Application to FSANZ for harmonisation of marine biotoxin standards for bivalve shellfish

Submitted by SafeFish on behalf of the Australian Shellfish Quality Assurance Advisory Committee

September 2021

Executive summary

This application is made on behalf of the Australian Shellfish Quality Assurance Advisory Committee: a committee consisting of one regulator and one industry representative from each shellfish producing state; a representative from the Department of Agriculture Water and the Environment; and observers from the Seafood Importers Association and SafeFish. The application requests that Food Standards Australia New Zealand (FSANZ) conducts a review on the current biotoxin maximum levels (ML) for bivalve molluscs in Schedule 19 of the Food Standards Code[1] (hereafter called the FSANZ Code) referenced in Standard 1.4.1[2], with a view to harmonising the Maximum Level (ML) for diarrhetic (DST) and paralytic shellfish toxins (PST) with those in the Codex Standard CAC 292-2008 Standard for Live and Raw Bivalve Molluscs[3] and the New Zealand Regulated Control Scheme - Bivalve Molluscan Shellfish for Human Consumption[4]. The proposed changes will harmonise Australia with the international Codex food standard (CAC 292-2008[3]), which is supported by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO). This application aligns with the objectives specified in Section 18(1) of the FSANZ Act[5] to "a) ensure the protection of public health and safety" and Section 18(2) to "have regard to a) the best available scientific evidence b) promotion of consistency between domestic and international food standards and c) the desirability for an efficient and internationally competitive food industry".

The MLs for marine biotoxins in seafood currently listed in the FSANZ Code were last reviewed between 1997 and 1999 (Proposal P158 'Review of the maximum permitted concentrations of non-metals in food'[6]). The assessment at the time stated:

"There are four major groups of shellfish toxins, namely, paralytic shellfish poisons, diarrhetic shellfish poisons, amnesic shellfish poisons and neurotoxic shellfish poisons, which can be found in bivalve molluscs and cause serious and, in some cases, long term toxicity in humans. There is, however, a poor understanding of the dose response relationship associated with this toxicity and the current regulatory levels are pragmatically derived on the basis of the limited information available on the dose levels which do not appear to cause toxic symptom in humans."

Since 1999, several significant studies have emerged that would inform a new risk assessment. These studies were considered in risk assessments by working groups from the FAO and WHO in 2004[7] and the European Food Safety Authority (EFSA) in 2008[8] and 2009[9, 10]. As a result, both working groups determined lowest observed adverse effect levels (LOAEL) and acute reference doses (ARfD) for DST and PST. Both working groups acknowledged the toxicity of these compounds and the low safety margin employed to set the regulatory levels. The FAO publication[7] was used to inform the Codex Committee of Fish and Fishery Products, which developed MLs for marine biotoxins in 2008[3]. The Codex MLs for DST and PST are lower than those listed in the FSANZ Code[1]. New Zealand has since adopted the Codex MLs through the Regulated Control Scheme - Bivalve Molluscan Shellfish for Human Consumption[4]. In addition, quantitative modelling of the dose response to PST by Arnich and Thebault[11] determined a significantly lower threshold of harm from PST than determined by both the FAO/WHO and EFSA working groups, highlighting the narrow safety margin associated with the ML of this toxin group.

The 1999 FSANZ risk assessment (Proposal P158[6]) reviewed industry data on marine biotoxins in a limited number of Australian shellfish samples because biotoxin testing was not readily available at that period and was only conducted in response to the presence of toxic algae. A commercial biotoxin analytical service started in Australian in 2012 and all states with commercial bivalve production have been monitoring for marine biotoxins since that date. Data from the monitoring programs have demonstrated that commercial farms can meet the lower Codex and NZ MLs for both DST and PST with minimal disruption to commercial production

(maximum impact was one additional DST closure for pipis in South Australia every year and an additional 3.5 PST closures per year in Tasmania, each affecting one oyster growing area for one week.

Adopting Codex MLs would involve:

- Lowering the DST ML from 0.20 mg Okadaic Acid equivalents/kg (OA equiv./kg) to 0.16 mg OA equiv./kg.
- Defining the PST ML in mg saxitoxin dihydrochloride equivalents/kg (mg STX.2HCl equiv./kg), rather than mg saxitoxin equivalents/kg (mg STX equiv./kg). As the STX dihydrochloride salt is 24% heavier than its free base, this results in a 24% difference between the standards, with the Codex standard being more conservative (0.8 mg STX.2HCl equiv./kg = 0.60 mg STX equiv./kg).

We recommend adoption of the Codex ML for DST and PST in bivalve shellfish based on:

- High toxicity of DST and PST and low safety margin associated with current MLs
- Harmonisation with Codex CAC 292-2008 Standard for Live and Raw Bivalve Molluscs
- Harmonisation with NZ Regulated Control Scheme - Bivalve Molluscan Shellfish for Human Consumption
- Consistent testing parameters for domestic and export production, with the ability to meet all market requirements
- Alignment with the FSANZ principle from Proposal P158 of keeping the levels of contamination from toxins in the food chain as low as reasonably achievable (ALARA)
- The proposed change would result in minimal economic disruption
- Support from Australian industry and regulators

This application has been written in accordance with the Food Standards Australia New Zealand Application Handbook, 1 July 2019. Each section of the Application is headed up with the corresponding Handbook reference. Information contained in this application is focused on new information since the last FSANZ review in 1999 (Proposal P158[6]).

Contents

Attachments:

Attachment 1: Executive Summary

Attachment 2a: Australian shellfish data for DST 2012-2017- Summary of impact from harmonisation of regulations

Attachment 2b: Australian shellfish data for PST 2012-2017- Summary of impact from harmonisation of regulations. This data set also includes a summary of PST in rock lobster and abalone.

Attachment 3: FSANZ product recall data 2008-2018.

Attachment 4: Statutory declaration to support application.

Attachment 5: Checklist for General requirements 3.1.1 application.

Attachment 6: Checklist for Applications for contaminants and natural toxicants 3.4.1

Attachment 7: Electronic folder containing all supporting documentation referenced within this proposal. Supporting documents are numbered as they appear within the proposal. Where access to the supporting information was only available through the internet (websites), the specific URL is given in the list of references at the end of the document.

3.1.1 General Requirements

A. Form of the Application

A.1 Language

The language of the application is in English.

A.2 Format

- The application provided contains a table of contents using the guideline heading titles.
- The application contains an Executive Summary in a separate document that is included as Attachment 1.
- The application is numbered sequentially on each page.

A.3 Copies

The application is submitted electronically on USB thumb drive device and all documents are searchable by word and phrase. Full electronic copies of all references are provided.

B. Applicant Details

(a) Applicant (Individual or organisation's name)

SafeFish, facilitated through the Institute of Marine and Antarctic Studies (IMAS), College of Sciences and Engineering at the University of Tasmania in conjunction with the South Australian Research & Development Institute (SARDI) Food Sciences division.

(b) Name of contact person (s)

[redacted]

(c) Address (Street and postal)

[redacted]

(d) Telephone numbers

[redacted]

(e) Email Address

[redacted]

(f) Nature of applicant's business

Advisory body to the Australian Seafood Industry facilitated through a Tertiary research and education institute (IMAS/The University of Tasmania) and a State Government research organization (SARDI Food Sciences) that provides applied research and development for the grains/cropping, wine, horticulture, fishing and aquaculture, livestock (including wool), poultry, pig and food sectors.

(g) Details of other individuals, companies or organization associated with the application

This application has been completed by the University of Tasmania (UTAS) and SARDI Food Sciences staff following a request from the Australian Shellfish Quality Assurance Advisory Committee (ASQAAC).

University of Tasmania

- [redacted]

SARDI Food Sciences Division

- [redacted]

ASQAAC Members:

- [redacted]

C. Purpose for the application:

The purpose of this application is to request that FSANZ review the current biotoxin maximum levels (MLs) for bivalve molluscs in Schedule 19 to Standard 1.4.1, of the FSANZ Food Standards Code[1] (herein referred to as 'the FSANZ Code'), with a view to harmonising the FSANZ Code with the Codex MLs for diarrhetic and paralytic shellfish toxins (DST and PST respectively) in seafood (CXS 292-2008)[3].

Adopting Codex MLs would involve:

- Lowering the DST ML from 0.20 mg Okadaic Acid equivalents/kg (mg OA equiv./kg) to 0.16 mg OA equiv./kg.
- Defining the PST ML in mg saxitoxin dihydrochloride equivalents/kg (mg STX.2HCl equiv./kg), rather than mg saxitoxin equivalents/kg (mg STX equiv./kg). As the STX dihydrochloride salt is 24% heavier than its free base, this results in a 24% difference between the standards, with the Codex standard being more conservative (0.8 mg STX.2HCl equiv./kg = 0.60 mg STX equiv./kg).

D. Justification for the application:

The MLs for marine biotoxins in seafood in the FSANZ Code were last reviewed between 1997 and 1999 (Proposal P158 'Review of the maximum permitted concentrations of non-metals in food'[6]). The assessment at the time stated:

"There are four major groups of shellfish toxins, namely, paralytic shellfish poisons, diarrhetic shellfish poisons, amnesic shellfish poisons and neurotoxic shellfish poisons, which can be found in bivalve molluscs and cause serious and, in some cases, long term toxicity in humans. There is, however, a poor understanding of the dose response relationship associated with this toxicity and the current regulatory levels are pragmatically derived on the basis of the limited information available on the dose levels which do not appear to cause toxic symptom in humans." (page 20).

"The available data suggests there is a potential for significant health risk from shellfish contaminated with PSP, ASP, NSP or DSP and that the level of contamination should be kept as low as reasonably achievable" (page 21).

"The serious and in some cases long-term nature of the toxicity associated with seafood toxins makes them a particularly important public health issue. However, there is still a very poor understanding of the target organs for toxicity and of the nature of any dose-response relationship associated with this toxicity. For these reasons, it is still difficult to identify a safe level of exposure to the respective toxins and, therefore, to provide an estimate of the margin of safety at various levels of exposure. Estimates of toxic dose levels have been made at times of algal blooms but it is difficult to get accurate estimates from this data. An acceptable daily intake (ADI) has not been established for any of the seafood toxins." (page 4).

"For PSP, the available data suggests that moderate symptoms of toxicity can occur at intake levels of 120 µg of saxitoxin. At the current regulatory level of 80 µg/100 g of edible shellfish flesh, this level could be reached after consuming 150 g of contaminated shellfish. The margin of safety in this case, therefore, is very small." (page 48).

Since 1999, several significant epidemiological studies have emerged that were considered in risk assessments by the Food and Agriculture Organisation and the World Health Organisation (FAO/WHO)[7] and the European Food Safety Authority[8-10]. As a result both working groups determined similar lowest observed adverse exposure levels (LOAELs) and acute reference doses (ARfD), as described below (Table 1 and Table 2). The FAO publication was used to inform the Codex Committee of Fish and Fishery Products, which developed MLs for marine biotoxins in 2008. The Codex MLs for DST and PST vary from those listed in the FSANZ Code. New Zealand has since adopted the Codex MLs through the Regulations for Bivalve Molluscan Shellfish[4]. In addition, quantitative modelling of the dose response of PST by Arnich and Thébault 2018[11] reviewed an additional seven publications of illness outbreaks of paralytic shellfish poisoning published since the 1999 FSANZ assessment[6]. They determined a significantly lower threshold of harm from PST than determined by both the FAO/WHO and EFSA working groups, highlighting the narrow safety margin associated with the ML of this toxin group.

Table 1. Lowest observed adverse effect levels (LOAELs), acute reference doses (ARfD) and tolerated daily intake for DST.

DST	FAO 2004[7] & Toyofuku 2006[12]	EFSA 2008 OA[8]	Aune 2001[13]
LOAEL	1.2 – 1.6 µg/kg bw	0.8 µg OA equiv/kg bw	0.67-0.83 µg/kg bw*
ARfD	0.33 µg OA equiv/kg bw	0.3 µg OA equiv/kg bw	
TDI	Insufficient data on chronic effects available		
*assumes 60 kg individual. bw = body weight			

Table 2. Lowest observed adverse effect levels (LOAELs), acute reference doses (ARfD) and tolerated daily intake for PST.

PST	FSANZ 2015[1]	EFSA 2009 STX[9]	Arnich and Thébault 2018[11]
LOAEL	2.0 µg/kg bw	1.5 µg STX equiv/kg bw	0.33 µg STX equiv/kg bw*
ARfD	0.7 µg STX equiv/kg bw	0.5 µg STX equiv/kg bw	
TDI	Insufficient data on chronic effects available		
**Determined from quantitative modelling that 10 % of individuals consuming this dose would exhibit symptoms*			

There are inconsistencies in how PST concentrations are calculated and expressed in different areas of research, testing and national regulation[14]. This is of great concern because of the potential for errors and misunderstandings that could result in adverse human health and trade implications. PST test methods have evolved from mouse bioassays (MBA) developed over 80 years ago (expressing results as total toxicity in Mouse Units or STX equivalents) to modern chemical analytical methods such as HPLC and LC-MS. Chemical methods are able to identify individual PST analogues with a diverse range of toxicities that require application of toxicity equivalence factors (TEFs) to express the overall toxicity of naturally occurring mixtures. The parent analogue, saxitoxin, is traded as a stand-alone reference material, or included in diagnostic test kits, mainly as the dihydrochloride salt or diacetate salt. This is primarily due to the saxitoxin hydrate (free base) form having poor stability. Care must be taken to ensure the correct molecular weight is then used in subsequent calculations for total toxicity. The current, inconsistent use of reporting units creates problems for the interpretation of data from testing laboratories and in the interpretation of research findings. It confuses the translation of risk assessment advice into regulatory limits, and causes diverse, often ambiguous, regulatory limits. Furthermore, recent epidemiological research suggests that the current acceptable daily intakes for PST on which these limits are based may be underestimating the potential for human illness from consumption of contaminated shellfish[11]. As such, a 24% difference in reporting is significant.

Currently in Australia, there are two tiers of regulatory standards, each stating different biotoxin MLs – one for domestic products[1] and one for exported products (set by the destination the product is exported to). This causes confusion to industry and regulators, and additional work for exporting companies to ensure they comply with both sets of standards. Adopting Codex MLs will reduce this inconsistency as it would mean the compliance with the FSANZ Code would equate to compliance with all international standards.

This application aligns with the objectives specified in Section 18(1) of the FSANZ Act to "a) ensure the protection of public health and safety" and Section 18(2) to "have regard to a) the best available scientific evidence b) promotion of consistency between domestic and international food standards and c) the desirability for an efficient and internationally competitive food industry".

Advantages:

- Consistency between domestic and international food standards

  Harmonised MLs will allow the Australian industry to follow the same rules as other international bodies, including New Zealand[4], resolving the current situation of two different MLs for domestic or international market access, and differing MLs between New Zealand and Australia.

- Additional safety factor

  The distinction between STX equivalents and STX.2HCl equivalents has only recently become an issue, with the rise in use of chemical methods of analysis[14]. As CXS 292-2008 reporting units are more conservative, adoption will enable compliance with all other countries and provide an additional safety factor in light of recent reviews revealing that PST are more toxic than originally assumed.

- Taking advantage of the best available science in the recent international reviews by expert working groups

  In 2004, the Joint FAO/IOC/WHO ad hoc Expert Consultation group released a risk assessment on Biotoxins in Bivalve Molluscs[15] to assist the Codex Committee on Fish and Fish Products (CCFFP) to revise MLs in shellfish for marine biotoxins. Based on the information from this risk assessment, in 2008 the CCFFP formally adopted MLs for a number of marine biotoxins, which included setting the ML for DST at 0.16 mg OA equiv./kg. Australia has not recently reviewed the ML for DST in the FSANZ Code which remains set at 0.20 mg OA equiv./kg. By harmonising DTS limits with Codex, Australia will be brought in line with what is considered a protective level.

- Application endorsed by Australian shellfish industry

  The Australian Shellfish Quality Assurance and Advisory Committee (ASQAAC) is the body that manages a government-industry co-operative program that provides the procedures and administrative practices that, if adhered to, enable food safety programs to comply with the FSANZ Code and Export Orders for bivalve mollusc production. The program is adopted by shellfish producing states of Australia, and consists of representatives from industry, FSANZ, Department of Agriculture Water and the Environment and State Government bodies responsible for shellfish safety. ASQAAC members have endorsed this proposal to harmonise biotoxin MLs within the FSANZ Code as this will provide clear guidance and consistency of regulation (currently there is confusion and additional requirements through the two tiered domestic/import system). It will also simplify when their harvest areas can be opened or must close.

Disadvantages:

- Marginal increase in shellfish zone closures

  A decrease in the ML could result in shellfish aquaculture zones being closed for harvest for a slightly higher proportion of the year as a result of toxic algal blooms. The potential scale of this impact has been calculated by investigating the test results of Australian shellfish data from 2012-2017 (8156 DST tests and 7044 PST tests; Attachments 2a/b). It has been determined that the average impact of changing reporting units for DST would result in a 0.16% average increase in the number of regular monitoring results that report above the ML (ranging from 0 – 3.9% impact per species per state),

whilst the impact of changing the ML for PST would be a 0.58% average increase (ranging from 0 – 5.1% impact per species per state). Correspondingly, there will be a minor increase in the number of days shellfish growing areas are closed for harvest. We estimate the maximum number of additional closures for any bivalve species in any state associated with this change would be 1 per annum in South Australia for DST in pipis and an additional three and a half PST closures per year in Tasmania, each affecting one oyster growing area for one week. These results indicate that if the regulations were harmonised, there would be a very low impact to the Australian shellfish industry based on the substantial data to date. ASQAAC are aware of this minimal impact and are unanimously in support of progressing this application for harmonisation.

D.1 Regulatory impact estimate of proposed changes to paralytic and diarrhetic shellfish toxin MLs (PST and DST)

This section provides data on Australian production and recreational harvest only. In 2002, New Zealand adopted the FSANZ Food Standards Code and all its amendments as joint food standards with the exception of the Australian-specific chapters and standards. New Zealand, however, follows a Regulated Control Scheme (issued under the Animal Products Act 1999) that specifies the requirements that must be met for harvesting bivalve molluscan shellfish for human consumption. The marine biotoxin limits in this legislation is currently the same as the new MLs proposed, thus New Zealand recreational and commercial bivalve shellfish harvesters would not be impacted by any change made in the FSANZ Code.

D.1.1 Cost and benefits of the application

Domestic production

The combined wild harvest and aquaculture commercial Australian bivalve industry production in Australia in 2020 was valued at approximately $140M. The distribution of this value across species and states is given in Table 3 below, sourced from ABARES[16]. Limited commercial harvest of cockles and pipis is listed in ABARES.

Table 3. Fisheries and aquaculture production in 2019/20 by state, Australia. Source: ABARES[16].

Commodity	Unit	NSW	Vic.	Qld	SA	WA	Tas.	NT	Comm.	Total
Oysters	$'000	58,242		500	24,948		30,758			114,448
Pipis	$'000	2,117			4,798					6,915
Mussels	$'000	282	5,189[b]		3,472		2,289			11,232
Other Molluscs	$'000	106[a]	1,555[c]		1537[c]		1,619[c]			4,817
Scallops	$'000			3,662		9,199	0			12,861

[a] Mainly includes Cockles, Periwinkles, Whelk and Blue Mussel

[b] 2017-18 figure as 2019-20 data not available

[c] Wild capture *Other Molluscs* type of mollusc not stated, may include non-bivalve species

South Australia, New South Wales and Tasmania account for the majority of bivalve shellfish production.

- In SA there are 92 aquaculture growers with an average 2-5 hectares per business. Approximately 10 companies operate leases of 10 hectares or more. There is one large company producing mussels, and 15 licences for wild caught pipis and cockles (personal comment, Clinton Wilkinson, PIRSA). All cockles and pipis come from one large harvest area (the Coorong region).

- In NSW there are 244 oyster businesses operating across 74 harvest areas. 29% of oyster producing companies are small producers (<5,000 dozen per annum), 59% are medium producers (5,000 – 50,000 dozen per annum) and 11% produce more than 50,000 dozen per annum[17]. There are also 2 mussel producing businesses operating in two harvest areas and 41 wild harvest licences for pipis extracted from10 harvest areas.

- In Tasmania there are 65 marine farming businesses harvesting Pacific oysters, and two business harvesting mussels from three growing areas. Approximately 10% of the oyster businesses are large producers (>150,000 dozen oysters per annum), the remaining are split equally between medium and small producers (150,000 – 50,000 or <50,000 dozen per annum respectively). There are 6 wild harvesters of oysters from 23 harvest areas, pipis and clams from 2 harvest areas (personal comment, David Balk, Oysters Tasmania).

- WA has issued 64 shellfish licences and 5 exemptions. Currently edible shellfish are produced, harvested and sold from 10 licences in two bioregions producing three species (Western (Sydney) Rock Oyster, Akoya and mussels). One large company holds all 10 licences and utilises 281 ha across four locations. Production is small compared to the eastern states with significant expansion planned. New species development includes tropical rock oysters, the blacklip rock oyster (*Saccostrea echinata* and *S. cucullata*) with trials underway in the Pilbara and the Kimberley by a single company. There is also interest in two scallop species. There are two businesses harvesting wild Venus Clams.

- The production listed above in Table 3 for Victoria comes from 8 mussel farmers in Port Phillip Bay and Western Port from 6 harvest areas. Small volumes of native oysters and scallops are also produced (from the same licences as the mussel farms), and there are small volumes harvested by four wild pipi fishers operating from three harvest in Discovery Bay, and one scallop dive fisher operating in Port Phillip Bay. The total value of bivalve shellfish production for human consumption from Victoria between 13 wild harvest and aquaculture licence holders is <$6M[18].

- The production of oysters listed above in Table 3 for Queensland comes from 89 active licences split between Pearl Oysters (Goldlip Pearl (*Pinctada maxima)* and Blacklip pearl oysters (*P. margaritfera)* 6 licences) and Wild-caught Oysters (Blacklip (*Striostrea mytiloides)* and milky oysters (*Saccostrea cucullata)* 83 licences). A small rock oyster (*Saccostrea glomerata)* aquaculture industry also operates based south of Hervey Bay, with most oyster growing areas in Moreton Bay[19].

- In the southern states, commercial scallops are caught in the Bass Strait Central Zone Scallop Fishery (BSCZSF), in the Bass Strait between Tasmania and Victoria (in 2020 there was 63 fishing permits issued with 12 active vessels operating in this zone). In 2019 BSCZSF were allowed a Total Allowable Catch (TAC) of 3897 t and when the fishery closed had landed 2931 t[20]. Commercial scallops are also caught in the Victorian and Tasmanian managed scallop fisheries that lie within 20 nautical miles off their respective coasts. The Victorian Scallop Fishery (also known as the Ocean Scallop Fishery) had a TAC of 135 t (with only a small proportion being reported as landed)[21]. There are 63 licenced fishers catching the Tasmanian production (DPIPWE data). Production of scallops from Victoria and Tasmania is below the volumes recorded by ABARES for the last three years (Table 3).

- Scallop producers in WA, NT and Qld do not conduct routine biotoxin analyses and are thus not impacted by this application.

Potential impact of change of DST and PST MLs to domestic production

The results of 8156 tests for DST and 7044 tests for PST in Australian bivalve shellfish from 2012-2017[a] are shown below in Table 4 and Table 5. Following the analysis of this data, it has been determined that the average impact of changing reporting units for PST would be a 0.58% increase in the number of monitoring results that report above the ML (ranging from 0 – 5.1% impact per species per state; Attachment 2b), whilst the impact of changing the ML for DST would result in a 0.2% average increase (ranging from 0 – 3.9% impact per species per state; Attachment 2a).

To estimate the potential increase in closure days associated with the proposed change to MLs we assumed weekly testing during the impacted period. Most states (Tasmania excepted) only sample biotoxins on a minimum monthly basis and increase sampling when phytoplankton or biotoxin data indicates biotoxins may be present. Thus, the results from all states except Tasmania are representative of the impact during rare high-risk periods, rather than the impact during normal conditions.

In most cases marine biotoxin events are short lived. Weekly sampling ensures areas are re-opened as soon as possible. Costs associated with growing area closures are dependent on the fishery. For all shellfish species this includes lost opportunity in the impacted harvest area. For scallops, pipis and mussels, costs also include lost stock and costs associated with harvesting the stock, as shellfish cannot be returned to the water after harvest. For the rarer, longer-term closures as might occur in some of the harvest areas on the east coast of Tasmania, costs include lost markets. However, for longer term closures, the loss of market would occur regardless of this proposed change to ML.

Table 4. Detections of Diarrhetic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by state. Source: ASQAP confidential data.

Bivalve samples tested by State	Number of samples analysed for DST in 2012-2017	Samples >0.16 mg OA equiv/kg	Samples >0.20 mg OA equiv./kg	Samples 0.16-0.20 mg OA equiv./kg	% of samples 0.16- 0.20 mg OA equiv./kg
NSW	2843	10	7	3	0.11
SA	426	22	16	6	1.41
WA	140	2	1	1	0.71
TAS	4457	9	8	1	0.02
VIC	268	10	8	2	0.75
QLD	18	0	0	0	0
NT	4	0	0	0	0
All States	*8156*	*53*	*40*	*13*	*0.16*

[a] Most Australian States have a low risk of biotoxin contamination, and therefore conduct biotoxin sampling on aquaculture leases on a minimum monthly frequency, changing to weekly testing during periods of elevated phytoplankton counts or biotoxin levels. Tasmania, having a high risk of biotoxin contamination in many growing areas, currently conducts weekly biotoxin monitoring in all growing areas. The scallop industry only samples prior to and during the fishing period on a frequency determined by the current risk (informed by results of testing).

Table 5. Detections of Paralytic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by state. Source: ASQAP confidential data.

Bivalve samples tested by State	Number of samples analysed for PST in 2012-2017	Samples >0.8 mg STX equiv./kg	Samples impacted if reporting units change to STX.2HCL equiv/kg	% of samples impacted if reporting units change
NSW	1616	7	4	0.2
ACT	6	5	0	0
SA	292	7	0	0
WA	126	1	0	0
TAS	4739	272	37	0.8
VIC	257	2	0	0
NT	4	0	0	0
QLD	4	0	0	0
All States	*7044*	*294*	*41*	*0.58*

When considering whether to apply to FSANZ to harmonise biotoxin standards with Codex, ASQAAC (representing oysters, mussels, pipis and clams) has considered the impact detailed in Table 6 and

Table 7. Industry and regulator representatives discussed the issue over several years, allowing enough time for consultation within each state. ASQAAC determined that the advantages of the proposed changes outweigh the disadvantages. ASQAAC's view is that the shellfish industry would benefit from harmonisation of biotoxin standards because it would mean that they would have consistent regulations for domestic and international trade, a reputation for supplying safe shellfish in accordance with all international standards, and greater protection from potential illness.

Table 6. The annual impact of the proposed change to the DST ML for each bivalve species in each state. Species that are not listed for each state are not impacted. Source: ASQAP confidential data.

Impacted species/state	Estimated additional DST failed samples per year[a]	No. harvest areas	Estimated additional DST failed sample per growing area per year
Pipis in NSW	0.5	10	0.05
Pipis in SA	1.0	1	1.00
Mussels in WA	0.2	4	0.05
Oysters in Tas	0.2	23	0.01
Mussels in Vic	0.2	6	0.03
Pipis in Vic	0.2	3	0.07

[a] *Calculated using the following: total number of samples analysed between 2012-2017 multiplied by the percentage of samples for that species detected above 0.16 and below 0.20 mg OA equivalent/kg divided by 100 then divided by the number of years testing occurred (N=6).*

Table 7. The annual impact of the proposed change to the PST ML for each bivalve species in each state. Species that are not listed for each state are not impacted. Source: ASQAP confidential data.

Impacted species/state	Estimated additional PST failed samples per year[b]	No. harvest areas	Additional PST failed sample per growing area per year
Oysters in NSW	0.2	74	0.003
Mussels in NSW	0.5	2	0.25
Pacific oysters in Tas	3.5	23	0.15
Mussels in Tas	1.8	3	0.60
Clams in Tas	0.3	2	0.15
Scallops in Tas	0.5	Variable (1 - 4)	0.5 – 0.12

[b] *Calculated using the following: total number of samples analysed between 2012-2017 multiplied by the percentage of samples for that species that is over the ML if the reporting unit is changed divided by 100 then divided by the number of years testing occurred (N=6).*

Other seafood industries were also consulted by SafeFish (see below for non-bivalves). Scallops are the only bivalve shellfish not covered by ASQAAC that may be impacted by this proposal. Scallops are exempt from ASQAAC as they are generally sold without the digestive tract[22]. The only scallop industry impacted by biotoxin closures is the Tasmanian scallop industry, as scallops from other states are not deemed to be at risk of PST accumulation. This industry is not supportive of harmonizing standards, as they believe additional closures would be an unnecessary impost. The impact of the proposed PST ML change to scallops is estimated to be one additional sample from Tasmania over the ML every second year (no change to other states: see data from PST monitoring in Australian shellfish above and below in Section 3.4.1.2; sub-section C.2). This represents 3% of the scallop samples tested in Tasmania across all harvest areas. The number of additional days lost by the change would depend on the frequency of testing, which is difficult to assess in the scallop industry due to the practicalities associated with testing and fishing remote areas at distance from shore bases.

Other seafoods

Although the FSANZ Code only covers bivalve molluscs, risk management programs exist in Tasmania and New Zealand that also cover PST in abalone and Southern Rock Lobster. In both Australia and NZ these fisheries are export focused. All programs in NZ already adhere to the proposed MLs in the Animal Products Notice: regulated control scheme for bivalve molluscan shellfish for human consumption and would therefore be unaffected by any change. The programs running in Tasmania are focused on PST contamination, with no DST testing occurring, as no DST risk has been identified to date.

The Tasmanian biotoxin monitoring plan for lobster employs mussel sentinels which trigger testing of lobster hepatopancreas from 5 individual lobsters per site when PST levels in the mussels are elevated. Zones are deemed safe for harvest if all hepatopancreas samples contain <0.5 mg STX equiv. kg^{-1}; unsafe for harvest if any hepatopancreas sample exceeds 0.8 mg STXequiv.kg^{-1}; and questionable if any hepatopancreas sample is between 0.5 and 0.8 mg STX equiv. kg^{-1}. In the latter case, further information is sought to allow an assessment of the risk (e.g. whether the bloom is increasing or decreasing). In addition, harvest zones are closed for fisheries management purposes during part of the high risk biotoxin season. Thus, the potential impact to lobster is more complicated to interpret than that for bivalves. The 2012-2017 data for PST in lobster presented in this proposal (Attachment 2b) is a combination of both research samples (targeting periods that would normally already be closed) and risk management samples. Using this data overestimates the number of rock lobster fishery closures expected from a reduction in the PST ML. The data indicates that 7% of samples taken would be impacted. As five lobster samples are used to assess the risk of each area, this could represent 1-7% additional area closures per year, dependent on what the new precautionary closure stance would be, and

when the fisheries management closures occur. The length of any additional closure period is likely to be at least 10 days due to sampling practicalities. Between 0 and 7 closures per annum have occurred for the lobster industry since monitoring began in 2012, meaning an increase of 1-7% area closures would result in between 0 and 0.5 additional area closures each year across the whole industry.

The only abalone industry in Australia that would be impacted by a reduction in the PST ML is the Tasmanian east coast abalone fishery as this is the only fishery with a biotoxin risk[23]. There has been no abalone harvest on the east coast of Tasmania where the high PST risk occurs for the last four years due to fisheries conservation reasons. When stock numbers allow reopening of the abalone fishery on the east coast of Tasmania, there could be an impact to some abalone fisheries' blocks in high risk areas due to prolonged retention of PST in abalone tissues.

Consultation has occurred with both the lobster and abalone industries in Tasmania, both of which are export focused. Neither have raised any concerns with harmonising the FSANZ Code biotoxin standards with Codex.

Recreational harvest

Recreational harvests in Australia are generally managed through results collected via the commercial programs. The exception is one pipi harvest area in Victoria that is monitored by the Victorian government. Recreational harvest is closed by public health order when results from adjoining commercial farms indicate it is not safe to consume the bivalve shellfish. Therefore, changing the ML for DST and PST in the FSANZ Code would have a similar impact timing wise on recreational harvest.

In Tasmania, recreational harvest of abalone and lobster from the east coast are managed in line with the commercial fisheries closures.

D.1.2 Impact on International trade

Imported bivalves and potential impact of DST and PST ML change

In 2018, Australia imported $12M mussels (96% from New Zealand, which already applies Codex MLs) and $50.2M scallops (59% from China, 20% from Japan and 6% from USA) – see Table 8 below. The food safety risks of imported products are managed by the Department of Agriculture, Water and the Environment[24]. Bivalve molluscs and PST are regarded as a high risk hazard:food pairing and are subjected to regular testing on arrival. Bivalve molluscs and DST are not considered a high risk hazard:food pairing and are therefore not subjected to regular testing. A review of the failed import testing reports from 2010 to April 2020 showed that imported product failed the current ML requirements of 0.8 mg STX equiv/kg on three occasions[25]. These shipments of mussels originating from Italy exceeded the current Australian ML by more than 50 times (41.5-43.5 mg STX equiv/kg). Were Australian biotoxin MLs harmonised with the Codex standard, the outcome of the testing on these occasions would have been identical and therefore no quantifiable impact of the here proposed biotoxin ML changes on international imports has been identified.

Exported bivalves and potential impact of DST and PST ML change

During 2018, the Australian oyster industry was in recovery from the Pacific Oyster Mortality Syndrome (POMS) virus, with production volumes from Tasmania and South Australia significantly reduced. As a result, exports of oysters were minimal, below the level required for listing in ABARES Fishery statistics. The latest ABARES Fishery statistics (2019/20) group bivalve mollusc import and export data across countries and do not give country specific import or Australian state specific information on export values. To provide a better representation of the quantities of bivalve molluscs shipped to and sourced from overseas countries, Tables

9-11 present the more detailed 2017/2018 ABARES data, while 2019/2020 total export values are given in the text and table headings. The only bivalve listed in the export statistics is scallops ($11M export value in 2017/18; Table 9 below, export value of 8.4 M in 2019/20). The main destinations for Australian exported scallops are Hong Kong, and Singapore, accounting for 44% and 49% of the export trade respectively (Table 9below).

Scallops are not considered to be at risk of DST accumulation in Australia and are not tested on a regular basis. Therefore, there will be no impact on scallop exports from a change to the DST standard.
Similarly, scallops are only considered to be at risk of PST accumulation if harvested from Tasmanian waters and are therefore only tested for PSTs if sourced from this area. During 2018, the export value of scallops from these destinations was $21,600 (Table 10).

Standardisation will simplify regulation and testing for the shellfish industry, as only one ML will need to be met for domestic and international trade. Compliance with the Codex ML will ensure all shellfish produced can meet all the international MLs listed in Section J.2. Harmonisation will simplify the job of regulators as well, as closures for domestic harvest will directly translate to closures for international access as well. Moving toward international harmonisation also clarifies research and food safety risk assessments. The current scenario, where both the DST and PST ML in Australia are around 25% less than those recommended in Codex, exposes industry and consumers alike to unnecessary risk. The data presented here highlights that the industry can meet the proposed MLs, and thus following ALARA (as low as reasonably achievable) principles, these MLs should be adopted.

Table 8. Imports of major mollusc products by source, Australia. Unit = $'000. Source: ABARES 2017/18[26]. Total imports in 2019/20 were 19.6 M for mussels and 38.9 M for scallops (ABARES 2019/20[16]).

Commodity	Chile	New Zealand	Vietnam	China	Japan	Thailand	United States	Other	Total
Frozen mussels	357	4,790	4.6	-	-	-	-	95.8	5,247.4
Unfrozen mussels	-	7,364	-	-	-	-	-	-	7,364
Frozen scallops	-	-	-	27,471	9,444	747	2,918	5,702	46,282
Unfrozen scallops	-	-	-	-	-	95	-	3,872	3,967
Total	357	12,154	4.6	27,471	9,444	842	2,918	9,669.8	62,860.4

Table 9. Exports of molluscs by destination, Australia. Unit = $'000. Source: ABARES 2017/18[26]. Total scallop exports in 2019/20 were $8.4 M (ABARES 2019/20[16]).

Commodity	Hong Kong	Indonesia	Malaysia	China	Singapore	Other	Total
Live fresh or chilled	-	-	-	-	-	3	3
Frozen or cooked	4,957	-	8	39	5,506	724	11,234

Table 10 Exports of molluscs by state, Australia. Unit = $'000. Source: ABARES 2017/18[26].

Commodity	NSW	Vic.	Qld	SA	WA	Tas.	NT	Australia[b]
Scallops							-	
	69	320	3,173	19	7,276	22		11,237
[b] *Australian total, including Australian Capital Territory and re-exports.*								

E. Information to support the application

Public Health and Safety Issues related to the Proposed Change:

Information addressing the public health and safety aspects relating to this submission are provided separately in Section 3.4.1.1 for DST and Section 3.4.1.2 for PST.

Consumer Choice related to the Proposed Change

Consumer choice is not expected to be changed directly by the proposed changes. It is unlikely that costs for testing would increase as industry is currently testing product for domestic trade/sale on a regular basis (frequency varies from weekly to monthly). It is also unlikely that product availability is impacted, as the number of additional closures are very small and will impact very few growing areas, leaving the majority of areas open to operate their business as normal.

E.1 Data Requirements

In accordance with the Guide to submitting request for ML proposals, the data requirements applicable to this application are presented in Attachment 7. All supporting References are numbered within this attachment as they appear within the proposal. Where information from websites is given, the URL is provided in the reference list at the end of this document.

F. Assessment Procedure

Feedback from consultation with FSANZ suggests that this application should be considered a General Assessment Procedure level 1 (up to 350 hours).

G. Confidential Commercial Information

This application contains no confidential commercial information.

H. Other Confidential Information

This application contains a summary of biotoxin monitoring data of the Australian commercial shellfish industry (Attachment 2a/b). The raw data may contain confidential commercial information; it details specific information that the seafood industry may consider sensitive in nature. As such, SafeFish would like to request that this data is treated as confidential and is not published publicly however, the data in the aggregated summaries worksheet should be made publicly available.

The FSANZ product recall data 2008-2018 (Attachment 3) was provided to SafeFish in confidence and we therefore request that this should also be treated as confidential.

We request that electronic reference number 23 be treated as confidential: McLeod C, Turnbull A, Hay B. Provisional risk assessment of paralytic shellfish toxins in Australian wild caught abalone. South Australian Research and Development Institute; 2014. This reference is given in the electronic reference package as: 23. McLeod et al. 2014 CONFIDENTIAL.pdf.

I. Exclusive Capturable Commercial Benefits

This application contains no quantifiable exclusive capturable commercial benefits that SafeFish is aware of.

J. International Standards

J.1 International Standards

Codex standards relevant to this application are:

Codex Alimentarius Commission Standard 292-2008 (CXS 292-2008[3]). 2008 Standard for live and raw bivalve molluscs. In Codex Alimentarius International Food Standards. Rome: Codex Alimentarius Commission. MLs for PST (saxitoxin group) and DST (okadaic acid group) can be found on page 2.

J.2 Other National Standards or Regulations

Table 11. Comparison of levels for biotoxins between the FSANZ Food Standards Code and other International Standards (differences highlighted in red). Note that Australia currently does not import from or export bivalve shellfish to the European Union or Canada, although some growing areas are approved for EU export and have exported in the past.

Country Regulation	Paralytic Shellfish Toxin (PST)	Diarrhetic Shellfish Toxin (DST)
FSANZ FS Code[1]	0.8 mg/kg (STX equivalents)	0.2 mg/kg (OA equivalents)
Codex Standard[3]	0.8 mg/kg (STX.2HCl equivalents)	0.16 mg/kg (OA equivalents)
United States of America[27]	0.8 mg/kg (0.8 ppm) (STX equivalents)	0.16 mg/kg (0.16 ppm) (total OA equivalents)
China & Hong Kong[28]	4 MU/g	0.05 MU/g
Canada[29*]	0.8 mg/kg (STX equivalents) (bivalve shellfish edible tissue)	1 mg/kg (bivalve shellfish digestive tissue)[6] 0.2 mg/kg (bivalve shellfish edible tissue)[6] (Sum of OA and dinophysis toxins)
European Union[30]	0.8 mg/kg	0.16 mg/kg (160 µg/kg) (OA equivalents)
New Zealand[31]	0.8 mg/kg (STX.2HCl equivalents/kg)	0.16 mg/kg (OA equivalents)
Singapore[32]	0.8 mg/kg (STX equivalents/kg)	0.16 mg/kg (OA equivalents)
Japan[33]	4 MU/g	0.05 MU/g

Key: mg = milligrams, kg = kilograms, µg = micrograms, STX = Saxitoxins, OA = Ocadaic acids, ppm = parts per million, MU = mouse unit: 1MU (Mouse Unit) represents the amount of toxin that causes death in a mouse of 20g body weight in 15 minutes in case of paralytic shellfish poisoning toxin, while in case of diarrrheal shellfish poisoning toxin 1MU represents the amount of poison that causes death in a mouse of 16-20g body weight in 24 hours.
*currently under review by Health Canada.

The Australian Shellfish Quality Assurance Program Operations Manual (Version 5) 2019 contains guidelines on meeting the requirements for biotoxin risk management for Australian bivalve production, referencing the FSANZ Code[22]. These guidelines include information on toxin analogues and their equivalency factors and methods of analysis.

K. Statutory Declaration

A signed statutory declaration is provided in Attachment 4 and includes the required statements, is signed by a senior officer and has been created using the applicable template as per the handbook.

L. Checklists

The completed checklist for 'General requirements 3.1.1' is provided in Attachment 5. As the application relates to a natural toxicant, the checklist for 'Applications for contaminants and natural toxicants 3.4.1' is also included in Attachment 6.

3.4.1.1 Chemical contaminant and natural toxicant: maximum levels for Diarrhetic shellfish toxins

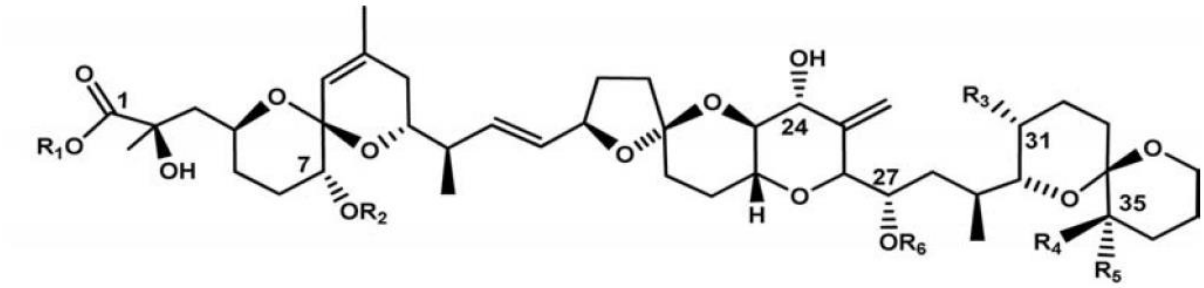
The information supplied below is divided into two sections: one for diarrhetic shellfish toxins, followed by another for paralytic shellfish toxins.

A General information on DST

A.1 Nature of the contaminant or natural toxicant including chemical and physical properties

A.1.1 Chemical and physical properties of DST

Diarrhetic shellfish toxins, also referred to as okadaic acid (OA) group toxins, are heat-stable, lipophilic cyclic polyether compounds. They include okadaic acid and the isomeric compounds 19-*epi*-okadaic acid and dinophysistoxin 2 (DTX-2), along with the methylated derivative dinophysistoxin 1 (DTX-1). There are also numerous esters formed from OA and the dinophysistoxins by conjugation of the terminal carboxylic acid group with poly-hydroxylated, sulphated or unsaturated alcohols[34]. The acylated derivatives of OA, DTX-1 and DTX-2 are together described as DTX-3[35].



	R1	R2	R3	R4	R5	R6
OA	H	H	CH3	H	H	H
DTX1	H	H	CH3	CH3	H	H
DTX2	H	H	H	H	CH3	H
DTX3	H	acyl	as parent	as parent	as parent	H
Diol-ester	diol	H/acyl	as parent	as parent	as parent	H
DTX4	Diol-ester	H	as parent	as parent	as parent	H
DTX5	Diol-ester	H	as parent	as parent	as parent	H
27-O-acyl	H	H	CH3	H	H	acyl

Figure 1. Outline of the structure of okadaic acid, dinophysistoxins and derivatives[36].

A.1.2 Sources of DST

DSTs are produced by certain species of marine phytoplankton in the genera *Dinophysis* (e.g. *Dinophysis acuta, D. acuminata, D. fortii*) and *Prorocentrum* (e.g. *Prorocentrum lima, P. hoffmanianum, P. concavum, P. belizeanum, P. rhathymum*)[37, 38]. *Dinophysis* species are planktonic, but DST-producing *Prorocentrum* species tend to be tycoplanktonic (i.e. benthic or epibenthic species that are found at some time in the water column).

A.1.3 Factors that influence the level of contamination of food with DST

Australian oysters and mussels are harvested from marine aquaculture zones, whilst scallops, pipis and clams come from wild harvest. Bivalves can bioaccumulate DST by filter feeding on naturally occurring toxin producing phytoplankton. The level of contamination depends upon the abundance of toxic phytoplankton cells and the quantity, as well as the type of DST analogues produced by the phytoplankton cells. These vary between phytoplankton strains and can be influenced by environmental conditions[39-44].

Shellfish aquaculture sites are found in Moreton Bay QLD, along the entire coast of NSW, Port Phillip Bay, along the north, east and south east coast of Tasmania, on the York and Eyre Peninsula in South Australia and Albany and Cockburn Sound in WA. DST producing species are known in all these areas[45], but rarely form harmful algal bloom (HAB) events. A graph of the number of harmful algal events (DST, PST and AST) that have occurred in Australia and New Zealand from 1985 to 2018 is presented below (Figure 2). Whilst the number of HAB events has remained relatively constant over that period, at between 1 and 6 events per year, there have been shifts in specific bloom events. These bloom events are not unique to Australia and overseas blooms may also lead to the accumulation of DST in bivalve molluscs that may be imported to Australia.

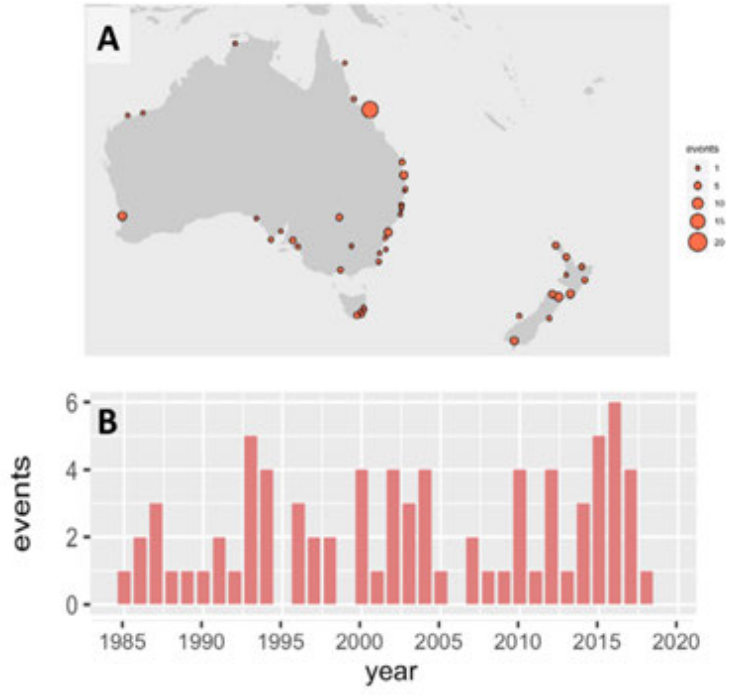


Figure 2. Overview of A. Geographic distribution and B. Total number of Harmful Algal Events impacting on human society (Harmful Algae Event Database, HAEDAT) in the combined Ocean Biodiversity Information System (OBIS) region 5 (Australia + New Zealand) between 1985 and 2018[45].

A.1.4 Interaction of DST with food

Following ingestion of DST producing phytoplankton by filter feeding bivalves, DST predominantly accumulate in the digestive gland-stomach complex or viscera of the shellfish as the initial repository of toxic phytoplankton cells (typically between 76-98% of total toxin burden)[44, 46]. DST contaminated bivalves cannot be distinguished from non-contaminated individuals by physical appearance or taste.

DST are generally considered temperature stable and are not destroyed by either cooking or freezing. McCarron et al. (2008)[47] note that fluid loss during cooking of bivalves can concentrate the biotoxins and distribute DST from the digestive gland to other tissues. EFSA acknowledges that processing of shellfish could lead to an approximate 2-fold increase in the concentration of DST in bivalve tissues[10].

A.1.5 Current control measures and their effectiveness

The Australian shellfish quality assurance program (ASQAP) Operations Manual[22] stipulates the marine biotoxin monitoring and risk assessment required in commercial bivalve operations in Australia (Sections 4-10). Under Primary Production and Processing (PPP) Standard 4.2.1 of the FSANZ Code[48], all bivalve shellfish must comply with ASQAP or an equivalent system. The ASQAP Operations Manual refers directly to the biotoxin maximum levels set in Standard 1.4.1[2] of the FSANZ Code. The rate of contamination of biotoxins in various bivalve species (pre-market) validates the requirement for this type of monitoring (Table 4, Application 3.1, section D.1).

Biotoxin risk management is usually a combination of analysis of shellfish for biotoxins and analysis of water for toxin producing phytoplankton. The minimum frequency of biotoxin monitoring stipulated in ASQAAC is monthly for low risk growing areas. Growing areas with a higher biotoxin risk will have an increased frequency of monitoring and monitoring increases during times of heightened risk (as indicated by either biotoxin results or elevated counts of toxin producing phytoplankton species). For example, in Tasmania biotoxin monitoring occurs on a weekly basis. Growing areas are closed for harvest when: marine biotoxins exceed the FSANZ Code ML; toxic algae exceed closure trigger levels; or shellfish poisoning in humans has occurred.

More than 200,000 tonnes of commercial bivalve shellfish have been harvested in Australian fishery and aquaculture operations in the last decade (2010-2020)[16 16 1616]. In that time period, no human illnesses from the consumption of commercial bivalves contaminated with DST have been reported in Australia (OzFoodNet data[50]). Two outbreaks of Diarrhetic Shellfish Poisoning (DSP) have been reported prior to the establishment of routine biotoxin monitoring in NSW. In both episodes (1997 and 1998), wild harvested pipis were implicated[51, 52]. Similarly, a single case of DSP from recreationally collected pipis from North Stradbroke Island, QLD was reported in 2000[53].

During the last decade, a total of 27 shipments of bivalve molluscs have been recalled within Australia, 3 of these shipments were recalled due to DST contamination (FSANZ recall data 2008-2018, Attachment 3). An end product market survey on DST in commercial wild harvest pipis and clams (*Plebidonax deltoides, Katelysia* spp., *Anadara granosa, Notocallista kingii*), obtained from the Sydney Fish Market over three harvest years (2012-2015), found that 99.38% of samples were within current regulatory levels (2 out of 271 Pipi samples contained DST >0.2 mg/kg; Farrell et al. 2018[54]).

A.2 Analytical methods for detection of DST

Analytical methods for the detection of marine biotoxins rely on the separation of toxin analogues and subsequent detection by fluorescence or mass spectroscopy. Each analogue is then adjusted for toxic

potency using known toxin equivalency factors (TEFs) and summed to reach a total toxin content for each toxin group. Codex Standard 292-2008[3] list the criteria for determination of toxin analogues in each biotoxin group by chemical methods. The performance criteria are met by the validated confirmative LC-MS chemical method described in Villar-González et al. (2011)[55]. The mouse bioassay for the detection of DST is being phased out internationally due to ethical concerns and performance issues. It is no longer applicable to routine monitoring of bivalve molluscs for DST in the European Union[56], Australia or New Zealand.

Codex Standard 292-2008 specifies that international, scientifically validated toxin equivalency factors (TEFs) must be used and refers to the FAO/WHO (2016)[57] website for current TEFs. The FAO/WHO most recently revised these TEFs in 2016 (summarised in

Table 12 for DST). DTX3 was not assigned a TEF during the FAO/WHO review, as the molecule represents a mixture of 7-O-acyl ester derivatives of OA, DTX1 and DTX2 that are converted back to their respective parent compounds (OA, DTX1, DTX2) during sample extraction[57].

Table 12 Toxin equivalency factors for diarrhetic shellfish toxin analogues recommended by the FAO/WHO expert group[57].

	TEF based on cytotoxicity	TEF based on PP2A inhibition	TEF based on membrane paracellular permeability	EFSA proposed TEF	Recommended TEF	Rationale
OA	1.0	1.0	1.0	1.0	1.0	
DTX1	3.1	1.6	2–15	1.0	1.0	In the case of the OA group of toxins, several case reports from human intoxication are available, and these reports are analogue specific. As outlined in the recent risk assessment by EFSA, a human poisoning event with DTX1 as main contaminant in Japan suggested a LOAEL of 48 µg DTX1 per person. This dose is similar to that reported in poisoning events in Sweden, Norway, UK and Portugal. Thus, it is not surprising that the currently used TEF of 1.0 in some countries appears protective for public health. Still, it should be noted that multiple *in vitro* studies suggest that the intrinsic potency of DTX1 could be higher than that of OA. However, large uncertainty is associated with these studies (a factor of approximately 5-fold difference between results depending on the cell line used). Therefore, the recommended TEF of 1.0 for DTX1 should be verified in future studies to corroborate the observations in humans, and also through more controlled studies *in vivo* (animals).
DTX2	0.52	0.5	0.6	0.6	0.5	Consistent among the different assays; based on acute oral and i.p. toxicity in mice, DTX2 is on average 0.5 times as toxic as DTX1). This value is also supported by the various *in vitro* data
DTX3						Explanation provided in main text as to why no TEF is recommended.

In addition to confirmed tests, analytical screening methods are available for marine biotoxins, and can be either qualitative or quantitative, although many have not been validated for use with Australian species. A qualitative lateral immunoflow assay (Neogen test kit) has been validated in mussel and oyster matrixes for the detection PST (qualitative screen only). Not designed for the confirmative analysis required for open/closure decisions of shellfish harvest areas, the ASQAP manual specifies the criteria to be met for its use as a screening tool. Efforts by the University of Technology Sydney are currently underway to validate the test kits use for screening of DST.

The Australian Shellfish Quality Assurance Program's Operational Manual directly refers to the FSANZ Code 1.4.1 for the biotoxin groups to be analysed and the maximum levels for each group, and refers

to Codex Standard 292-2008 for detail on the biotoxin analogues to be analysed and the toxin equivalency factors (TEFs) to be used to account for the differing potencies of DST analogues.

B.1 Information on the toxicokinetic and metabolism of DST

B.1.1 Toxicokinetics

The mode of action of OA group toxins remains uncertain. At the time of the original FSANZ assessment in 1999[6] it was thought that Okadaic acid inhibited protein phosphatases in vitro and it was suggested that this effect could be responsible for the diarrhoeagenic nature of OA[58]. A review by Munday (2013)[59] stated there is no *in vivo* evidence to support this and suggested that the other observed effects of OA were not directly associated with inhibition of protein phosphatases.

B.2 Toxicity studies (animal studies) of DST

Since the FSANZ review of marine biotoxins in 1999 (Proposal P158[6]) several new studies and toxicity assessments have occurred. This discussion focuses on data collected since 1999.

B.2.1 Acute and short-term toxicity of DST

A review into the toxic effects of OA by Munday (2013)[59] states:

- OA is highly toxic to mice by IP injection; LD50 = 192 - 225 µg/kg[8, 60].
- DTX-1 is similarly toxic: minimum lethal dose of 160 µg/kg[62].
- DTX-2, DTX-3 and DTX-4 are less acutely toxic; LD50 = 352 - 600 µg/kg[63-65].
- OA appears to be between 2- and 5-fold less toxic by oral administration than by injection.
- The median lethal dose of OA by gavage was reported as 400 µg/kg[65] and 880 µg/kg[66], while Tubaro et al. (2008)[67]et al. observed no deaths at 1,000 µg/kg.
- DTX-1 is only slightly less toxic when administered orally than when given by intraperitoneal injection[68, 69].
- Damage is caused to the intestinal epithelium following ip injection and oral administration of OA and DTX-1, while little effect was observed in animals receiving the same dose of DTX-3[68]. Oral administration of OA also caused oedema and mucosal erosion in the stomach of mice, accompanied by acute inflammatory changes in the submucosa[70].
- The cause of death after oral administration of lethal doses of OA is unclear.

B.2.2 Long term toxicity and carcinogenicity of DST

The tumour-promoting effect of OA and DTX-1 has been well-documented (see Munday 2013 and Valdiglesias et al. 2013 for reviews[38, 59]).

- Munday and Reeve (2013)[71] suggest that the tumour-promoting activity of OA and DTX-1 may simply be due to their irritant effect, which is unlikely to be expressed following exposure to small amounts of these substances in food.
- Two human population studies based on information gathered by questionnaires have been undertaken[72, 73] and are mentioned in EFSA (2008)[8] and Valdiglesias et al. (2013)[38]. These studies indicated a possible association between diarrheic intoxication (DSP) and several types of cancer, specifically cancers of the oesophagus, stomach, colon, pancreas and liver, leading

to the suggestion that a continued exposure to OA could induce different types of cancer in humans.

Valdiglesias et al. (2013)[38] summarise:

- In rats, DST was observed to induce hyperexcitability and neuronal stress[74], deficits in spatial memory[75], cognitive deficits[76], and astroglial alterations and spatial cognitive deficits, even 12 days after OA exposure[77]. Subsequent dose- and time-dependent neurodegeneration was observed in all cases.
- *C*hronic OA injection into rat brain ventricles for up to eight weeks generated a neuronal protein redistribution that led to severe memory impairment[78]. This memory-impairment associated to OA exposure was also observed in other rodent studies[79, 80]. Kamat et al. (2012)[80] found that memory-impairment was related to an increased expression of proinflammatory cytokines and total nitrite in several brain regions, indicating that neuroinflammation may play a vital role in OA-induced memory impairment.

B.2.3 Reproductive toxicity of DST

Munday and Reeve (2013)[71] observe that "No evaluation of the potential reproductive and developmental toxicity of shellfish toxins by use of standard tests has been reported". In vitro and in vivo studies in animals (mainly fish and amphibians) suggest OA intoxication may cause retardation of embryo development, malformation and reduction in embryo survival rate[38]. OA was detected in the foetuses of pregnant mice dosed orally at day 11 of gestation[81]. The risk of orally consumed OA on human unborn life is not known.

B.2.4 Developmental toxicity of DST

There is no evidence of reproductive or developmental toxicity of OA on humans in the literature. Valdiglesias (2013)[38] summarise the developmental toxicity of DST as follows:

- Microinjection of OA in frog (Xenopus laevis) oocytes and starfishes (Marthasterias glacialis and Astropecten aranciacus) induced the meiotic maturation and the activation of the mitosis promoter factor[82, 83].
- Medaka fish (*Oryzias latipes*) embryos incubated in a medium containing OA and observed retardation of embryo development and dose-dependent reduction in survival rate and caused malformations and delayed growth[84].
- Casarini et al. (2007)[85] examined the effects of OA on some genes involved in the neural and muscular specification and patterning.
- OA induced important alterations in the expression of several development-related genes of *X. laevis* with embryonic development stage more sensitive to the toxin than the larval stages[86].
- Bioassays show OA to exhibit a weak teratogenicity on cultured murine embryonic cells[87], increase the meiotic resumption and maturation rates of canine oocytes[88], affect meiotic resumption of blue fox oocytes in vitro[89], and to resume meiosis with fast kinetics of germinal vesicle breakdown through the MEK-MAPK pathway in incompetent growing mouse oocytes[90].

- OA was found to induce irreversible damage to porcine embryonic development by causing premature chromosome condensation, meiosis resumption, pronucleus breakdown, inhibition of spindle organization, and microtubule assembly suppression by sperm centrosomes in oocytes and fertilized eggs[91].

B.2.5 Genotoxicity of DST

The genotoxicity of OA is controversial, with some studies showing mutagenic effects and others not. Valdiglesias et al. (2013)[38] suggest that this might be because OA genotoxic effects are highly dependent on cell type and experimental conditions. The authors concluded that OA is able to cause damage to the genetic material and to mechanisms that would normally repair such damage. Alterations in DNA repair mechanisms may affect the susceptibility of individuals exposed to a particular mutagen and have been linked to a large number of diseases and cancer. Exposure may increase the susceptibility to other genotoxic agents, increasing the risk of adverse health outcomes.

Munday and Reeve (2013)[71] note that OA has been tested for mutagenicity in the standard Ames test[2], with a negative result. It has been suggested that some of the observed effects may reflect the cytotoxic activity of OA rather than a specific action on DNA[5].

B.2.6 Neurotoxicity and immunotoxicity of DST

Much work on the neurotoxicity of DST would have been noted in the 1999 assessment of DST by FSANZ (Proposal P158[6]), however significant new findings are now available. Impacts include:

- neuronal apoptosis, protein tau hyperphosphorylation, and morphological alterations, on both neuronal cells and animal systems[74, 92, 93].
- apoptosis in TR14 and NT2-N human neuroblastoma cells[92], mouse neuroblastoma cells[94], and rat cerebellum neurons[95] and in SHSY5Y human neuroblastoma cells[96].
- Changes that could lead to neural cell death: differentiation of neuronal cells into the mitotic cycle[92], caused changes in microtubule associated proteins and neuronal cytoskeleton of cultured cortical neurons[74], and induced disintegration of neurites and swelling of cell bodies in cultured cerebellum neurons[97].
- phosphorylation and accumulation of tau protein (associated with multiple brain diseases) in several neural cell types including mouse[98] and human neuroblastoma cells[99], rat cortical neurons[100], and neuronal cultures derived from cerebral cortex of early postnatal rats[101].
- modify the expression of genes related to cytoskeleton and neurotransmission in neuronal cells[102].

[b] The Ames test is a biological assay to determine the mutagenic potential of chemical compounds.

OA effects on immune system of animals and humans have been poorly studied. Observed impacts include:

- thymus morpho-functional modifications and atrophy, depletion in the lymphoid compartment, and angiogenesis[103]
- important alterations on interleukin production by human peripheral monocytes in-vitro[104] and modulation of IL-1β gene expression[105]
- down-regulation of T cell receptor (TCR) expression levels in mouse T lymphocytes in vitro, compromising the T cell activation and, consequently, the immune response[106], suggesting that low oral doses of OA are able to induce immunostimulation and systemic immunotoxicity. An inflammatory cell response was activated in this study
- intracerebroventricular administration of OA caused memory impairment in rats and neuroinflammatory changes in the hippocampus and cortex brain regions[80].

B.3 Information from human studies that is relevant to the toxicity of DST

B.3.1 Observations of DST in humans

DSP incidents have been reported in many countries around the world. Since the 1999 review (FSANZ Proposal P158[6]), incidents have been recorded in Norway[107] , Belgium[109], Portugal[110, 111], the UK[112, 113], France[114] and Chile[115]. The predominant symptoms induced by OA are nausea, vomiting, diarrhoea and abdominal pain, beginning from 30 min to a few hours after consumption of contaminated shellfish. Fever, chill and headache have also been reported in some incidents. Symptoms usually resolve within 2-3 days of consumption. No information is available relating to possible longer-term effects or repeated exposure.

A review of available information on OA and its analogues with respect to the risk of marine biotoxins in shellfish was undertaken by the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain[8]. The panel found that information on the doses and profiles of OA-related toxins that cause illness is limited. Many illness reports do not provide information on the toxin levels in the shellfish nor the amount of contaminated shellfish required to be consumed to cause toxicity, and where exposure assessments are reported, little information is given on how these estimates have been derived. Whilst the illness reports provide no information on the effects of cooking on levels of OA toxins in shellfish, it is generally accepted that the toxins are not readily degraded by heat and that loss of fluid during cooking can result in a 25-80% increase in the concentration of DST[47]. Where DST concentrations have been determined by mouse bioassay, a variety of protocols have been used (e.g. observation times, extraction solvents) and results may be reported as either mouse units (MU) or OA equivalents. This results in additional uncertainty in the estimation of dietary intakes associated with human illness. The risk assessment summarises reports from Sweden, Norway, Portugal and the UK, indicating that cases of human illness have been associated with LOAELs generally in the region of 50 µg OA equiv./person[8].

The 2008 EFSA Panel report which undertook a DST risk assessment also examined the tumour promoting effects of OA and DTX1 that have been demonstrated in animal studies, and the attempts to assess whether there may be a link between cancer risk and exposure to OA-group toxins in humans. Cordier et al. (2000)[72] assessed mortality rates in French coastal areas with differing numbers of DST closures, hypothesising that residual levels of toxins may be present in shellfish harvested from

beds recently re-opened. A possible association between living in areas with a high rate of closures and some digestive cancers was found, but the authors acknowledged the large number of assumptions that had been made in the study. A statistically significant correlation between consumption of molluscs and incidence of total and colorectal cancer was found in different regions of Spain[73]. A 7-fold increase in bivalve molluscs consumption was associated with a two-fold increase in colorectal cancer but it was not possible to determine if OA was the causative agent.

B.3.2 Tolerable intake levels DST

The 2008 EFSA Panel report concluded that a lowest observed adverse effect level (LOAEL) for human illness is in the region of 50 µg OA equivalents per person, equating to 0.8 µg OA equiv./kg body weight (bw) for adults based on 60 kg body weight (bw). The results of a subsequent investigation of 11 cases in an outbreak of DSP linked to the consumption of mussels contaminated with OA and DTX-3 in France in 2009 found a LOAEL consistent with this[114]. Usually, an uncertainty factor between three and 10 is applied to convert a LOAEL into a "no observed adverse effect level" (NOAEL). The scientific opinion provided by the Panel to EFSA advised the application of a factor of three because the symptoms are relatively mild and reversible. This results in a NOAEL of 0.3 µg OA equiv./kg bw.

In calculating an ARfD for OA, the EFSA panel considered it unnecessary to apply an additional safety factor for the variation among humans as "the data were based on observations in a rather large number of affected shellfish consumers, originating from various countries, and considered to comprise the most sensitive individuals". Thus the EFSA Panel derived an ARfD of 0.3 µg OA equiv./kg bw based on the NOAEL with no safety factor applied[8]. Similarly, scientific advice provided to the Codex Committee on Fish and Fishery Products by an Expert Panel from FAO/WHO/IOC assessed the ARfD as 0.33 µg OA equiv./kg bw[12].

The standard international maximum level of OA is 0.16 mg OA equiv./kg shellfish tissue[12]. However, internationally there is some discussion about whether this is low enough – Toyofuku (2006)[12] notes that the consumption of 250 or 380 g of shellfish meat by adults would lead to a derived guidance level of 0.08 or 0.05 mg OA equiv./kg shellfish meat respectively.

C. Information on dietary exposure of DST

C.1 Food Group where maximum level is proposed

All bivalve molluscs are potentially at risk of contamination with marine biotoxins. The following table lists major commercial bivalve species grown in Australia (Table 13).

Table 13 Major commercial Australian bivalve mollusc species.

Common name	Scientific name	Raw/ Processed
Mussels	*Mytilus edulis*	Both
Clams/Pipis/Cockles	*Katelysia* spp., *Dosinia* spp., *Venerupis* spp., *Donax* spp. *Plebidonax* spp., *Anadara* spp. *Notocallista* spp.	Both
Oysters	*Ostrea spp., Crassostrea gigas, Saccostrea spp., Pinctada maxima*	Both
Scallops	*Pecten* spp., *Chlamys* spp., *Equichlamys* spp., *Mimachlamys* spp., *Amusium spp., Ylistrum* spp.	Both

C.2 Surveys on the levels of the DST in foods

As biotoxin contamination levels are monitored before product enters the market, few end-product surveys on the prevalence of DST in Australian bivalves are available. An exception is a survey of DST in pipis (*Plebidonax deltoides*, n=271), cockles (*Anadara granosa* and *Katelysia* spp., n=47) and Strawberry Cockles (*Notocallista kingii,* n=3) harvested in New South Wales[54]. Of the samples tested, 99.38% were within current Australian regulatory limits of the FSANZ Code Schedule 19[1]. DST were present in 34.27% of pipi samples (*P. deltoides*), with two samples above the regulatory limit. Comparison of these market survey data to samples (phytoplankton in water and biotoxins in shellfish tissue) collected during the same period at wild harvest beaches, demonstrated that, while elevated concentrations of *Dinophysis* (DST producing dinoflagellate) were detected, a lag in detecting bloom events on two occasions meant that wild harvest shellfish with DSTs above the regulatory limit entered the marketplace.

As described in Section 'A.1.5 Current controls and their effectiveness', biotoxin monitoring in bivalve shellfish pre-harvest occurs regularly as part of the state shellfish quality assurance programs. Results from the monitoring of DST pre-harvest are given in Table 14 and Table 15 (by state and species respectively).

Table 14 Detections of Diarrhetic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by species monitored. Source: ASQAP confidential data.

Bivalve samples tested for DST by Species	Number of samples analysed for DST in 2012-2017	Samples >0.20 mg OA equiv./kg	% of samples > 0.20 mg OA equiv./kg
Oysters	6484	2	0.03
Clams	93	1	1.08
Cockles/Pipi	568	28	4.93
Mussels	799	9	1.13
Scallops	212	0	0.00
Total	8156	40	0.49

Table 15. Detections of Diarrhetic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by state. Source: ASQAP confidential data.

Bivalve samples tested for DST by State	Number of samples analysed for DST in 2012-2017	Samples >0.20 mg OA equiv./kg	% of samples > 0.20 mg OA equiv./kg
NSW	2843	7	0.25
SA	426	16	3.76
WA	140	1	0.72
TAS	4457	8	0.02
VIC	268	8	2.99
QLD	18	0	0.00
NT	4	0	0.00
All States	8156	40	0.49

Surveys on international markets and imported shellfish

In the European Union, the Rapid Alert System for Food and Feed recorded 10 incidents where bivalve shellfish were found to be contaminated with unacceptable levels of DST and product had to be withdrawn from market between 2010 and 2021 (Table 16). DST is not listed in the US National Outbreak Reporting System so no information is available from this source. As described in the risk impact statement above (D.1.2), the Australian Agriculture Department's Food Inspection Scheme currently does not include testing for DST and there is therefore no data available from this source[24].

Table 16. Shipments of bivalve molluscs withdrawn from market in the European Union due to high DST levels. Source: Rapid Alert System for Foods and Feed 2010 - 2021. The ML for DST in seafood in the EU is 0.16 mg/kg.

Biotoxin	Product	Date	Country of origin	Notifying country	Concentration
DST	Live cockles	23/07/2021	Portugal	Spain	0.26 mg/kg
DST	Live slipper clam	19/07/2021	Portugal	Spain	0.41 mg/kg
DST	Frozen stuffed mussels	24/06/2021	Greece	Turkey	0.29 mg/kg
DST	Live cockles	1/06/2021	Portugal	Spain	0.32 mg/kg
DST	Mussels	26/05/2021	Spain	Spain	0.22 mg/kg
DST	Frozen mussels	10/03/2021	Spain	Italy	0.25 mg/kg
DST	Frozen mussels	12/01/2021	Spain	France	>0.16 mg/kg
DST	Canned cockles	26/11/2020	Portugal	Spain	>0.32 mg/kg
DST	Live mussels	17/08/2020	Italy	Spain	0.15 mg/kg
DST	Mussels	14/08/2020	Italy	Italy	>0.32 mg/kg
DST	Cockles	18/06/2020	Italy	France	0.5 mg/kg
DST	Mussels	25/05/2020	Italy	Italy	0.13±0.04 mg/kg
DST	Cockles	12/02/2020	Portugal	Spain	0.44 mg/kg

C.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

The Australian or New Zealand National Nutrition surveys broadly group seafood into one category and do not provide specific information on the consumption of bivalve shellfish. During the 2004 Report of the joint FAO/WHO Ad hoc expert consultation on biotoxins in bivalve molluscs[7], the following Australian consumption details were provided: The frequency of consumption of bivalve molluscs estimated that 2% of the Australian population consume bivalve molluscs (from 1 day survey). While no specific data are provided in the same report on frequency of consumption, details are provided for France and the USA, where consumption is estimated to be around 8.6 and 4 eating occasions per year, respectively. The 2011-2012 Australian Health Survey (Nutrition First Results – Foods and Nutrients) reports a daily intake of molluscs (bivalves and all other molluscs) of 0.6 and 0.2 g for females and males, respectively, with a standard error of 25-50% for the male statistics. The survey does not provide details on serving size, which would be more relevant to estimating biotoxin exposure in a single sitting. Based on the 2004 FAO/WHO report[7], the 97.5th percentile consumption figures for edible portions were calculated to be 181 g for adults and 70 g for children in Australia. Table 17 and Table 18 below summarise the available molluscan shellfish consumer survey data by frequency (Table 17) and serving size (Table 18).

Table 17. Frequency of bivalve consumption summarised from Australian and New Zealand consumer surveys.

Country	Metric	Survey reference
Australia	2% of the Australian population consume bivalve molluscs	FAO 2004[7]
Australia	0.5 g per person per day for entire Australian population	2011-2012 Nutritional Survey
Australia	0.6 and 0.2 g of bivalve and other molluscs consumed per day for males and females respectively (25-50% standard error for male statistic)	2011-2012 Australian Health Survey (Nutrition First Results – Foods and Nutrients)
New Zealand	9% of children aged 5-14 reported eating shellfish at least once per week	1999 survey entitled NZ Food: NZ People (Russell et al., 1999)[116]
	6% of 4576 respondents aged 15 or over reported eating shellfish at least once per week	
	15 and 14% of Maori people reported eating shellfish at least once per week (males and females, respectively).	
	26 and 31% of Pacific Islander people reported eating shellfish at least once per week (males and females, respectively).	
New Zealand	8 g per person per day for New Zealand population	King and Leake (2013)[117]
France	Bivalve molluscs consumed at 4.2 eating occasions per year	FAO 2004[7]
USA	Bivalve molluscs consumed at 8.6 eating occasions per year	FAO 2004[7]

Table 18. Bivalve mollusc serving size summarised from Australian and New Zealand consumer surveys.

Country	Metric	Reference
Australia	97.5th percentile consumption for edible bivalve portions were calculated to be 181 g for adults and 70 g for children	FAO 2004[7]
Australia	79 g per person per day for bivalve shellfish consumers. The 50th, 90th, 95th and 97th percentiles for consumers only was 63, 146, 180, and 248 g/day respectively.	2011-2012 Australian National Nutrition and Physical Activity Survey (Australian Bureau of Statistics, 2014; Williamtown Contamination Expert Panel, 2015)[118]
New Zealand	407 g per person per day for Shellfish consumers	King and Leake (2013)[117]
New Zealand	Mussels: average portion size = 82 g 97.5th percentile portion size = 256 g	2008/09 New Zealand Adult Nutrition Survey Parnell et al. (2011)[119] summarised in Boundy et al. 2020[120]
	Oysters: average portion size = 6 g 97.5th percentile portion size = 94 g	
	Scallops: average portion size = 51 g 97.5th percentile portion size = 91 g	
	Clams (Tuatua): average portion size = 240 g 97.5th percentile portion size = 240 g	

C.4 For foods where consumption has changed in recent years, information on likely current food consumption

There is no data to suggest that consumption patterns of bivalve molluscan shellfish have changed in recent years and domestic production of shellfish remained relatively constant over the last 5 years (ABARES 2019[16]).

3.4.1.2 Chemical contaminant and natural toxicant maximum levels – for Paralytic shellfish toxins

A.General information on PST

A.1 Nature of the contaminant or natural toxicant including chemical and physical properties

A.1.1 Chemical and physical properties PST

The paralytic shellfish toxins (PST) are a group of non-proteinaceous toxins composed of related analogues that are produced by algae (predominantly dinoflagellates[121, 122]). Fifty seven analogues have been identified from various organisms[122]. Saxitoxin is the parent analogue, consisting of a 3,4-propinioperhydropurine tricyclic structure with the molecular formula $C_{10}H_{17}N_7O_4$ (Figure 3). The saxitoxin analogues are classified structurally based on the presence of various side chains such as carbamate, sulphate, hydroxyl, hydroxybenzoate or acetate. The level of toxicity of each analogue varies depending on the configuration of side chains and analogues with carbamate side chains (e.g. STX, NEO and GTX1-4) are considered the most important because they are of the highest toxicity in mammalian assays[11, 122-124].

The most common PSTs are hydrophilic (water soluble), but some analogues with hydrophobic side chains have been described[122, 125]. PSTs are also often described as heat stable at acidic pH. However, EFSA (2009)[9] note that when heated at pH 2-4 analogues with the N-sulfo-carbamoyl side (e.g. GTX5) chain could be converted to their more potent corresponding carbamate toxins (e.g. STX) through hydrolysis of the N-sulphated group.

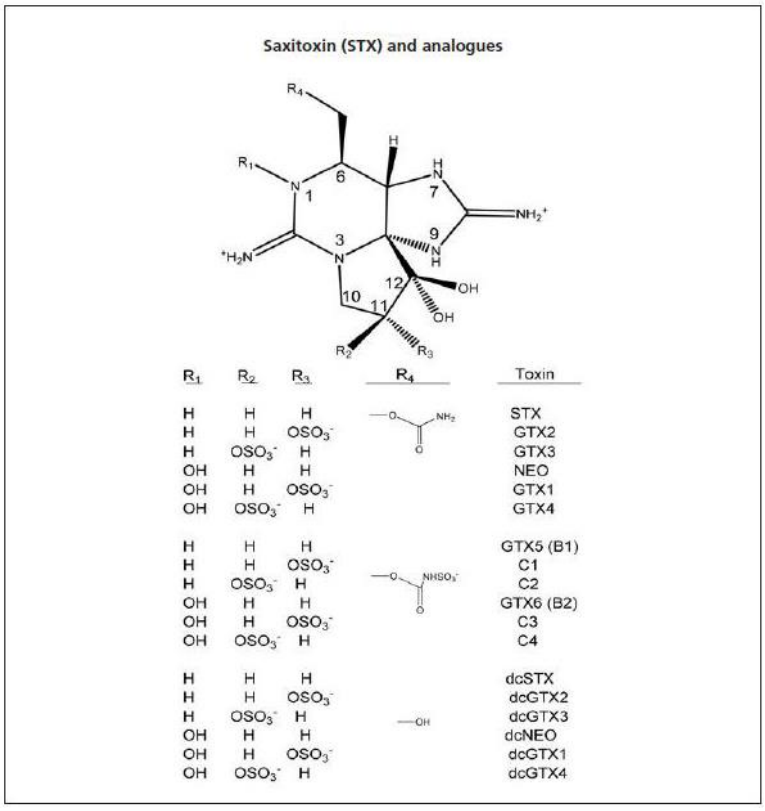


Figure 3. Structure of saxitoxin and analogues[36].

A.1.2 Sources of PST

PSTs are produced by certain species of marine dinoflagellates in the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium*[126]. PST production has also been demonstrated in certain species of freshwater cyanobacteria belonging to the genera *Anabaena*, *Aphanizomenon, Cylindrospermopsis, Lyngbya* and *Planktothrix*[122, 127, 128]. The main known dinoflagellate sources of PSTs of concern to the marine seafood-producing sector in Australia include *Alexandrium minutum*, *A. catenella*, *A. tamarense* and *G. catenatum*[129-131]. *A. affine* and *A. ostenfeldii* are also capable of producing PSTs[130, 132] and have been identified in the south-eastern waters of Australia, however they have not yet been conclusively linked to the presence of PSTs in shellfish in Australian coastal waters[130].

In the past, PST have also been detected in the macroalgae *Ecklonia maxima*[133] and *Jania* spp[134]. However, the production of PST by these species remains unconfirmed, as the presence of PST producing dinoflagellates or cyanobacteria in the vicinity of collected macroalgae at the time of sampling could not be excluded[23].

A.1.3 Factors that influence the level of contamination of food with PST

Australian bivalve shellfish are mostly harvested from marine aquaculture, with some wild harvest of pipis and clams. Bivalves can bioaccumulate PST by filter feeding on naturally occurring toxin producing phytoplankton. The level of contamination depends upon the abundance of toxic phytoplankton cells and the quantity, as well as the type of PST analogues produced by the phytoplankton cells. These vary between phytoplankton strains and can be influenced by environmental conditions[39-44].

The distribution of shellfish aquaculture sites and PST producing species are given in Hallegraeff et al. (2021)[45], along with the total number of harmful algal bloom (HAB) events in Australia and New Zealand from 1985 to 2018. Whilst the number of HAB events has remained relatively constant over that period, at between 1 and 6 events per year, there have been shifts in specific bloom events. The algal bloom PST events shown in Tasmania in Figure 4 have changed from *Gymnodinium catenatum* events in the D'Entrecasteaux Channel (1987-2003), to *Alexandrium catenella* events on the east coast (2012/13 -present). Figure 4 also summarises the number of biotoxin events in NSW. These bloom events are not unique to Australia and overseas blooms may also lead to the accumulation of PST in bivalve molluscs that may be imported to Australia.

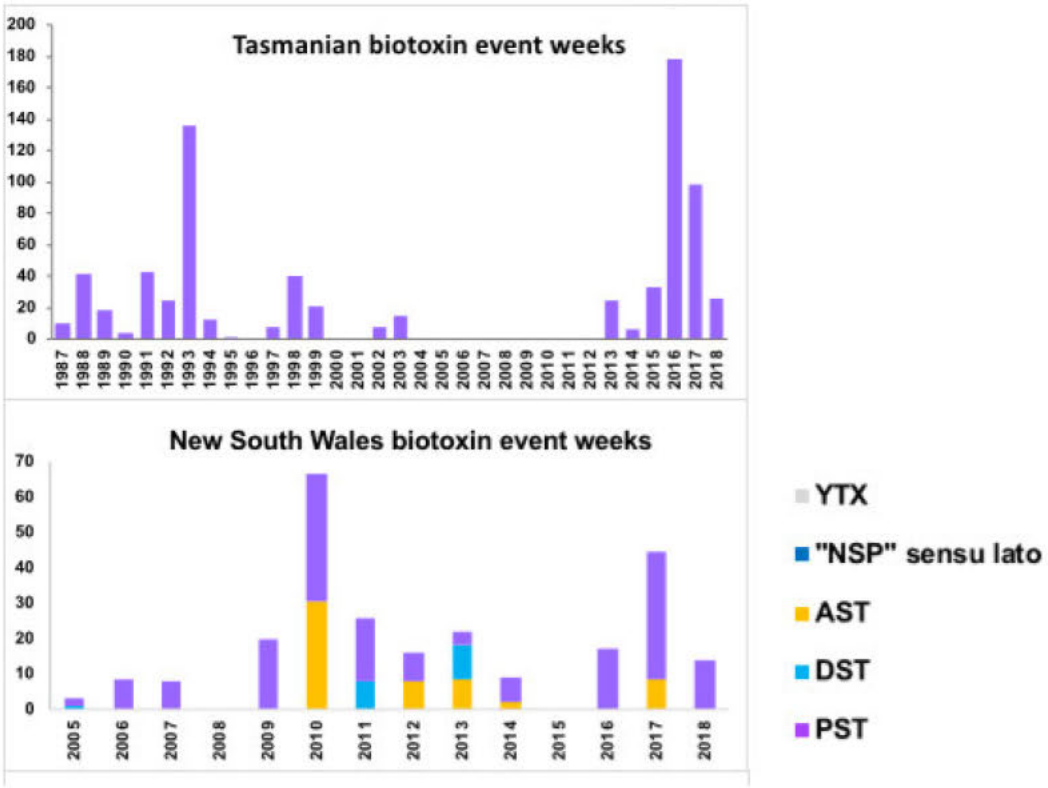


Figure 4. Summary of the marine biotoxin events in Australia, specifically Tasmania and New South Wales. Source: Hallegraeff et al. (2021)[45].

A.1.4 Interaction of the toxicants with food

Following ingestion of PST producing phytoplankton by filter feeding bivalves, PST predominantly accumulate in the digestive gland-stomach complex or viscera of the shellfish as the initial repository of toxic phytoplankton cells (typically between 76-98% of total toxin burden)[44, 46]. PST contaminated bivalves cannot be distinguished from non-contaminated individuals by physical appearance or taste.

PST are generally considered temperature stable and are not destroyed by either cooking or freezing. However, water loss during household processing (cooking, steaming) of shellfish could lead to leaching-out of STX-group toxins from the flesh into the cooking fluid[10]. The European Food Safety Authority's (EFSA) Panel on Contaminants in the Food Chain concluded that the available data made it difficult to draw firm conclusions on possible interconversion or destruction of STX-group toxins occurring during commercial processing[10].

A.1.5 Current control measures and their effectiveness

The Australian shellfish quality assurance program (ASQAP) Operations Manual[22] stipulates the marine biotoxin monitoring and risk assessment required in commercial bivalve operations in Australia (Sections 4-10). Under Primary Production and Processing (PPP) Standard 4.2.1[48] of the FSANZ Code, all bivalve shellfish must adhere to ASQAP or an equivalent system. The ASQAP Operations Manual refers directly to the biotoxin maximum levels set in Standard 1.4.1[2] of the FSANZ Code. The rate of contamination of biotoxins in various bivalve species (pre-market) validates the requirement for this type of monitoring (Table 5, Application 3.1, section D.1).

Biotoxin risk management is usually a combination of analysis of shellfish for biotoxins and analysis of water for toxin producing phytoplankton. The minimum frequency of biotoxin monitoring stipulated in ASQAAC is monthly for low risk growing areas. Growing areas with a higher biotoxin risk will have an increased frequency of monitoring and monitoring increases during times of heightened risk (as indicated by either biotoxin results or elevated counts of toxin producing phytoplankton species). For example, in Tasmania biotoxin monitoring occurs on a weekly basis. Growing areas are closed for harvest when: marine biotoxins exceed the FSANZ Code ML; toxic algae exceed closure trigger levels; or shellfish poisoning in humans has occurred.

More than 175,000 tonnes of commercial bivalve shellfish have been harvested in Australian fishery and aquaculture operations in the last decade (2010-2020, ABARES[16]) In that time period, no human illnesses from the consumption of commercial bivalves contaminated with PST have been reported in Australia (OzFoodNet data[50]). However, consumption of recreationally harvested, wild bivalve shellfish contaminated with PST has been associated with several human poisonings in New Zealand and Australia and anecdotal reports of Tasmanian cases reach as far back as the 80's[135-138].

During the last decade, a total of 27 shipments of bivalve molluscs have been recalled within Australia, 25 of these shipments were recalled due to biotoxins (FSANZ recall data 2008-2018, Attachment 3). In 2012, Japanese import authorities (MHLW) recalled a shipment of Blue Mussels (*Mytilus galloprovincialis*) derived from the east coast of Tasmania due to the presence of unacceptable levels of PST[139]. A review of the ASQAP manual has since been conducted to include detailed guidance on biotoxin management, with minimum frequencies for flesh testing of biotoxins.

A.2 Analytical methods for PST detection

Validated analytical methods for PST detection that meet the Codex requirements are listed in Table 19 below. The latest guidance by the FAO/WHO specifies TEFs for PST quantification of total PST in saxitoxin dihydrochloride equivalents[57]. This includes a revised TEF for the PST analogue neosaxitoxin. All PST analogues listed in Figure 3 have been detected either in Australian microalgae or shellfish samples[140, 141].

Table 19. Validated PST chemical methods.

Biotoxin group	Chemical method	Type of analysis	References
PST	HILIC MS/MS	Confirmative	Boundy et al. (2015)[142] & Turner et al. (2015)[143]
PST	LC-FLD	Screen & confirmative	Lawrence et al. (2005), AOAC 2005.06[144]

The mouse bioassay is a Codex accepted technique for the detection of PST, but is being phased out internationally due to ethical concerns and performance issues. It is no longer applicable to routine monitoring of bivalve molluscs for PST in the European Union, Australia or New Zealand.

B.1 Information on the Safety of PST

B.1.1 Toxicokinetics of PST

Although the basic understanding of the mode of action of PST has not changed since the FSANZ assessment in 1999 (Proposal P158[6]), research has progressed to understanding this in more detail. Various models for the mode of action of saxitoxins have been reviewed by Zhang et al (2013)[145]. Put

simply, the binding of STX to voltage gate sodium channel at site one of the α subunit within the cellular membrane blocks the inward flow of Na^+ to the cell. This inhibits action potential and prevents nerve transmission impulses being passed from cell to cell, leading to the reported paralytic effects of PSTs in humans e.g. muscular paralysis, respiratory distress etc.[123, 146, 147].

Different forms of the α subunits of the sodium channel exist in humans. These have different binding affinities to PSTs[147] and it has been suggested that differences in sensitivity to the PSTs may occur as a result[9]. This may indicate that some groups of people have immunity to PSTs; however further research is required to fully evaluate this possibility.

B.1.2 Metabolism of PST

In a review by Munday & Reeve in 2013[71], it is noted that saxitoxins are well absorbed from the gastrointestinal tract following oral administration and then distributed among internal organs. In 2002, two fishermen working in the Patagonia Chilean fjords consumed the bivalve shellfish species *Aulacomya ater* (ribbed mussel). Within 3 - 4 h of shellfish consumption the fishermen died. A forensic examination of the fishermen was undertaken and HPLC analysis for PSTs was carried out on various body fluids and tissue samples (liver, kidney, lung, stomach, spleen, heart, brain, adrenal glands, pancreas and thyroid glands). A wide variety of PSTs were detected in all tissue types tested as well as the bile and urine. Due to the rapid time to onset of symptoms reported (within 30 minutes of consumption the fishermen displayed symptoms) and time to death (3 - 4 hours) it is clear that PSTs are rapidly absorbed in the blood and transported efficiently throughout the body[148].

B.2 Toxicity studies (animal studies) of PST

Since the FSANZ review of marine biotoxins in 1999 (Proposal P158[6]) several new studies and toxicity assessment have occurred. This discussion focuses on data collected since 1999.

B.2.1 Acute and short-term toxicity of PST

Several recent works have studied the relative toxicities of PST analogues to rodents via intraperitoneal injection or gavage[123, 149, 150]. These will not be discussed in detail here, as sufficient data from human intoxications is available to calculate adverse effect levels in humans. Section B3 below details the acute and short term toxicity of PST derived from human studies, and extensive reviews on the derivation of toxin equivalency factors are available (see FAO/WHO, 2016 for latest TEFS)[57].

B.2.2 Long term toxicity and carcinogenicity of PST

No data derived from studies employing standard tests have been reported on long-term toxicity (chronic toxicity or carcinogenicity) of PST[71, 121, 151]. The lack of repeat oral dosing studies in animals and humans led the EFSA CONTAM panel to conclude that a tolerable daily intake (TDI) could not be established[9].

B.2.3 Reproductive toxicity of PST

No data derived from studies employing standard tests have been reported on the reproductive toxicity of PST[9, 36, 71, 151].

B.2.4 Developmental toxicity of PST

No data derived from studies employing standard tests have been reported on the developmental toxicity of PST[9, 36, 71, 151].

B.2.5 Genotoxicity of PST

No data derived from studies employing standard tests have been reported on the genotoxicity of PST[9, 36, 71, 151].

B.2.6 Neurotoxicity and immunotoxicity of PST

See section B.2.1 above on acute toxicity for neurotoxicity. No data could be found on immunotoxicity of PST.

B.3 Information from human studies that is relevant to the toxicity of PST

B.3.1 Observations of PST in humans

Paralytic Shellfish Poisoning (PSP) results in a variety of symptoms in humans, ranging from mild to severe, and may result in death (Table 20). Following the consumption of toxic shellfish, the time to onset of PSP symptoms can be as short as several minutes (paraesthesia and numbness around the lips, tongue and mouth), but may begin within 12 hours following a latent period. These symptoms have been summarised in detail in the following reviews and are briefly outlined below:

- Chung et al. (2006)[152]
- EFSA (2009c)[9]
- Gessner and Middaugh (1995)[153]
- Sumner (2000)[154]
- van Dolah (2000)[155]

A retrospective analysis of 54 outbreaks of PSP involving 117 persons in Alaska over the period 1973 to 1992 shows the time from ingestion of shellfish to recovery from illness ranged from 30 minutes to 24 hours[153]. However, in a study of a large outbreak (58 cases) of PSP caused by the consumption of scallops in Hong Kong in 2005, the duration of symptoms in some cases was found to be much longer, with a reported range of 1 to 228 hours[152].

In the review of PSP outbreaks in Alaska (1973 – 1992) it was found that death occurred in 0.85 % of affected people[153]. However in some outbreaks the fatality rate has been higher - for example in Guatemala in 1987, 187 cases of PSP resulted from the consumption of clams (meat and soup) causing 26 people to die. The overall fatality rate was 14%. The fatality rate for victims under the age of 6 was 50% and for those older than 18 the fatality rate was 7%[156]. In fatal cases, death is caused by respiratory paralysis.

Several reviews note that patients surviving beyond 24 hours have a higher probability of full recovery[9, 154].

Table 20 Symptoms of Paralytic Shellfish Poisoning in humans[9, 153, 155].

Mild	Moderate	Severe
Prickly sensation in fingers and toes	Extremity numbness and tingling	Muscular/limb paralysis
Tingling sensation or numbness around lips	Incoherent speech	Pronounced respiratory difficulty
Headache	Stiffness and non-coordination of limbs	Choking sensation
Dizziness	General weakness and feeling of lightness (floating sensation)	
Nausea	Slight respiratory difficulty/ shortness of breath and rapid pulse plus backache	
Vomiting		
Dry mouth		
Diarrhoea		

B.3.2 Tolerable intake levels PST

In 2004 and 2009 the World Health Organisation, Intergovernmental Oceanographic Commission and Food and Agricultural Organisation (WHO/IOC/FAO)[3], and the European Food Safety Authority (EFSA)[4] respectively, reviewed data related to human poisonings from PSTs in order to develop an ARfD for PSTs. This involved reviewing approximately 20 illness outbreaks in Canada[7, 12, 15] and around 500 reported cases of illness[9]. Data from the illness cases were used to establish a lowest-observed-adverse-effect-level (LOAEL). The LOAELs derived by the WHO/IOC/FAO and EFSA were slightly different i.e. 2.0 and 1.5 µg kg^{-1} bw respectively.

The WHO/IOC/FAO Expert Consultation and EFSA both utilised a safety factor of 3.0 to arrive at a no-observed-adverse-effect level (NOAEL) and an acute reference dose (ARfD). The EFSA Panel (2009) described how this was done: "From the available reports on intoxications in humans, comprising more than 500 individuals, a lowest-observed-adverse-effect-level (LOAEL) in the region of 1.5 µg STX equiv./kg bw could be established. Because many individuals did not suffer adverse reactions at higher intakes it is expected that this LOAEL is close to the threshold for effects in sensitive individuals. Therefore the CONTAM Panel concluded that a factor of 3 was sufficient to move from this LOAEL to an estimated no-observed-adverse-effect level (NOAEL) of 0.5 µg STX equiv./kg bw" Table 2 above shows the LOAEL's and ARfD's estimated by the EFSA Panel and the WHO/IOC/FAO Expert Consultation.

C. Information on dietary exposure of PST

C.1 Food Group where maximum level is proposed

All bivalve molluscs are potentially at risk of contamination with marine biotoxins. The following table lists major commercial bivalve species grown in Australia (Table 21). During bloom periods it is possible that non-traditional vectors such as abalone and lobster will also accumulate marine biotoxins. A national survey over two years demonstrated biotoxin contamination in abalone to be rare[157]; the only known exceedances of the bivalve ML are in Tasmania[158]. For lobster this is only known to occur in Southern Rock Lobster on the east of Tasmania during bloom events[159].

Table 21. Major commercial Australian bivalve mollusc species.

Common name	Scientific name	Raw/ Processed
Mussels	*Mytilus edulis*	Both
Clams/Pipis/Cockles	*Katelysia* spp., *Dosinia* spp., *Venerupis* spp., *Donax* spp. *Plebidonax* spp., *Anadara* spp. *Notocallista* spp.	Both
Oysters	*Ostrea* spp., *Crassostrea gigas, Saccostrea* spp., *Pinctada maxima*	Both
Scallops	*Pecten* spp., *Chlamys* spp., *Equichlamys* spp., *Mimachlamys* spp., *Amusium spp., Ylistrum* spp.	Both

C.2 Surveys on the levels of PST in seafood

Biotoxin monitoring in Australian bivalve species at risk of contamination with marine biotoxins is regularly conducted in commercial harvest areas following ASQAP requirements[22]. The rate of occurrence of PST contamination in various bivalve species (pre-market; Table 22 and Table 23) validates the requirement for this type of monitoring. Note that biotoxin monitoring in NSW, Vic, SA and WA is conducted on a minimum monthly basis and increases during rare times of heightened risk (as indicated by elevated levels of toxin or counts of toxin producing phytoplankton species). Tasmania is the only state with weekly, ongoing monitoring. As biotoxin contamination levels are monitored before product enters the market, few end-product surveys on the prevalence of PST in Australian bivalves are available.

Table 22. Detections of Paralytic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by species monitored. Source: ASQAP confidential data.

Bivalve samples testing for PST nationally by species	Number of samples analysed for PST in 2012-2017	Samples > 0.8 mg STX equiv./kg	% of samples > 0.8 mg STX equiv./kg
Oysters	5356	106	1.98
Clams	107	6	5.61
Pipis/Cockles	422	0	0.00
Mussels	936	170	18.16
Scallops	223	12	5.38
Total	**7044**	**294**	**4.17**

Table 23. Detections of Paralytic Shellfish Toxin in commercial Australian bivalve mollusc species between 2012-2017 tested as part of ASQAP biotoxin monitoring programs. Data is presented by state. Source: ASQAP confidential data.

Bivalve samples tested for PST by State	Number of samples analysed for PST in 2012-2017	Samples > 0.8 mg STX equiv./kg	% of samples > 0.8 mg STX equiv./kg
NSW	1616	7	0.43
ACT	6	5	83.33
SA	292	7	2.40
WA	126	1	0.79
TAS	4739	272	5.74
VIC	257	2	0.78
NT	4	0	0.00
QLD	4	0	0.00
All States	7044	294	4.17

Surveys on international markets and imported shellfish

In the European Union, the Rapid Alert System for Food and Feed recorded 2 incidents where bivalve shellfish were found to be contaminated with unacceptable levels of PST and product had to be withdrawn from market between 2010 and 2021 (Table 24). The US National Outbreak Reporting System reported 20 illnesses, including 6 hospitalisations due to consumption of PST contaminated mussels or clams (Table 25).

Table 24. Shipments of bivalve molluscs withdrawn from market in the European Union due to high PST levels. Source: Rapid Alert System for Foods and Feed 2010 -2021. The ML for PST in seafood in the EU is 0.8 mg/kg.

Biotoxin	Product	Date	Country of origin	Notifying country	Concentration
PST	Live scallops	9/06/2021	Norway	Norway	2.84 mg/kg
PST	Scallops	20/03/2020	Norway	Norway	1.12 mg/kg

Table 25. Number of illnesses and hospitalisations due to PST in the United States (National Outbreak Reporting System, 2009-2018). Note that this database does not distinguish between commercial or recreational harvest of bivalve molluscs.

Year	Food vehicle	Illnesses	Hospitalisations
2010	Butter clams	3	2
2012	Mussels	7	4
2013	Clams	2	0
2017	Mussels	5	0
2017	Clams, steamed	3	0

C.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

See section 3.4.1.1 subsection C.3. Information provided for DST applies here.

C.4 For foods where consumption has changed in recent years, information on likely current food consumption

See section 3.4.1.1 subsection C.4. Information provided for DST applies here.

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