Meeting

Advice on the risk to human health from consumption of bivalve molluscs (shellfish) harvested from UK waters associated with marine biotoxins

This is a paper for discussion.

This does not represent the views of the Committee and should not be cited.

Introduction

1. The Food Standards Agency (FSA) is considering the current advice and monitoring programme for marine biotoxins and whether there is a need to update or change existing legislative standards.

2. The main purpose of this work is to identify any emerging marine biotoxins in UK waters, including considerations on increasing occurrence with increasing temperatures due to climate change. The views of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) are sought whether any of these emerging marine biotoxins would pose a risk to human health.

3. Given the recent availability of additional analytical standards for pinnatoxin (PnTX), the work also includes considerations of the toxicological database for PnTX and whether there is a public health risk present that justifies including PnTX in current FSA England and Wales biotoxin monitoring programme. A discussion paper for PnTX was presented to the COT at the July 2023 meeting (TOX/2023/37). Members noted that the toxicological database for PnTX was limited, especially regarding human exposure data and concluded that it may be reasonable to include PnTX in any monitoring programme. Occurrence data for UK

waters would be useful to establish potential exposures of the UK population.

4. EFSA’s 2010 scientific assessment of pectenotoxin (PTX) resulted in its recent removal from the EU monitoring programme. Hence FSA policy colleagues are considering whether to follow suit and have asked for a review of the current database on pectenotoxins and whether the COT agrees with EFSA’s conclusions. The discussion paper is also being presented at the December 2023 meeting (TOX/2023/58).

5. The current scoping paper provides an overview of potentially emerging biotoxins, brief summaries of any available toxicological information, occurrence data, with an emphasis on UK waters, and any additional relevant information, such as proposed or current limits/monitoring and considerations in other countries.

Background

6. Marine biotoxins are natural toxic metabolites produced by marine phytoplankton and can bioconcentrated in shellfish, and along the food chain. If concentrations of these toxins in shellfish are sufficiently high, then consumption of these shellfish can result in human illness.

7. Marine biotoxins have previously been categorised on the basis of clinical signs but are increasingly being categorised by chemical structure. The structural toxin groups that are generally considered to be of relevance to shellfish harvested in European waters are:

- Domoic acid group (DA),
- Saxitoxin group (STX),
- Okadaic acid group (OA),
- Pectenotoxin group (PTX),
- Azaspiracid group (AZA),
- Yessotoxin group (YTX),
- Cyclic imine group (CI).

8. Marine biotoxins can also be categorised according to their water solubility which determines the extraction protocol required for analysis. The domoic acid and saxitoxin groups are hydrophilic, while the okadaic acid, pectenotoxin, azaspiracid, yessotoxin and cyclic imine groups are lipophilic. The domoic acid group is associated with amnesic shellfish poisoning (ASP), the saxitoxin group with paralytic shellfish poisoning (PSP) and the okadaic acid group with diarrhetic shellfish poisoning (DSP).

9. In the United Kingdom (UK) and European Union (EU), there are currently three major biotoxin groups that are regulated in shellfish, and which are subject to statutory testing to protect human health. The biotoxins specified within the Retained EU Regulation (EC) No. 853/2004 (E&W, and Scotland) and EU Regulation (EC) No. 853/2004 (NI) are PSP toxins (saxitoxin and relevant analogues), the lipophilic toxin group (okadaic acid, azaspiracid, pectenotoxin and yessotoxin) and ASP toxin (domoic acid).

10. In the UK the Agri-Food and Biosciences Institute (AFBI) is the GB National Reference Laboratory (NRL) for marine biotoxins. The Centre for Environment, Fisheries and Aquaculture Science (Cefas) are designated as the Official Laboratory (OL) for marine biotoxins in England, Wales and Scotland. Northern Ireland's NRL for Marine Biotoxins is Wageningen Food Safety Research (WFSR) and the designated OL AFBI who undertake analysis and reporting of shellfish official controls (OCs). A move from biologically based assays (such as the mouse bioassay (MBA)) for marine biotoxin testing to chemical-based assays has been implemented due to their increased specificity.

11. The purpose of this scoping paper is therefore to identify information to help inform risk managements decisions on the benefits of, or need to, gather monitoring data for emerging toxins and whether there is a need to change the existing legislative standards, based on an assessment of risk and likelihood of occurrence in UK waters.

Evaluations by other authorities and published literature

EFSA

12. Under Article 34 of the European Food Safety Authority's (EFSA) Founding Regulation 178/2022, EFSA is required to identify, assess and disseminate information on emerging issues. Each year EFSA publishes a report detailing its activities on emerging risks in food and feed. This work has included assessments on the brevetoxin group (2010), the ciguatoxin (CTX) group (2010), cyclic imines, including spirolides, gymnodimines, pinnetoxins and pteriatoxins, and cyanobacteria toxins in food (2016).

CEFAS (2014)

13. In 2014, CEFAS published a report to support the development of a monitoring programme for new and emerging marine biotoxins in shellfish in UK waters. The report identified six genera/species of potentially new/emerging toxic algae in UK waters that were cause for concern, **Gymnodinium catenatum**, a PSP causative species, known to be capable of translocation and present in ballast water in UK ports, **Karenia, Ostreopsis, Coolia** and **Alexandrium catenella**, which have the potential to establish viable populations in UK waters of advected/introduced and **Vulcanodium** sp., which has been identified to produce pinnatoxins. Phytoplankton monitoring methods were found to be generally fit for purpose and in line with other countries but unable to detect **Karenia** and *A.* **catenella** which are benthic species and while **Azadinium** spp. has previously been found in UK waters, the current methods cannot detect it due to its small size.

14. The report also CEFAS review also identified new and emerging toxins, such as novel azaspiracid and PSP (domoic acid) analogues, brevetoxin (BTX), cyclic imines (including pinnatoxins and spirolides), tetrodotoxins (TTXs), and recommended to develop a testing method for cyanobacterial toxins to help assess the potential risk, while noting that the latter were currently not prevalent in UK waters.

FSAI (2016;2020)

15. In 2016, the Food Safety Authority Ireland (FSAI) published a report on the occurrence of marine biotoxins, focusing not only on current risks but also novel and emerging toxins. The report recommended to continue the investigation of azaspiracid analogues, other than azaspiracid 1-3, the need for toxicity and epidemiological data for pectenotoxin to evaluate the proposed deregulation of pectenotoxin and to collect long term data for new and emerging toxins. The report further recommended that monitoring should address the levels of PSP toxins in areas where they had previously not been encountered.

16. In the research needs published in 2020, FSAI noted the increased risk of PSP events in Ireland, with saxitoxin (STX) having been observed in increasing abundance and geographical distribution and occurrence in Irish coastal waters. FSAI further highlighted tetrodotoxins, ciguatoxins, pinnatoxin and palytoxins, as they have recently emerged in several coastal waters in Europe.

ANSES (2021)

17. In 2021, the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) was asked to assess brevotoxins in light of their recent discovery in French Mediterranean mussels from Corsica.

IFREMER (2021)

18. In 2023, the French Research Institute for Exploitation of the Sea (IFREMER) published a report on the monitoring of emerging of unregulated toxins in shellfish in France (Amzil et al., 2023). The results from the five year (2018-2022) monitoring programme showed that unregulated lipophilic toxins, i.e. saxitoxins, pectenotoxins, pinnatoxins, gymnodimine, brevetoxins, mycrocystins, were quantified in various species of shellfish every year. Microcystins (MC-RR and/or dmMR-RR and/or MC-LR), gymnodimine (GYM-A), and brevetoxins were found for the first time. While toxins of the palytoxin group (palytoxins, ovatoxins) were not detected in shellfish, they were detected in other seafood organisms, e.g. sea urchins, fish, gastropods and crustaceans.

19. Tetrodotoxins were not detected. However, β-N-methylamino-L-alanine (BMAA), a non-protein amino acid produced by cyanobacteria and microalgae and 2,4-diaminobutyric acid (DAB) were continuously present in all shellfish samples analysed. Concentrations of BMMA varied between shellfish species, suggesting differences in trophic status and geological and seasonal factors, while the relatively constant concentration of DAB in shellfish suggested that DAB could be present in primary producers or bacteria and/or naturally present in shellfish flesh. The environmental origin and metabolism in molluscs is as yet, unknown.

Literature

20. A paper by Davidson et al. (2015) on cyclic imines (CIs) noted that while the CI producing phytoplankton is known to be present in UK waters, these co-exist with saxitoxin (STX) producing and benign species and current monitoring practises do not allow for discrimination.

21. A review by Vilarino et al. (2018) on human poisonings from marine toxins focused on several toxin classes including paralytic toxins, amnesic toxins, ciguatoxins, brevetoxins, tetrodotoxins, diarrhetic toxins, azaspiracids and palytoxins (PITX).

22. A paper by Estevez et al. (2019) on emerging marine biotoxins in seafood from marine coasts identified recent incidence of ciguatera toxins and tetrodotoxins in the EU, not only in fish where it had previously been reported but

also in bivalve molluscs.

23. A paper by Gerssen and Gago-Martinez (2019) noted that several marine biotoxins, i.e. tetrodotoxins, ciguatoxins and palytoxins, have recently emerged among coastal areas in Europe, especially around Macaronesia such as Azores, Madeira Islands (Portugal) and the Canary Islands (Spain). In addition, cyanobacterial toxins such as microcystins have been reported to cause problems in drinking water with the potential to end up in freshwater fish and shellfish produced in estuaries.

24. A paper by Otero and Silve (2022) discusses emerging marine biotoxins in European waters, focusing on the presence of emerging azaspiracids, cyclic imines, ciguatoxins, brevetoxins and tetrodotoxins.

Emerging marine biotoxins

25. Based on the published assessments and reports on emerging marine biotoxins by other authorities, as well as a brief literature search, the following overview of emerging marine biotoxins of potential concern to human health has been provided.

26. Please note that PnTX was discussed in separate discussion paper and hence has not been included in the overview here.

27. This paper focuses solely on the potential risk from emerging marine biotoxins in shellfish. While ciguatoxins have been reported more frequently in European waters, they occur in fish and therefore have been excluded from discussion here.

Brevetoxin group

28. Brevetoxin (BTX) group toxins are primarily produced by the dinoflagellate **Karenia brevis** and are grouped into two chemical structures (A and B) based on their backbone. BTX-1 (type A) and BTX-2 (type B) are considered the parent toxins, with BTX-2 being the most abundant (EFSA, 2010). In total, BTX have around 30 metabolites of which the structure is known, and approximately 30 other metabolites whose structural formulas have not yet been chemically characterised. BTX are lipid soluble and can accumulate in shellfish and fish. Consumers however are predominantly exposed to BTX metabolites, rather than the parent algal BTX-group toxins, as BTX undergo metabolism in fish and shellfish (EFSA, 2010; ANSES, 2021).

29. BTX group toxins cause neurological/neurotoxic shellfish poisoning (NSP). Symptoms include nausea, vomiting, diarrhoea, paraesthesia, cramps, bronchoconstriction, paralysis, seizure and coma. The onset of symptoms occurs rapidly, usually within 30 minutes to 3 hours after consumption of contaminated shellfish and lasts for a few days. Dermal exposure or inhalation can result in irritant effects. Inhalation occurs predominantly through breathing in aerosol from wave action. Persistent symptoms or fatalities have not been reported (EFSA, 2010; CEFAS, 2014). Only a few hundred cases of human poisoning have been reported to date, however the number of cases remains underestimated (ANSES, 2021).

30. The toxicological database for BTX is limited.

31. Following intraperitoneal (i.p.) injection BTX-2 and BTX-3 peak blood levels were reached in rats after one hour, with levels for BTX-2 being approximately three times higher than those for BTX-3. BTX-2 was excreted within 24 hours, predominantly in urine, as the cysteine conjugate. BTX-3 was cleared from circulation within one minute and eliminated within 24 hours, primarily through biliary excretion in faeces. After oral administration, BTX-3 was rapidly absorbed and widely distributed to all organs with the highest concentration in the liver. Elimination was approximately equal between urine and faeces. In both, mice and human plasma, BTX-3 has been reported to bind to lipoproteins, particularly to high-density lipoproteins. In humans diagnosed with NSP, the urine showed a number of metabolites, including BTX-3, methylsulfoxy-BTX-3, 27-epoxyBTX-3, cysteine conjugates and reduced BTX-5, plus several minor hydrolysis metabolites of BTX-1 (EFSA, 2010).

32. No long-term studies in experimental animals were available, only studies of acute effects following i.p., intravenous (i.v.) or oral exposure. The limited data show that BTX bind to the voltage-gated sodium channels in cell membranes and thereby cause depolarization of neuronal and muscle cell membranes resulting in impairment of the central and peripheral nervous system, including neurovegetative effects, neuromuscular effects, cardiorespiratory symptoms and central signs such as ataxia, seizure and decreased body temperature (EFSA, 2010; ANSES 2021).

33. After i.p. or i.v. administration, no lethal dose (LD50) data were available for BTX-1 , however LD50s after i.v. administration range from 94 µg/kg bw for BTX-3 to 200 µg/kg bw for BTX-2, while LD50s after i.p. administration range from 170 - > 300 µg/kg bw for BTX-3, 200 – 400 µg/kg bw for BTX-(B)2 and 211 µg/kg bw for S-deoxy-BTX-B2, after 24 hours. Minimum lethal doses (MLD) were also

reported for BTX-4 and BTX-5 at 100 µg/kg bw (i.p. 6-24 hours) and 300 – 500 µg/kg bw (i.p., time of death not reported), respectively. LD50s after oral administration were only available for BTX-2 and BTX-3 in mice, with a concentration of 6600 mg/kg and 520 mg/kg bw, respectively. Whereas signs of toxicity, including death, occurred almost instantly after i.v. and i.p. administration signs of toxicity after oral administration occurred after approximately five hours.

34. Based on the limited data available, the oral potency of BTX-3 is about 10-fold higher than that of BTX-2, while based on i.p. administration, BTX-2, BTX-3, BTX-B2 and S-deoxy-BTX-B2 appear to have similar toxic potencies.

35. *In vitro* data indicated clastogenic activity of BTX; BTX-2 induced chromosomal aberrations in Chinese hamster ovary cells while BTX-2, BTX-3 and BTX-9 induced DNA damage in human lymphocytes and BTX-2, BTX-3 and BTX-6 in Jurkat E6-1 cells. Further *in vitro* studies with BTX-2 in isolated rat lung cells and *in vivo* in lung tissue following intratracheal administration in rats showed evidence of DNA adduct formation. However, neither BTX-2 nor BTX-6 were mutagenic in a bacterial reverse mutation assay (Ames test).

36. No data on reproductive or developmental effects of BTX were available.

37. No health based guidance values (HBGVs) for BTX have been set. However, EFSA (2010) noted that due to the acute toxicity of BTX group toxins, an acute reference dose (ARfD) should be established, however they were unable to do so, due to the limited quantitative data both from experimental animals and related human intoxications.

38. NSP appears to be prevalent to the Gulf of Mexico, the east coast of the United States (USA), and the New Zealand Hauraki Gulf region, with concentrations of BTX group toxins ranging from 880 – 49,000 µg BTX-2 equivalents/kg (with a conversion of 1 MU to 4 µg BTX-2 equivalent) in shellfish. Levels of BTX-3 in fish varied from 580 – 6000 µg BTX-3 equivalents/kg (EFSA, 2010). In their respective reports in 2010 and 2014, EFSA and CEFAS noted that no BTX toxins have been reported to date in European fish or shellfish. However, the discovery of new BTX-producing algae and the expansion of bloom distributions suggest the toxin may emerge in other regions, including UK waters where conditions for the toxin producing algae are favourable (EFSA, 2010; CEFAS, 2014). In 2018, BTX was detected for the first time in French Mediterranean mussels from Corsica and regularly ever since during the autumn and winter season, with concentrations ranging from 82 - 345 µg (BTX-2 + BTX-

3)/kg (Amzil et al., 2021).

39. No data were available on the influence of processing on the level of BTX in shellfish or fish.

40. There are currently no regulatory limits for BTX group toxins in fish or shellfish in Europe. However, some countries have set action levels, maximum levels or guidance levels for BTX. The USA set an action level of ≥ 0.8 mg BTX-2 equivalents/kg shellfish (based on 20 mouse units (MUs)/100 g) or 5,000 cells/L (US FDA and EPA, 2021). While in Australia and New Zealand the maximum level is also 20 MUs/100g, the BTX analogues is not specified (NZFSA, 2006 (Reference from CEFAS, 2014; original could not be found); FSANZ, 2010). A MU is the amount of raw extract that kills 50% of mice within 930 minutes/15.5 hours. Following the recent detection of BTX in shellfish in France (Corsica), ANSES proposed a guidance level of 180 µg BTX-3 equivalent/kg shellfish meat, considering a protective standard portion size of 400 g shellfish meat and considering the sum of all tested BTX metabolites. The guidance level was based on cases of food poisoning which had occurred in other countries and the minimum dose of BTX associated with symptoms (acute lowest adverse effect level (LOAEL)) of 27-40.5 MU/person or 92-138 µg BTX-3 eq./person (McFarren et al., 1965) and 0.3-0.4 MU/kg bw or 1.02-1.36 µg BTX-3 eq./person (Hemmert, 1975). The species of BTX producing algae in Corsica however is yet unknown (ANSES, 2021).

Cyclic imines

41. Cyclic imines (CIs) are a family of marine biotoxins, including spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs), pteriatoxins (PtTXs) and prorocentrolides and spiro-prorocentrimines, which are macrocyclic compounds with imine and spiro-linked ether moieties. SPX, GYMs and PnTX are produced by **Alexandrium ostenfeldii/Alexandrium peruvianum, Karenia selliformis/Alexandrium** sp. and **Vulcanodinium rugosum**, respectively. PtTX has been suggested to be biotransformed from PnTX in shellfish (EFSA, 2010; CEFAS, 2014; Davidson, 2015, Stivala et al., 2015).

42. Please note, the following information on CIs excludes PnTX and portimine, they were discussed separately.

43. Due to similarities in chemical structure and toxicity in mice, the toxins have been grouped together. SPX are the largest group, with currently 16 analogues having been identified and detected in Europe with 13-desmethyl SPX

C being the most commonly found in shellfish. Eight analogues of GYM have been characterised, PtTX and PnTX are almost structurally identical, and it has been suggested that PnTX-F and PnTX-G are progenitors of all known PnTX and PtTX via metabolic and hydrolytic transformation in shellfish. Three analogues of PtTX have been identified (A-C) (EFSA, 2010; FSAI, 2016).

44. No reports were available linking this toxin group to poisoning events in humans.

45. The toxicological database for CIs is limited.

46. Based on the available data, SPX and GYM are neurotoxic and have a similar mechanism of toxicity, with evidence pointing to both inhibiting the muscarinic and nicotinic acetylcholine receptors (mAChR, nAChR) in the central and peripheral nervous system and the neuromuscular junction. No mechanistic data was available for PtTX (EFSA, 2010; Stivala et al., 2015).

47. No long term studies were available, however the acute toxicity of CIs is characterised by the rapid onset of systemic neurotoxicity and death within minutes. LD50s for STX after i.p. injection range from 8 µg/kg bw (SPX-C) to 40 µg/kg bw (SPX crude extract), LD100s were 250 µg/kg bw for SPX-B and SPX-D, while at doses of 1000 µg/kg bw no effects were reported for SPX-E and SPX-F. SPX demonstrated a higher toxicity by gavage than administration on feed, overall the LD50 values ranged from 500-1005 µg/kg bw. In general, the LD0 values were close to the LD50 values, indicating a steep dose response relationship. Symptoms of toxicity were similar after i.p. and oral administration and if animals survived for more than 20 minutes, a full recovery occurred, and subsequent behaviour and appearance were normal (EFSA, 2010).

48. GYM-A was highly toxic to mice by i.p injection with an LD50 of 80-96 µg/kg bw and was 10-fold more toxic than GYM-B (LD50 800 µg/kg bw). After intra-cerebroventricular injection (i.c.) the LD50 for GYM-A was 3 µg/kg bw. The oral (gavage) LD50 (755 µg/kg bw) was 8-times higher than from i.p. administration, no toxicity was observed when GYM-A was administered by food at doses up to 7500 µg/kg bw. At toxic but sub-lethal doses, mice showed symptoms of prostration and respiratory distress but recovered and no adverse effects were detected thereafter (EFSA, 2010).

49. While the interactions with the receptors may differ between the different CIs, some bindings are reversible others are not, overall effects of CIs appear to be mediated by similar modes of actions (MOA). In the absence of data

on combined exposure EFSA assumed additive toxicity of the different analogues within each group of CIs. By i.p. injection 13-desmethyl SPC-C, SPX-C and 20-methyl-SPX-C had equal potency. For all other analogues/groups of CIs insufficient information was available to derive relative potencies (EFSA, 2010).

50. No HBGVs for CIs have been set. However, EFSA (2010) noted that due to the acute toxicity of CIs an acute reference dose (ARfD) should be established, however they were unable to do so, due to the limited quantitative data on acute oral toxicity and the lack of a no observed adverse effect level (NOAEL). However, EFSA calculated a margin of exposure (MOE) between the lowest oral LD50 values for SPX (50 and 500 µg/kg bw) in mice and the estimated 95th percentile of exposure (0.06 µg/kg bw) from consumption of shellfish currently on the market. The resulting margin of exposure (MOE) was in the range of 1000-10,000. The lower end of the range was based on administration via gavage, while the higher end of the range was based on administration in feed. The latter is therefore more likely to be relevant to human exposure. In 2010, EFSA concluded that at the estimated exposures, SPX did not raise concern for health in consumers. Exposures to the group of CIs could not be estimated.

51. SPX has been identified in several European countries bordering the Mediterranean Sea, Atlantic cost and North Sea, i.e. in the toxin producing organism has been identified in Scotland, Italy, Denmark, Ireland and SPX in shellfish in Norway, Spain and Italy. PtTX and GYM have not yet been detected in shellfish from Europe, but the latter has been found in imported shellfish (EFSA, 2010; Rambla-Alegra, 2018).

52. No data were available on the influence of processing on the level of CIs in shellfish.

53. Currently there are no regulatory limits for CIs in shellfish in Europe or other regions of the world. However, the EU Community Reference Laboratory for marine biotoxins (CRLBM)/EU Regulatory Reference Laboratory (EURL) proposed a guidance level of 400 µg sum of SPXs/kg shellfish meat (CRLMB, 2005) and an oral and i.p. LD50 of 130 µg/kg bw and 7-28 µg/kg bw for SPX-13, respectively (Otera and Silva, 2022).

Palytoxins

54. Palytoxin (PlTX) group toxins have mainly been detected in marine zoanthids (soft corals) of the genus **Playthoa** and benthic dinoflagellates of the genius **Ostreopsis** spp (**O. siamensis, O. mascarenensis, O. ovata**). Of the

eight known PITX group toxins, i.e. PITX, ostreocin-D, ovatoxin-A, homopalytoxin, bishomopalytoxin, neopalytoxin, deopalytoxin and 42-hydroxypalytoxin, only PITX and ostreocin-D have been characterised (structurally).

55. Symptoms of PITX group toxins are not well defined and most cases of PITX toxicity are anecdotal. Reported symptoms include, myalgia and weakness, possibly accompanied by fever, nausea and vomiting, and rhabdomyolysis, characterised by injury to skeletal muscle, muscle breakdown and leakage of myocytes into plasma. In severe cases renal failure and disseminated intravascular coagulation were described. Fatalities appear to be rare although there have been some reports of severe cases with death, due to respiratory arrest, after 15 hours. Recovery can take up to several months. In addition to consumption of contaminated shellfish, poisoning cases have also been reported due to exposure through injured skin and inhalation, leading to skin, eye and respiratory irritation (EFSA, 2009; FSAI, 2016; ANSES, 2021; Otera and Silva, 2022). For some cases of human intoxication, the involvement of PITX remains unconfirmed as it was unclear whether the incident could solely be attributed to PITX (CEFAS, 2014).

56. The toxicological data for PITX group toxins are limited.

57. While limited data were available on the toxicokinetics of PITX group toxins, the systemic toxicity observed after i.p administration, inhalation and gavage, suggested that PITX group toxins can cross the pulmonary and gastrointestinal (GI) barriers and be distributed to various organs. Oral toxicity data also indicated that PITX group toxins are absorbed in different areas of the gastrointestinal (GI) tract, with higher absorption in the oral cavity than after gavage. However, no data was available indicating whether PITX group toxins crossing epithelial barriers also lead to potential alterations of said epithelial. The (acute) toxicity of PITX group toxins appears to be strongly dependent on the route of administration, with PITX and ostreocin-D being less toxic after oral than parenteral administration. No data were available on the metabolism or elimination of PITX group toxins (EFSA, 2009; ANSES, 2021).

58. The main targets of PITX group toxins are skeletal, heart and smooth muscle cells. PITX group toxins interfere with the sodium/potassium-ATPase ion-pump resulting in the depolarisation of membranes in excitable and non-excitable cells and contraction of muscle cells. A number of secondary effects due to the increased sodium in cells have been reported, such as the induction of sodium-dependent transport of calcium ions into cells and the exchange of intracellular sodium for extracellular protons, leading to a reduced intracellular pH. In addition,

exposure to PITX group toxins in rabbits lead to an increased metabolism of arachidonic acid and the production of eicosanoids; arachidonic acid was then metabolised to different prostaglandins, resulting in the release of norepinephrine and contraction of the aorta (EFSA, 2009).

59. A study by Wiles et al. (1974) demonstrated large differences in susceptibility between species following i.p. administration of PITX. Rabbit (LD50 0.025 µg/kg bw), dog (LD50 0.33 µg/kg bw) and monkey (LD50 0.078 µg/kg bw) were the most susceptible to PITX group toxins, while the mouse was the least susceptible (LD50 0.45 µg/kg bw). The LD50s for rats and guinea pigs were 0.089 µg/kg bw and 0.11 µg/kg bw, respectively. Intramuscular (i.m.) and s.c. administration of PITX resulted in higher LD50s. Oral administration (gavage) resulted in LD50s of 510-767 µg/kg bw in mice and 40 µg/kg bw (> 24 hours) in rats (EFSA, 2009; ANSES, 2021).

60. PITX group toxins were cytotoxic in a number of *in vitro* systems, with EC50s of 5×10^{-10} M and 2×10^{-7} M for rat and cattle erythrocytes, respectively. Ostreocin-D was approximately 27-fold and 67-fold less toxic than PITX in mouse erythrocytes and human MCF-7 cells, respectively. No relative potencies were available comparing ovatoxin-A and PITX *in vitro*. *In vivo* studies in mice indicated that the relative potency of ostreocin-D ranged from 0.4-1.0, after i.p administration, while oral administration suggested ostreocin-D to be slightly less toxic than PITX. Given that both compounds are structurally similar, act on the same target and caused similar signs of toxicity PITX and ostreocin-D could be considered equipotent by the oral route and dose-addition would be anticipated upon co-exposure (EFSA, 2009).

61. Gemin et al. (2022) exposed CaCo-2 cells to PITX group toxins, demonstrating that PITX and ovatoxins-a (OVTX) affect the integrity of the intestinal barrier at concentrations of 0.5 and 5 ng/mL in Caco-2 cells, while OVTX-d did not. OVTX-d was less toxic than OVTX-a and PITX but neither OVTX-a or OVTX-d induced cytotoxicity up to concentrations of 20 ng/mL. All three PITX group toxins resulted in increased IL-8, however to a lesser extent after OVTX-a and OVTX-d than PITX exposure.

62. Following i.p. administration, mice showed acute toxicity, characterised by stretching of hind limbs, lower back and concave curvature of the spinal column, muscle spasms, respiratory distress, dyspnoea and progressive muscular paralysis. Animals which survived 24 hours but died without morphological signs, showed several histopathological changes in the heart, kidney, liver, pancreas, intestines and lymphoid tissues, e.g. necrosis in the spleen and thymus,

peritonitis, congestion and bleeding. In mice receiving 0.25 µg/kg bw PlTX five times a week up to 29 times the number of lymphocytes was reduced in tissues and blood, however, mice recovered within a month. Following intra-tracheal administration with lethal doses mice showed paralytic signs and lung pathology showed extensive bleeding in the alveoli and oedema around blood vessels, as well as gastro-intestinal erosions and atrophy of kidney glomeruli. At a sublethal dose neurological signs occurred within one to two hours but disappeared after 24 hours. Following single sublingual administration, animals showed inactivity and rapid respiration. Upon autopsy, bleeding, interstitial inflammation, oedema and alveolar destruction in the lungs, gastrointestinal erosions and kidney glomerular atrophy were observed. Administration of ostreocin-D caused similar symptoms than PITX but less severe. Repeat administration of PITX resulted in scratching and severe pathology, particularly in the heart, but also congestions in the stomach and the intestine, but not in the lungs. No symptoms or injuries were seen for ostreocin-D (EFSA, 2009; ANSES, 2021).

63. Symptoms after oral dosing were similar to the ones described above. In addition, clinical blood chemistry showed a significant, and dose related increase in creatine phosphokinase, lactate dehydrogenase and aspartate transaminase. Histopathological alterations were observed in the forestomach, liver and pancreas (EFSA, 2009; ANSES, 2021).

64. Del Favero et al. (2013) exposed mice (by gavage) to concentrations of 30, 90 and 180 µg/kg bw PITX extract per day for seven consecutive days, with a recovery time of two weeks. Mortality rates were 33, 33 and 83%, respectively, with toxicological symptoms apparent right before death. In the lungs, histological alterations were observed, irrespectively of the dose, and alterations of the heart muscle could be seen at the two highest doses, and to a lesser extend at the lower dose. In a second experiment, Del Falvero et al. (2013) exposed mice to 3 or 30 µg/kg bw for seven days. No mortalities, abnormalities or clinical signs were observed in mice exposed to the lower dose, mice at the higher dose showed mild symptoms of toxicity. During the recovery period, weight and food intake returned to normal (for the higher dosing group). The authors hypothesised that PITX can be lethal after seven days at concentrations of 30 µg/kg bw, a dose which is 17-times lower than the LD50 after single administration. The authors further proposed a NOAEL of 3 µg/kg bw day and a lowest observed adverse effect level (LOAEL) of 30 µg/kg bw per day. At this LOAEL, the lungs, heart, liver and gastrointestinal tract are major targets for PITX.

65. Sosa et al. (2022) exposed mice to PITXs at concentrations of 30, 90 and 270 µg/kg bw, with signs of toxicity/lethal effects observed after 24 hours at concentrations ≥ 90 µg/kg bw. PITX at the highest concentration also resulted in decreased liver/body weight ratio and decreased hepatocyte glycogen.

66. Boente-Juncal et al. (2020) exposed mice to PITX daily (gavage) for 28-days at concentrations of 0.03, 0.1, 0.3, 1, 3.5 or 10 µg/kg, resulting in an LD50 of 0.44 µg/kg and a NOAEL of 0.03 µg/kg. Animals dosed with PITX ≥ 1 µg/kg bw demonstrated a significant weight loss, while animals exposed to ≥ 0.1 µg/kg bw showed symptoms of toxicity, such as lethargy, piloerection and facial swelling. Stomach and intestinal alterations were observed, as well as liver and kidney damage. PITX did not alter blood glucose or cholesterol levels. However, plasma concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) increased significantly after administration of PITX at doses of ≥ 36 µg/kg bw. Low doses of PITX did not cause any significant changes in blood sodium and chloride levels; a significant increase was however observed at 300 µg/kg bw, while at 1200 µg/kg bw a decrease in blood sodium levels was observed. An increase in blood potassium levels was observed at high doses (750 and 1200 µg/kg bw). Please note, ANSES (2021) converted the exposures by Boente-Juncal et al. on a bw basis resulting in doses equivalent to 15, 36, 100, 350, 500, 750 and 1200 µg/kg bw, with an LD50 of 599 µg/kg bw. These values have been referred to in parts here.

67. PITX was reported to activate extracellular signal-regulated kinases (ERK), the c-Jun-NH2-terminal protein kinase (JNK) and p38 kinase. The functional implications of these findings are not fully understood but it has been suggested that PITX plays a possible role in carcinogenesis. Repeat dermal exposure in a two-stage mouse skin carcinogenesis model resulted in tumour promotion (EFSA, 2010). However, PITX was negative in the Ames test and did not act as an initiator in the *in vitro* BALB/c 3T3 cell transformation assay, or on mouse skin *in vivo* (ANSES, 2021).

68. In 2010, EFSA established an ARfD for the sum of PITX and ostreocin-D of 0.2 µg/kg bw, based on the LOAEL for oral toxicity (gavage) in mice. EFSA applied the default uncertainty factors (UF) for intra- and interspecies variability, plus an additional UF of 10 to account for the apparent lower sensitivity of mice compared to other species. The sublingual administration of PITX in the region of 200 µg/kg bw caused toxicity of the internal organs and transmucosal transport in the mouth could not be excluded. No chronic HBGV could be established at the

time due to the lack of chronic toxicity studies. The Secretariat is not aware of any chronic HBGVs being established since then.

69. In 2021, ANSES proposed a short term toxicity reference value for PITX of 0.08 µg/kg bw per day. ANSES considered lethargy, ataxia, abdominal pain, dyspnoea and piloerection observed in experimental animals as critical effects as they were consistent with symptoms of acute exposure/poisonings. Diverging from EFSA's assessment, ANSES selected the study by Boente-Juncal et al. (2020) as key study and adopted the NOAEL of 15 µg/kg bw as point of departure (POD). An overall UF of 25 was applied, comprising of an UF of 2.5 for inter-species variability, 10 for inter-individual variability, 1 for use of a POD and 1 for inadequacy in the data. ANSES noted that this is a TRV for single exposure by the oral route, and therefore should be considered as an acute TRV.

70. In 2016, a report produced by ANSES and the Istituto Superiore di Sanita-Rome for EFSA (Testai et al., 2016) also considered a short-term repeat study in mice, potentially representative of human exposure scenarios. The study showed that repeat administration over seven days of PITX doses of 30 mg/kg per day (below the LD50) caused lethality and toxic effects. A NOAEL of 3 mg/kg per day and a LOAEL of 30 30 mg/kg per day was estimated, with lungs, heart, liver and GI tract as the main target organs.

71. PITX and ostreocin-D were reported in mussels and sea urchins from several European countries, such as France, Greece, Italy and Spain. Concentrations of PITXs ranged from 300-625 µg/kg shellfish meat (EFSA, 2010).

72. No data were available on the influence of processing on the level of CIs in shellfish.

73. Currently there are no regulatory limits for PITX in the EU, or in other countries around the word. However, in 2005, the NRL for Marine Biotoxins proposed a provisional limit of 250 µg/kg shellfish (CRLMB, 2005). EFSA noted in 2010 that for a 60 kg adult not to exceed the ARfD, a 400 g portion of shellfish meat should not contain more than 12 µg of the sum of PITX and ostreocin-D, corresponding to 30 µg/kg shellfish meat. However, based on worst case estimates of PITX group toxins reported in mussels and sea urchins, a 60 kg adult would be exposed to 3 µg/kg bw of the sum of PITX and ostreocin-D exceeding the ARfD; if the potency of OVTX-a was similar to PITX and ostreocin-D, then the resulting exposure would be well above the ARfD (EFSA, 2010).

Saxitoxins

74. Saxitoxin (STX) group toxins have been detected in filter-feeding bivalves, such as oysters, mussels, scallops and clams, and are produced by dinoflagellate of the genius **Alexandrium** (e.g. **Alexandrium tamarensis, A. minutum/A. excavata, A. catenella, A. fraterculus, A. fundyense and A. cohorticula** as well as **Pyrodinium bahamense** and **Gymnodinium catenatum.** The toxicity of the dinoflagellates is due to a mixture of STX analogues, with more than thirty different analogues identified. Of the analogues identified STX, NeoSTX, GTX1 and dc-STX appear to be the most toxic (FAO, 2004; EFSA, 2009). The composition differs per algae species and/or per region. In addition, STX group toxins have also been identified in some cyanobacteria, which may occur in fresh and brackish water.

75. STX group toxins cause PSP in humans, with symptoms varying from mild, i.e. a slight tingling sensation or numbness, mostly around the lips but spreading to face and neck, headache, dizziness and nausea, to moderately severe, i.e. incoherent speech, progression of prickling sensation to arms and legs, stiffness, non-coordination of limbs, general weakness and feeling of lightness, slight respiratory difficulties and rapid pulse to sever, to extremely severe, i.e. muscular paralysis, pronounced respiratory difficulties to fetal respiratory paralysis. In fatal cases, respiratory arrest occurred within 2-12 hours following consumption. Patients who survive PSP for 24 hours have a high probability of a full and rapid recovery. Doses associated with severe illness generally ranged from 5.6-2058 µg STX equivalents/kg bw, with a more recent meta-analysis establishing a critical minimal dose of 37 µg STX equivalents/kg bw (EFSA, 2009; ANSES, 2020). Among individuals who did not develop symptoms after consumption of contaminated shellfish, estimated STX group toxin intake ranged from 0.3-90 µg STX equivalents/kg bw, although one study reported a concentration of 610 µg STX equivalents/kg bw (EFSA, 2009). According to one case study, children appear to be more sensitive to STX, with a higher mortality rate (ANSES, 2020).

76. STX also causes skin and eye irritation (ANSES, 2020). Exposure in freshwater comes through recreational contact, e.g. swimming, surfing, water/jet skiing, and contaminated drinking water.

77. The toxicological data based for STX toxins is limited and mostly comprised of acute studies following i.p. administration.

78. The reported symptoms following consumption of contaminated shellfish indicate local absorption through the buccal mucous membranes, within a short time frame. The reports also suggest that the toxins are rapidly transported via

the bloodstream to the rest of the body, including the brain (Garcia et al., 2004). A study by Andrinolo et al. (2002a) demonstrated transport of GTX2 and GTX3 across the epithelium by the paracellular route in human and rat derived epithelial cell lines. When administered orally STX crosses the intestinal barrier and enters the bloodstream despite the first-pass effect through the liver, and hence giving it access to the peripheral nervous system (ANSES, 2020). Data on distribution is limited, but postmortem examination showed trace amounts of STX toxins in the liver but fairly large amounts in the gut contents, blood and urine. In cats, STX disappeared quickly from the blood (after injection) with a serum half life of 22 minutes (Adrinolo et al., 2022b). However, STX was detected in the blood and urine but also in the spleen, liver, medulla oblongata and brain. In rats, saxitoxinol (the reduced analogue of STX) was detected in the muscle, liver, kidney, small/large intestine, lung, heart and spleen one hour after administration, but disappeared quickly from blood with a serum half life of 29 minutes (Naseem, 1996). In humans, STX and its analogues, e.g. neoSTX and gonyautoxins (GTX) were distributed to numerous organs, tissues and fluids, regardless of exposure route (ANSES, 2020).

79. Based on the different toxin profiles between causative foods and human specimens, STX toxins have been suggested to undergo metabolic biotransformation. B1 was incubated at modelled conditions of the human stomach (pH 1.1) and conversion of the toxin was observed; in rat gastric juice (pH 2.2) no conversion was observed. Similar experiments on C1 and C2 showed that the toxins were converted to GTX2,3 (5.5% at pH 1.6, 1.5% at pH 2.2) after 4 hours. Chemical transformations have also been detected in shellfish, but not in warm blooded animals. No apparent change was observed in GTX2 and GTX3 and GTX2/3 and C1/2 when incubated with cat liver homogenate or liver enzyme preparations from rats/mice, respectively (EFSA, 2009). Study results have also reported STX and its analogues initially found in gastric contents to undergo metabolic transformation, following poisonings in humans. Glucuronides are often the metabolic end products (ANSES, 2020).

80. Given the high levels of STX group toxins detected in urine, urine is most likely the primary route of elimination. This is supported by animal data, where orally or intravenously administered STX group toxins were detected at 40% in the urine of dogs (after 2 hours; half life 90 minutes). Studies with cats and rats showed slower eliminations from the body, with estimated half lives of 17.8 hours (rats and cats) and 12.3 hours (rats). Faecal elimination is unlikely as STX was not detected in the bile of cats and no TSTX was detected in faeces of rats (EFSA, 2009). Other studies corroborate these results, showing that GTX2

and GTX3 are primarily excreted by glomerular filtration in animals and humans. Depending on the study, species and matrix, the half-life for STX varied from 1.5-10 hours (ANSES, 2020).

81. The available information suggested that STX group toxins bind to the voltage-gated sodium channels, resulting in a blockade of ion conductance through these channels, thereby acting on nerve and muscle fibres. The loss of membrane depolarisation and transmission of action potentials leads to a progressive loss of neuromuscular function resulting in the reported neurotoxic (paralytic) symptoms. While STX for example also binds to potassium and calcium channels, the effective dose was three to four orders of magnitude higher than those affecting sodium channels. The significant homologies among the channels for different cations, and the high number of STX analogues, could be the basis for the wide array of biological effects but the toxicological relevance of the interactions remains to be established.

82. The i.p. LD50 for STX is in the order of 10 µg/kg bw. The acute i.p toxicity of other analogues has also been measured in mice and can be found in Table 1 (EFSA, 2009). Following i.v. administration of 1-2 µg STX/kg bw caused decreased respiratory activity as well as weakening of muscle contractions, both direct and indirect stimulation, in the cat and rabbit. This dose also resulted in decreased action potential amplitude and longer latency time in the peripheral nervous system. Both motor and sensory nerves were affected. At doses of 4-5 µg STX/kg bw, a strong respiratory depression was observed resulting in death. Doses > 1 µg STX/kg bw in can provoke hypertension, with paralysis of muscles already reported at lower doses. However, this effect has not been reported in humans following intoxication (Mons et al., 1998). The oral LD50 in mice was 260-263 µg/kg bw, which is significantly lower than the i.p. LD50. While no firm conclusions could be drawn, a study by Andrinolo et al. (2002b) suggested that the GTX analogues may be more toxic to cats than STX via the oral route. A study in rats suggested that pre-exposure to sub-lethal levels of STX group toxins lowers the susceptibility to the toxins; the LD50 of pre-treated rats was 50% higher than that of untreated rats (McFarran et al., 1960). A study by Munday et al. (2013) found that the order of acute toxicity of all STX analogues was > i.p. > gavage > feed.

83. In 2009, EFSA proposed the toxic equivalent factors (TEFs), based on acute toxicity in mice (i.p. administration) as seen in Table 1.

Saxitoxin	TEF	Specific toxicity (MU/μM)
STX	1	2483
NeoSTX	1	2295
GTX1	1	2468
GTX2	0.4	892
GTX3	0.6	1584
GTX4	0.7	1803
GTX5 (B1)	0.1	160
GTX6 (B2)	0.1	No data
C1	No data	15
C2 (GTX8)	0.1	239
C3	No data	33
C4	0.1	144
dc-STX	1	1274
dc-NeoSTX (GTX7)	0.4	No data
dc GTX2	0.2	382

dc GTX3	0.4	935
11-hydroxy-STX	0.3	No data

84. Subchronic and chronic studies with STX group toxins also demonstrated neurotoxicity in the form of changes in total antioxidant capacity (TAC) and the production of reactive oxygen species (ROS) in the brain and liver. Furthermore, a decrease in glutamate cysteine ligase (GCL), aversive and special memory performance and an increase in GST and amino acid neurotransmitters (aspartate, glutamate, GABA) has been reported, as well as acute alterations of the production of dopamine and its metabolite. Given that neuronal excitability plays a major role in neuronal development, prolonged exposure may affect neurogenesis. A reduction in bodyweight and feed intake were also reported after repeat administration of 6 µg/kg neoSTX in rats (ANSES, 2020).

85. No reproductive or developmental studies were available for humans or animals. However, a study in zebra fish noted sublethal reversible morphological and sensory motor effects. Fish exposed during larval development additionally showed reduced growth and survival. However, concentrations in these studies were high (ANSES, 2020).

86. The limited data available did not demonstrate any significant changes in micronuclei (MN) frequency in binucleated cells in N2A and Vero cells, indicating no genotoxic effects of STX. In contrast, genotoxic effects, i.e. increased DNA damage in the COMET assay, were seen in fish neuronal cells at a concentration of 3 µg/L STX equivalent.

87. In 2004, the FAO/IOC/WHO determined a provisional LOAEL of 2 µg/kg bw STX equivalents and established a provisional ARfD of 0.7 µg STX equivalents/kg bw by applying an UF of 3, to account for the wide spectrum of people and reversibility of mild symptoms.

88. EFSA (2009) was unable to establish a chronic HBGV due to the lack of chronic data. However, in view of the acute toxicity displayed by STX group toxins, EFSA established an ARfD of 0.5 µg STX equivalents/kg bw, based on a LOAEL in the region of 1.5 µg STX equivalents/kg bw from human reports of intoxication (n=500). As many individuals did not show adverse effects at higher intakes, EFSA considered the LOAEL to be close to the threshold for sensitive individuals. Therefore, an UF of 3 was sufficient for the extrapolation from a

LOAEL to a NOAEL. No additional UF were deemed necessary as the data covered a large number of affected consumers, including sensitive individuals.

89. ANSES (2020) assessed STX within their work on the risks related to cyanobacteria and their toxins in drinking water, water for bathing and other recreational use. Due the uncertainties in the epidemiological studies estimating the exposure, ANSES used skeletal muscle disfunction generated by blocked sodium channels, observed in experimental studies as the critical effect. Despite the lack of a dose-response relationship in the selected key study (Munday et al., 2013) the NOAEL of 544 nM/kg (163 µg/kg) was considered as a starting point, due to the narrow dose interval between the observed NOAEL and the LD50 given in the study; the NOAEL was considered the threshold below which sodium channel blockage was not expected to be sufficient to induce observable muscular paralysis in animals. ANSES performed an allometric adjustment to reduce the uncertainty regarding interspecies variability and calculate a human equivalent dose (HED) dose resulting in a NOAELHED of 22 µg/kg bw. Furthermore, an overall UF of 250, 2.5 for interspecies variability, 10 for inter-individual variability and 10 for inadequacy in the data was applied to derive a TRV of ~ 0.1 µg/kg bw.

90. The Oregon Health Authority (OAH) considered the ARfD established by EFSA as a representative estimated NOAEL and applying a total UF of 10 for limitations in the database derived a TDI of 0.05 µg/kg bw per day. No UF for human interspecies variability or individual variability was applied, as the study selected covered the general population, as well as sensitive individuals (Farrer et al., 2015).

91. The toxin producing algae occurs worldwide, both in tropical and moderate climates. In Europe this includes the Atlantic coast and the North Sea (Norway, Portugal, France, Germany), the Mediterranean (Italy), but also Turkey and Egypt.

92. Processing (cooking, steaming) of shellfish, and subsequent water loss lead to the leaching of STX group toxins. In lobster hepatopancreas this resulted in a reduction of STX group toxins of about 40-65%, indicating that leaching occurred at a higher percentage than through water loss only. A study by Lawrence et al. (1994) suggested that some analogues were less absorbed in the hepatopancreas than others, and hence were reduced more (i.e. GTX toxins). In shellfish, STX group toxins are heat stable at temperatures of about 100°C, temperatures relevant to cooking and steaming. A reduction of toxins during commercial processing (115-120C) was suggested to be due to leaching out but

also partially to the interconversion of STX analogues. However, EFSA concluded that the data did not allow for a firm conclusion on possible interconversion or destruction during commercial processing.

93. The current legislative EU limit for STX group toxins is 800 µg STX equivalents/kg shellfish meat. In order for a 60 kg adult to avoid exceeding the ARfD, a 400 g portion of shellfish should not contain more than 30 µg STX equivalents or 75 µg STX equivalents/kg shellfish meat. This is significantly below the current EU limit.

94. A number of countries have set interim drinking water guidance levels for STX ranging from 1 µg/L (Australia, New Zealand) to 3 µg/L (Brazil, Australia). The World Health Organisation (WHO, 2022) also set a drinking water guidance level of 3 µg/L. Washington State (2011) has a recreational guidance value of 75 µg/L, the Californian Office of Environmental Health Hazard Assessment (OEHHA, 2021) recommended an interim notification level (NL) for STX of 0.6 µg/L, based on the EFSA (2009) ARfD. The OAH set a guidance level of 1 µg/L and 10 µg/L for drinking and recreational water, respectively.

Tetrodotoxins

95. Tetrodotoxin (TTX) and TTX analogues have been detected in marine bivalves and gastropods. TTX is hydrophilic and produced by bacteria, such as **Pseudoalteromonas, Pseudomonas, Vibrio, Aeromonas, Alteromonas, Shewanella, Roseobacter, Raoultella, Actinomycetes, Microbacterium, and Serratia** (EFSA, 2017; Katikou, 2019; Kotipoyina et al., 2023). TTX occurrence has also been linked to phytoplankton species such as **Alexandrium tamarense** (Kodama et al., 1996) and **Prorocentrum cordatum** (Rodríguez et al., 2017; Vlamis et al., 2015) but no direct link has been established so far (Antonelli et al., 2021).

96. TTX is acutely toxic in humans, with symptoms such as perioral numbness and paraesthesia, with or without GI symptoms to lingual numbness, early motor paralysis, incoordination, slurred speech with normal reflexes, to generalised flaccid paralysis, aphonia and fixed/dilated pupils to hypoxia, hypotension, bradycardia, cardiac dysrhythmias and unconsciousness and death. Death is caused by respiratory failure and cardiac collapse. Onset of symptoms occurs within 10-45 minutes of ingestion, although delayed responses of 3-6 hours have also been reported. There is no antidote to TTX poisoning (EFSA, 2017; Lago et al, 2015).

97. Limited information was available on the toxicokinetics of TTX and its analogues. The fast onset of symptoms in humans after ingestion indicates that TTXs are rapidly absorbed in the human digestive tract and TTX has been detected in blood (1-320 ng/mL plasma/serum) and urine (0.4-650 ng/mL) of patients several days after exposure. The concentrations in plasma/serum falls rapidly and may be undetectable after 6-24 hours. No information was available on the distribution or metabolism of TTXs. No studies were available on the toxicokinetics in animals, however, LD50 values are 25-50 times higher after i.p. than oral administration (EFSA, 2017). A study by Rambla-Alegre (2019) confirmed the presence of TTX in urine, after a puffer fish poisoning incident, but also 4-epiTTX, 4,9-anhydroTTX and 5,6,11-trideoxyTTX. Analysis of serum and plasma revealed the presence of TTX and 5,6,11-trideoxyTTX.

98. Hong et al. (2017) exposed rats to a single dose (6 µg/(16µCi/kg) of radiolabelled 11-[^{3}H]TTX resulting in maximum radioactivity in 10 minutes, with levels below detection in plasma after 24 hours. The main residence time was 1.62 hours and half-life of 2.31 hours. Bile secretion accounted for 0.43%, while urine was the main route of elimination (51%). The radioactivity in stomach, lung, kidney and intestine was higher than in plasma. The only identified metabolite was the oxidised form of TTX, and could be found in plasma, urine and faeces.

99. TTX blocks the extracellular channel pore of voltage-gated sodium channels, thus affecting both action potential generation and impulse conduction. Therefore, the neuron action potential is blocked resulting in muscle paralysis.

100. TTX is acutely toxic in experimental animals, with exposures resulting in skeletal muscle fasciculation, apathy, lethargy, ataxia, ascending progressive paralysis and death. Acute i.p. and s.c. LD50s in mice range from 8-13 µg/kg bw, LD50s following intragastric/oral administrations were much higher with values of 232 µg/kg bw and 532 µg/kg bw, the oral LD100 was 1000 µg/kg bw.

101. According to EFSA (2017) a minimum lethal dose of 2 mg (40 µg/kg bw, 50 kg Japanese adult) was mentioned in the literature. However, EFSA was unable to retrieve the underlying data/original source. While the doses at which TTX is acutely toxic remain unclear, case reports indicated acute poisoning from doses of 4- ≥ 42 µg/kg.

102. After i.p. administration TTX analogues exhibit similar effects as TTX, but no data were available to derive a NOAEL or LOAEL. EFSA however applied the LD50 and LD99 (i.p. administration) and MLD to derive relative potencies for a number of analogues, i.e. 0.75 for 11-oxoTTX, 0.17 for 11-norTTX-6(R)-ol, 0.14 for

11-deoxyTTX, 0.19 for 11-norTTX-6-(S)-ol, 0.16 for 4-epiTTX, 0.02 for 4,9-anhydroTTX and 0.01 for 5,6,11-deoxyTTX. Potencies for some analogues are supported by *in vitro* Neuro-2a bioassays, however, overall the derivation of potencies is associated with high uncertainties in the underlying method and data (EFSA, 2017; Alkassar et al., 2023).

103. Kasteel et al. (2017) used micro-electrode array (MEA) recordings as an integrated measure of neurotransmission to demonstrate that TTX inhibited neuronal electric activity in rat primary cortical cultures and human-induced pluripotent stem cell-derived iCell neurons. The authors concluded that interspecies differences are limited.

104. Boente-Juncal et al. (2019) exposed mice to TTX at concentrations ranging from 25 µg/kg, 75 µg/kg and 125 µg/kg via gavage, for 28 days. Animals dosed with 25 µg/kg survived the duration of the experiment and showed no behavioural alterations or toxicity. Mortality rates at 75 µg/kg and 125 µg/kg were 50% and 40%, respectively. The deaths occurred generally rapidly after dosing (1-2 minutes), but in one instance after seven days. No effects on food consumption or body weight gain were reported, however at 75 µg/kg and 125 µg/kg daily urine production was significantly decreased. No effects on blood glucose, cholesterol, ALT or AST were observed but increases in LDH and CK levels could be seen, although still within normal physiological ranges. Exposure at the highest concentration resulted in changes to the kidney and myocardium.

105. No genotoxicity was observed in a number of good laboratory (GLP) compliant *in vitro* and *in vivo* assays, following OECD guidelines by Guzman et al. (2007). A study by Lokesh et al. (2016) demonstrated spindle fibre aberration in the human lymphocyte chromosome aberration test following exposure of with crude extracts from skin/liver of the porcupine fish containing TTX at 0.5 mg/mL. However, no effect was observed with extracts from intestine, eyes and gonads, and no DNA damage was observed in the COMET assay with any of the abstracts. No structural alerts were seen for 4,9-anhydroTTX and 11-oxoTTX, following quantitative structure activity relationship (QSAR) analysis (EFSA, 2017).

106. No chronic HBGVs have been set either in the EU or other countries around the world. However, in 2017 EFSA derived a group ARfD of 0.25 µg/kg bw, based on apathy as the sensitive endpoint. Whereas a benchmark dose (BMD) could not be derived for apathy detected in mice after oral exposure to ≥ 125 µg TTX/kg bw, a BMDL10 of 112 µg/kg bw could be calculated for lethality (at 250 µg TTX/kg bw). This was slightly above the NOAEL for apathy of 75 µg/kg bw. The standard UF of 100 was applied. Limitations in the human data related to the

estimation of the ingested dose, did not allow for it to be used to derive a ARfD but it, together with the ARfD for saxitoxin (STX) was used as supportive information. TTX showed similar effects and potencies to STX, and both have a similar MOA. EFSA noted that the ARfD is 4.5-fold lower than the BMDL10 calculated for lethality and 16-fold lower than the lowest dose reported for sever effects in humans (4 µg/kg bw) and 2-fold lower than the ARfD for STX.

107. Kasteel et al. (2017) derived a human ARfD of 1.33 µg/kg bw. The authors thereby converted the mammalian LD50 of 400 µg/kg to a LOAEL (40 µg/kg) by applying a conversion factor of 10 and a NOAEL (13.33 µg/kg) for TTX in humans by applying an additional conversion factor of 3. A further factor of 10 was applied to take into account intra-species differences due to, e.g. poor renal clearance.

108. Finch et al. (2018) demonstrated that STX and TTX are additive and hence concluded it was appropriate to treat TTX as a member of the PSP toxin group. Since, the toxicity of TTX was found to be the same as STX by feeding, the authors established a toxic equivalent factor of 1.0 for TTX. The authors further derived a NOAEL of 1294 nmol/kg by feeding and applying the same considerations as EFSA, derived an ARfD of 10.1 nmol/kg (3.2 µg/kg). As the NOAELs in the study were the same for STX and TTX, the authors concluded that the ARfD for TTX could alternatively be set at the same level as that for STX, equating to 0.43 µg/kg for TTX.

109. According to a review by Katikou (2019), Dutch and Belgian experts concluded in 2017 that the information from an *in vitro* study published by Kasteel et al. (2017) after the EFSA evaluation, justified the reduction of uncertainty of 2.5, with a safe level of 25 x 44 = 110 µg TTX equivalent/kg shellfish meat. The Secretariat was unable to find the reference.

110. TTX and its analogues have been detected in gastropods and bivalves form European waters, such as France, Spain, Italy, Greece, the Netherlands Ireland and the UK (Northern Ireland, England, Scotland, Wales) at concentrations ranging from 0.0003-0.541 mg/kg (EFSA, 2017; Gerssen et al., 2018; Bacciocchi et al., 2019; Blanko et al., 2019; Bordin et al., 2021; Dhanji-Rapkova et al., 2020; Hort et al., 2020). TTX was the most dominant analogue found in all regions (Katikou, 2019).

111. The limited information available showed TTX was heat stable and did not decompose during cooking (Islam et al., 2011; Bane et al., 2014; Turner et al., 2015; FAO/WHO, 2016). Levels of STX and domoic acid, both hydrophilic and

similar to TTX, were reduced in shellfish meat during home cooking, such as boiling or steaming, due to partial leaching into the cooking liquid (EFSA, 2009; 2010).

112. There are no maximum levels for TTX in seafood in the EU. Based on the available data in EU shellfish, EFSA estimated that average and 95th percentile exposure levels did not exceed the group ARfD, exception for the consumption of a large portion of oysters. When combining the large portion size (400 g) with the ARfD, the highest estimated level in shellfish not expected to result in adverse effects would be 44 µg/kg raw shellfish meat. This level was exceeded in some samples reported to EFSA.

Novel azaspiracid analogues

113. In their 2014 review, CEFAS considered the potential presence of novel azaspiracids (AZAs) to contribute to the overall risk of emerging biotoxins. However, there are currently no certified standards and the development and validation of the current LC-MS/MS reference method would be required, together with formally assessed toxicity equivalence factors from oral toxicity studies.

114. AZA analogues can be formed through shellfish metabolism, but work is still ongoing as to their presence and levels in shellfish tissues, and occurrence around the world.

115. A paper by Krock et al. (2019) identified two novel AZAs from Pacific strains of **Azadinium poporum**, AZA-42 and AZA-62. Ozawa et al. (2021) recently reported a number of AZA analogues from several strains of **Azadinium poporum from** Japanese coastal waters, 13 of which were newly discovered analogues.

116. The Secretariat was unable to find any further information on potential emerging AZA analogues or any toxicological data or risk to human health.

Novel PSP analogues domoic acid analogues

117. In their 2014 review, CEFAS considered the potential presence of novel PSP analogues to contribute to the overall risk of emerging biotoxins. These included most notably the presence of PSP toxins from **Gymnodinium catenatum. G. catenatum** produces a range of hydrophilic and hydrophobic toxins, including gonyautoxin 6 (GTX6), for which currently no certified standard is available.

118. In 2018, Maeno et al. isolated six novel domoic acid (DA) related compounds from red algae (**Chondria armata),** i.e. 7’-methyl-isodomoic acid A and B, N-geranyl-L-glutamic acid, 7—hydrxymethu;-isodomoic acid A and B, N-geranyl-3(R)-hydroxy-L-glutamic acid. And determined their structures.

119. The Secretariat was unable to find any further information on potential emerging PSP/DA analogues or any toxicological data or risk to human health.

Cyanobacteria toxins

120. Cyanobacteria are photosynthetic procaryotes that occupy a wide range of niches, some can produce toxins as secondary metabolites. Cyanotoxins are produced within the cyanobacterial cell and are diverse in chemical structure and toxicity, but fall into three broad groups structurally, i.e. peptides, alkaloids and lipopolysaccharides. Cyanotoxins are also classified by their toxicity, see Table 2. In the aquatic environment, the release of toxins occurs mostly during cell death and lysis, however some species are capable of releasing toxins into the water without rupture/cell death (Testai et al., 2016).

Toxicity	**Cyanotoxin**
Hepatotoxicity	Microcystic (MC)
	Nodularin
Neurotoxicity	Anatoxin-a
	Homanotoxin
	Saxitoxin (STX)
	B-N-methylamino-L-alanine (BMAA)
Cytotoxicity	Cylindrospermopsin
Dermal toxicity	Lyngbyatoxin
Irritation	Lipopolysaccharides

121. The most investigated group of toxins are mycrocystins (MCs), produced by several cyanobacteria genera, i.e. **Mycrocystis, Anabaena, Nostoc, Oscillatoria**. Of the more than 250 MCs identified, only a few occur frequently and at high concentrations. The MC-LR congener is the most common and most studied and is also considered the most potent on the basis of its acute toxicity (Testai et al., 2016; WHO, 2020;2022).

122. Anatoxins (ATX) are produced by cyanobacteria of the genus **Anabaena, Dolichospermum, Aphanizomenon** and **Cuspidothrix**, many of which are primarily benthic. Cylindrospermopsins (CYN) and its four variants are predominantly produced by cyanobacteria of the genus **Raphidiopsis, Aphanizomenon and Chrysosporum.** Unlike MC, a major fraction of CYN is often dissolved in water.

123. Human exposure can occur via consumption of fish, crops or food supplements based on algae, but also contaminated drinking water. However, many different aquatic species may accumulate cyanotoxins via ingestion of cyanobacteria or contaminated water, e.g. fish, bivalves, snails. While CEFAS concluded in 2014 that there were no occurrences of cyanotoxins in UK waters, and occurrence of/exposure from cyanotoxins is predominantly from contaminated freshwater and freshwater species, a recent incident of cyanobacteria in Lough Neagh in Northern Ireland (NI) resulted in a policy ask to include cyanobacteria more broadly in this review. Policy colleagues noted that the incident itself was in a freshwater Lough, but water exiting the Lough was near marine shellfish beds along the coast. To date, work is still underway to determine levels in fish.

124. One limitation for toxicity studies of cyanotoxins, is the lack of available standards/purified toxins. Hence, extracts were often used, which are poorly characterised.

125. In humans, acute illness following consumption of drinking water contaminated by cyanobacteria is most commonly gastroenteritis (Percival and Williams, 2023)

Microcystins

126. The available toxicological data is predominantly on MCs, and one particular congener (MC-LR) but they are usually from single dose studies where the toxin is administered via i.p. injection. The available data on MCs indicated an up to 30-fold difference in acute toxicity, following i.p administration compared to

the oral route (Testai et al., 2016).

127. MCs are actively transported into the cells by specific organic anion transport proteins (OATPs) and due to the high number of OATPs in the liver MCs are primarily hepatotoxic. However, distribution to other organs and tissues, which have OATPs also occurs. Once in the cell, MCs bind to certain protein phosphatases involved in a range of regulatory pathways, e.g. those responsible for cytoskeletal structures, cell replication, stress response and DNA repair (Testai et al., 2016; WHO, 2020;2022).

128. Acute effects of MC poisonings include intrahepatic haemorrhage.

129. While the liver has been identified as the target organ, with effects such as e.g. enlargement, inflammation, necrosis of hepatocyte, recent studies have suggested that MCs may also be toxic to reproductive organs and other organs, e.g. the thyroid, after prolonged exposure to lower concentrations (Testai et al., 2016; WHO, 2020;2022). Several sub-chronic and chronic effects have been described in the literature following oral exposure in animal studies, including lung effects (thickening of the alveolar septum, disruption of cell junctions, alveolar collapse and lung apoptosis), serum profile changes (increase in transaminases, decrease in total proteins), effects on the nervous system (cognitive impairment, lesions, oxidative injury, inflammation in brain regions) and reproductive and developmental effects (decreased sperm number and motility, abnormal sperm morphology, lesions in the testes, testicular atrophy, change in serum hormone concentrations, impact on the ovaries) (OEHHA, 2021).

130. Chronic effects of MCs include reduced efficiency of proliferation control mechanisms within tumour cells. The International Agency for Research on Cancer (IARC, 2010) classified MC-LR as a possible carcinogen to humans (Group 2B) due to its reported tumour promotion in rodents.

131. In humans MC exposure has resulted in fatalities. However, these have occurred after mistreated water was used in renal dialysis (WHO, 2020).

Anatoxins

132. ATX has led to rapid animal deaths, following ingestion of cyanobacterial cells in beached scum material or benthic mats. In contrast, high concentrations have not been reported in drinking water, most likely due to ATX being rapidly diluted after release.

133. The (+) enantiomer of ATX-a binds to the nicotinic acetylcholine receptor of nerve cells leading to chronic overstimulation resulting in increased heart rate and blood pressure, fatigue and eventual muscle paralysis and potential death. Limited data suggest that homoanatoxin-a and the dihydro derivates bind to the same receptor and may have a similar potency as ATX-a (WHO, 2020).

Cylindrospermopsin

134. CYN is a potent inhibitor of protein synthesis and also has cytochrome P450 dependant effects on other processes, e.g. DNA damage and induction of cellular stress responses. Various studies demonstrated the effect of CYN on different organs, with the kidney being the most sensitive (WHO, 2020).

β-methylamino-L-alanine

135. BMAA is a neurotoxin leading to motor neuron dysfunction and death. To date three isomers have been described, 2,4-diaminobutyric acid (DAB), N-(2-aminoethly)glycine and β-amino-N-methylalanine (BAMA). While Metcalf et al. (2008) found BMAA to present among other cyanotoxins in samples from various waterbodies, Krueger et al. (2010) reported no BMAA in cyanobacterial samples, but the presence of DAB. BMAA however was present in seeds of *Cycas revoluta*. BMAA has also been reported in other groups of microalgae (Lopicic et al., 2022).

136. BMAA has been implicated in neurodegenerative diseases such as aAmyotropic lateral sclerosis (ALS)/Parkinsonism-dementia complex, ALS and Alzheimer (Testai et al., 2016; ANSES, 2017, Lopicic et al., 2022). However, the mechanisms underlying BMAAs effects, the pharmacokinetic parameters regulating BMAA uptake into the brain, its permeability across the blood brain barrier (BBB) and metabolic pathways involved are not yet clearly understood. Hence, BMAAs potential role in some of these diseases is still being debated with some of the criticism stemming from exposures in animals (monkeys in the case of ALS-PDC) corresponding to an unrealistic consumption in humans (Testai et al., 2016).

137. Oral bioavailability, distribution and brain uptake has been demonstrated after i.v and oral administration in rats and primates, with BMAA suggested to be transported into the brain by the large neutral amino acid carrier (L-system) of the BBB. More recent studies demonstrated that BMAA could be mis-incorporated into proteins and then released and reincorporated into newly synthesised proteins. The level of protein-associated BMAA was higher in the liver of neonates than in the brain regions (10-fold), potentially due to the higher protein synthesis

in the liver (Testai et al., 2016; ANSES, 2017). Administration of a single oral dose of radiolabelled BMAA resulted in radioactivity in the liver, adipose tissue, muscles and skin, with less than 0.01% of the dose found in the brain. It would appear that after single or repeated administration, 50-60% of the radioactivity is present in the tissues in protein bound form. Protein incorporation of BMAA may thus result in misfolding and therefore an accumulation of protein in the lysosome. Associated with an input of calcium leads to a stress reaction in the endoplasmic reticulum, the deregulation of the reduction/oxidation system and activation of pro-apoptotic caspases and ultimately cell death. In addition, an accumulation of neuromelanin modified by BMAA could disrupt the function of dopaminergic neurons, leading to early neural aging common to many neurogenerative pathologies (ANSES, 2017).

138. The secretion of BMMA into milk of lactating mice and subsequent transfer into the developing brain has also been demonstrated in rats, highlighting lactation as an important elimination pathway on the one hand and efficient exposure route on the other hand. Excretion via urine has suggested to be low. Given that the influx of BMMA into the brain has been demonstrated to be saturable and low sodium dependent *in vitro* (mouse mammalian epithelial cells) suggested that the amino acid transporters LAT1 and LAT2 were involved in BMMAs transport (Testai, et al., 2016; ANSES, 2017).

139. Limited data are available on acute and repeat toxicity and concentrations tested were (often) very high and administration done via non-oral routes. ANSES (2017) further noted that the administration period did not reflect the reality of consumer exposure.

140. Neurotoxicity of BMAA has been demonstrated in rats and monkeys. While BMMA was considered to have low neurotoxic potency in adult rodents, long term cognitive impairments and histopathological changes in the brain have been reported in adult brains after exposure during developmental stages. Metabolic profiling of serum in neonatal rats showed significant alterations of the intermediate metabolites, i.e. d-glucose, lactate, 3-hydroxybutyrate, acetate, creatine, all of which are associated with changes in energy and amino acid metabolism (Testai et al., 2016).

141. The neurotoxicity of BMAA has also been shown *in vitro* in cells from rodents and leeches and in human cells lines. The observed dose-dependent cell death has been proposed to result from the activation of excitatory amino acids (glutamate). It has been further suggested that the interaction of BMAA with bicarbonate produces molecules “resembling” glutamate which in turn act as

glutamate receptor antagonists, in particular ionotropic receptors such as N-methyl-D-aspartic acid (NMDA). BMAA has also been suggested to be capable of acting at low concentrations (e.g. 10 µM) as co-actor of a neurodegenerative phenomenon involving other molecules. As this neurotoxicity involves ionotropic (NMDA) and metabotropic (mGluR5) glutamate receptors, BMAA may be excitotoxic via different molecular targets (Lobner et al., 2007; ANSES, 2017, Lopicic et al., 2022). BMMA further plays a role in phosphorylation of the tubulin-associated unit (Tau) protein and may cause an overexpression of the TAR DNA binding protein 43 (TDP-43), as well as the formation of aggregates of this protein.

142. BMMA has also been shown to disturb undifferentiated cells (B1 and C cells) at 100 µM and promotes their proliferation, affects the organisation of neuroblasts, indicating that the subventricular zone (SVZ) function could be impaired. BMAA further affected neuroinflammatory processes by increasing the release of proinflammatory cytokines (IL-1β, IL-6, TNFα) and targeted the central nervous system homeostasis via glial cells (Meresse et al., 2022).

143. While no data on chronic exposures were available, the report by ANSES (2017) noted that BMAA's interaction with neuromelanin without incorporation therein, could lead to BMAA being stored for years with the possibility of leaching throughout a life time, potentially resulting in chronic damage to the brain, including permanent inflammation and a reactive gliosis contributing to the development of neurodegenerative pathologies (Krone et al., 2016; ANSES et al., 2017). A recent study by Soto et al. (2023) exposed cultured amacrine and photoreceptor rat neurons (PHRs) and Mueller glial cells (MGCs) to BMAA at concentrations of 0.4 µM (3 days) and 0.4,1 and 10 µM (3 and 9 days), respectively. Results indicated that BMAA promotes cell death and induces subcellular changes in neurons and MGCs, affecting viability in neurons but not in MGCs.

144. Limited data on genotoxicity were available.

145. A study by Novak et al. (2016) showed no significant rise in the number of revertants in the Ames test at concentrations ranging from 11-900 µg of BMAA per plate. A second test on **Salmonella typhimuriom** (TA1535/pSK1002) with concentrations between 0.32-100 µg of BMAA/mL also produced negative results, with and without metabolic activation.

HBGVs

146. In 2016, EFSA derived a provisional reference value for subchronic exposure of 0.4 µg/kg bw per day for MCs and provisional TDI of 0.04 µg/kg bw per day, based on a NAOEL from a subchronic toxicity study with MC-LR. An acute NOAEL of 25 µg/kg bw was also derived, as this was the highest dose at which no hepatic effects were observed from an i.p. injection study in mice.

147. In 2019, ANSES derived a subchronic toxicity reference value (TRV) of 1 ng/kg bw per day for MCs based on the effects shown on the male reproductive system, including decreased sperm motility and count and increased sperm abnormalities in the study by Chen et al. (2011). The value was based on a NOAEL of 0.15 µg/kg bw per day and a total UF of 25 (2.5 and 10; the text did not specify for what). Based on the available studies, ANSES was unable to establish an acute TRV.

148. The WHO (1998; 2006; 2022) proposed a provisional TDI of 0.04 µg/kg bw for MC, based on a NOAEL of 40 µg/kg bw per day for liver pathology observed in a 13-week study in mice. A total UF of 1000 was applied, 10 each for inter- and intraspecies variability and an additional UF of 10 for deficiencies in the database.

149. The database for other cyanotoxins on repeated, long-term oral exposures was limited and often not sufficient to derive a TDI. A tentative HBGV has been proposed by the WHO (2022) for ATX, based on the no effect level from a 28-day mouse study, but the levels of uncertainty were even higher than for MC. The WHO (2022) also derived a TDI of 0.03 µg/kg bw for CYN, based on a NOAEL of 30 µg/kg bw per day for renal pathology in an 11-week study in mice. The WHO also applied a total UF of 1000, 10 each for inter- and intraspecies variability and an additional 10 for limitations in the database.

150. ANSES derived a subchronic TRV of 0.14 µg/kg bw per day for CYN based on increased kidney and liver weight. The LOAEL was adjusted for the HED resulting in a value of 10 µg/kg bw per day and a total UF of 75 was applied (an UF of 2.5, 10 and 3; it was not specified for what these were applied). The data on ATX-a were too limited to characterise a hazard to humans. However, ATX-a generally causes rapid paralysis of muscles and the respiratory system. While ANSES considered the neurotoxicity of BMAA to have been demonstrated in rats and monkeys, the toxicological data did not allow the identification of a no effect dose applicable to humans.

151. The Oregon Health Authority (OHA) developed guideline values (TDIs) for the most common cyanotoxins, i.e. ATX-a, CYN, MCs, STX in fresh water (Farrer et al. 2015). For ATX-a a TDI of 0.1 µg/kg per day was derived, based on a 28 day

study in mice. While the cause of death was not entirely clear, the death at 500 µg/kg per day was considered as a LOAEL, while a NOAEL of 100 µg/kg per day was selected as POD. A total uncertainty factor of 1000 was applied, the default UF of 100 for inter- and intraspecies variability and an additional UF of 10 for limitation sin the database. For CYN the EPAs subchronic oral reference dose (RfD) of 0.03 µg/kg per day was applied. The RfD was based on an 11 week study in mice, which reported kidney weight as sensitive endpoint, with a LOAEL of 60 µg/kg per day and a NOAEL of 30 µg/kg per day. A total UF of 1000 was applied, the default factor of 100 for inter- and intraspecies variability and an additional UF of 10 for limitations in the database. For MCs OAH derived a TDI of 0.05 µg/kg per day for MC-LR, the only MC for which sufficient data were available. The POD was a LOAEL of 50 µg/kg per day for intrahepatic haemorrhage in mice exposed to MC-LR for 28 days. A total UF of 1000 was applied, the default UF of 100 for inter- and intraspecies variability and an extra UF of 10 for the extrapolation from a LAOEL to a NOAEL (Farrer et al., 2015).

Occurrence and regulation

152. Cyanobacteria, especially MC have been reported in European countries, with the frequency of occurrence increasing in recent years (ANSES, 2019). Liver and viscera of fish have been reported to have higher levels than muscle tissue, but no relationship could be found due to variability in results and species. The available data also did not indicate a direct relationship between fish species and feeding strategy and concentration measured in tissue (Testai et al., 2016). However, a recent study by Falfushynska et al. (2023) reported higher levels in planktivorous compared to carnivorous fish. The WHO (2022) noted that MCs concentrations in fish muscle are generally 100 µg/kg fresh weight. Vareli et al. (2012) reported levels ranging from 45-142 µg MC-LR/kg fresh weight in saltwater mussels from Greece.

153. Numerous data on cyanotoxins other than MCs come from laboratory studies, only half of the information available however comes from occurrence data in field studies. Anatoxins (ATX) and CYN have only been detected in fish, none in Europe. Nodularins (NOD) have been detected in shellfish, with reported levels ranging from 80 µg/kg dw in soft tissue of mussels (Finland; Sipia et al., 2008) to 817 µg/kg dw in mussels (Poland; Marzur-Marzec et al., 2013). β-N-methylamino-L-alanine (BMMA) was detected at a level of up to 900 µg/kg in soft tissue of oysters from several European countries, i.e. France, Sweden, Greece. Some reports from mussels and oyster in France reported levels of 3,300 -14,000 µg/kg BMMA and 1,100 -9,700 µg/kg 2 DAB (mussels), 1,300 – 10,000 µg/kg

BMMA and 1,300- 8,800 DAB (oysters) and 1,400-1,700 µg/kg AEG (Testai et al., 2016; ANSES, 2019; Amzil et al., 2023).

154. The five year monitoring programme by IFREMER (Amzil, 2023) detected MC-RR and dmMC-RR produced by freshwater cyanobacteria in marine bivalve molluscs (mussels, oysters), particularly during winter on the English Channel and Atlantic coast. MCs were quantified in caged mussels/oysters placed in several sites along the freshwater/estuary/seawater continuum. The environmental origin of MC in marine bivalves was not known, due to the absence of any blooms. However, the report suggested that the MC could originate from planktonic cyanobacteria sinking in the sediment or from benthic MC-producing cyanobacteria. The report also found that marine mussels accumulate more dmMC-RR than MC-RR, with the proportion of dmMC-RR significantly increasing (3-50%) with increasing salinity.

155. Cooking (boiling, microwave) may alter MC concentrations, but the data is inconsistent, with some studies showing various degrees of increase, while others show no effect or even a decrease in concentration.

156. Only a limited number of countries have set guidelines for acceptable levels of cyanotoxins in fish and shellfish. California and Washington have health advisories in place for MCs but also for ATX-a and CYN. The OEHHA (2021) recommended interim notification levels (NLs) of 0.03 µg/L and 0.3 µg/L for MCs and CYN, respectively. Australia (Victoria State) has defined acceptable levels of cyanotoxins, i.e. MCs (51 µg/kg), NOD (51 µg/kg) and cylindrospermopsin and deoxyCYN (39 µg/kg), in seafood harvested from lakes. The WHO (1998; 2006) proposed a provisional upper limit for drinking water of 1 µg/L. However, the scientific literature suggests that cyanotoxins can be found at higher concentrations in food than the provisional limits set for water. The OAH established guideline values of 3.0 µg/L (ATX-a), 1.0 µg/L (CYN) and 1.0 µg/L (MCs) for drinking water, as well as additional values for recreational water and dog specific (Farrer et al., 2015).

Summary

157. The Food Standards Agency (FSA) is considering the current advice and monitoring programme for marine biotoxins and whether there is a need to update or change existing legislative standards or include monitoring of additional emerging biotoxins. As part of this work the COT have been asked whether any of the identified potential emerging marine biotoxins, i.e

brevetoxins, cyclic imines, palytoxins, saxitoxins, tetrodotoxins, novel azaspiracid analogues, novel PSP analogues and domoic acid analogues, or freshwater cyanobacteria toxins, would pose a toxicological risk to UK consumers. The selection of potential emerging biotoxins was based on reports from the literature and other authorities.

158. For many of the discussed biotoxins limited information or data was available on the toxicology or occurrence in EU, or more specifically UK waters.

159. While human poisonings were reported for BTX, STX, TTX and PITX, no human poisoning have been reported to date for CIs. However, for PITX the majority of the information on human intoxication is anecdotal and the effect could not always be linked directly to PITX.

160. No HBGVs have been set for BTX and CIs, but other authorities, including EFSA, derived ARfDs for PITX (sum of PITX and ostreocin-D), STX and TTX (group ARfD). No chronic HBGVs were set with the exception of a TDI derived by OAH for STX.

161. BTX, CIs, PITX, STX and TTX have been detected either directly in shellfish from EU countries or the toxin producing organism has been reported in EU waters. TTX has also been reported from shellfish in the UK.

162. No toxicological data or information on risk to humans were available for novel AZA and PSP/DA analogues.

163. While cyanobacteria toxins are usually found in freshwater, cyanotoxins have also been reported in coastal marine shellfish and MCs in particular have been detected in fish with increasing frequency in EU countries in recent years. In addition, a recent cyanobacteria bloom incident during the summer of 2023 in Lough Neagh in Northern Ireland led to the inclusion here, water exiting the Lough was near marine shellfish beds along the coast and there are concerns regarding uptake of toxins by commercial fish species in the Lough and potential implications for coastal shellfish.

164. The available toxicological data was predominantly on MCs, with gastroenteritis being the main symptom of human intoxication. BMAA on the other hand is neurotoxic. Subchronic reference values for MCs were derived for MCs by EFSA and ANSES, and provisional TDIs by EFSA and the WHO. For other cyanotoxins the database was not sufficient to derive HBGVs per se. The WHO derived a tentative HBGV for ATX and a TDI for CYNs, while ANSES derived a subchronic TRV for CYN. OAH derived a TDI for the most common cyanotoxins, i.e.

ATX-a, CYN, MCs and STX.

165. MCs, BMAA/DAB have been reported in shellfish from EU countries, while ATX and CYN have only been detected to date in fish in non-EU countries.

Questions to the Committee

i. Given the available toxicological data and occurrence data, do any of the emerging marine biotoxins (brevetoxins, cyclic imines, palytoxins, saxitoxins, tetrodotoxins, novel azaspiracid analogues, novel PSP analogues and domoic acid analogues, cyanobacteria toxins) discussed in this scoping paper raise concerns for UK consumers?

a. If any of these emerging marine biotoxins are of concern, are there any particular risks the Committee would like to highlight?

ii. Are there any data gaps the Committee thinks are pertinent to be filled?

iii. Are Members aware of any other emerging marine biotoxins not discussed in this scoping paper?

iv. Does the Committee have any comments?

Secretariat

December 2023

List of Abbreviations

m/n AChR	muscarinic/nicotinic acetylcholine receptors
ALT	Aminotransferase
ARfD	Acute reference dose
ASP	Amnesic shellfish poisoning

AST	Aspartate aminotransferase
ATX	Anatoxin
AZA	Azaspiracid gorup
BMAA	β-N-methylamino-L-alanine
BMD	Benchmark dose
BTX	Brevetoxin group
CI	Cyclic imine group
CPK	Creatine phosphokinase
CTX	Ciguatoxin
CYN	Cylindrospermopsins
DA	Domoic acid group
DAB	2,4, diaminobutyric acid
DSP	Diarrhetic shellfish poisoning
EC	Effect concentration
ERK	Extracellular signal-regulated kinases
GCL	Glutamate cysteine ligase

GI tract	Gastrointestinal tract
GTX	Gonyautoxins
GYM	Gymnodimine
HBGV	Health based guidance value
i.c.	Intra-cerebroventricular
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
JNK	c-Jun-NH2-terminal protein kinase
LD	Lethal dose
LDH	Lactate dehydrogenase
LOAEL	Lowest observed adverse effect level
M	Mol
MBA	Mouse bioassay
MC	Microcystins
MDL	Minimum lethal dose

MEA	Micro-electrode array
MN	Micronuclei
MOA	Mode of action
MOE	Margin of exposure
MU	Mouse units
NL	Notification level
NOAEL	No observed adverse effect level
NOD	Nodularins
NSP	Neurological/neurotoxic shellfish poisoning
OA	Okadaic acid group
OATPs	Organic anion transport proteins
OVTX	Ovatoxin
PITX	Palytoxin group
PnTX	Pinnatoxin
POD	Point of departure
PtTX	Pteriatoxins

PSP	Paralytic shellfish poisoning
PTX	Pectenotoxin group
QSAR	Quantitative structure activity relationship
ROS	Reactive oxygen species
RP	Reference point
s.c.	Subcutanious
SPX	Spirolides
STX	Saxitoxin group
TAC	Total antioxidant capacity
TRV	Toxicity reference valyue
TTX	Tetrodotoxin group
UF	Uncertainty factor
YTX	Yessotoxin group

AFBI	UK Agri-Food and Biosciences Institute

ANSES	French Agency for Food, Environmental and Occupational Health and Safety
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CRLBM	EU Community Reference Laboratory for marine biotoxins
EFSA	European Food Safety Authority
EU	European Union
EURL	EU Regulatory Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
FSA	UK Food Standards Agency
FSAI	Food Standards Agency Ireland
FSANZ	Food Standards Australia New Zealand
GB	Great Britain
GLP	Good laboratory practise
IARC	International Agency for Research on Cancer

IOC	Intergovernmental Oceanographic Commission
IFREMER	French Research Institute for Exploitation of the Sea
NI	Northern Ireland
NRL	National Reference Laboratory
OAH	Oregon Health Authority
OC	Official controls
OECD	Organisation for Economic Co-operation and Development
OEHHA	Californian Office of Environmental Health Hazard Assessment
OL	Official laboratory
UK	United Kingdom
US	United States
WFSR	Marine Biotoxins is Wageningen Food Safety Research
WHO	World Health Organisation

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