Presence of Tetrodotoxin in shellfish

Joint Advice of the Aquaculture Advisory Council and the Market Advisory Council

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Follow up and comments received	

Background

Tetrodotoxin (TTX) is a potent neurotoxin that is found in the organs of a variety of marine species and some terrestrial species [1]; it is responsible for the highest fatality rate of all marine intoxications [2]. Over 20 species of pufferfish, or fugu, have been found to harbour the toxin, especially in the liver, ovaries and skin. Besides pufferfish, other species known to harbour TTX include the following: gastropods, newts, crabs, frogs, sea slugs, starfishes, blue-ringed octopuses, ribbon worms and bacteria. The distribution of TTX and its analogues (there are 26 naturally occurring analogues of TTX) are known to be organism and/or tissue specific [1].

It has been demonstrated that TTX and its analogues in marine species are produced by a wide range of host-associated bacterial species, such as Vibrio, Bacillus, Aeromonas, Shewanella, Alteromonas and Pseudomonas, which naturally inhabit the animals' gut [1, 3]. A correlation between the TTX occurrence in shellfish, and the prevalence of Prorocentrum minutum in seawaters has been noted [4]; TTX-like compounds have also been found in P. minutum cultures [5].

TTX is a sodium channel blocker. It binds to the sodium channels of the excitable tissues of the victim (muscles and nerves) and immobilises them [1]. In humans, the onset and severity of the symptoms of TTX poisoning after ingestion is dose dependent. Initial symptoms include tingling (paraesthesias) of the tongue and lips, followed by or concurrent with headache and vomiting, which may progress to muscle weakness and ataxia [1]. In severe cases, death may occur due to respiratory and/or heart failure. The only treatment for TTX intoxication is observation and appropriate supportive care. Plus, TTX is both water soluble and heat stable, so cooking does not negate its toxicity [1].

Usually common in tropical warm waters (Japan, Taiwan, Bangladesh and Southeast Asia) [1], TTX has recently been found in pufferfish [6, 7, 8] and gastropods [7, 9, 10] collected from European countries. Detection of TTX in European bivalve molluscs was first reported in the United Kingdom in 2014 for shellfish in England harvested in 2013 and 2014 at a maximum level of 137 µg TTX equivalent/kg of shellfish meat [11], then in Greece in 2015 for samples obtained in 2012 at a maximum level of 223 µg TTX eq/kg [4]. Moreover, a survey carried out in the Netherlands in 2015 revealed that TTX can be found in mussels and oysters from Dutch production areas [12]. Several researchers have formulated the theory of 'Lessepsian migration' (influx of red sea biota into the Mediterranean Sea by way of the Suez Canal) for explaining the new occurrence of TTX in European regions. Ballast water can also cause the transfer of TTX-containing organisms from Asian to European waters [1].

The study conducted in Greece by Vlamis and al. [4] on the presence of TTX in European bivalve molluscs raises the question of a link between TTX in temperate waters and climate change, with the warming of the oceans. This study suggests a possible link between the presence of TTX in the temperate zone and the presence of algal blooms of Prorocentrum minimum, microalgae that produce TTX. It also showed that TTX were already present in Greek shellfish since 2006, in concentrations ranging between 61.0 and 197.7 µg TTX eq/kg. To our knowledge, this is the earliest reported detection of TTXs in European bivalve shellfish [4].

Live bivalve molluscs are biofilters and bioaccumulators. Thus, they can accumulate toxins present in waters and transmit them to consumers.

In contemporary reports, the only European case of TTX intoxication occurred in 2007 in a man who purchased an edible trumpet shell of the species Charonia lampas lampas from a market in southern Spain [13]. He ingested some of the white meat of the shellfish and reached the dark digestive gland. The patient suffered general paralysis, including the respiratory muscles, a few minutes after the consumption, without any other symptoms or consequences [13]. However, it is important to specify that the mollusc bought and consumed is not a species usually fished or raised in European countries.

Context of this advice

In 2015, the Institute for Food Safety in the Netherlands (RIKILT) carried out a survey into TTX in live bivalve molluscs from Dutch production areas [12]. It revealed that TTX can be found in mussels and oysters from Dutch production areas. In July and August 2015, values in the range of 13.7–124.1 µg TTX equivalent/kg of shellfish meat were detected [12]. The Netherlands Food and Consumer Product Safety Authority (NVWA) considers it likely that, even in low concentrations, TTX may constitute a risk when live bivalve molluscs containing the toxin are consumed. The advisory research (NVWA/BuRO/2016/79) of the Office for Risk Assessment (BuRO) has confirmed this and advised the NVWA to act from a precautionary standpoint and ensure that live bivalve molluscs containing TTX are not put on the market. The advice does not propose a maximum permitted limit, because one cannot be identified based on the current research. Thus, BuRO assumes that any presence of TTX in live bivalve molluscs may pose health risks if they are consumed [14].

On April 18, 2016, the Netherlands issued a national draft regulation concerning the TTX in live bivalve molluscs [14]. This project stated that the NVWA needed to adopt the following measures in that year [14]:

- Setting up monitoring of Dutch shellfish production areas for TTX;
- Preventing placing on the market of live bivalve molluscs containing a concentration equal to or greater than 20 µg TTX eq/kg; this concentration corresponds to the limit of detection of the TTX by the liquid chromatography–mass spectrometry (LC-MS/MS) method (Dutch method) [15].

In their motion of May 3, 2016, to members of the ‘Live Bivalve Molluscs’ Working Group, the Netherlands wish to extend the measures taken by the NVWA to the European Member States. These measures are overly stringent, and they are not based on practical experience of illness. If this becomes the EU rule, Dutch trading companies can ask the producers from states exporting to the Netherlands to certify the absence of TTX in their oysters and mussels prior to any purchase. This certificate must be provided by a recognised laboratory according to the LC-MS method. However, this analysis has not been validated in Europe.

Toxicological data on TTX are limited, making it difficult to identify the risks and propose a regulatory threshold. Further research on TTX is required to take evidence-based effective restrictive measures; this must be done primarily for the health of the consumers, but another important issue is addressing the economic consequences of this ‘unfounded epidemic’.

With the support of the NVWA, on June 15, 2016, the European Commission’s DG HEALTH asked the European Food Safety Authority (EFSA) to prepare a scientific opinion on the following:

- The presence, accumulation and toxicity for humans of TTX and its analogues found in bivalve molluscs and marine gastropods in and outside European waters, including, where possible, the establishment of a toxicity threshold [16]; and
- The most effective method that could be used to detect and quantify this group of toxins, including parameters like a limit of detection and limit of quantification [16].

The EFSA accepted this request on July 26, 2016, and it provided a scientific opinion entitled 'Risks for public health related to the presence of tetrodotoxin (TTX) and TTX analogues in marine bivalves and gastropods' on April 20, 2017 [17].

The United Kingdom, Greece and the Netherlands submitted 1677 samples of bivalves collected between 2006 and 2016 to the EFSA, including mussels, oysters, cockles, clams, scallops and razor clams. In 92% of the samples, using the LC-MS method, TTX was not detected or quantified, with limits of detection in the range of 0.5–5 µg TTX equivalent/kg of shellfish meat and limits of quantification in the range of 1–25 µg TTX equivalent/kg of shellfish meat. TTX analogues were detected in about 5% of the samples [17, Part 3.3].

Based on a large portion size of shellfish meat (400 g), an adult body weight of 70 kg and a group acute reference dose (ARfD) of 0.25 µg TTX equivalent/kg of body weight, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that a concentration lower than 44 µg of TTX and/or the equivalent toxic amount of its analogues per kilogramme of shellfish meat is not expected to lead to adverse effects in humans [17].

After the EFSA published its opinion, a Dutch in vitro study (from 2017) [18] gave further information on the regularly used uncertainty factors (UFs). The regular procedure for defining an ARfD would be to select the No Observed Adverse Effect Level (NOAEL) for the most critical endpoint in an acute animal study and divide that by an uncertainty factor of 10 for interspecies differences (between the experimental animals and human) and by another uncertainty factor of 10 for intraspecies differences (between humans). At an expert meeting on May 2, 2017 [19], with Dutch and Belgian experts, it was concluded that the information of this in vitro study results in a reduction of uncertainty of 2.5, with a safe level of 2.5 * 44 = 110 µg TTX equivalent/kg shellfish meat.

The EFSA CONTAM Panel concluded that chemical-analytical methods, especially LC-MS/MS), are the most suitable detection methods because they allow identification and quantification of TTX and its analogues. Limits of quantification vary between 0.1 and 25 µg TTX equivalent/kg of shellfish meat. Although they had been validated in laboratories, the EFSA CONTAM Panel suggested that LC-MS/MS methods required further validation in interlaboratory trials [17]. At present, the validation of the method by the national reference laboratories is ongoing [20, 21].

The EFSA CONTAM Panel recommended obtaining more occurrence data on TTX and its analogues in edible parts of marine bivalves and gastropods from different EU waters to provide a more reliable exposure assessment. In addition, certified standards and reference materials for TTX and its analogues are needed to improve the quality of the occurrence data [17].

Since 2017, the Netherlands has used 44 µg TTX equivalent/kg of shellfish meat as a standard for measures to close production areas. They follow the standard from the EFSA advice instead of the level of 20 µg TTX equivalent/kg of shellfish meat that was initially chosen [22]. During summer 2017, there was no TTX crisis in Holland. The Netherlands currently continue to follow their national

regulation concerning TTX, but it applies only to the Dutch products rather than all European products [21].

In 2018, to meet the ESFA recommendation, Leao and al. [2] investigated the possible presence of TTX in different bivalve molluscs from the Galician Rias, in the Atlantic on the west coast of Spain, in which the production of bivalves, and especially, mussels, is the most representative in the EU [2]. They also studied the determination of the non-ribosomal peptide synthetase (NRPS) gene, after isolation of the major *Vibrio spp*., because this gene is involved in the biosynthesis of toxins [23, 24]. The presence of the NRPS gene has been observed in samples were TTX was detected; nevertheless, this presence was also observed in other *Vibrio* species isolated from samples where TTX was not detected. Therefore, the association of this gene with the production of TTX is not clear and needs further investigation. Only two samples (out of 1279) from infaunal areas (cockle and oyster) showed a TTX toxicity response; their TTX concentration levels were significantly lower than those recommended by the EFSA; and no TTX analogues were found in these samples, nor in any other samples. It should be noted that the samples were collected in areas where the environmental conditions are similar to those described by Turner and al. [25] for the majority of TTX-positive shellfish found in the United Kingdom. Therefore, the data on TTX occurrence obtained in this study do not allow the conclusion that TTX represents a risk for public health in Galicia; nevertheless, additional data are still required, and more studies are being performed to obtain additional occurrence data [2].

Aquaculture Advisory Council (AAC) and Market Advisory Council (MAC) can put forward the following standpoint on this issue:

1. Reaffirm that the health of consumers of their products remains a major priority;
2. Underline the threat to intra-Community trade and the image of shellfish in general in connection with an unfounded ‘epidemic’;
3. Underline the need for more *evidence-based* information on TTX toxicity (toxicokinetics, oral toxicity, chronic effects, combination with STX) to reduce the uncertainty factors in a responsible way;
4. Stress the need for more occurrence data on TTX from different EU waters to provide a more reliable exposure assessment, as well as the need for studies on the source and accumulation of TTX;
5. Welcome the ongoing definition of a validated European reference method between the Member States with regard to the introduction of this toxin in the monitoring system; and
6. Request the European Commission to conduct a risk assessment when sufficient evidence-based data on TTX toxicity, source and accumulation are available.

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