COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT ON RISK ASSESSMENT AND MONITORING OF PARALYTIC SHELLFISH POISONING (PSP) TOXINS IN SUPPORT OF HUMAN HEALTH

Introduction

1. A number of marine phytoplankton produce biotoxins that can be bioconcentrated by shellfish. Consumption of shellfish sufficiently contaminated with these toxins can result in human illness. Marine biotoxins can be categorised on the basis of clinical signs or chemical structure. Based on clinical signs, the main categories of shellfish poisoning are:

- Amnesic shellfish poisoning (ASP)
- Paralytic shellfish poisoning (PSP)
- Diarrhetic shellfish poisoning (DSP)
- Neurotoxic shellfish poisoning (NSP)

2. The Committee was asked for its view on the risk assessment of PSP toxins, and on the best method(s) of testing for biotoxins responsible for PSP in order to support protection of the health of the consumer.

Background

3. Paralytic shellfish poisoning (PSP) is a neurotoxic syndrome with signs including tingling and numbness of extremities, muscular incoordination, respiratory distress and muscular paralysis leading to death by asphyxiation. The signs of PSP are the result of blockade of voltage-gated sodium channels on excitable membrane[1].

4. The toxins responsible for PSP are saxitoxins (STXs), of which there are around 20 known analogues. STXs have been found worldwide.

5. STXs have varying toxicities, and the relative intraperitoneal (i.p) toxicities of the major PSP toxins, as determined in mice, have been used to sum the toxicity of the different toxins as STX equivalents (eq).

6. In 2004, a Joint FAO/IOC/WHO *ad hoc* Expert Consultation on Biotoxins in Bivalve Molluscs was asked by the Codex Committee on Fish and Fishery Products (CCFFP) to perform risk assessments for a number of biotoxins that are present in bivalve molluscs, and to provide guidance on methods of analysis

and monitoring of these toxins[2]. The COT has also been provided with a copy of the background document that supported the consultation[3].

Previous COT evaluations

7. The COT considered PSP toxins in 1994, when it reviewed a MAFF food surveillance paper on Naturally Occurring Toxicants in Food. The Committee noted that the development of chemical assays, immunological or other *in vitro* methods which are more sensitive and more specific than the bioassays currently used in monitoring for marine biotoxins in the UK, would not only be beneficial from an analytical viewpoint but would also avoid the use of experimental animals. The COT recommended:

- That the surveillance programme for detecting PSP toxins as described in the surveillance paper be continued

- That research to develop an assay to complement and/or replace the MBA for PSP be continued

Toxicology

Toxicokinetics

8. A study of PSP patients detected PSP toxin levels of 2.8-47 nM in serum during acute illness and of 65-372 nM in urine following acute symptom resolution, suggesting that urine is a major route of excretion[4]. Clearance of PSP toxins from serum was evident within 24 hours. Compared with cooked mussel samples, serum from individuals that had consumed them had a larger proportion of C1 and a lower proportion of gonyautoxin 2. In a post mortem analysis of tissues and body fluids obtained from two victims of PSP, toxins were detected in the gastric content, body fluids (urine, bile and cerebrospinal fluid), and in tissue samples (liver, kidney, lung, stomach, spleen, heart, brain, adrenal glands, pancreas and thyroid glands)[5]. The PSP toxins found in body fluids appeared to have undergone metabolism in the 3-4 hours following ingestion.

9. Rapid excretion in urine has been observed in rats after i.v. administration of STX at a sub-lethal dose (ca. 2 µg/kg). By 24 hours, approximately 58 percent of the administered dose had been excreted[6]. Experiments in cats indicate that STX excretion primarily involves glomerular filtration [7]. Studies investigating the potential for biotransformation of B1 to its carabamoyl form (STX) indicate that conversion occurs in artificial gastric juice (pH 1.1) but not in rat gastric juice (pH 2.2) [8].

Acute toxicity

10. The LD_{50} values for STX in mouse by different routes of administration are shown in Table 1. The oral LD_{50} values for species other than the mouse are shown in Table 2.

Table 1 Acute toxicity of STX in mice[9]

Route	LD50 in µg/kg bw
oral	260-263
intravenous	2.4-3.4
intraperitoneal	9.0-11.6

Table 2 Oral LD50 values of STX in various species[9]

Species	LD50 in µg/kg bw
rat	192-212
monkey	277-800
cat	254-280
rabbit	181-200
dog	180-200
guinea pig	128-135
pigeon	91-100

11. An i.p. mouse bioassay (MBA) has been used to determine the relative potencies of PSP toxins (see Table 3). Toxins were extracted from contaminated shellfish and separated by chromatographic methods. Each toxin was then tested using an MBA and the toxicity relative to STX (assigned as 1) calculated[3].

12. PSP causes death by asphyxiation due to progressive respiratory muscle paralysis. In animals (cat, rabbit) STX causes a decreased respiratory activity reflected in both a decline in the amplitude and velocity. Death can be prevented by artificial respiration, and depending on the dose, respiration may return spontaneously [9].

13. An intravenous dose of 1-2 µg STX causes a rapid weakening of muscle contractions, affecting contractions by direct stimulation and by indirect motorneurone stimulation in all skeletal muscle tissues. This dose level also induces a decrease of the action potential-amplitude and a longer latency time in the peripheral nervous tissue. Both motor and sensory neurones are influenced but the sensory neurones are inhibited at lower dose levels[9].

14. There are uncertainties about the possible effects of PSP toxins on the central nervous system. Most symptoms can be attributed to peripheral effects. However central effects may occur [9].

Table 3 Specific i.p. toxicities of saxitoxin analogues [3]

Toxin	Relative Toxicity
Saxitoxin (STX)	1
Neosaxitoxin (neoSTX)	0.92
Gonyautoxin 1 (GTX1)	0.99
Gonyautoxin 2 (GTX2)	0.36
Gonyautoxin 3 (GTX3)	0.64
Gonyautoxin 4 (GTX4)	0.73
Decarbamoyl saxitoxin (dcSTX)	0.51
Decarbamoyl GTX 2 (dcGTX2)	0.15
Decarbamoyl GTX 3 (dcGTX3)	0.38
B1 (GTX5)	0.064
C1	0.006
C2	0.096
C3*	0.013
C4*	0.058

* Estimated by the measurement of GTX1, GTX4 formed by acid hydrolysis

15. No data are available on repeat dose toxicity, mutagenicity, carcinogenicity, reproductive toxicity or developmental toxicity.

Human data

Symptomalogy

16. Human PSP cases have been defined as mild, moderately severe and extremely severe[10]. Typical symptoms for each category are:

- *Mild:* Tingling sensation or numbness around lips, gradually spreading to face and neck. Prickly sensation in fingertips and toes. Headache, dizziness, nausea.

- *Moderately severe:* Incoherent speech. Progression of prickly sensation to arms and legs. Stiffness and noncoordination of limbs. General weakness and feeling of lightness. Slight respiratory difficulty. Rapid pulse.

- *Extremely severe:* Muscular paralysis. Pronounced respiratory difficulty. Choking sensation.

17. Patients who survive PSP for 24 hours, with or without mechanical ventilation, have a high probability of a full and rapid recovery. Whether a severe response leads to death will be influenced by medical intervention, and this is likely to affect estimates of lethal doses.

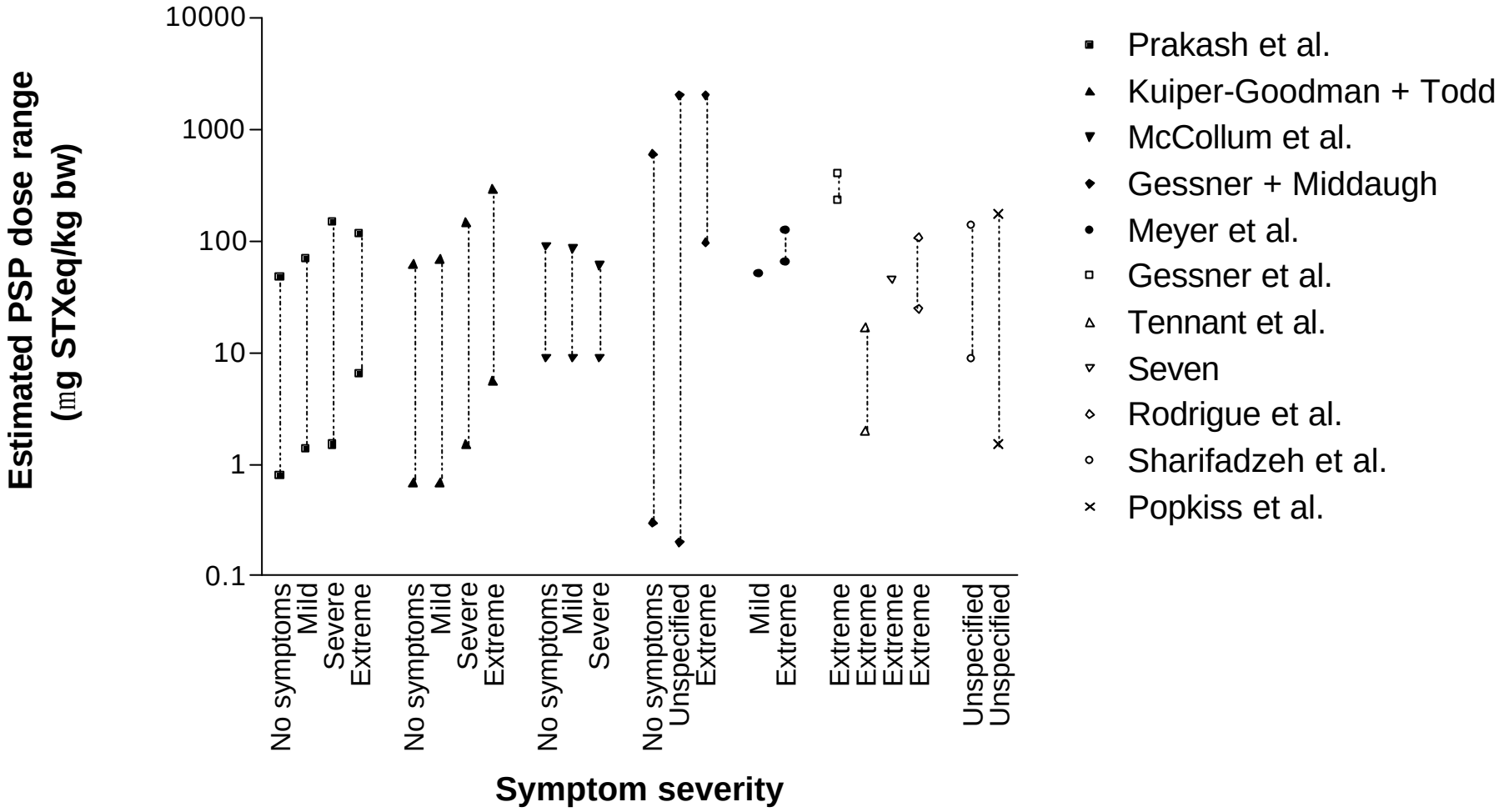
Epidemiology studies

18. A number of reports of PSP cases from a range of countries were reviewed by FAO/IOC/WHO[2,3]. The Committee reviewed these data as a possible basis for setting an acute reference dose. The data reviewed by the Committee are summarised in table 4 and Figure 1.

19. Except where noted otherwise, toxin levels were determined by MBA, using either leftover food or shellfish samples of a similar origin. Some authors applied a correction factor for the effects of cooking on PSP toxin levels, as studies have indicated that cooking can reduce the toxicity of contaminated shellfish by as much as 70%[10]. The toxins are not completely destroyed but are, in part, leached into the cooking fluids. Steaming tests with mussels and clams indicate that 50% of the toxin present in the raw tissue is present in the bouillon (the liquid left after cooking). While the bouillon is commonly discarded following steaming substantive amounts of PSP toxins may still be ingesting when eating a chowder, as the bouillon forms part of the chowder.

20. Most shellfish contaminated with PSP toxins contain a mixture of several saxitoxins. The toxicity of different toxins may be expressed in mouse units (MU). One MU is defined as the amount of toxin required to kill a 20g mouse 15 minutes after i.p. injection, and has been reported to be approximately equivalent to 0.18 µg STX eq[11]. To aid comparison, values that were reported in mouse units (MU) were converted to STX eq, assuming a conversion factor of 0.18 µg STX eq per MU.

Figure 1. Range of PSP toxin intakes associated with human illness



Symbols indicate the highest and lowest intake of PSP toxins associated with illness of varying severity, as noted in the human case reports. Values reported in mouse units have been converted to STX eq, as described in paragraph 20.

21. From the human case reports, the intake of STXs required to induce PSP symptoms varies greatly. This may be due to differences in susceptibility among individuals, and inaccuracies in exposure assessments due to differences in sampling and analysis of contaminated shellfish at the time of poisoning incidents and uncertainty with respect to amounts consumed.

22. Prakash *et al.*[10] reported data on PSP cases that occurred in New Brunswick, Canada from 1945 to 1957. Records were analysed for 131 individuals who had consumed contaminated shellfish from areas where shellfish toxicities were being monitored and included dates and the size, species and number of shellfish consumed by an individual. This information was combined with data on toxin levels determined by MBA and meat yields of shellfish to estimate the amount of toxins ingested by each person. The authors applied a conversion factor of 0.3 to the toxin concentration of raw shellfish when calculating the intake of individuals who had eaten known quantities of cooked shellfish, when no samples of the cooked shellfish were available.

23. Forty-nine cases, including several children, were categorised as having mild, severe, and extreme poisoning. These individuals had ingested toxins within the range of 85-4128, 90-9000 and 390-7000 µg STX eq per person, respectively. Assuming an adult bodyweight of 60 kg, the estimated toxin dose would be 1.4-69, 1.5-150 and 6.5-117 µg STX eq/kg bw, respectively. Only six patients had consumed less than 2 µg STX eq/kg bw. The report also includes information for 82 individuals who did not show any PSP symptoms, who had ingested between 50-2800 µg STX eq per person (0.8-47 µg STX eq/kg). The authors noted that because of the large person-to-person variation in sensitivity, average toxin consumption values are of limited significance.

24. The authors of the report suggested a number of factors that may affect sensitivity to PSP. Noting that two young children aged 2 and 8 years became ill after eating lower than average amounts, they speculated that young children may be more sensitive than adults. It was also suggested that sex may influence susceptibility. However, the COT considered that the variability of the data does not support these conclusions, and any differences in susceptibility related to age or sex appear to be within the range of uncertainty regarding the overall variability in human sensitivity to PSP.

25. The authors also noted that their records suggest PSP symptoms are more acute when contaminated shellfish are eaten alone on an empty stomach than when they are eaten as part of a normal meal, and that consumption of alcohol alongside shellfish accentuates symptoms. However, no details are provided to support these statements.

26. Several other reports have also assessed the effect of alcohol upon PSP. Gessner and Middaugh[17] applied a backward unconditional multiple logistic regression model to information relating to 30 ill and nine non-ill individuals for whom data on alcohol consumption, cooking history and race/ethnicity were available. While the analysis suggested that alcohol consumption may protect against PSP, no details on age or amounts of alcohol consumed are provided in

the paper, and the significance of the findings are unclear given the limitations of the study as noted in paragraph 29 below. Popkiss *et al.*[16] did not observe an association between symptom severity and estimated alcohol consumption in a report of a PSP outbreak in South Africa involving 17 individuals (see paragraph 31).

27. An unpublished Health Canada report[14] analysed data on Canadian cases of PSP from 1970-1990, together with information on outbreaks in Canada from 1944-1970, and from Guatemala in 1987 (see paragraph 31). Case histories were used to classify cases as mild, moderately severe or extremely severe. A number of assumptions were made in order to derive PSP toxin intakes when the data were incomplete. When the actual consumption of shellfish by an individual was unknown, typical values from the literature were used. Toxin concentrations in raw shellfish were adjusted for the effects of cooking. Cases for which the overall information was judged inadequate were not included in the final assessment. Intakes were reported as µg STX eq/kg bw, but it is not clear what bodyweight measurements or assumptions were made. However, in one section of the report, adult and child bodyweights of 60 and 25 kg, respectively, are mentioned.

28. Intakes for patients with mild, moderately severe and extremely severe PSP ranged from 0.7-70, 1.5-150 and 5.6-300 µg STX eq/kg bw, respectively. One patient with moderately severe symptoms had a reported intake of 0.3 µg STX eq/kg bw, but this was considered an outlier by the authors of the report. In addition, there were some individuals who did not develop symptoms after apparently consuming doses up to approximately 63 µg STX eq/kg bw. The authors noted that only two cases, both non-fatal, were reported where the PSP toxin dose was less than approximately 1.4 µg STX eq/kg bw. Therefore, they proposed a tolerable single intake of 1.4 µg STX eq/kg bw.

29. Gessner and Middaugh[17] reviewed 54 outbreaks of PSP that had occurred in Alaska between 1973 and 1992, involving 117 patients. PSP toxin levels were determined by MBA from either leftover shellfish collected from persons involved in an outbreak, or from shellfish collected from the same beach where shellfish implicated in an outbreak had been harvested. Data collection was performed over 20 years by several individuals and was not standardised. In addition, no correction appears to have been applied for the effects of cooking, and the authors acknowledged that they may have miscalculated the amount of toxin consumed by assuming that toxin levels from tested shellfish were identical to levels in ingested shellfish, and by assuming uniform weight within shellfish species. The estimated amounts of toxin ingested were reported as µg STX eq per person, and have been converted to µg STX eq/kg bw assuming an adult bodyweight of 60 kg. The estimated dose for 33 ill people (mean age 38 yrs) ranged from 0.2-2058 µg STX eq/kg bw. For 10 non-ill people (mean age 36 yrs), the estimated toxin dose was 0.3-610 µg STX eq/kg bw. Two persons with respiratory arrest ingested 98-2058 µg STX eq/kg bw.

30. A large-scale outbreak of PSP occurred in Guatemala in July-August 1987, affecting 187 individuals between 9 months and 86 years of age[18]. The

overall case fatality rate was 14%, but was highest in children under 6 years of age, at 50%. A case study involving 57 patients and 43 healthy family members from 19 households implicated clams harvested from local beaches as the source of the PSP toxins. Of the controls, five had consumed clams, but no information on their dose of PSP was provided. Analysis of clams harvested from the affected area on 1 August by MBA indicated a PSP concentration of 30,000 mouse units (MU)/100 g. Assuming a conversion factor of 0.18 µg STX eq per MU, this corresponds to a concentration of 5400 µg STX eq/100 g clam meat. HPLC analysis of a clam sample indicated a concentration of 7500 µg STX eq/100 g clam meat. A sample of soup collected from one of the affected households was analysed by MBA. The estimated intake of PSP toxins from this soup for one child who died, reported as MU and converted to STX eq, was 25 µg STX eq/kg bw. Four adult patients who died had each consumed 30-85 g clam meat. The authors calculated the amount of PSP toxins consumed by these individuals using the HPLC estimate of 7500 µg STX eq/100 g clam meat, but there appears to be an error in their calculations. The dose range for these individuals has been therefore been recalculated, assuming a 60 kg bodyweight, as 38-106 µg STX eq/kg bw.

31. An outbreak of 17 cases of PSP, none of which were fatal, occurred in South Africa in 1978[16]. The amount of PSP toxins ingested were based on MBA analysis of toxin concentrations in mussels collected from restaurants or affected coastal areas, and estimated mussel consumption. A factor of 0.3 was applied to adjust for the effects of cooking. The estimated dose ranged from 500-58,500 MU per person. Assuming an adult bodyweight of 60 kg and using the conversion factor of 0.18 µg STX eq per MU, these doses equate to 1.5-176 µg STX eq/kg bw. Only one patient had ingested less than 5 µg STX eq/kg bw. The authors did not observe an association between symptom severity and ingested dose of PSP toxins.

32. In 1954, a family of six adults and one child aged 12 years collected and consumed clams containing PSP toxins[20]. All members of the family experienced PSP symptoms, and two of the adults died. The authors of the report had sampled shellfish from the area where the family had obtained the clams on several of the days preceding and following the day that shellfish implicated in the incident were collected. PSP toxin levels within these samples were calculated by MBA and graphed. As no shellfish had been sampled on the day when the clams involved in the incident had been collected, toxin levels were estimated by interpolation on the graph, and a correction factor of 0.3 was applied to adjust for the effects of cooking. The amount of PSP toxins ingested by the patients was calculated by applying the estimated PSP toxin concentrations to the estimated shellfish consumption of the patients.

33. The authors estimated that one of the patients who died had consumed approximately 5800 MU, while the second fatality and one surviving adult had probably consumed 2400 MU. Assuming an adult bodyweight of 60 kg and using the conversion factor of 0.18 µg STX eq per MU, these doses equate to 17 and 7 µg STX eq/kg bw, respectively. The remaining patients were estimated to have probably consumed between 650 and 1000 MU (2 and 3 µg STX eq/kg bw).

34. In 1994, four outbreaks of PSP due to mussel consumption involving two, two, one and six ill individuals were reported in Alaska, USA [4]. Mussel toxin concentrations were calculated by MBA. For outbreaks 1-3, PSP toxin levels were determined from mussels collected within 24 hours of the onset of the outbreak from the implicated beach, while for outbreak 4, all persons had eaten boiled mussels and toxin concentrations were determined from left-over cooked and uncooked mussels.

35. Shellfish toxin concentrations for the four outbreaks ranged from 1778-19,418 µg STX eq/100 g. For 10 individuals for whom dose estimates were available, the lowest dose that caused illness was estimated to be 21 µg STX eq/kg bw, with a median dose of 167 µg STX eq/kg bw. Among four persons with respiratory arrest, who the authors suggested may be considered to have consumed a lethal dose, the estimated dose ranged from 230-411 µg STX eq/kg bw.

36. An outbreak of PSP occurred in the summer of 1968 in 78 individuals who had eaten mussels harvested from the Northumbrian coast, UK[15]. In total, the authors conducted interviews with 71 of the affected individuals. MBA analysis was performed on raw mussels obtained from the retailer that supplied 65 of the individuals, and also on samples that were cooked by the same method the retailer had employed. Toxin concentrations were estimated in MU, and converted to STX eq/kg bw using the conversion factor of 0.18 µg STX eq per MU and an adult bodyweight of 60 kg. Of the 22 persons who consumed an estimated dose of 9-30 µg STX eq/kg bw, 18% did not experience symptoms, while 23% and 59% reported mild and moderate symptoms, respectively. For the 42 individuals estimated to have ingested 30-60 µg STX eq/kg bw, 19% did not report symptoms, while 19% and 62% experienced mild and moderate symptoms, respectively. Only seven people ingested more than 60 µg STX eq/kg bw. Five experienced moderately severe symptoms, while two experienced no symptoms whatsoever. No fatalities were reported in this incident.

37. The Australia New Zealand Food Authority published a risk assessment on shellfish toxins in 2001[22]. This report claims that 2-3 µg STX eq/kg bw can produce moderate symptoms, 6.7-18 µg STX eq/kg bw can cause death, but 33-167 µg STX eq/kg bw is more likely to constitute a fatal dose, although no references are cited to support this statement. These values have been converted from STX eq per person, assuming an adult bodyweight of 60 kg.

38. The case fatality rate for PSP varies considerably. In relatively recent outbreaks in North America and Western Europe involving over 200 people, there were no deaths. However, in similar outbreaks in Southeast Asia and Latin America, case fatality rates of 2-14% have been recorded. Part of this difference may be related to how readily victims have access to hospital care[1].

39. Other estimated intakes resulting in illness or death, derived from case studies describing a single or small number of incidents or review papers are summarised in table 4.

FAO/IOC/WHO Evaluation

40. The unpublished data from Health Canada[14] (see paragraphs 27-28) was specifically mentioned in the FAO/IOC/WHO Consultation's conclusions[2]. It was noted that analysis of this report indicated that mild cases had generally consumed 2-30 µg STX eq/kg bw, while more severe cases generally involved an exposure of >10-300 µg STX eq/kg bw. On this basis, the Consultation proposed a provisional lowest observed adverse effect level (LOAEL) of 2.0 µg STX eq/kg bw. Considering that mild illness is readily reversible, and the epidemiology data represents a range of individuals with varying susceptibilities, the Consultation applied a safety factor of 3 to this LOAEL, establishing a provisional acute reference dose of 0.7 µg STX eq/kg bw.

COT Evaluation of toxicological data

41. The Committee noted that the available animal and human data are limited. A tolerable daily intake (TDI) could not be derived as the data all related to acute exposure. The acute exposure data were assessed in order to consider establishment of an acute reference dose.

42. The COT noted a large number of uncertainties in the human data. These relate to uncertainties in exposure assessments, for example due to disparities between toxin levels in tested shellfish compared with the levels present in shellfish that were actually consumed. While leftover cooked shellfish were analysed in some incidents, other reports were based on toxin concentrations determined in uncooked shellfish, either from the same batch of shellfish that had been consumed, or that had been collected from areas where consumed shellfish were obtained. In some reports, samples were collected on the same day as the shellfish implicated in the PSP outbreak, while in others shellfish had been collected on a different day.

43. Further uncertainties in exposure assessments relate to uncertainties with respect to amounts of shellfish reportedly consumed, and assumptions regarding the weight of the edible portions of specific shellfish species. While some studies had applied a correction factor to adjust for the effects of cooking, the precise effects of individual cooking practices on toxin levels are uncertain. In the majority of studies, PSP toxin levels in the shellfish were calculated using an MBA, and the identity of the specific toxins that had been consumed was unknown.

44. The Committee observed that some PSP cases have been reported following consumption of PSP toxins below the FAO/IOC/WHO's provisional LOAEL of 2 µg STX eq/kg bw. However, relatively few patients had been ill after consuming such amounts, and these studies were subject to the uncertainties noted above. FAO/IOC/WHO had considered that mild cases had generally consumed 2-30 µg STX eq/kg bw while more severe cases involved an exposure of >10-300 µg STX eq/kg bw[2].

45. Based on an overview of all the available data, and given the limitations regarding the exposure data, the Committee concluded that the FAO/IOC/WHO approach was reasonable.

46. FAO/IOC/WHO had applied a safety factor of 3 to the LOAEL of 2 µg STX eq/kg bw cited for mild effects to establish a provisional acute reference dose of 0.7 µg STX eq/kg bw. The value of 3 had been selected rather than a larger value because the epidemiological data on PSP represented a wide range of individuals with varying susceptibilities, and because mild illness is readily reversible. In addition, the COT noted that the reported dose range was likely to represent individuals at the extreme ends of sensitivity. The Committee noted that the proposed acute reference dose was about one-tenth of the dose range associated with severe illness and was therefore unlikely to be overly conservative.

47. The limited animal data would appear to support this approach. Applying an uncertainty factor of 1000 to allow for differences between species, for human variability and for extrapolation from a lethal dose to the oral LD_{50} of STX in monkeys of 277-800 µg/kg bw (table 2) would indicate an acute reference dose in the region of 0.3-0.8 µg/kg bw.

48. FAO/IOC/WHO assumed a portion size of 250 g would cover 97.5% of consumers in most countries. The Committee noted that this value was a reasonable estimate for high level shellfish consumption in the UK, based on analysis of information on consumption of cockles, mussels, oysters and whelks from the UK National Diet and Nutrition Survey Programme (NDNS)[23]. Given the acute effects of PSP, the Committee considered it essential to refer to high level portion size as the comparator in the risk assessment.

49. For a 60 kg adult, consumption of 250 g of shellfish containing 17 µg STX eq/100 g shellfish meat would result in an intake of PSP at the acute reference dose of 0.7 µg STX eq/kg bw. Because of the uncertainty and lack of precision in the data, the COT concluded that this value should be rounded to a single significant figure of 20 µg STX eq/100 g shellfish meat, which would be the maximum concentration considered to be without appreciable health risk.

50. The current regulatory limit for PSP toxins in shellfish is 80 µg STX eq/100 g shellfish meat, which could result in some individuals consuming greater than the proposed acute reference dose. There have been no reported incidents of PSP resulting from consumption of UK shellfish since the official UK monitoring programme was introduced. This could be interpreted as suggesting the current regulatory limit may provide adequate protection for human health. However, the Committee agreed that it would be imprudent to conclude that mild cases of PSP have not occurred in the UK, as they may go unreported. Furthermore, given the potential for PSP to result in severe illness or even death, the proposed acute reference dose should be supported.

51. Although some reports had suggested factors that may affect sensitivity to PSP, the variability in the available data does not allow identification of any specific susceptibility factors.

52. The Committee agreed with FAO/IOC/WHO that that there is a need for better collection of implicated samples and patient information in future PSP outbreaks, as well as more detailed information on the effects of food processing on toxin levels.

Monitoring of PSP toxins and regulatory levels

53. Current legislation requires shellfish containing 80 µg STX eq/100 g shellfish meat to be withdrawn from sale. A maximum concentration of 20 µg STX eq/100 g shellfish meat would be required in order to ensure that a 60 kg adult consuming 250 g of shellfish would not exceed the proposed acute reference dose of 0.7 µg STX eq/kg bw.

54. Mouse bioassays (MBAs), involving intraperitoneal injection of shellfish extract, are prescribed as the reference methods in EU legislation (Commission Regulations (EC) No 854/2004 and (EC) No 2074/2005) for detection of PSP biotoxins, and are used in the UK PSP monitoring programme. MBAs were developed in the 1930s for the detection of marine biotoxins in protection of public health, when specific analytical methodology was not available. Recent progress in development of certified reference material and alternative methods means it is timely to reconsider the most appropriate way of protecting public health.

55. The COT was asked to comment on the extent to which the available methods for detecting PSP toxins are appropriate for protecting public health. The COT consideration focussed on the MBA and two alternative methods, a high performance liquid chromatography (HPLC) technique and an immunoassay known as the Jellett Rapid Test (JRT).

56. The current MBA for PSP toxins in the UK is carried out using a method based on the updated Association of Analytical Chemists (AOAC) official method, and has a limit of detection of approximately 30 µg STX eq/100 g shellfish meat[24]. The HPLC method, developed by Lawrence et al. [25,26], has a substantially lower detection limit than the MBA currently employed, and is able to identify and quantify a range of PSP toxins. This method has recently undergone interlaboratory validation and has received AOAC approval for the determination of STX, GTX2,3 (together), GTX1,4 (together), dcSTX, B1, C1,C2 (together) and C3,4 (together) in some shellfish species (mussels, clams, oysters and scallops)[27].

57. The JRT, unlike the MBA and HPLC methods, is not a quantitative assay, which the manufacturer claims can be used to screen out samples found to contain approximately =40 µg STX eq/100 g shellfish flesh.

58. Tables 5-7 show the available data on the performance characteristics of the three methods. In general, these have been generated from testing of a relatively small number of samples.

Standards and reference materials

59. The use of methods based on HPLC for PSP monitoring programmes has previously been limited by a lack of availability of commercial standards for all the known PSP toxins. Since 2003, however, standards covering all the carbamate and most of the decarbamoyl saxitoxin families, which comprise the PSP compounds that are most toxic in the MBA, have been available. The predominant toxins that have been detected in UK samples are STX, GTX2 and 3, GTX 1 and 4, C1, C2, and NEO[28,29].

Comparative data

60. The Committee noted that a number of trials had been published in which two or three of the methods had been performed concurrently[26 28 37 38 39 40,24,41].

61. Evaluation of alternatives to the MBA by comparison with the MBA is problematic, given the MBA's inherent variability and that the method is unable to identity the specific toxins present within a sample.

62. A further complexity is evident in studies comparing the MBA with HPLC. The authors of the various studies have converted toxin levels determined by HPLC into STX eq, by multiplying the measured toxin concentrations by a relative toxicity factor, as determined by MBA. Although the same source has generally been cited for these relative toxicity values[42], the precise figures used differ in several reports.

63. As part of the interlaboratory study on the Lawrence pre-column method[26], a set of samples used in the study was also analysed by the MBA and by Jellett Rapid Test (JRT) in a single laboratory. To compare with the MBA result, individual PSP toxin levels obtained by HPLC were converted to STX eq using relative toxicity values[42]. In this study, similar results were generally obtained with the MBA and HPLC methods, although one sample that was negative in the MBA was found to contain 54 µg STX eq/100 g shellfish meat by HPLC.

64. In 2005, FSA Scotland funded a short project, which employed the Lawrence HPLC method to verify the presence or absence of PSP toxins in 147 extracts giving positive and negative results using the JRT in Scotland[28]. HPLC results agreed with the absence of toxins in JRT negative extracts, and revealed that the predominant toxins in JRT positive extracts were saxitoxin and GTX 2,3. Higher toxicity values were recorded using HPLC when compared to the MBA data. Similar results have been observed in previous comparisons of HPLC and MBA data, and are considered to be due to the underestimation of

PSP toxicity by the MBA[38,43,42]. Underestimation by MBA is thought to be due to high salt levels in the extracts and matrix effects[44,45].

65. Comparative HPLC and MBA data are available from the Portuguese PSP monitoring programme, where the Lawrence HPLC method has been implemented alongside an MBA since 1996. Concentrations of the different PSP toxins were summed as STX eq by conversion of measured PSP toxin levels to their relative toxicity in the MBA. For 79 tested samples, agreement between the MBA and HPLC was 87.3%, with a 12.7% incidence of a 'negative' MBA (defined in the report as = 80 µg STX eq/100 g shellfish meat) alongside a 'positive' (defined as = 80 µg STX eq/100 g shellfish meat) HPLC result. There were no incidences of a 'positive' MBA combined with a 'negative' HPLC result[38]. The authors of this report noted that problems had been experienced with the HPLC method due to the presence of two interfering compounds, one eluting close to STX and the other eluting close to dcSTX. However, introduction of solid-phase extraction, as recommended in the Lawrence method, removed one of the interfering peaks completely while the other was reduced by approximately 80%.

66. Data are also available from parallel trials of the JRT alongside the MBA comprising over 2000 samples including a wide range of shellfish species sampled from Alaska, Maine, Washington State, British Columbia, New Zealand and the UK[37]. In these trials, the JRT detected 100% of toxic extracts, defined as those containing =80 µg STX eq/100 g shellfish meat. One borderline toxic sample, determined to contain 78 and 86 µg STX eq/100 g shellfish meat in two separate MBAs, was interpreted as positive in one JRT and negative in the second. In addition, between 85-100% of extracts found to contain 32-80 µg STX eq/100 g in the MBA were also positive in the JRT test. The overall rate of JRT positives that were MBA negative was around 14%.

67. To date, 2939 shellfish extracts have been tested in tandem by MBA and JRT by the three UK shellfish monitoring laboratories. Of these samples, 70 were found to contain levels = 40 µg STX eq per 100 g of shellfish flesh by MBA, all of which tested positive by JRT. Of the remaining 2869 extracts, 350 tested positive by JRT, but were negative in the MBA.

68. New data comparing MBA and JRT results from the Californian PSP monitoring programme have recently been published[39]. The JRT was introduced to screen for shellfish containing PSP toxins in California following an initial study involving parallel testing of 232 mussel and oyster extracts by MBA and JRT. There were no instances of a negative JRT for a sample positive in the MBA, while 29% had a positive JRT result and a negative MBA result.

COT Evaluation of PSP detection methods

69. The Committee concluded that HPLC was currently the only method sensitive enough to detect PSP toxins at the concentration of 20 µg STX eq/100 g shellfish meat, considered by the Committee to be necessary for protection of

public health. It was important for the methodology to support detection of all toxins considered likely to be relevant to public health.

70. Potency of the different PSP toxins is currently compared based on the i.p. toxicity by MBA. However, it is not known how this relates to the oral toxicity of these toxins.

71. The COT considered that HPLC should be used for quantification of PSP toxins, subject to appropriate quality control measures and method validation in the testing laboratories, including investigation of possible interfering peaks that could mask the presence of toxins in different matrices.

72. The existing data comparing MBA and HPLC at the current regulatory limit provided reassurance that public health would not be compromised by not using the MBA. However, taking into account the inherent variability in results from bioassays, uncertainty with respect to the relevance to health of discordant results and the inability of the MBA to identify individual PSP toxins, comparative testing was not considered appropriate for validation of alternative methods.

73. As HPLC detects individual PSP toxins, relative i.p. toxicity values have been used to calculate the STX eq concentration within shellfish samples for comparison with a regulatory limit. Consideration should be given to the most appropriate method of summing the concentration of PSP toxins within shellfish samples.

74. The Committee was informed that it might be possible for the JRT to be re-engineered to detect lower concentrations of PSP. The Committee agreed that if this was possible it could be used as a screen, using HPLC for quantification of positive results.

75. At the current regulatory limit of 80 µg STX eq/100 g shellfish meat, the COT considered that, based on the data presented, the JRT was appropriate for use as a pre-screen to identify samples for quantitative testing, subject to appropriate quality control measures.

Conclusions

76. We note that the available animal and human data relate to acute exposure, and are therefore not suitable for the derivation of a tolerable daily intake for PSP toxins. The potential for long-term health effects arising from repeated exposure to PSP toxins is unknown.

77. We consider that human case reports should be used as a basis for risk assessment, while noting the uncertainties related to the amount and nature of PSP toxins actually consumed in cases of human illness.

78. We consider that 2 µg STX eq/kg bw is the best estimate of a LOAEL for mild illness in humans, taking into account the uncertainties in the available data. More severe cases may occur above 10 µg STX eq/kg bw.

79. We conclude that the LOAEL can be used as the basis for deriving an acute reference dose of 0.7 µg STX eq/kg bw, by applying an uncertainty factor of 3 to the LOAEL in order to allow for the absence of a no observed adverse effect level (NOAEL). A larger uncertainty factor is not required because the epidemiological data on PSP represent a wide range of individuals and are likely to include information relating to those who are most sensitive. This value for the acute reference dose is supported by the available data relating to oral toxicity in animals.

80. We note that a portion size of 250 g is a reasonable estimate for high level consumption of shellfish in the UK. We conclude that a PSP toxin concentration of 20 µg STX eq/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

81. HPLC is currently the only method sensitive enough for the detection of PSP toxins at a concentration of 20 µg STX eq/100 g shellfish meat.

82. We conclude that HPLC should be used for quantification of PSP toxins subject to appropriate quality control measures and method validation in the testing laboratories, including investigation of possible interfering peaks for different matrices. The methodology should support detection of all toxins that are likely to be relevant to public health.

83. At the current regulatory limit, the JRT could be used as a pre-screen to identify samples for quantitative testing, subject to appropriate quality control measures.

84. We agree that it would be appropriate to review this advice when information on the distribution of PSP toxins in UK shellfish becomes available from the more sensitive HPLC analyses.

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Table 4. Summary of PSP epidemiology data

Cases	Reported intake of PSP toxins	Derived dose of PSP calculated as µg STX eq/kg bw*	Assumptions
Reports based on measurement of PSP toxins in cooked shellfish samples left over from meal.			
3 adult cases, 2 men and 1 woman[12]	Mild symptoms (male patient): 17,000 MU ingested Respiratory failure (female): 22,000 MU ingested Fatality (male): 42,000 MU ingested	Mild symptoms: 51 Respiratory failure: 66 Fatality: 126	Exact number of mussels eaten known by number of shells left. Cooked and raw shellfish analysed by MBA. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
6 male cases. [13,3]	Consumption of 3-48 cooked mussels containing 4280 µg STX eq/100 g.	9-137	Assuming an edible mass of 4 g per mussel, and a bodyweight of 60 kg. Method of analysis unspecified.
Reports based on measurement of toxins in shellfish samples, exact source of shellfish unspecified. Adjustment made for effects of cooking on toxin levels.			
49 cases, male and female, including a child of 2 years old. 82 individuals without symptoms [10]	Mild symptoms: 85-4128 µg STX eq/person Severe symptoms: 90-9000 µg STX eq/person Extreme symptoms: 390-7000 µg STX eq/person No symptoms: 50-2800 µg STX eq/person Illness in 2-year old: 96 µg STX eq/person	Mild symptoms: 1.4-69 Severe symptoms: 1.5-150 Extreme symptoms: 6.5-117 No symptoms: 0.8-47 Illness in 2-year old: 8	PSP intake calculated by combining information on species, size and number of shellfish consumed with data on toxicity (from MBA data) and meat yield of shellfish. Correction factor applied for effects of cooking. Estimated dose calculated assuming an adult bodyweight of 60 kg, and 12 kg for a 2-year old.
91 cases in Canada from 1944-1990, and the outbreak in Guatemala detailed below. Details on age and sex not provided. [14]	Mild symptoms: 0.7-70 µg STX eq/kg bw Severe symptoms: 1.5-150 µg STX eq/kg bw Extreme symptoms: 5.6-300 µg STX eq/kg bw No symptoms: 0.7-63 µg STX eq/kg bw		Assumptions included: edible portion sizes for various species; if number of shellfish consumed unknown literature values used; toxin levels corrected for effects of cooking. Adult bodyweights of 60 and 25 kg appear to have been assumed. Only cases judged to have adequate data included in the assessment. **NB**: only 2 cases reported where toxin doses <1.4 µg STX eq/kg bw, tolerable single intake of 1.4 µg STX eq/kg bw proposed

Reports based on measurement of toxins in shellfish samples collected from affected beach, restaurant or retailer. Testing performed on cooked samples or results for raw samples adjusted for effects of cooking.			
71 patients, details of age and gender not provided.[15] NB Only UK PSP outbreak identified.	Mild symptoms: 3000-20,000 MU per person Moderate symptoms: 3000-28,700 MU per person No symptoms: 3000-30,000 MU per person	Mild symptoms: 9-60 Moderate symptoms: 9-86 No symptoms: 9-90	Toxin concentrations determined by MBA analysis of samples supplied by the retailer who supplied majority of patients. Samples analysed when raw and cooked. Intake assessment appears to be based on patient interviews. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
17 cases, 10 male, 7 female.[16]	500-58,500 MU per person	1.5-176	Toxin levels determined by MBA in shellfish collected from restaurants or affected coastal areas. Correction applied for effects of cooking. Intake assessment appears to be based on patient interviews. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Reports based on measurement of toxins in leftover cooked or raw shellfish samples, or shellfish collected from affected beach or restaurant. Some samples were collected after the incident. No reported adjustment for effects of cooking.			
Dose estimates provided for 33 ill people (mean age 38 yrs) and 10 non-ill people (mean age 36 yrs)[17]	Ill persons: 13-123,427 µg STX eq/person Respiratory arrest: 5863 and 123,427 µg STX eq/person Non-ill persons: 17-36,580 µg STX eq/person	Ill persons: 0.2-2058 Respiratory arrest: 98-2058 Non-ill persons: 0.3-610	Estimated PSP levels determined by MBA from either leftover shellfish or shellfish collected from affected beaches. Estimated dose calculated assuming an adult bodyweight of 60 kg. Authors acknowledged that toxin intakes might be miscalculated due to assumption that toxin levels in tested shellfish identical to those in ingested shellfish.
Four outbreaks involving 11 patients, 6 female, age ranging from 13-61 years. Dose estimates provided for 10 individuals.[4]	Median intake: 167 µg STX eq/kg bw (9176 µg STX eq) Respiratory arrest: 230-411 µg STX eq/kg bw Lowest dose causing illness: 21 µg STX eq/kg bw		Toxin levels in mussels determined by MBA from samples collected from affected beach within 24 hours of outbreak (3 outbreaks), or from left-over cooked and uncooked mussels (1outbreak). Conversion of median intake to dose, based on a 55 kg bodyweight; details not provided for other dose estimates.
Outbreak in Guatemala affecting 187 individuals; intakes estimated for 1 child and 4 adults, all of whom died.[18]	Fatality (adult): consumption of 30-85 g shellfish meat containing 7500 µg STX eq/100 g shellfish meat. Fatality (child): 140 MU/kg bw	Fatality (adult): 38-106 Fatality (child): 25	Intake of adults based on HPLC analysis of a clam sample; 60 kg bodyweight assumed. Intake in child based on MBA analysis of soup consumed. Weight of patient given as 25 kg. MU converted to STX eq using conversion factor of 0.18 µg STX eq per MU.
2 adult patients, 1	>15,000 MU per person	>45	Unconsumed shellfish analysed by MBA (cooked or uncooked unspecified).

male and 1 female.[19]			Empty shells used to estimate shellfish consumption. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Toxin levels extrapolated from graph of levels measured in shellfish harvested from the affected area before and after the incident. Correction factor applied for effect of cooking.			
6 adult patients aged 27-69 yrs (2 male and 4 female) 1 female child aged 12 yrs. 2 adult fatalities.[20]	Fatalities and 1 surviving patient: Approximately 2400--5800 MU per person Other patients: Approximately 650-1000 MU per person	7-17 2-3	Estimated PSP levels determined by MBA in samples collected from the implicated beach on days preceding and following the day shellfish involved in incident were collected. Values graphed and toxin concentrations estimated by interpolation. Correction applied for effects of cooking. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Review data; source of estimate unspecified.			
Review data[21]	Lethal dose: 500-12,400 µg STX eq/person	8-207	No details provided on source of data. Estimated dose calculated assuming an adult bodyweight of 60 kg.
Australia New Zealand Food Authority[22]	Moderate symptoms: 120-180 µg STX eq per person 'May cause death': 400-1060 µg STX eq per person Fatal dose: 2000-10,000 µg STX eq per person	2-3 6.7-18 33-167	No reference given for estimate. Estimated dose calculated assuming an adult bodyweight of 60 kg.

***Derived PSP toxin doses (µg STX eq/kg bw) estimated by COT based on reported intake data, where data on doses was not included in the original paper.**

Table 5 Performance characteristics reported for the MBA

Method	Specificity	Within lab precision	Between lab precision	HORRAT value	Recovery %	Standard of validation	LOQ (µg/100 g)	Reference materials available
Mouse Bioassay (MBA)	Used for detection of PSP toxins in shellfish	5-10% (RSD_r)*[30] 95% confidence interval for result of 77 µg STX eq/100g shellfish flesh reported as 65-94 µg[++ 10] Results ranging from 0-202 µg STX eq/100g observed in 9 mice injected with 140 µg STX eq/100g [31]	8-40% (RSD_R)* [30] 14-27 % (CV)[+ 32] Statistical evaluation not performed in a FAPAS interlab study[33] due to variable nature of results. (Results in 9 labs for samples spiked with 80 µg STX eq/100 g shellfish flesh ranged from 1-383 µg STX eq/100 g.) **NB** - MBA protocol not standardised between laboratories.	None reported	35-47[**] [30]	AOAC standardised method published [32]	LOD = 33-40 µg STX eq/100 g shellfish meat [24,34]	Assay standardised using STX dihydrochloride standard solution

Key:
RSD_r = relative standard deviation of repeatability (within laboratory variation)
RSD_R = relative standard deviation of reproducibility (between lab variation)
NB – Values in table have been rounded to whole numbers.
HORRAT value: HORRAT values for interlaboratory studies provide a measure of the acceptability of the reproducibility of a method. They compare the observed reproducibility (RSD_R) with a theoretical value calculated from the Horwitz equation, which was derived from observed reproducibility values from thousands of collaborative trials. Values below 2 are considered acceptable for between-laboratory precision (Horwitz, 1982).
* Based on a proficiency study involving eight French laboratories. PSP toxin levels were determined in four shellfish (oyster) samples; one control sample, one naturally contaminated with PSP toxins, and two samples spiked with low (152.8 µg STX/100 g shellfish meat) and moderate (334.7 µg STX/100 g shellfish meat) amounts of STX. Samples were analysed in duplicate. **NB** - Variation in between-laboratory results postulated to be due to variation in the toxin dilution factors selected by the different laboratories.
** Mean recovery reported by the eight laboratories involved in the above proficiency study for shellfish samples spiked with 152.8 and 334.7 µg STX/100 g shellfish meat.
+ Coefficient of variation calculated for determination of PSP toxins in a collaborative study involving 11 laboratories. PSP levels were determined in 8 shellfish (clam) samples spiked with 0, 100, 400 or 800 µg purified PSP standard solution/100 g shellfish meat (2 samples per toxin concentration). Details were not provided on this standard solution used in the studies, STX.2HCl is used now.
++ 95% Confidence interval calculated from the statistical analysis of 120 MBAs performed with 18 shellfish extracts giving median times to death of 4.0-6.5 minutes.

Outline of method:
Shellfish samples (100 g) are extracted by boiling in 0.1M HCl (1:1; pH should be <4.0, preferably ca. 3.0) for 5 minutes, and adjusted to pH 2.0-4.0. Supernatant can be separated from solid particles by centrifugation or filtration. The principle is for three mice to be injected i.p with 1 ml of shellfish extract. Median time to death used to calculate toxin level. In practice fewer mice are used in some laboratories.

Table 6 Performance characteristics reported for HPLC[26]

Method	Specificity	Toxin	Mean (µg/kg)	Within lab precision (RSD_r [%])*	Between lab precision (RSD_R [%])*	HORRAT value*	Recovery (%) ***	Standard of validation	LOQ (µg/100 g)	Reference materials available
HPLC	Applicable for determination of STX NEO GTX1,4 GTX2,3 DcSTX B1 C1,2 C3,4 in shellfish (Interlaboratory validation study performed in mussels, clams, oysters and scallops)	STX	Clams: 520 Mussel: 313 Oyster: 140	Clams: 6 Mussel: 22 Oyster: 18	Clams: 14 Mussel: 23 Oyster: 31	Clams: 0.78 Mussel: 1.23 Oyster: 1.46	74-93	Validated through collaborative trial to AOAC standard	2	STX NEO GTX1,4 GTX2,3 DcSTX B1 C1,2[++]
		NEO	Clams: 41 Mussel: 263	Clams: 19 Mussel: 26	Clams: 39 Mussel: 34	Clams: 1.78[$] Mussel: 1.75	38-77		4	
		GTX1,4	Clams: 74 Mussel: 660	Clams: 15 Mussel: 13	Clams: 26 Mussel: 20	Clams: 1.19[$] Mussel: 1.20	67-79		5	
		GTX2,3	Clams: 117 Mussel: 785 Oyster: 347	Clams: 14 Mussel: 17 Oyster: 19	Clams: 20 Mussel: 28 Oyster: 23	Clams: 0.90[$] Mussel: 1.69 Oyster: 1.25	76-88		13[+]	
		DcSTX	Clams: 8	Clams: 9	Clams: 27	Clams: 1.22[$]	64-84		1	
		B1	Clams: 42 Mussel: 331 Oyster: 39	Clams: 13 Mussel: 8 Oyster: 8	Clams: 19 Mussel: 15 Oyster: 30	Clams: 0.85[$] Mussel: 0.79 Oyster: 1.38[$]	76-86		3	
		C1,2	Clams: 241 Mussel: 101 Oyster: 169	Clams: 16 Mussel: 28 Oyster: 32	Clams: 22 Mussel: 38 Oyster: 54	Clams: 1.12 Mussel:1.75[$] Oyster: 1.52	74-78		9	
		C3,4	Mussel (spikeA): 725 Mussel (spikeB): 1425	N/A	Mussel (spikeA): 25 Mussel (spikeB): 21	Mussel (spikeA): 1.49 Mussel (spikeB): 1.40	79-81		73	

* PSP toxins determined in blind duplicate samples by 8-16 laboratories. **NB** - It should be noted that not all laboratories were able to detect all toxins at the limits in the test materials. These data were selectively excluded from the analysis.
** Based on determination in spiked mussel samples.
*** Recovery based on interlaboratory data for spiked mussel samples.
[+] Lowest concentration tested.
[++] Standard for C1,2 not currently available commercially.

RSDr = relative standard deviation of repeatability (within laboratory variation)
RSD_R = relative standard deviation of reproducibility (between lab variation)
NB – Values in table have been rounded to whole numbers.
HORRAT value: HORRAT values for interlaboratory studies provide a measure of the acceptability of the reproducibility of a method. They compare the observed reproducibility (RSD_R) with a theoretical value calculated from the Horwitz equation, which was derived from observed reproducibility values from thousands of collaborative trials. Values below 2 are considered acceptable for between-laboratory precision[35].
NB: HORRAT values marked [$] have been adjusted according to recent guidelines for concentrations below 120 µg/kg.

Outline of method:

Test portions are extracted by heating with acetic acid solution. Extracts are cleaned up using solid phase extraction (SPE) C18 cartridges. After periodate and peroxide oxidation, they are analysed by high performance liquid chromatography (HPLC) with fluorescence detection. Most toxins (STX, C1,2, B1, dcSTX and GTX2,3) can be quantified after simple SPE-C18 cleanup. Extracts containing the toxins NEO, GTX1,4, C3,4 and B2 must be further purified by using SPE-COOH cleanup/separation. This method is also suitable for shellfish samples extracted by the MBA HCl extraction protocol[36] (Jim Lawrence, personal communication).

NB: A standard is also available for dcGTX2,3; production of a dcNEO standard is in progress.

Table 7 Performance characteristics reported for the JRT

Method	Specificity	Within lab precision	Between lab precision	HORRAT value* #	Recovery %	Standard of validation	LOQ (µg/100 g)	Reference materials available
Jellett Rapid Test	Cross-reactivity to all known PSP toxins is claimed. Shown to detect PSP toxin profiles in UK shellfish. Cannot be used for individual toxins	3-9% CV* [37] 86% agreement for triplicate analyses** [2] (100% agreement for samples >80 µg STX eq/ 100 g shellfish meat[24])	No difference in results for 72 samples tested at two separate laboratories [37]	None reported	Not applicable to qualitative assay	Not internationally validated. Accepted by some competent authorities for screening out negative samples	Qualitative assay: LOD = approximately 40 µg STX eq/ 100 g shellfish meat. Sensitivity varies with toxin profile, but studies indicate able to detect all samples with toxin levels >80 µg STX eq/ 100 g shellfish meat.	Routine use not specified, but those available for HPLC could be used if required

NB: Majority of data generated by manufacturer of kit

* Coefficient of variation in line intensity, determined by testing of shellfish extracts spiked with a STX standard during company QC procedures. Values indicate the coefficient of variation of line intensity over ten replicate tests, and the range indicates results across nine production lots.

** Percentage of sixty-four shellfish extracts giving identical responses when tested in triplicate[24].

NB – Values in table have been rounded to whole numbers.

Outline of method:

The HCl extraction method employed for MBA is also used for JRT. An alternative method for field use, using 10 g of shellfish tissue rather than the 100 g recommended by AOAC, has been developed and is awaiting validation.

The test works on the principle of lateral flow immunochromatography using a strip format. The assay uses a mixture of polyclonal antibodies raised against PSP toxins, and provides a qualitative (yes/no) indication of the presence of PSP toxins within a shellfish extract within 20 minutes.

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