Food and Agriculture Organization of the United Nations

Intergovernmental Oceanographic Commission of UNESCO (IOC)

World Health Organization

Report of the Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs

Oslo, Norway, Sept. 26-30, 2004

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DECLARATIONS OF INTEREST

Two out of the 30 experts who participated in this meeting declared an interest in the topics under consideration:

Dr Quilliam: His research is related to a commercially available tool for rapid testing .

Prof Rossini: His research on "Process for the measurement of dinophysistoxin and of yessotoxin", that is protected by a patent application (International Publication number WO 02/03060 A2), whose applicant is Università degli Studi di Modena e Reggio Emilia, which is the employer of Prof Rossini.

ABBREVIATIONS

(Well-known abbreviations in general use are not included)

AOAC	Association of Official Analytical Chemists
APEC	Asia Pacific Economic Cooperation
APHA	American Public Health Association
AZA	Azaspiracid
bw	Body weight
CCFFP	Codex Committee on Fish & Fishery Products
CCPR	Codex Committee on Pesticide Residues
CEN	Comité Européen de Normalisation
CNS	Central Nervous System
CRL	European Community Reference Laboratory
CRM	Certified Reference Material
DA	Domoic Acid
DG	Digestive Gland of shellfish; hepato-pancreas
ELISA	Enzyme-Linked ImmunoSorbent Assay
FAO	Food and Agricultural Organization of the United Nations
GEMS/Food	Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme
IOC	International Oceanographic Commission of UNESCO
ISO	International Standards Organisation
IRMM	Institute for Reference Materials and Measurements
IUPAC	International Union of Pure & Applied Chemistry
i.p.	intraperitoneal administration
i.v.	intervenous injection
JECFA	Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
JRC	Joint Research Centre Director General of European Commission
LC	Liquid Chromatography
LC-FL	Liquid Chromatography with Fluorescence detection
LC-MS	Liquid Chromatography with Mass Spectrometric detection
LC-UV	Liquid Chromatography with UltraViolet absorption detection
LD_{50}	Median Lethal Dose
LFIC	Lateral Flow Immuno-Chromatography
LOAEL	Lowest Observable Adverse Effect Level
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
MBA	Mouse Bioassay
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NOAEL	No Observable Adverse Effect Level
NRC	National Research Council of Canada
OA	Okadaic Acid
PTX	Pectenotoxins
p.o.	per os, oral administration
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
RBA	Receptor Binding Assay

RfD	Reference Dose
STX	Saxitoxin
TDI	Tolerable Daily Intake;
TEF	Toxicity Equivalence Factor
UV	Ultraviolet radiation
US FDA CFSAN	Centre for Food Safety and Applied Nutrition, Food and Drug Administration of the United State of America
WF	Whole Flesh of shellfish
YTX	Yessotoxin
WHO	World Health Organization

EXECUTIVE SUMMARY

The expert consultation met in Oslo, Norway, Sept. 26-30, 2004 to review the technical reports prepared and to provide scientific responses to the following specific questions posed by the CCFFP (see the report of the 26th session, ALINORM 04/27/18, paragraph 130):

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels in shellfish for shellfish toxins;
- 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 geographical distribution of biotoxin-forming marine phytoplankton.

The Expert Consultation was asked to perform risk assessments for a number of biotoxins that are present in bivalve molluscs. Since exposure to biotoxins 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 dose (acute RfD)[1], and generally insufficient data were available to establish a tolerable daily intake (TDI)[2]. A crucial issue when deriving either of these values from the most relevant toxicological information is the size of safety factors. Generally, default values of 10 and 100 on the basis of human and animal data are applied, respectively. Furthermore, the safety factor is usually increased if for the critical effect there is a lowest observable adverse effect level (LOAEL) instead of a no observable adverse effect level (NOAEL). On the other hand, a safety factor between 1 and 10 for human data may be used depending upon the magnitude and severity of the effect, the steepness of the dose-response curve, the amount of information, and whether the human data include data on a large spectrum of people. The acute RfD usually has a higher value than the TDI, and some safety factors used in its derivation may be smaller than those used for the derivation of the TDI. Based on the available information, the Expert Consultation established LOAELs for the AZA, OA, STX, and DA toxin groups, and suggested safety factors of 10, 3, 3 and 10, respectively. The derived provisional acute RfDs for the AZA, OA, STX, and DA groups are 0.04 μg/kg, 0.33 μg/kg , 0.7 μg/kg and 100 μg/kg bw, respectively. For the YTX group, a NOAEL was established, based on animal studies. Applying a safety factor of 100, a provisional acute reference dose of 50 μg/kg bw was suggested for the YTX group (summarized in the table 2). The Expert Consultation considered that the database for the cyclic imines, brevetoxins and pectenotoxins was insufficient to establish provisional acute RfDs for these three toxin groups.

Marine biotoxin management for growing areas is generally documented in a Marine Biotoxin Management Plan (MBMP). Generic MBMPs are presented in UNESCO publications and the concepts have been in use for over 10 years in a number of countries. Integrated shellfish and micro-algal monitoring programmes, as part of MBMPs, are highly recommended to provide expanded management capability and enhanced consumer protection.

[1] The acute RfD is the 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 in a period of 24 hours 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

In the development of an MBMP, information on the micro-algae known to cause toxicity needs to be considered. In general this is well documented, and many micro-algae have a worldwide distribution. Evaluation of environmental conditions also plays a key part in marine biotoxin management. Through expert judgment the location of sampling stations and frequency of sampling for micro-algae and shellfish can be decided. The number and location of sample stations must be sufficient to address spatial - temporal changes.

Significant inter- and intra-species variability in toxin profile and toxin content for many micro-algal species as well as persistence of toxins in shellfish, after the toxic micro-algae have gone, should also be considered. Results from micro-algal monitoring programmes, along with other information, may be used to define "switching factors" to initiate management actions as detailed in Action Plans included in the MBMP.

Test methods for the 8 toxin groups were reviewed and recommendations for Codex purposes have been made. Mouse bioassays are widely used for shellfish testing but for technical and ethical reasons it is highly desirable to move to new technologies which can also better meet Codex requirements. Most methods currently available do not strictly meet criteria for Codex type II[3] or III[4] methods and have not necessarily been widely used in routine shellfish monitoring. However, the recommendations made by the Expert Consultation represent the best currently available methods. LC-MS has much potential for multi-toxin analysis and has been recommended for consideration and recommendation by Codex. The expert consultation is of the opinion that the complexity and chemical diversity of some toxin groups is such that validated quantitative methods to measure all toxins within a group will be extremely difficult. Thus the implementation of a marker compound concept and the use of functional assays should be explored. There is an urgent need to develop additional certified analytical standards and reference materials since these are critical in applying test methods for Codex purposes. The Expert Consultation recommends that, with some exceptions, only edible portions (normally whole tissue) of shellfish be taken for biotoxin analysis. Guidance has been provided on the management of test results and the effects of processing on shellfish detoxification.

It must be pointed out that the Expert Consultation did not have enough time to fully evaluate epidemiological data or to assess the effects of cooking or processing for deriving the provisional guidance levels/maximum levels for several toxin groups (especially the AZA and STX groups). The Consultation agreed that there is a need for a further in-depth review of these data to better derive the guidance levels/maximum levels.

[3] A Type II method is the one designated Reference Method where Type I methods do not apply. It should be selected from Type III methods (as defined below). It should be recommended for use in cases of dispute and for calibration purposes

[4] A Type III Method is one which meets the criteria required by the Codex Committee on Methods of Analysis and Sampling for methods that may be used for control, inspection or regulatory purposes.

1. Introduction

At its 25th session, the Codex Committee on Fish and Fishery Products (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, at its 26th session, elaborated the following specific questions to be covered through this advice:

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels in shellfish for shellfish toxins;
- 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 geographical distribution of biotoxin-forming marine phytoplankton.

FAO/WHO/IOC agreed to organize an Expert Consultation to address this request. First, a joint FAO/IOC/WHO workshop on biotoxins in bivalve molluscs was held in Dublin in March 2004 to identify the scope, content of the work, candidates for the electronic drafting groups and information needed for compiling scientific advice to be discussed at the Expert Consultation. To facilitate the discussion, the workshop classified the toxins into 8 groups based on chemical structure (the Azaspiracid (AZA) group, Brevetoxin group, Cyclic Imines group, Domoic Acid (DA) group, Okadaic Acid (OA) group, Pectenotoxin (PTX) group, Saxitoxin (STX) group, and the Yessotoxin (YTX) group). The reason for this was that for enforcement of Codex standards chemical classification is more appropriate for analytical purposes than classification based on clinical symptoms.

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 drafting groups examined the available relevant information and prepared technical documents.

The Expert Consultation met in the National Veterinary Institute, Oslo, Norway, Sept. 26-30, 2004 to review the technical reports and prepare the report for the CCFFP. The experts were selected according to their scientific and technical expertise and to provide a balanced regional point of view.

The Minister of Fisheries of Norway, Mr. Svein Ludvigsen, opened the expert consultation. In his opening remarks, he stressed the importance of the scientific advice that the expert consultation will provide to the CCFFP to facilitate the further elaboration of the draft Code of Practice and the draft Standard for bivalve molluscs.

The Expert Consultation appointed Mr. Phil Busby as Chairperson and Dr. Jim Lawrence as rapporteur of the Expert Consultation. Dr. Philipp Hess was appointed Chairperson and Dr. Pat Holland as rapporteur of the working group on analytical methods. Dr. Tore Aune was appointed Chairperson and Dr. Tine Kuiper-Goodman as rapporteur of working group on toxicology, and Mr. Phil Busby was appointed Chairperson and Mr. David Lyons as rapporteur of working group on marine biotoxin management.

2. Approach taken

2.1 Risk assessment

The Expert Consultation was asked to perform risk assessments for a number of biotoxins that are present in bivalve molluscs. Since exposure generally involves only occasional consumption, and because most of the available toxicological data concerns only acute and short-term studies, priority was given to the establishment of an acute RfD. Although more frequent exposure may also occur, the Expert Consultation could not establish TDI values, due to the lack of appropriate toxicological 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 implemented within existing monitoring plans contribute to maintaining the probability of adverse health effects at an extremely low level.

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 whole population, 35% consume bivalve molluscs both in Norway (Fish and Game study 1999) and in France (SECODIP[5] 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 consumption for those consumers is 4.2 eating occasions per year. In the USA the frequency of consumption is 8.6 eating occasion per year (USFDA-Market Research Corporation of America. In Norway 33 % of consumers are eating bivalve molluscs between 1 and 11 times a year and 2 % of consumers eat these molluscs between 1 to 8 times a month.

Short-term dietary intake assessment should be carried out 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, this 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 USA and 263 g in New-Zealand. A consumption of 182 g has been reported in Norway as a maximum level of consumption. For children the highest reported 97.5th percentile consumption figure is 70 g for (Australia) and 27 g was reported for Japan.

It should be noted that the standard portion of 100 g, which is sometimes used in risk assessment, is not adequate to assess an acute risk; a portion of 250 g would cover 97.5% of

[5] SECODIP = Société d'Etudes de la Consommation, de la DIstribution et de la Publicité

the consumers of most countries for which data were available. Three simulations were done using, respectively, portion sizes of 100, 250 and 380 grams.

2.3 Occurrence and concentrations of toxins in bivalve molluscs

Because of insufficient data, the occurrence and the concentrations of toxin in bivalve molluscs was not fully evaluated during the consultation. However, for the purpose of exposure assessment, the Expert Consultation developed the typical range of toxin levels that may lead to closure of the harvesting area and maximum reported level in shellfish in the table1.

Table 1: The typical range of toxin levels that may lead to closure of the harvesting area and maximum reported level in shellfish

Toxin Group	Typical level when toxins occur at levels that may lead to closure of the area (mg/kg)	Maximum reported level (mg/kg)
AZA	0.16-0.3	1.4
Brevetoxins	0.8 mg/kg (as PbTx-2)	40
Cyclic Imines	0.1	2
DA	20-200	1280
OA	0.16-1	36
PTX	LOD-0.2	0.9
STX	0.8-10	800
YTX	1-2	8

3. General considerations on analytical methodology

3.1 Implementation of improved test methods

Contamination by marine biotoxins often involves more than one toxin group, and monitoring programs typically cover a range of toxins. Mouse bioassays have been the traditional means of overcoming these complexities. However, these assays have severe technical and ethical limitations and generally lack adequate validation for Codex purposes. Therefore multi-toxin instrumental methods are required for more cost-effective screening and these increasingly utilise LC-MS. However, many toxin groups also encompass numerous analogues which may be impracticable to be individually measured. Choice of suitable marker compounds can ease the analytical burden but positive samples will generally require more detailed follow-up analyses. Functional assays based on the common biochemical activity of a group of toxins are attractive alternatives to multi-toxin methods but few thoroughly validated assays are widely available. Rapid screening tests, generally based on immuno techniques, are already in widespread use for the saxitoxin group and can be effective management tools. However, an alternative quantitative test is generally required for opening closed shellfish growing areas. All areas of marine biotoxin method development, validation and testing require certified calibration standards and reference materials. There is an urgent need to expand the currently available CRMs and Codex should encourage Member States to fund the necessary efforts.

3.2 Method validation and performance

For Codex purposes, the preference is for methods to be validated by collaborative study according to the IUPAC/AOAC/ISO harmonised protocol. Labs must demonstrate adequate performance of their methods through proficiency testing. However, there is a lack of inter-lab studied methods and proficiency testing schemes for phycotoxins. Therefore there should be particular emphasis on thorough within-lab validations and internal QC procedures (Thompson & Wood 1995). Codex has issued guidelines for single lab method validation based on IUPAC recommendations. For the management of the analytical result it is particularly important to establish the uncertainty of measurement as outlined in ALINORM 04/27/23.

3.3 Portion of the shellfish sample to be analysed

In principle, it is preferable to analyse the part of the shellfish, which is considered edible (normally whole tissue). Only the parts analysed should be marketed, except when there are possible analytical method interferences or detectability issues, or other practical issues e.g. large scallop species with fibrous tissues. In these cases, the most contaminated tissues (e.g. the digestive gland (DG)) may be dissected and analysed, and the result should then be converted to an edible tissue basis. Data should be obtained to determine an appropriate conversion factor, based on weights of dissected parts and the extent of transfer of toxin into other tissues.

4. Effects of Processing

Several procedures to detoxify shellfish have been developed to mitigate the negative economic impact of toxic contamination. Initial efforts had limited success and were hampered by factors such as operational costs, depuration characteristics of different shellfish species and the effects of treatments on the organoleptic properties of shellfish (Anderson et al, 2001).

The concentration of toxins in digestive glands has enabled commercial evisceration procedures for several shellfish species, principally scallop, to produce edible portions with acceptable toxin levels. This is especially applicable to lipophilic toxins. In contrast, concentrations of water-soluble toxins and/or heat labile toxins in shellfish are decreased by thermal treatment.

Procedures combining evisceration and conventional canning can successfully reduce the levels of the STX group and DA group in a number of shellfish species of commercial interest without affecting organoleptic and quality properties. Some countries have issued exceptions to harvest action limits for lots destined for such processing, thereby shortening closure periods.

However, to ensure public health safety it is still necessary to determine the specific effects of post-harvest processing on toxin levels, interconversions and redistribution. In addition, all processed lots should be subjected to final product testing before marketing.

5. Toxin group specific section

5.1 Azaspiracid (AZA) group

Background information

The syndrome that later was named azaspiracid poisoning (AZP) was detected 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 with the mouse bioassay (MBA) as the reference method. However, a MBA protocol with adequate specificity or detectability has not been validated. Current testing is based on preliminary LC-MS methods using a limited supply of AZA1 reference standard.

Biological Data

Absorption, Distribution, Metabolism and Excretion

No data are available

Mechanism of Action

No data are available

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.

In a preliminary long term experiment, repeated administration once or twice a week by gavage of 20 µg/kg bw 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 1 year in survivors. 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 on the basis of i.p. studies in mice, it would appear that AZA 2 and 3 are somewhat more toxic than AZA 1, and AZA 4 and 5 are less toxic.

Observations in humans

Limited data in humans indicate a LOAEL between 23 and 86 µg/person for acute gastrointestinal effects.

Evaluation

The Expert Consultation established a provisional acute reference dose of 0.04 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 to take into consideration the small number of people involved.

The Expert Consultation found that because of insufficient data on the chronic effects of AZA, no TDI could be established.

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

Gaps in the Data

The preliminary studies, in which AZA was administered by gavage, indicate the possibility of severe and prolonged toxic effects at low doses. Administration by gavage may, however, have contributed to the observed severe erosive effects in the gastrointestinal tract. Repeat studies involving administration of the test material by feeding are urgently required.

To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are needed. Information on absorption, excretion and metabolism is also required.

Analytical Methodology

Available methods

In vivo Bioassays

Mouse or rat bioassays can detect AZAs with an LOD of ca 0.16 mg/kg but there are potential interferences from other lipophilic toxins. Further method development and validation is required, particularly to achieve lower LODs.

Instrumental Methods

AZAs lack a chromophore for LC-UV determination and conditions for fluorescence derivatisation have not been established. However, LC-MS has shown great promise as a highly specific and sensitive technique for detection of AZAs. One multi-toxin protocol (McNabb et al 2004) has been subjected to a full within lab validation (4 shellfish species) and a limited inter-lab study. The LOQ for this method was 0.05 mg/kg but lower limits would be readily achievable, which will be necessary to enforce the proposed levels.

Recommendation for choice of Reference Method (Type II)

Because an LC-MS method is the best available option, a collaborative study of a multi-toxin method that includes AZAs should be conducted to fully meet Type II criteria. However, applicability of the technique is currently limited by the lack of certified analytical standards.

Management of Analytical Results

Analytical data for all methods should be expressed as mg AZA1 equivalents per kg of whole flesh, using TEFs for AZA2 and AZA3. Other analogues are considered of low relevance.

Standards and Reference Materials

Lack of reference materials and standards is a severe limitation to research, development, method validation and management of AZA contamination. Codex should encourage member states to participate and fund initiatives such as those of NRC, Halifax to develop standards and CRMs for AZAs

5.2 Brevetoxin group

Background information

Florida red tides annually occur in the Gulf of Mexico, and result from blooms of the dinoflagellate *Karenia brevis*. The algae produce neurotoxins named brevetoxins. In humans, brevetoxins may induce acute gastrointestinal and neurologic symptoms after ingestion of contaminated shellfish (oysters, clams). The syndrome was named neurotoxic shellfish poisoning (NSP). Neurotoxic shellfish poisoning due to brevetoxins has limited geographical distribution (USA, New Zealand). Brevetoxins have been shown to be responsible for the death of fish and some marine mammals. The analysis of brevetoxins poses considerable difficulties due to their extensive metabolism in shellfish. The APHA protocol for the mouse bioassay of an ether extract of shellfish is currently the basis for regulation of shellfish. A regulatory level of 20 MU/100 g shellfish meat (1MU = 4.0 µg PbTx-2) is implemented in some countries.

Biological data

Absorption, Distribution, Metabolism and Excretion

Brevetoxins are rapidly absorbed with distribution throughout the body (including 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). They are excreted both in urine and bile.

Mechanism of Action

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

Toxicity in Animals

In mice i.p. LD_{50}s of 100, 200 and 170 µg/kg bw have been reported for PbTx-1, PbTx-2 and PbTx3, respectively. The oral LD_{50} in mice ranges from 520 µg/kg bw for PbTx-3 to 6600 µg/kg bw for PbTx-2. The subchronic/chronic toxicity of the brevetoxins is unknown, and there is no information on reproductive toxicity and genotoxicity.

Observations in humans

In one episode, brevetoxins were responsible for acute neurotoxic shellfish poisoning (NSP) at concentrations of 120-472 µg PbTx3 equivalents/100g shellfish. It is not known whether there are chronic toxicities associated with brevetoxin ingestion.

Evaluation

Based on a reported incident in humans, with an assumed consumption of 100-150 g shellfish at 120 µg PbTx-3 equivalents/100g, and a 60 kg body weight, an exposure of 2-3 µg PbTx-3 equivalents/kg bw was estimated. However, there is uncertainty whether this actually represents the dose experienced by consumers, because of possible underestimation of the toxins actually present in shellfish (toxins from *K.brevis* metabolites produced in some bivalves) and because the metabolites are not reliably extracted by the method used for regulatory monitoring. The Expert Consultation decided that there are currently insufficient data to complete the risk assessment.

Gaps in the Data

The relevant brevetoxins and their metabolites need to be identified and estimates of their oral potencies are needed, before an acute RfD can be established. To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are needed. Information on absorption, excretion and metabolism is also required.

Analytical Methodology

Available methods

In vivo Bioassays

The APHA MBA protocol[6] has protected human health in the south eastern USA from the regular contamination of shellfish by brevetoxins over a period of 30 years and has shown good reproducibility. However, it has only been validated for Eastern oyster and clam. It is not a quantitative method with death of >2 of 5 mice in less than 360 minutes[7] taken as the toxic threshold (ca 0.8 mg/kg PbTx-2). The ether extraction procedure is of unknown efficiency in recovering the full range of parent brevetoxins and metabolites that might be significant for human toxicity. The specificity is also not well established with respect to other potentially co-occurring lipophilic toxin groups.

In vitro Functional Assays

Neuroblastoma assays have been developed that can detect brevetoxins but the methods have performed poorly in inter-lab studies. An RBA using tritiated- PbTx-3 and sodium channel receptor preparations performed well in the same studies. The LOD is 0.03 mg/kg PbTx-3 equivalents. A full collaborative study has not been carried out. These assays are currently all type IV[8] category.

Immunochemical Methods

Two ELISA methods have been developed that detect a wide range of brevetoxins (Naar et al 1998; Briggs et al 2004). On the basis of the current limited evaluations, including some inter-method comparison exercises, they appear sensitive screening tests (Type IV).

Instrumental Methods

LC-MS methods have been developed to detect a wide range of brevetoxins. However, validation has been carried out for only a few toxins for which analytical standards were available. Good recoveries and precision, and LOQs of 0.03 mg/kg have been reported for several brevetoxin metabolites. LC-MS testing for these toxins as markers of brevetoxin contamination has been recommended. However, further method development and validation is required for LC-MS methods that quantitatively determine a wider range of toxicologically relevant metabolites. This would enable regulation of brevetoxin contamination in shellfish.

[6] Recommended Procedures for the examination of Seawater and Shellfish, 4th edition, published by the American Public Health Association

[7] 20 mouse units per 100 gram shellfish meat is measured by using a 360 minute observation period, or 10 mouse units per 100 grams shellfish meat using a 930 minute observation period

[8] A Type IV Method is a method which has been used traditionally or else has been recently introduced but for which the criteria required for acceptance by the Codex Committee on Methods of Analysis and Sampling have not yet been determined.

Recommendation for choice of Reference Method (Type II)
No method currently meets all the criteria for a reference method. An LC-MS method or a functional assay that detects a range of the toxicologically relevant brevetoxin metabolites should be collaboratively studied to enable Type II criteria to be fully met.

Management of Analytical Results
APHA MBA results should be reported as passing or failing the method criterion for shellfish toxicity. Analytical data for all other methods should be expressed as mg PbTx-3 equivalents per kg of whole flesh. Crude TEFs (i.p.) for some brevetoxin analogues and metabolites are available.

Standards and Reference Materials
The University of North Carolina, Wilmington, USA, can supply standard materials for parent brevetoxins. Calibration solution CRMs and naturally contaminated shellfish tissue CRMs are also required. Calibration standards for brevetoxin metabolites are also required, depending on toxicological significance, especially for LC-MS methods.

5.3 Cyclic Imines group

Background information

The cyclic imines group includes gymnodimine, spirolides, pinnatoxins, prorocentrolide and spirocentrimine. The presence of this group of 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, they rapidly kill mice, and their presence may interfere with the MBA for OA, brevetoxins, and AZA groups. At sublethal doses, the mice recover rapidly. The toxic potential of the cyclic imines is much lower via the oral route. The regulatory significance of the cyclic imine toxins is still unclear. Although gymnodimine and spirolides are now 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), there have been no reports of adverse effects in humans.

Biological Data

Absorption, Distribution, Metabolism and Excretion
There are no data available for any of the cyclic imines.

Mechanism of Action

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.

Toxicity in Animals

All the cyclic imines for which data are available are toxic to mice after i.p. administration. For gymnodimine the LD_{50} values after ip injection, oral gavage and feeding in fasted mice are 100, 755 and >7500 µg/kg bw, respectively. For desmethyl spirolide C the respective values are 6.5, 157, and 500 µg/kg bw, and 1050 µg in mice fed *ad libitum*, with all values indicating high toxicity. There are no data 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.

Observations in humans

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

Evaluation

The Expert Consultation considered that the database was insufficient to establish an acute RfD or TDI for the cyclic imines.

Gaps in the Data

More data on the subchronic oral toxicity are needed before an acute RfD can be established. To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are needed. Information on absorption, excretion and metabolism is also required.

Analytical Methodology

Available methods

In vivo Bioassays

The high i.p. toxicity of cyclic imines has created problems with the use of the MBA for the OA, brevetoxin and AZA groups. The high and fast-acting i.p. toxicity of cyclic imines means this group can potentially be detected by MBA using short observation times. However, no validation has been carried out on the recovery of these toxins through the standard extraction and partitioning protocol (Yasumoto 1984). MBA protocols require validation before they can progress beyond Type IV status.

Instrumental Methods

LC-MS methods have been developed for gymnodimine and spirolides which are suitable for screening and for confirmation. LOQs are below 0.03 mg/kg. One multi-toxin method that includes gymnodimine has received a full within-lab validation (4 matrices; 2 fortification levels) and limited inter-laboratory study (McNabb et al 2004). A small inter-lab study of spirolides in algal extracts has also been completed. LC-MS methods are excellent candidate methods that could be moved from Type IV to Type II status when regulatory requirements are defined and further interlab studies are completed.

Recommendation for choice of Reference Method (Type II)

No method currently meets the criteria for a reference method. An LC-MS multi-toxin method that includes gymnodimine and some spirolides should be collaboratively studied to enable Type II criteria to be fully met.

Management of Analytical Results

Analytical data for all methods should be expressed as mg gymnodimine per kg of whole flesh or mg desmethyl spirolide C equivalents per kg of whole flesh. Oral TEFs for the spirolide analogues are not currently available and are assumed as 1.0 in the interim.

Standards and Reference Materials

Calibration solution CRMs for gymnodimine and desmethyl spirolide-C are available (NRC, Halifax). Naturally contaminated shellfish tissue CRMs are also required. Calibration

solutions for other spirolide analogues are also required, depending on toxicological significance, especially for LC-MS methods. LC-MS relative response factors of 1.0 for spirolide analogues can be assumed in the interim.

5.4 Domoic Acid (DA) group

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 the nervous system were observed. Since some of those affected experienced memory loss, the syndrome was named amnesic shellfish poisoning (ASP). As a result of the episode of human illness 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 in various regions of the world. There have been numerous reports of toxicity in a variety of wildlife species indicating that domoic acid moves up the food chain in marine ecosystems. Routine monitoring using LC-UV is well established in most monitoring programs and has adequate detection limits to regulate DA at current limits. More rapid techniques such as ELISA would be useful. The recent finding of significant amounts of certain naturally occurring DA isomers requires an investigation of their toxicological significance for potential inclusion in monitoring.

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. The distribution of DA is largely to the blood compartment (volume of distribution ~ 0.25 l/kg) and studies indicate that there is very poor penetration of the blood-brain barrier in normal animals. Consequently, any condition that impairs blood-brain barrier integrity confers additional risk. There is no evidence that DA is metabolized and it appears to be almost entirely excreted unchanged in the urine 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

Mechanism of Action

DA produces excitoxicity 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.

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.

There is currently no evidence of cumulative toxicity on repeat exposure or of genotoxicity. There are no studies on long-term toxicity or carcinogenicity.

There is evidence in rodents that s.c. exposure to sub-convulsive doses of domoic acid *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 to domoic acid toxicity than adults.

Observations in humans

There is one well-documented episode of human toxicity (Canada, 1987) in which 107 persons (all adult) met the case definition. Dose-related symptoms included nausea, vomiting, abdominal cramps, diarrhoea, headache, memory loss and convulsions and several deaths were attributed to the toxin. Patients with moderate memory loss showed a very selective deficit in delayed recall and had difficulty in learning verbal and visuospatial material. These findings are consistent with the results of animal studies. Hospital charts for 16 patients indicated that all severely ill persons less than 65 years of age had pre-existing illness.

Consumption data for 9 patients indicated that only 1 of 6 who consumed between 60 and 110 mg of DA showed memory loss whereas 3 of 3 patients consuming 270-290 mg suffered neurological symptoms. Based on these data a LOAEL of 1.0 mg DA/kg bw was estimated. No adverse effects were observed in one person at 0.33 mg DA/kg bw

Evaluation

Based on the LOAEL of 1 mg DA/kg bw observed in humans, and a safety factor of 10 to take account of inter-human variability and the relatively small numbers of individuals on which this LOAEL is based, a provisional acute reference dose of 0.1 mg DA/kg bw was established by the Expert Consultation.

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 (> 65 years of age) people 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.

Gaps in the Data

Chronic toxicity studies over a range of doses are required and there is an urgent need for studies on risk during pregnancy, long-term developmental effects, neurological deficits induced by doses below the acute toxic dose and toxicity in health-compromised individuals.

To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are also needed.

Analytical Methodology

Available methods

In vitro Functional Assays

The kainic acid RBA provides sensitive detection of glutamate receptor ligands such as DA and isomers. However, it has not been evaluated in inter-laboratory studies (Type IV category).

Immunochemical Methods

An ELISA kit for DA (Biosense AS) has been subjected to full within- and inter-laboratory studies and is currently at the final stages of AOAC acceptance; it may be recommended as a

suitable alternative method (Type III). A Lateral Flow Immuno Chromatography (LFIC) strip test (Jellett Rapid Testing) has received limited evaluation but also appears a suitable screening test (Type IV).

Instrumental Assays

LC-UV is the current basis for regulatory testing of the DA group. The older AOAC method using acid extraction has been collaboratively studied and meets Type II criteria. However, stability of DA in the extract is poor and the method using 50% methanol extraction (Quilliam 1995) is now preferred. Two inter-laboratory trials and proficiency testing (QUASIMEME) support this method as meeting Type III criteria.

LC-MS methods have been developed which are suitable for screening and for confirmation, particularly where isomers of DA are present. One method has received a full within-lab validation and is a good candidate for moving from Type IV to Type III status when further interlab studies are completed. A TLC method is available as an inexpensive screening test (Type IV).

Recommendation for choice of Reference Method (Type II)

The Quilliam LC-UVD method is expected to receive full acceptance by CEN in 2005 and is recommended as the reference method.

Management of Analytical Results

Analytical data for all methods should be expressed as mg DA equivalents per kg of whole flesh. Toxicity equivalence factors for the epi-DA and other isomers of DA are not currently available and are assumed as 1.0 in the interim.

Standards and Reference Materials

A calibration solution CRM and a mussel tissue CRM are available (NRC, Halifax).

5.5 Okadaic Acid (OA) group

Background information

Toxins from the okadaic acid group have been known to cause human illness since the late 1970´s. The syndrome was named diarrhoeic shellfish poisoning (DSP) due to the dominating symptom. The OA group has been detected in microalgae and/or bivalve molluscs globally. Analyses for this group have been a key part of many biotoxin monitoring programs. However, contamination by the OA group has been generally accompanied by other lipophilic toxins which often cause positives in animal bioassays and require further confirmatory testing to evaluate actual risks. This is leading to development of multi-toxin methods based on LC-MS so that the OA group can be regulated more accurately and quickly. [The importance of ester forms is now widely recognised and has implications for testing programs. Hydrolysis is required for detection of ester forms in methods other than *in vivo* assays] A regulatory level of 0.16 mg OA-eq/kg shellfish is implemented in some countries.

Biological Data

Absorption, Distribution, Metabolism and Excretion

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. There are no data on metabolism *in vivo*.

Mechanism of Action

OA, DTX-1 and 2 are potent inhibitors of the serine/threonine protein phosphatases 1 and 2A.

Toxicity in Animals

The lethal dose following oral administration of the DSP toxins is 3-6 times higher than the lethal dose required by the i.p. route. DTX1 and DTX3 have a toxicity similar to OA.

OA was found to be 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 the data are insufficient to take this effect into account in the risk assessment. No data are available for DTX2.

Observations in humans

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 is usually complete in three days. Human data from Japan (8 persons from 3 families, age 10-68) indicate a LOAEL of 1.2 to 1.6µg/kg bw. In a second study from Norway, 38 of 70 adults were affected at levels ranging from 1.0 to 1.5µg/kg bw.

Evaluation

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, and a safety factor of 3 because of documentation of human cases including more than 40 persons and because DSP symptoms are readily reversible.

The Expert Consultation found that because of insufficient data on the chronic effects of OA, no TDI could be established.

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. 5 mg OA equivalent/kg shellfish meat, respectively.

Gaps in the Data

More studies on pharmacokinetics are needed. To establish a TDI, data on long-term/carcinogenicity and further studies on genotoxicity (i.e. clastogenicity) and reproductive toxicity are needed. Further data on absorption, excretion and metabolism are also required.

Analytical Methodology

Available methods

In vivo Bioassays

Although several MBA methods have been established, the Yasumoto (1984) protocol is the most widely used (acetone extraction, diethyl ether partition, 3 mice, 24 hour observation). The long observation time enables detection of slow acting ester forms. The partition step eliminates some potential interferences, including STXs and DA, but not those from other lipophilic toxins. A more detailed procedure has been developed by Yasumoto (2002) to overcome YTX interference. However, ambiguity with respect to PTXs and cyclic imines remain. None of the MBA protocols have had formal inter-lab study. However, there is some

comparability data from several countries for MBA and LC-MS or LC-FL methods. The assay can only serve as a screening test, which creates difficulties for carrying out an inter-lab study to the harmonised protocol. Large-scale studies are required to accurately establish the rates of false positives and false negatives. MBAs are Type IV methods.
A rat bioassay (Kat, 1983) can detect OA group toxins through diarrhetic effects and food refusal (16 hr observation). Validation studies are limited. Sensitivity is adequate to enforce a 0.16 mg/kg limit. AZAs potentially interfere but not PTXs or YTXs. (Type IV method).

In vitro Functional Assays
Protein phosphatase inhibition (PP2A) assays are attractive because they give an integrated toxic response for the OA group without interference from other toxins. Two forms of the assay using either fluorimetric detection or colorimetric detection have undergone limited inter-lab study. These assays are being commercialised. A further variant using PP2A enzyme in a competitive displacement format is also being commercialised. LOQs are ca 0.05-0.1 mg/kg. Hydrolysis is required to detect ester forms. These assays are still at tentative (Type IV) status.

Immunochemical Methods
A commercial ELISA kit (DSP Check; UBE Industries, Tokyo) is quite widely used but has not received full validation. There is some uncertainty as to the cross-reactivity to DTX1 and DTX2. LOQ is 0.1 mg/kg. Another kit has recently been released commercially that can also detect DTX3 (Iyatron Co., Japan).

Instrumental Methods
A derivatisation LC-FL method (Lee et al 1987) has been routinely used in several laboratories and achieved good results for the OA group. The method has been inter-laboratory validated for OA in mussel DG and results for a collaborative study have been submitted for CEN approval. The LOQ is 0.1mg/kg. Formal validation data is lacking for DTX1 and DTX2 but the method has been used for these analogues. LC-MS methods are increasingly being used in routine monitoring programs. One method (McNabb et al 2004) has under gone an intensive single-lab validation (4 matrices; 2 fortification levels; natural contamination) and a limited inter-lab study. The LOQ is 0.05 mg/kg. These methods are type III candidates.

Recommendation for choice of Reference Method (Type II)
Because an LC-MS method is the best available option, a collaborative study of a multi-toxin method that includes the OA group should be conducted to fully meet Type II criteria.

Management of Analytical Results
Analytical data for all methods should be expressed as mg OA equivalents per kg of whole flesh. Toxic equivalents are 1.0 for analogues. For functional, ELISA and instrumental assays the result should include toxins released by hydrolysis of ester forms. For MBA, death of 2 or 3 mice within 24 hours is presumptive for presence of OA group toxins exceeding 0.16 mg/kg.

Standards and Reference Materials
An OA calibration solution CRM and mussel tissue CRM (OA and DTX1) are available (NRC, Halifax). It is highly desirable to also have calibration solution CRMs for DTX1 and DTX2, and further shellfish tissue CRMs containing this toxin group. As an interim measure, relative response factors of 1.0 have been used for LC-FL and LC-MS methods.

5.6 Pectenotoxins (PTX) group

Background information

The presence of pectenotoxins in shellfish was discovered due to their high acute toxicity in the 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 via the oral route and that they do not induce diarrhoea. There are no data indicating adverse effects in humans associated with pectenotoxins in shellfish. PTXs exclusively arise from *Dinophysis spp* and are always accompanied by toxins from the OA group. Therefore, analytical methods must reliably distinguish these toxins, since they should be regulated separately. The provisional action level for PTX seco-acids (20 mg/100 g shellfish) is implemented in some countries.

Biological Data

Absorption, Distribution, Metabolism and Excretion

No data are available

Mechanism of Action

No data are available

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, PTX-11, 219-411 µg/kg; PTX -4, PTX-6, 500-770 µg/kg; PTX-7, PTX-8, PTX-9, PTX-seco acid, >5,000 µg/kg.

The acute oral toxicity of PTX-2 and PTX-2 seco acid is > 5,000 µg/kg in the mouse.

Although diarrhoea has sometimes 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.

Observations in humans

Although it has been suggested that PTX toxins were responsible for gastrointestinal effects in Australia, the observed effects were later attributed to okadaic acid esters.
Therefore there is no evidence of an adverse effect of PTX in humans.

Evaluation

The expert consultation considered that the database was insufficient to establish an acute RfD or TDI for the PTX toxins. Nevertheless, the human exposure of 0.6 µg/kg bw for a 60 kg person (Canada), and 1.6 µg/kg bw (Norway) is > 8,300 and > 3,100 times lower than the LD_{50} by gavage in mice.

Gaps in the Data

More data on the subchronic oral toxicity are needed before an acute RfD can be established. To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are needed. Information on absorption, excretion and metabolism is also required.

Analytical Methodology

Available methods

In vivo Bioassays

PTXs, but not seco acid metabolites, are toxic to mice by i.p. injection but the symptoms do not involve diarrhoea. The MBA (Yasumoto 1984) will respond to PTXs but is subject to interference from the OA group and YTXs. The revised protocol (Yasumoto 2002) can remove the interference from YTX but not OA group toxins.

Immunochemical Methods

A highly sensitive ELISA has been developed that detects PTX and analogues. It is being commercialised. Cross-reactivity to PTX2 seco acids is low. Full within-lab validation is not yet available. On the basis of the current limited evaluation it appears a suitable screening test (Type IV).

Instrumental Methods

LC-MS methods have been developed for PTXs which are suitable for screening and for confirmation. One multi-toxin method has received a full within-lab validation (4 matrices, 2 fortification levels) and limited inter-laboratory study (McNabb et al 2004). Good recovery data has been reported for PTX2, PTX1 and PTX6 from scallop tissues using LC-MS detection. An LC-MS method is a good candidate for moving from Type III to Type II status when further interlab studies are completed.

Recommendation for choice of Reference Method (Type II)

Because an LC-MS method is the best available option, a collaborative study of a multi-toxin method that includes PTXs should be conducted to fully meet Type II criteria.

Management of Analytical Results

Analytical data for all methods should be expressed as mg PTX2 equivalents per kg of whole flesh. Toxicity equivalence factors for the analogues of PTX2 are not currently available and are assumed as 1.0 in the interim.

Standards and reference materials

A calibration solution CRM is available for PTX2, and one for PTX2sa is under development (NRC Halifax).

5.7 Saxitoxin (STX) group

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. On the basis of case reports, the intake of toxins necessary to induce various 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 have been found worldwide. They are produced by *Alexandrium spp* 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 about fifty years, and generally no PSP has been associated with commercially harvested shellfish. The

same regulatory limit is presently used in many other countries. The MBA has been widely used in monitoring programs.

Biological Data

Absorption, Distribution, Metabolism and Excretion

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 hours. 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.

Mechanism of Action

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, though the affinities differ greatly.

Toxicity in Animals

The potency of different saxitoxins varies widely. From i.p. studies in mice, the carbamates and the decarbamoyls are the most toxic, while the sulfocarbamoyls exert lower acute toxicity. Based on LD_{50} data in mice, the saxitoxins are four times less toxic by i.p. injection than when given i.v., and about 100 times less toxic orally than i.v. Significant species differences in oral toxicity have not been observed.

Observations in humans

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

In an examination of several case series on PSP in Canada, involving about 60 persons, age 3-72, and covering some 20 incidents of poisoning between 1970 and 1990, the exposure to STX was estimated. For the affected persons, the symptoms of PSP were classified as mild, moderately severe or extremely severe. Mild cases generally had consumed 2-30 µg/kg bw, while the more severe cases generally involved exposure > 10-300 µg/kg bw. 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.

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 STXequ/kg bw and a safety factor of 3 because documentation of human cases includes a wide spectrum of people (occupation, age and sex) and mild illness is readily reversible.

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

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 equ./kg, respectively.

Gaps in the Data

In cases of PSP, there is a need for better collection of implicated samples and detailed collection of patient information, as well as the effects of food processing, in order to improve the quality of data on which to base future evaluations.

The Expert Consultation strongly recommended that all data on human intoxication be critically and thoroughly examined by an expert panel, including epidemiologists and toxin chemists, in order to select the most reliable data with the goal of increasing the accuracy of the estimate of the lowest acute toxic dose. In addition, the group should also examine how the history of use of the established tolerance limit (0.8 mg STX equ/kg) can be put into perspective.

Analytical Methodology

Available methods

In vivo Bioassays

The AOAC protocol for MBA is widely used and has provided health protection in many member states when used within a biotoxin monitoring program. However, performance was highly variable in an inter-lab study involving 9 European labs (FAPAS 2003). Some tightening of the protocol (e.g. pH adjustment of the extract) has been recommended to improve the reproducibility (CRL, APEC). Accurate conversion from MU to mg/kg requires calibration of mouse strain sensitivity to STX according to the AOAC protocol. The detection limit of the MBA is 0.4 mg/kg STX.2HCl equivalents and considerable uncertainties exist at levels close to this limit. The method may underestimate true levels, e.g. when levels of ca. 0.8 mg/kg are detected by the MBA, the actual concentration present may range from 1.2 to 2.1mg/kg (due to salt effects). Ethical issues, relating to the use of live animals, affect the acceptance and use of MBA in many member states.

In vitro Functional Assays

The receptor binding assay (RBA) using tritiated-STX and rat brain membrane preparations with shellfish extracts prepared by the AOAC protocol has shown excellent correlations to the MBA (van Dolah). The LOQ is 0.001 mg/kg STX.2HCl equivalents. Interlaboratory trials are proceeding to establish the full performance characteristics. Availability of the labelled reagent and use of radioisotopes are on-going issues for routine use of this RBA.

Other functional assays are at a preliminary stage of validation including a saxiphylin receptor binding assay and a fluorescence sodium channel activation cell assay (Botana).

Immunochemical Methods

Antibodies are not available with binding characteristics that match the toxicity spectrum of all the STXs. Therefore immunoassays cannot deliver quantitative toxicity data for mixtures, particularly over wide geographical regions and different algal species. The PSP strip test (Jellett Rapid Testing) has delivered promising data in extensive studies in North America and Europe (UK) with a low false negative rate. This has led to its approval as a screening test within biotoxin monitoring programs in several countries.

Instrumental Methods

For LC detection, oxidative conversion of STXs to fluorescent derivatives is required. As the fluorescence yield is compound dependent, individual toxin calibration is required and TEFs are required to calculate the total STX equivalents. The Lawrence pre-column LC-FL method can provide a full coverage of STXs. The method has been inter-lab studied in Europe (mussel and two toxins) and is pending approval by CEN. A collaborative study had wider scope (four matrices and 12 toxins) and has been submitted to AOAC for approval. Accuracy and precision were good and correlation to MBA data was high. The LOQ is ca 0.1 mg/kg STX equivalents, dependent on the composition of toxins. The Oshima post-column LC-FL method is widely used but has not had a full inter-laboratory study. Correlations with MBA have been given favourable results. It is recommended as a Codex type IV method but should be promoted to type III through further within- and inter-lab validations. Other instrumental assays such as LC-MS are at a tentative stage (Type IV).

Recommendation for choice of Reference Method (Type II)

The AOAC International MBA protocol has been widely used and has protected public health for over 60 years. However, if the Codex limit is set below 0.8 mg/kg this method will not be applicable. The Lawrence LC-FL method is recommended as a possible reference method. Final AOAC International acceptance of this method is pending.

Management of Analytical Results

Analytical data for all methods should be expressed as mg STX.2HCl equivalents per kg of whole flesh. The Oshima TEFs should be used with instrumental methods but accuracy of results may be limited by the availability of some of the standards necessary to evaluate total toxicity in shellfish.

Standards and Reference Materials

Calibration solution CRMs for STX and 12 analogues are available (NRC, Halifax). An STX reference material for calibration is distributed by US-FDA CFSAN. Two mussel tissue CRMs are available (JRC/IRMM, Geel, Belgium).

5.8 Yessotoxin (YTX) group

Background information

Yessotoxins are produced by *Protoceratium reticulatum*, and they 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 they do not induce diarrhoea. There are no reports of human intoxications caused by yessotoxins. Consequently, yessotoxins should be regulated separately from the okadaic acid toxin group (DSP toxins) The analysis of YTXs pose considerable potential problems due to the large number of analogues produced by the algae and their extensive metabolism in shellfish. YTXs are persistent in shellfish tissues and therefore, depending on the regulatory significance, may require long-term monitoring in management programs. Regulatory level of 1 mg/kg shellfish has been implemented in some countries.

Biological Data

Absorption, Distribution, Metabolism and Excretion

Limited absorption of the toxin from the gastrointestinal tract has been observed, but no further data on absorption, distribution, metabolism and excretion are available.

Mechanism of Action

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.

Toxicity in Animals

Acute toxicity data, based on ip administration, are available for 9 YTX analogues. Of these, 7 were of similar toxicity to YTX itself, with LD_{50} values between 100 and 750 µg/kg bw. Two analogues were much less toxic, with no effects being recorded at a dose of 5000 µg/kg bw. 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.

Observations in humans

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

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 YTX eq/kg bw.

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

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

Gaps in the Data

Low concentrations of YTX (0.5 ng/ml) cause the disruption of the tumour suppressor E-cadherin *in vitro*, indicating a possible risk that YTX might favour tumour spreading and metastasis formation *in vivo*. To establish a TDI, data on long-term/carcinogenicity and genotoxicity and reproductive toxicity are needed. Information on absorption, excretion and metabolism is also required, as are studies on the mechanism of action of YTX.

Analytical Methodology

Available methods

In vivo Bioassays

The modification of the MBA protocol by Yasumoto (2002) prepares two fractions that separates YTXs from other lipophilic toxins but this has not solved all problems, including

variability due to matrix effects. However, a higher regulatory limit for YTX would enable use of more dilute extracts to reduce these effects.

In vitro Functional Assays

Research papers document the development of functional assays based on effects of YTXs on E-cadherin and phosphodiesterases. While applicability to analysis of shellfish extracts was demonstrated, these assays have not yet been developed or validated as routine assays and therefore are currently tentative methods - type IV category.

Immunochemical Methods

An ELISA has been developed that detects YTX and a wide range of YTX analogues (Briggs et al 2004). It is being commercialised and has been subjected to within-lab validation and a limited inter-laboratory study. The precision characteristics appear very good and should be confirmed in a full collaborative study. A high correlation of data from ELISA and LC-MS analyses has been obtained. However, ELISA detects more analogues than LC-MS and the relationship of the 'Total YTX' data from ELISA to the levels of YTXs to be regulated must be resolved. On the basis of the current limited evaluation it appears a suitable screening test (Type IV).

Instrumental Methods

A derivatisation LC-FL method (Yasumoto & Takizawa 1995) has been routinely used in several laboratories and achieved good results for YTX, homoYTX and the 45OH metabolites. It cannot detect carboxy-YTX and other analogues lacking the conjugated diene. The recommended status is Type IV until further validation data is available, including inter-lab study.

LC-MS methods have been developed for YTXs which are suitable for screening and for confirmation. One multi-toxin method has received a full within-lab validation (4 matrices; 2 fortification levels; naturally contaminated samples) and limited inter-laboratory study (McNabb et al 2004). The method is a good candidate for moving from Type III to Type II status if further interlab studies are completed.

Recommendation for choice of Reference Method (Type II)

Because an LC-MS method is the best available option, a collaborative study of a multi-toxin method that includes YTXs should be conducted to fully meet Type II criteria.

Management of Analytical Results

Analytical data for all methods should be expressed as mg YTX equivalents per kg of whole flesh. Toxicity equivalence factors are only available (i.p.) for a few analogues of YTX.

Standards and Reference Materials

A YTX calibration solution CRM should be available by end of 2004 (NRC, Halifax). A naturally contaminated shellfish tissue CRM is also required. Calibration standards for other analogues are also required, depending on toxicological significance, especially for LC-MS methods.

5.9 Summary Tables of Toxicology and Methods of Analysis

Table 2: Summary data used in the derivation of the acute RfD, as well as derived and current guidance levels.

Toxin Group	LOAEL(1) NOAEL(2) µg/kg bw	Safety Factor (Human data (H) Animal data (A))	Provisional Acute RfD[a]	Derived Guidance Level/ Max Level based on consumption of 100g (1), 250g (2) and 380g (3)	Guidance Level/Max Level currently implemented in some countries[b]
AZA	0.4 (1)	10(H)	0.04 µg/kg 2.4 µg/adult	0.024 mg/kg Shellfish Meat(1) 0.0096 mg/kg SM (2) 0.0063 mg/kg SM (3)	0.16 mg/kg SM
BTX			N/A		0.8 mg/kg SM as PbTx-2
Cyclic Imines			N/A		
DA	1,000 (1)	10(H)	100 µg/kg 6mg/adult[a]	60 mg/kg SM(1) 24 mg/kg SM(2) 16 mg/kg SM(3)	20 mg/kg SM
OA	1 (1)	3(H)	0.33µg/kg 20 µg/adult[a]	0.2 mg/kg SM (1) 0.08 mg/kg SM (2) 0.05 mg/kg SM(3)	0.16 mg/kg SM
PTX			N/A		
STX	2 (1)	3(H)	0.7 µg/kg 42 µg/adult[a]	0.42 mg/kg SM(1) 0.17 mg/kg SM(2) 0.11 mg/kg SM(3)	0.8 mg/kg SM
YTX	5,000 (2)	100(A)	50 µg/kg 3 mg/adult[a]	30 mg/kg SM(1) 12 mg/kg SM(2) 8 mg/kg SM(3)	1 mg/kg SM

[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 3: Summary of Methods for Analysis of Marine Biotoxins and Methods Recommended as Reference Methods

Toxin Group	Animal assays	Functional assays	Immunoassays	Analytical tests
Azaspiracid	MBA/RBA	Cell morphology	Non applicable (N/a)	*LC-MS*†
Brevetoxin	APHA-MBA	Na-channel receptor binding assay, Neuroblastoma	ELISA	*LC-MS*†
Cyclic imine	MBA	N/a	N/a	*LC-MS*†
Domoic Acid	N/a	Receptor-binding assay	ELISA, Immunobiosensor	LC-UV*, LC-FL, LC-MS, TLC
Okadaic acid	(S)MBA/RBA	PP2a, PP1, F-actin	ELISA	*LC-MS*† LC-FL
Pectenotoxin	MBA	F-actin	N/a	*LC-MS*† LC-FL, LC-UV
Saxitoxin	AOAC-MBA	Na-channel receptor binding assay Saxiphilin receptor binding assay, Neuroblastoma	ELISA, FLIC	LC-FL*, LC-MS, FIFLD
Yessotoxin	MBA	E-cadherin fragmentation PDE-enhancement,	ELISA	*LC-MS*†, LC-FL

* Recommended as reference method;
† Recommended as reference method after completion of successful collaborative trial

6. Monitoring

6.1 The Role of Micro-Algal Monitoring in Marine Biotoxin Management

Micro-algae (including planktonic and benthic organisms) are the primary source of biotoxins in bivalve molluscs.

A marine biotoxin management programme should be described in a marine biotoxin management plan (MBMP). The MBMP should include marine biotoxin action plans (MBAPs) for growing areas containing, for example, sampling strategy and requirements (frequency, sample size and composition), analyses to be carried out, and management action to be based on monitoring results and expert judgment.

Toxicity monitoring cannot be replaced solely by micro-algae monitoring. Information from micro-algal monitoring, especially if it is carried out regularly (for example weekly during

harvesting), as part of a bivalve mollusc biotoxin management programme, has particular strengths, including:

- Generally, observable concentrations of toxic micro-algae precede critical levels of toxins in bivalve molluscs and, therefore, allows management options to be considered, such as precautionary closures, intensified monitoring or depth-specific sampling.
- Micro-algal monitoring can also help focus shellfish testing, for example on likely toxins, at the right location, at the appropriate time and when new toxin-producing species of micro-algae are found in an area.
- Micro-algal monitoring as part of an integrated biotoxin management programme, is cost effective and operationally efficient.
- It may be used to investigate unknown, unusual or atypical toxic events.
- It may be used to provide information to set or use switching factors. These may activate associated management options.
- It may provide information not only on the onset of a toxic event but on the duration of any intensified management action.

Therefore, for early warning purposes and direct risk management activities it is recommended to have a programme to monitor growing areas for species of toxin-producing micro-algae. The programme should also include evaluation of other environmental conditions, for example wind, water temperature and salinity, which may suggest upwelling, stratification or mixing. These conditions may indicate that favourable conditions for a toxic event are developing.

However the weaknesses of such a system may include:

- Micro-algal observations may not accurately reflect the actual level of toxins in shellfish. In part this may be due to significant inter- and intra-species variability in toxin profile and toxin content for many micro-algal species even from the same area and over a short period.
- While micro-algae are the primary source of toxicity in shellfish, the toxins may remain in shellfish long after the toxic micro-algae are gone. Thus, the absence of toxic micro-algae cannot be taken as an indication that the shellfish are safe.
- Micro-algae are not always distributed uniformly in either time or space. “Patchy” distribution of micro-algae may make representative sampling difficult.
- The logistics of sampling offshore or remote areas, where scallops or clams for example are fished, may make micro-algal monitoring less cost effective.
- Special monitoring arrangements may be necessary to address the problems posed by benthic species of toxic micro-algae, for example *Prorocentrum lima.*

In conclusion, decisions made on the safety of shellfish can only be based on the direct measurement of toxins in shellfish flesh. However, an integrated shellfish and micro-algal monitoring programme is highly recommended to provide expanded management capability and enhanced consumer protection.

Furthermore, recent developments indicate that micro-algal monitoring coupled with operational oceanographic, meteorological, and remote sensing data, including modelling and other measurements may be used to base advice on the imminent onset of harmful events.

6.2 Indicator Micro-algal Species

Table 4. Examples of source indicator organisms for some of the toxin groups.

Toxin Group	Genus	Example species
Azaspiracid	*Protoperidinium**	*crassipes**
Brevetoxin	*Karenia*	*brevis*
Cyclic Imines	*Alexandrium*	*ostenfeldii* (for spirolides)
Domoic Acid	*Pseudo-nitzschia*	*australis, seriata, pungens, multiseries*
Okadaic Acid	*Dinophysis*	*acuta, acuminata, sacculus, fortii, caudata*
	Phalachroma	*rotundatum*
	Prorocentrum	*lima*
Saxitoxin	*Alexandrium,*	*tamarense, minutum, catenella*
	Gymnodinium,	*catenatum*
	Pyrodinium	*bahamense*
Yessotoxin	*Protoceratium*	*reticulatum*
	Lingulodinium	*polyedrum*

*Suggested source of Azaspiracid

6.3 Indicator Shellfish Species

The selection of an indicator shellfish species for each toxin group is problematic because the rate of toxin uptake and depuration is unique to the combination of species, toxin and geographic location.

It is important to note that, using an indicator shellfish species, the absence of toxicity in the indicator species is assumed to imply the absence of toxicity in other species in the growing area. This implication must be verified for each shellfish species and for each group of toxins before defining a particular shellfish species as an indicator for that growing area.

6.4 Sampling

A micro-algal and shellfish sampling protocol over time and space should include the adequate location and number of sampling sites. Sampling frequency must be sufficient to address spatial-temporal changes in micro-algae, toxins in shellfish and to cover the risks of rapid rises in shellfish toxicity.

Spatial Representational Sampling

The selection of sampling stations for both benthic and suspended culture should be based on sites which have historically presented toxicity in the early stages of a toxic event. It is recognised that sampling, generally, cannot be carried out in a statistically valid way without excessive cost. In order to protect public health, the selection of sampling stations should give

appropriate coverage of the extent of a toxic event or the likely "worst case scenario" in a growing area. This should be based on expert judgment using the following factors:

- Hydrography, known upwellings, fronts, current patterns and tidal effects.
- Access to sampling stations in all weather conditions during harvesting.
- Desirability of toxin and micro-algal sampling at the same sampling station.
- In addition to primary (routine) stations, the need for secondary (complementary) and offshore stations.
- Existence of *in-situ* growth (for example, toxic micro-algae from cyst beds).
- The advection of offshore toxic micro-algal blooms into growing areas.

Routine sampling for micro-algae will generally mean taking an integrated sample from the water column. When a toxic event is in progress or developing, targeted, depth-specific sampling should be considered.

Sampling for shellfish grown in suspension, should at least involve an integrated sample composed of shellfish taken from the top, middle and bottom of the lines.

Temporal Representational Sampling

Minimum weekly sampling frequencies are adopted by most monitoring programmes in areas where toxicity is prevalent and where harvesting is taking place or about to take place. Decisions on the frequency of sampling should be based on risk evaluation. Inputs into the decision may include factors such as seasonality (toxicity and / or harvesting), accessibility, historical baseline information, including toxin and micro-algal data, and the effects of environmental factors such as wind, tide and currents.

Sampling frequency and the factors that may lead to it being changed should be described in a "Marine Biotoxin Action Plan" for the growing area.

Shellfish Sample Size

There is no internationally agreed sample size for different shellfish species. There may be high variability of toxicity among individual shellfish. The number of shellfish sampled should be sufficient to address this variability. For this reason, the number of shellfish in the sample, rather than the mass of the shellfish flesh should be the determining factor for the sample size. Additionally, the size of the sample should be sufficient to allow the test or tests for which the sample is being taken to be carried out, and the shellfish sampled should be of the size marketed.

7. Replies to Specific questions posed by CCFFP

Q1 Provisions of Scientific Advice for the Establishment of Safe Upper Limits:

Review of toxicological information and provisional scientific advice to define which toxins belong in which toxin group, and recommendations for the establishment of upper safety limits for the following toxin groups: PSP-, DSP-, ASP-, AZP- and NSP-toxins, and YTXs and PTXs .

Please find the evaluation section in each toxin specific section

Q2. Provide advice on management of ' new toxins' and 'newly discovered analogues of existing toxins' where either:

i. There is no epidemiological evidence of illness resulting, or

ii. Where epidemiological evidence exists.

New classes of compounds

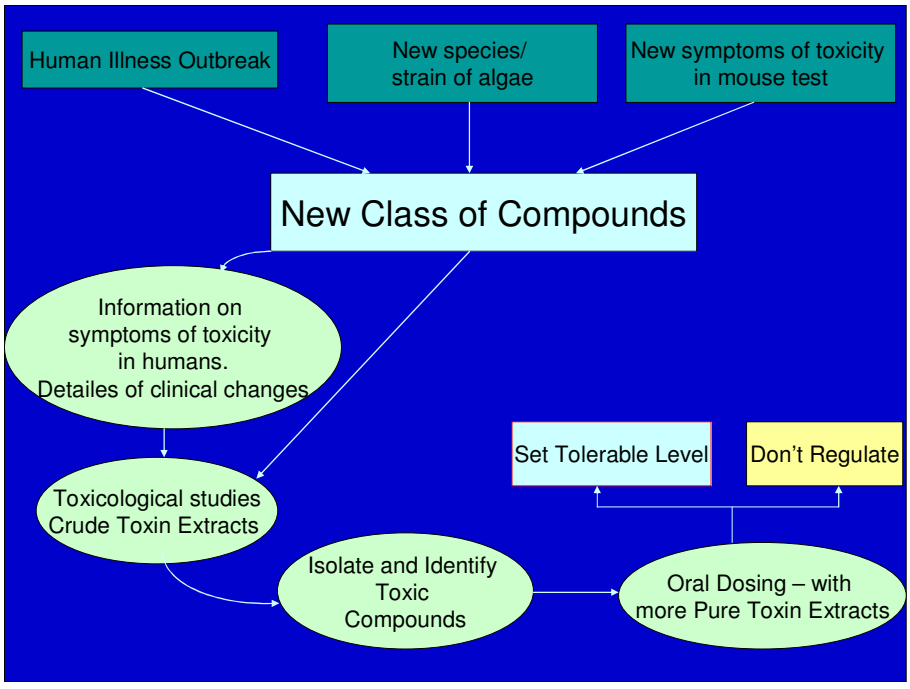
The Consultation envisaged three situations in which new toxins may be identified (Fig. 1):

1) An outbreak of poisoning in humans that is not associated with known toxins.
2) The identification of a new species or strain of algae.
3) Unusual clinical signs in the mouse bioassay.

In the case of human intoxication, the Expert Consultation recommends that every effort should be made to identify the symptoms and clinical changes in affected individuals, in order to give information on the target site of the new toxin. Samples of the material associated with the intoxication should be gathered and stored.

Initial evaluation of new toxins should be via oral administration in mice. Subsequently, the major toxin(s) should be separated and identified. These should then be evaluated for toxicity again by the oral route in order to establish acute RfDs and TDIs.

Figure 1: Proposed process for the management of new toxins

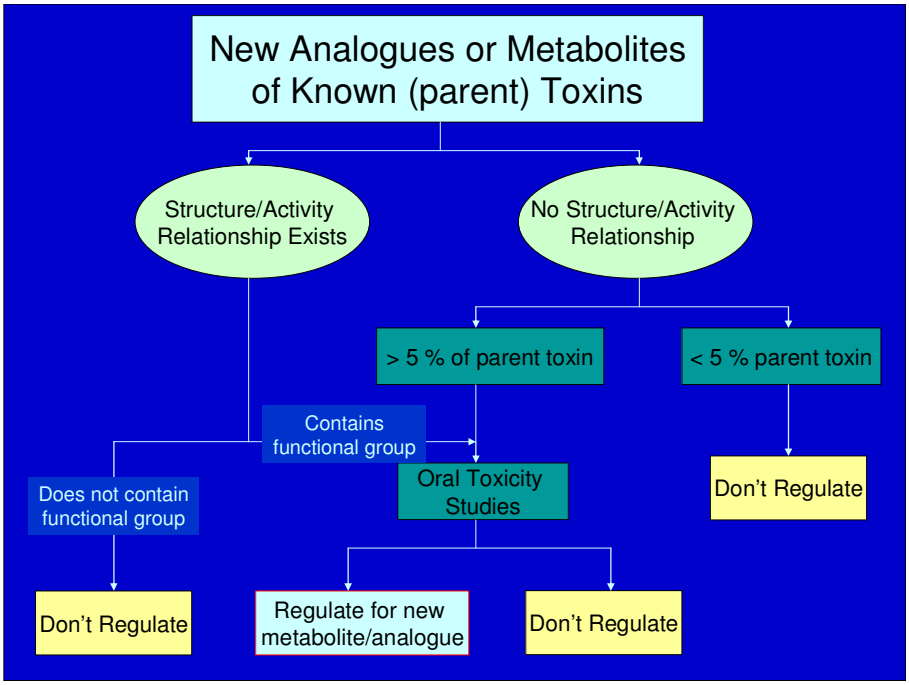


New analogues of existing toxins

For toxins for which adequate structure-activity data are available (Fig. 2), a decision with regard to regulation can be made on the basis of structure. If no adequate information is available, the Expert Consultation proposes that new analogues that are present in shellfish at less than 5% of the parent toxin should not be regulated against. Compounds present at a

concentration greater than 5% of the parent compound should be isolated, characterized and then toxicological properties investigated in order to establish an acute RfD and TDI.

Figure 2: Proposed process for the management of newly discovered analogues of existing toxins.



Q3. Provide guidance on the application of different methods of analysis concerning each toxin group:

- **Bioassays, analytical instrumental methods (HPLC, LC-MS…), immunological methods, other rapid methods – which methods should be considered reliable for each toxin group to ensure safety of product.**
- **Recommend choice of reference method in case of conflicting results**
- **Discuss needs for standards and reference materials**
- **Suggest management of analytical results, concerning precision, standard deviation, acceptance levels etc**

Please find the information described in each toxin specific section

Q4. Monitoring:

- **Provide guidance on which part of the seafood (shellfish or other) should be used for analysis (whole meat, different edible parts, digestive organs…)**

Please find the information described in the section 3.3

- **Provide guidance on sampling methods; suggest minimum representative sampling (size of sample, number of samples, different depths, frequency etc)**
- **Provide guidance on use of phytoplankton monitoring (strengths and weaknesses) as part of a shellfish biotoxin control program.**

- **Provide guidance on indicator organisms for the different toxin groups**

Please find the information described in the section 6.1 to 6.3.

Q5. Geographic Distribution:
Provide information on the existence of biotoxin forming marine algae in various geographical regions of the world.

Micro-algae responsible for the production of the toxins within the major toxin groups saxitoxins, domoic acid and okadaic acid, have a world wide distribution. Some species have restricted geographical distribution but toxic representatives from the different genera are known world wide.

Micro-algae responsible for the production of the rest of the toxins within the defined toxin groups listed in Table 3, have a more restricted geographical distribution, such as *Karenia brevis* which is mainly reported from the Gulf of Mexico..

In the case of the Azaspiracid toxin group the identity of the micro-algae responsible for the production of the toxins is uncertain. The very restricted distribution of Azaspiracid toxicity to Irish and Norwegian coastal waters, points to either an endemic species or the existence of endemic toxicity within a species with an otherwise more global distribution.

It is suggested that representative micro-algal species responsible for producing toxins from all defined toxin-groups are regarded as potentially worldwide.

8. Recommendations

8.1 To Member States, FAO, WHO

- Encourage Member states to implement public health programs that ensure that shellfish poisonings are captured 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 perform more accurate risk assessments.
- Promote increased international effort 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 relationship 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

8.2 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 ~~s~~ should be given to the situation in developing countries

8.3 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.