were present at the time (Todd, 2002). No human fatalities associated with consumption of shellfish contaminated with brevetoxins have been reported. Brevetoxins have been responsible for the death of fish and some marine mammals. Currently, the APHA protocol for the mouse bioassay of an ether extract of shellfish is the basis for regulation of shellfish.
The regulatory limit presently is set at 20 mouse units per 100 g of shellfish tissue (1 MU = 4.0 μg PbTx-2) in the US (US FDA, 2001).

3.2.2. Biological data

Brevetoxins are rapidly absorbed and distributed throughout the body (including throughout the CNS). They are metabolised in the liver. They have a very short serum half-life of less than one minute, but total body clearance appears to be much more prolonged (up to 6 days). Brevetoxins are excreted both in the urine and bile.

Brevetoxins bind to the alpha-subunit of the voltage-sensitive sodium channel, resulting in sustained sodium influx and consequent depolarisation of neural membranes.

3.2.3. Toxicity in animals

In mice i.p. LD₅₀ values after i.p. injection, oral gavage, and feeding in fasted mice are 100, 755, and >7500 μg/kg bw for PbTx-3, respectively (Munday et al., 2004b); an LD₅₀ value of 1005 g/kg in mice fed ad libitum also has been reported (Munday et al., 2004b). All values indicate high toxicity. No data exists on the oral toxicity of the pinnatoxins, prorocentrolide, or spirocentrimine. No information on the subacute or chronic toxicity of any of the cyclic imines is available.

3.3. Cyclic imines group

3.3.1. Background information

The cyclic imines group includes gymnodimine, spirolides, pinnatoxins, prorocentrolide, and spirocentrimine. The presence of these compounds in shellfish was discovered because of their very high acute toxicity in mice upon i.p. injections of lipophilic extracts. When present at elevated levels, the toxins rapidly killed mice, and their presence may interfere with the MBA for OA, brevetoxins, and AZA groups. At sublethal doses, the exposed mice recovered rapidly. The toxic potential of the cyclic imines is much lower via the oral route. The regulatory significance of the cyclic imine toxins remains unclear. Although gymnodimine and spirolides are known to commonly occur in microalgae and/or bivalve molluscs from several parts of the world (Canada, Denmark, New Zealand, Norway, Scotland, Tunisia and the United States of America), no adverse effects in humans have been reported.

3.3.2. Biological data

There are no absorption, distribution, metabolism, and excretion data available for any of the cyclic imines. The cyclic imines are fast-acting toxins. The imine function is essential for toxicity but detailed information on the mechanism(s) of action is not available.

3.3.3. Toxicity in animals

All of the cyclic imines for which data are available are toxic to mice after i.p. administration. For gymnodimine, LD₅₀ values after i.p. injection, oral gavage, and feeding in fasted mice are 100, 755, and >7500 μg/kg bw, respectively (Munday et al., 2004a). For desmethyl spirolide C, the respective values are 6.5, 157, and 500 μg/kg bw, respectively (Munday et al., 2004b); an LD₅₀ value of 1005 g/kg in mice fed ad libitum also has been reported (Munday et al., 2004b). All values indicate high toxicity. No data exists on the oral toxicity of the pinnatoxins, prorocentrolide, or spirocentrimine. No information on the subacute or chronic toxicity of any of the cyclic imines is available.

3.3.4. Human epidemiological data

There is no evidence of a harmful effect in consumers of shellfish contaminated with gymnodimine from New Zealand or Tunisia. Gastric distress and tachycardia were associated with spirolide-contaminated mussels in Canada, but the causative agent was not a spirolide. In Japan and China, some poisoning episodes were initially attributed to pinnatoxin, but later were shown to be due to Vibrio species. Consequently, there is no evidence that any of the cyclic imines have toxic effects in humans.

3.3.5. Evaluation

The Expert Consultation considered that the database was insufficient to establish an acute RfD or TDI for the cyclic imines.
3.4. Domoic acid (DA) group

3.4.1. Background information

Domoic acid (DA) was identified as the toxin responsible for an outbreak of illness in Canada in 1987, caused by eating blue mussels that had accumulated DA as a result of the presence of *Pseudo-nitzschia pungens*. Effects on both the gastrointestinal tract and nervous system were observed. Since some of the people affected experienced memory loss, the syndrome was named amnesic shellfish poisoning (ASP). As a result of this episode in Canada, a regulatory level of 20 mg DA/kg of shellfish meat was established, and no further incidences of ASP have been reported. The presence of DA in shellfish has been reported throughout the world. Numerous reports of toxicity in a variety of wildlife species indicate that DA moves up the food chain in marine ecosystems. Routine monitoring using LC-UV is well established in most monitoring programs and the detection limits are adequate to monitor and regulate DA at current limits.

3.4.2. Biological data

Absorption, Distribution, Metabolism and Excretion

The oral absorption of DA is 5–10% of the administered dose in all species studied, including non-human primates. DA is mainly distributed to the blood compartment (volume of distribution ～0.25 l/kg) and studies indicate that very poor penetration of the blood-brain barrier occurs in normal animals. Consequently, any condition that impairs blood-brain barrier integrity confers additional risk. No evidence exists that DA is metabolized; it appears to be excreted in the urine nearly unchanged with an elimination half-life ranging from 20 min in rodents to 114 min in monkeys. Impaired renal function results in significant increases in serum concentration and residence time, conferring additional risk. DA produces excitotoxicity by activation of glutamate receptors leading to excess accumulation of calcium, resulting in cell death. A subclass of glutamate receptors, the kainate receptors, is the primary target.

3.4.3. Toxicity in animals

Acute administration (i.p., i.v., and p.o.) of DA in experimental animals causes dose-dependent toxicity with predictable behavioural and histopathological sequelae that are consistent across species. No immediately observable adverse effects were seen in a 15-day study in monkeys given 0.5 mg DA/kg bw by gavage (Truelove et al., 1997).

No evidence of cumulative toxicity on repeat exposure or of genotoxicity exists. No studies have been published on long-term toxicity or carcinogenicity.

There is evidence in rodents that s.c. exposure to sub-convulsive doses of DA in utero or in neonatal animals results in immediate and long-term alterations in electrical discharges and learning behaviour, with newborn rats being at least 40 times more sensitive than adults to DA toxicity (Xi et al., 1997).

3.4.4. Human epidemiological data

Late in 1987, an outbreak of a newly recognized acute illness caused by eating blue mussels and characterized by gastrointestinal and unusual neurological symptoms occurred in Canada. More than 107 people (47 men and 60 women) were affected, most from Quebec. A case was defined as the presence of gastrointestinal symptoms (vomiting, abdominal cramps, diarrhoea) within 24 h and neurological symptoms within 48 h (severe headache and memory loss) of eating the mussels. Theetiologic agent was DA, an excitatory neurotransmitter amino acid, produced by *Nitzschia pungens f. multiseries* (now called *Pseudo-nitzschia multiseries*). Both *N. multiseries* and DA were present in the digestive glands of the associated cultivated mussels, harvested from the eastern coast of Prince Edward Island, and shipped to other parts of Canada (Perl et al., 1990a,b; Teitelbaum et al., 1990). For the nine patients and one person who did not become ill, analytical information on the unconsumed portion of the mussels and recall information on portion size were available, and this was used to estimate exposure. The concentration of DA in these mussels was determined by mouse bioassay (characteristic hindleg scratching), and ranged between 31 and 128 mg/100 g shellfish. Increasing exposure correlated with the clinical course of events (Table 1). All patients reported gastrointestinal illness, but only one of six patients who consumed between 60 and 110 mg DA suffered memory loss; none required hospitalization. All three patients who had consumed 270–290 mg DA suffered neurological symptoms and were hospitalized. One person who consumed only 20 mg DA did not become ill. The cognitive impairment observed in this new disease, attributed to DA, appeared persistent and led to the term ‘amnesic shellfish poisoning’ (Perl et al., 1990a,b; Todd, 1990). Based on this dose response relationship, and assuming a body weight of 60 kg, the LOAEL in humans was estimated to be 1 mg/kg bw, and no ill effects were observed in a person who consumed 0.33 mg/kg bw.

3.4.5. Evaluation

The results of the first outbreak of amnesic shellfish poisoning that occurred in 1987 in Canada, provide the best basis for developing an acute reference dose (tolerable single day intake, acute TD1). During this outbreak, a dose-related increase in the severity of signs and symptoms was observed in patients consuming between 1 mg/kg bw (the LOAEL) and 5 mg/kg bw. Studies in rodents and cynomolgous monkeys have generally supported these findings.

To cover the full spectrum of intra-human susceptibility, and account for the fact that 1 mg/kg bw was a LOAEL, this value was divided by a safety factor of 10, to derive a provisional acute reference dose of 0.1 mg/kg bw. This value seems reasonable, as one person who consumed 0.33 mg/kg bw did not become ill. The provisional acute reference dose of 0.1 mg/kg bw provided the basis for the establishment of the maximum residue limit (MRL) for
DA by Canadian authorities, which on the basis of an intake of 250 g shellfish and a body weight of 60 kg, was 24, rounded down to 20 \( \mu \)g DA/g shellfish. If instead of 250 g shellfish, a value of 300 g shellfish was used, the MRL would be exactly 20 \( \mu \)g DA/g shellfish.

Very few animal studies have been conducted on the subchronic and chronic toxicity of DA, and these limited data suggest that cumulative effects of low doses of DA are unlikely. In this regard, studies based on subacute mouse studies revealed no differences in behavioural toxicity scores upon re-exposure to DA compared to a single dose (i.e., behavioural equivalent of kindling). The available data indicate that chronic sequelae such as epilepsy and memory deficit were observed only in those patients who had suffered severe acute neurological effects (examined up to 3.5 years post-event) after they had ingested a single high dose of DA. It is therefore unlikely that people who habitually consume small amounts of DA (exposures less than 0.1 mg DA/kg bw) would experience any chronic effects. Thus, this acute RfD also may be considered a provisional chronic TDI.

For chronic effects, the available toxicity data are not sufficient to support the derivation of a TDI. Pregnant women, infants, and children, people with premorbid pathology, and elderly adults (>65 years of age) may be more susceptible.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would lead to a derived guidance level of 24 or 16 mg DA/kg shellfish meat, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Estimated weight of mussels consumedb (g/person)</th>
<th>Domoic acid in sample (mg/100 g)</th>
<th>Estimated domoic acid consumed (mg/person)</th>
<th>Clinical symptoms and treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>60</td>
<td>35</td>
<td>52</td>
<td>20</td>
<td>- + - -</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>120</td>
<td>52</td>
<td>60</td>
<td>+ - + -</td>
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<td>150</td>
<td>45</td>
<td>70</td>
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<td>31</td>
<td>90</td>
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<td>290</td>
<td>+ + + +</td>
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<td>84</td>
<td>375</td>
<td>76</td>
<td>290</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

a Adapted from Perl et al. (1990a,b).
b From patient’s recall or estimated as 375 g when amount consumed was not known.
c GI refers to gastrointestinal symptoms.

dominoic acid (OA) group

#### 3.5.1. Background information

Toxins from the okadaic acid group have been known to cause human illness since the late 1970s. The syndrome was named diarrhoeic shellfish poisoning (DSP) due to the dominant symptom. The OA group has been detected in microalgae and/or bivalve molluscs throughout the world. Analyses for this group have been a key part of many bio-toxin monitoring programs. However, contamination by the OA group generally occurs along with other lipophilic toxins, which can cause false positives in animal bioassays and require confirmatory testing to evaluate actual risks. A regulatory level of 0.16 mg OA-eq/kg shellfish has been implemented in some countries.

#### 3.5.2. Biological data

Data are limited but indicate limited absorption after oral administration in mice with a relative distribution of intestinal content > urine > feces > intestine tissue > lung > liver > stomach > kidney > blood. OA can be detected in blood and some organs for several weeks following exposure. No data on metabolism in vivo have been reported. OA and DTX-1 and -2 are potent inhibitors of the serine/threonine protein phosphatases 1 and 2A.

#### 3.5.3. Toxicity in animals

The lethal dose following oral administration of DSP toxins is 3–6 times greater than the lethal dose obtained after i.p. administration. DTX1 and DTX3 have a toxicity pattern similar to OA.

OA is a threshold (indirect) genotoxic compound in various cell types in vitro. No genotoxicity data are available for DTX2 and -3. Animal data indicate that OA and DTX1 are potential tumour promoters, but data are insufficient to account for this effect in the risk assessment. No data are available for DTX2.

#### 3.5.4. Human epidemiological data

Clinical symptoms of DSP are mainly gastrointestinal distress, diarrhoea, nausea, vomiting, and abdominal pain. These symptoms appear between 30 min and several hours after intake. Recovery usually is complete within three days. Human data from Japan (eight people from three families, ages 10–68) indicate a LOAEL of 1.2–1.6 \( \mu \)g/kg bw. In a second study from Norway, 38 of 70 adults were affected at levels ranging from 1.0 to 1.5 \( \mu \)g/kg bw (Aune, 2001).
3.5.5. Evaluation

OA and DTXs possess tumour-promoting activity; OA also possesses genotoxic and immunotoxic activity. These effects raise questions as to the human health risks of (sub)-chronic exposure to low levels of these compounds. A pressing problem is the lack of sufficient quantities of purified toxins to perform (sub)chronic animal toxicity studies. The Expert Consultation determined no TDI could be established because of insufficient data on the chronic effects of OA.

The Expert Consultation established a provisional acute reference dose of 0.33 µg OA equ/kg bw, based on the LOAEL of 1.0 µg OA/kg bw. A safety factor of 3 was chosen because of documented human cases involving more than 40 people and because DSP symptoms are readily reversible.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would lead to a derived guidance level of 0.08 or 0.05 mg OA equivalent/kg shellfish meat, respectively.

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As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would lead to a derived guidance level of 0.08 or 0.05 mg OA equivalent/kg shellfish meat, respectively.

### 3.6. Pectenotoxins (PTX) group

#### 3.6.1. Background information

The presence of pectenotoxins in shellfish was discovered due to their high acute toxicity in mouse bioassay after i.p. injections of lipophilic extracts. Pectenotoxins have been detected in microalgae and/or bivalve molluscs in Australia, Italy, Japan, New Zealand, Norway, Portugal, and Spain. Animal studies indicate that they are much less potent than OA and are readily reversible.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would lead to a derived guidance level of 0.08 or 0.05 mg OA equivalent/kg shellfish meat, respectively.

### 3.6.2. Biological data

No data on absorption, distribution, metabolism, excretion, and mechanism of action are available.

### 3.6.3. Toxicity in animals

Several pectenotoxins are acutely toxic in mice following i.p. administration in the following dosage range: PTX-1, PTX-2, PTX-3, and PTX-11 at 219–411 µg/kg; PTX-4 and PTX-6 at 500–770 µg/kg; PTX-7, PTX-8, PTX-9, and PTX-seco acid at >5000 µg/kg (Yasumoto et al., 1989; Miles et al., 2004a).

The acute oral toxicity of PTX-2 and PTX-2 seco acid is >5000 µg/kg in the mouse (Miles et al., 2004a). Although diarrhoea has been reported in animals dosed with PTX-2 and PTX-2 seco acids, recent studies have shown that pectenotoxins are not diarrhoeagenic.

No information is available on the chronic toxicity of PTXs.

### 3.6.4. Human epidemiological data

Although it has been suggested that PTX toxins were responsible for gastrointestinal effects in Australia, the observed effects later were attributed to okadaic acid esters (Burgess and Shaw, 2003). Therefore, no evidence of an adverse effect of PTX in humans exists.

<table>
<thead>
<tr>
<th>Toxin group</th>
<th>LOAEL(1) µg/kg bw</th>
<th>NOAEL(2) µg/kg bw</th>
<th>Safety factor (human data (H) animal data (A))</th>
<th>Provisional acute RfD*</th>
<th>Derived guidance level/max level based on consumption of 100 g (1), 250 g (2) and 380 g (3)</th>
<th>Guidance level/max level currently implemented in some countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZA</td>
<td>0.4 (1)</td>
<td>10(H)</td>
<td>0.04 µg/kg 2.4 µg/adult</td>
<td>0.024 mg/kg Shellfish Meat(1) 0.0063 mg/kg SM(3)</td>
<td>0.16 mg/kg SM</td>
<td></td>
</tr>
<tr>
<td>BTX Cyclic imines</td>
<td>N/A</td>
<td>N/A</td>
<td>60 mg/kg SM(1) 24 mg/kg SM(2) 16 mg/kg SM(3) 0.2 mg/kg SM(1)</td>
<td>0.08 mg/kg SM(2) 0.05 mg/kg SM(3)</td>
<td>0.8 mg/kg SM as PbTx-2 20 mg/kg SM</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>1000 (1)</td>
<td>10(H)</td>
<td>100 µg/kg 6 µg/adult*</td>
<td>60 mg/kg SM(1) 24 mg/kg SM(2) 16 mg/kg SM(3)</td>
<td>0.16 mg/kg SM</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>1 (1)</td>
<td>3(H)</td>
<td>0.33 µg/kg 20 µg/adult*</td>
<td>0.5 mg/kg SM(3)</td>
<td>0.8 mg/kg SM</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>2 (1)</td>
<td>3(H)</td>
<td>0.7 µg/kg 42 µg/adult*</td>
<td>0.17 mg/kg SM(2) 0.11 mg/kg SM(3)</td>
<td>0.8 mg/kg SM</td>
<td></td>
</tr>
<tr>
<td>STX</td>
<td>5000 (2)</td>
<td>100(A)</td>
<td>50 µg/kg 3 µg/adult*</td>
<td>30 mg/kg SM(1) 12 mg/kg SM(2) 8 mg/kg SM(3)</td>
<td>1 mg/kg SM</td>
<td></td>
</tr>
</tbody>
</table>

*a Based on an adult bw of 60 kg.

*b These levels are considered as standard international regulatory levels, even though some countries might have different levels.

#### Table 2

Summary data used in the derivation of the acute RfD, as well as derived and current guidance levels.
3.6.5. Evaluation
The Expert Consultation considered that the database was insufficient to establish an acute RfD or TDI for PTX toxins. Nevertheless, the calculated acute human intake of pectenotoxins in Canada is 0.61 µg/kg bw (Gully and Kuiper-Goodman, 2004) while Norwegian data indicate an intake of 1.63 µg/kg bw. Thus, the acutely toxic dose in the mouse is between approximately 3000 and 8000 times the estimated acute human intake.

3.7. Saxitoxin (STX) group

3.7.1. Background information
Paralytic shellfish poisoning (PSP), associated with intake of toxins from the saxitoxin group, has been known for a long time, and has caused many fatalities. Based on case reports, the intake of toxins necessary to induce PSP symptoms varies greatly. This may be due to differences in susceptibility among individuals, as well as a lack of precision in exposure assessments due to problems with sampling and analysis of contaminated shellfish at the time of intoxication. Saxitoxins are found worldwide. They are produced by Alexandrium and other species and affect a wide variety of shellfish. A regulatory level of 0.8 mg/kg shellfish meat as STX equivalents has existed in North America for approximately 50 years, and generally no PSP has been associated with commercially harvested shellfish. The same regulatory limit is used in many other countries. The mouse bioassay is widely used in monitoring programs.

3.7.2. Biological data
In cats, STX injected i.v. was widely distributed in the body and disappeared quickly from the blood, with a serum half-life of 22 min. Based on i.v. studies in rats and cats, residence times in the body appear to be much longer, with a half-life of 12–18 h. There are no data on STX metabolism in humans. Hydrolysis of N-sulfocarbamoyl toxins to the more toxic carbamates may not be significant for human health. Urine is the primary route of toxin excretion in humans. STXs selectively bind to receptors and subsequently block voltage-gated sodium channels on excitable membranes. All of the analogues of STX occupy the same receptor, although the affinities differ greatly.

3.7.3. Toxicity in animals
The potency of saxitoxins varies widely. The i.p. studies in mice have revealed that the carbamates and decarbamoyls are the most toxic, while the sulfocarbamoyls possess a lower acute toxicity. Based on LD₉₀ data in mice, the saxitoxins are four times less toxic by i.p. injection than when given i.v., and are about 100 times less toxic orally than intravenously. Significant species differences in oral toxicity have not been observed.

3.7.4. Human epidemiological data
Typically, a tingling sensation around the lips, gums, and tongue develops within 5–30 min of consumption. In more severe cases, these are followed by a feeling of numbness in fingertips and toes, which progresses to the arms, legs, and neck within 4–6 h. Death usually is caused by respiratory paralysis within 2–12 h; without medical intervention the case fatality rate is 5–10%. If patients survive for 24 h, either with or without mechanical ventilation, chances for rapid and full recovery are excellent. Continuous mechanical support of respiration is advisable in severe cases.

Canadian data on paralytic shellfish poisonings from 1970 to 1990 were analyzed and compared with selected outbreaks and case series in an unpublished Health Canada report by Kuiper-Goodman and Todd. These authors assessed the clinical information based on the original case histories of people for which occurrence data of PSP toxins in shellfish (raw or cooked) also were available, and they determined the corresponding exposure, adjusted for the effect of cooking. Similar to Prakash et al. (1971), cases were rated as mild, moderately severe, or extremely severe. Although some overlap existed in the doses associated with the three categories of severity, a clear and steep dose response was apparent. The more severe cases generally involved exposure >10–300 µg/kg bw. With one exception, people with mild cases had consumed between 2 and 30 µg/kg bw. The moderately severe category exposure was between these two ranges. Based on these data, a provisional LOAEL of 2.0 µg/kg bw was established by the Expert Consultation. Additional cases from other countries support these findings.

3.7.5. Evaluation
The Expert Consultation established a provisional acute reference dose of 0.7 µg STX equivalents/kg bw, based on an LOAEL of 2 µg STX eq/kg bw. A safety factor of 3 was chosen because documented human cases included a wide spectrum of people (occupation, age, and sex) and mild illness is readily reversible.

The Expert Consultation determined no TDI could be established because of insufficient data on the chronic effects of STX.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat by adults would lead to a derived guidance level of 0.17 or 0.11 mg STX eq/kg, respectively.

3.8. Yessotoxin (YTX) group

3.8.1. Background information
Yessotoxins are produced by Protoceratium reticulatum, and have been detected in microalgae and/or bivalve molluscs in Australia, Canada, Italy, Japan, New Zealand, Norway, and the United Kingdom. Their presence in shellfish was discovered due to their high acute toxicity in mice after i.p. injection of lipophilic extracts. They are much less potent via the oral route, and do not induce diarrhoea.
There are no reports of human intoxication caused by yessotoxins. Consequently, yessotoxins should be regulated separately from the okadaic acid toxin group (DSP toxins). Analysis of YTXs is complicated by the large number of analogues produced by the algae and their extensive metabolism in shellfish. YTXs are persistent in shellfish tissues and therefore, depending upon regulatory significance, may require long-term monitoring in management programs. A regulatory level of 1 mg/kg shellfish has been implemented in some countries.

3.8.2. Biological data

Limited absorption of toxin from the gastrointestinal tract has been observed, but no further data on absorption, distribution, metabolism, and excretion are available. Based on available data, YTX appears to exert effects in living systems by multiple mechanisms of action, but detailed information on the mechanism(s) of toxic action is not available.

3.8.3. Toxicity in animals

Acute toxicity data, based on i.p. administration, are available for nine YTX analogues. Of these, seven possessed toxicity similar to YTX, with LD$_{50}$ values between 100 and 750 μg/kg bw (Murata et al., 1987; Satake et al., 1997; Tubaro et al., 2003). Two analogues were much less toxic, with no effects recorded at a dose of 5000 μg/kg bw (Miles et al., 2004b, 2005). The oral toxicity of YTX in mice is much lower, with no effects observed at an acute dose level of 50 mg/kg bw. Data from one short-term gavage study in mice revealed no toxicity of YTX at 5 mg/kg bw.

No data are available on the long-term toxicity, reproductive toxicity, carcinogenicity, or genotoxicity of YTX.

3.8.4. Human epidemiological data

There have been no reports of ill effects in humans attributable to YTX.

3.8.5. Evaluation

By applying a safety factor of 100 to the dose of 5 mg YTX/kg bw that showed no toxicity in an oral short-term mouse study, and in the absence of human data, the Expert Consultation established a provisional acute reference dose of 50 μg/kg bw.

The Expert Consultation decided no TDI could be established because of insufficient data on the chronic effects of YTX.

As shown in Table 2, the consumption of 250 or 380 g shellfish meat would lead to a derived guidance level of 12 or 8 mg/kg, respectively.

4. Recommendations

The Consultation encouraged Member States to generate more toxicological data to allow more accurate risk assessments, and elaborated the following recommendations to Member States, FAO, WHO, and Codex.

4.1. Recommendations to Member States, FAO, WHO

- Encourage Member States to implement public health programs that ensure shellfish poisonings are detected in a more systematic way:
  - reportable disease (physicians),
  - public awareness programmes,
  - rapid outbreak-response teams (timely sample capture + analysis and pre-defined communication channels, questionnaire).
- Encourage Member States to generate more toxicological data to allow more accurate risk assessments.
- Promote international efforts for the production of certified reference materials and calibration standards.
- Encourage Member States to improve and validate toxin detection methods in shellfish.
- Promote toxicological studies conducted according to OECD guidelines.
- Encourage studies to clarify the mechanism of action for a number of toxin groups.
- Encourage Member States to implement an integrated shellfish and micro-algae monitoring program.
- Consider the position of developing countries regarding implementation of chemical analytical methods.
- Encourage Member States to determine the relation between quantitative occurrence of toxin producing micro-algae (planktonic and epiphytic) and the accumulation of biotoxins in bivalve molluscs.
- Encourage Member States to develop operational models for forecasting blooms of toxin producing micro-algae in time and space.

4.2. To codex

- Codex should continue to work on risk management recommendations (e.g., Standards and Code of Practice) to address issues related to biotoxins in bivalve molluscs.
- When selecting detection methods, consideration should be given to the situation in developing countries.

4.3. To FAO, WHO

- Establish a standing expert panel to periodically review scientific data and information at the international level. This panel should be convened soon to review epidemiological and cooking/processing data to more accurately derive guidance levels/maximum levels for some toxin groups.

5. Future

As mentioned initially, this report provides an overview of toxicological evaluations conducted by the joint FAO/IOC/
WHO Expert Consultation in 2004. Comparison of guidance levels proposed by the Expert Consultation with current regulatory maximum levels established by some countries showed differences for certain toxin groups. At the 27th session of the CCFFP (2005), the scientific summary prepared by the Expert Consultation was presented by FAO/WHO secretariats (Codex, 2005b). The Committee agreed to establish a Working Group, chaired by a Canadian representative, to examine the report from the Joint FAO/WHO/IOC ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs and prepare a discussion paper for consideration by the CCFFP with the following terms of reference:

- Assess how the CCFFP might use the expert advice and make recommendations that the CCFFP could consider to integrate into the Proposed Draft Standard for Live and [Raw] Molluscs and the section of the Code on Live and [Raw] Bivalve Molluscs.
- Identify new questions that the CCFFP may wish to pose to FAO/WHO.
- Identify areas in the report that may need further clarification.
- As appropriate, make recommendations on the validation of methodology (e.g., identifying other international organisations working in this area).
- As appropriate, make recommendations on possible changes to the Proposed Draft Standard for Live and [Raw] Molluscs and the section of the Code on Live and [Raw] Bivalve Molluscs arising from expert advice and other issues raised by the deliberations of the Working Group.

Therefore, the CCFFP working group should critically review the full technical report and discuss how the scientific advice could be utilized by the CCFFP.

The results of the Expert Consultation should assist the CCFFP in the development of a Standards and Codes of Practice to address issues related to biotoxins in bivalve molluscs.

References


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