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| **Joint advice (AAC & MAC) 2017/002 of [date of adoption] concerning the presence of Tetrodotoxin (TTX) in shellfish** | |
| **Legal basis**  **Procedure** | Art. 44 of regulation (UE) 1380/2013  2.b (initiative to EC) |
| **References / documents**  **CELEX main document**  **Other ID** | [European Commission, 2016. Dutch Policy Guideline on tetrodotoxin in live bivalve molluscs](http://ec.europa.eu/growth/tools-databases/tris/en/search/?trisaction=search.detail&year=2016&num=175)  2016/175/NL |
| **Working Group** responsible  **Rapporteur**  **Internal legal basis**  **Internal ID** | Shellfish Working Group  Bruno Guillaumie  Art. 3 of AAC statutes and 6 of MAC statutes  2017/002 |
| **WG Meetings / Written consultation dates**   * SWG meeting, 14/03/2017 * SWG meeting, 13/06/2017 * SWG written consultation, 10/07/2017 * SWG meeting, 10/10/2017 * SWG meeting, 28/02/2018 * SWG meeting, 16/05/2018 * MAC WG3, 24/05/2018 * MAC and AAC WG written consultation, June 2018 | Version / revision  Second advice of EMPA v6  Third advice of EMPA v7  Joint advice of AAC-MAC v1rev1  Joint advice of AAC-MAC v1rev4  TTX state of play  Joint advice of AAC-MAC v2rev1  Joint advice of AAC-MAC v2rev2  Joint advice of AAC-MAC v2rev2 |
| **EXCOM meetings**   * AAC 06/07/2017 * AAC 13/09/2018 * MAC 18-19/10/2018 | Version / revision / final release  State of play and provisional calendar  Possible adoption?  Possible adoption? |
| **Notifications**  EC – DG MARE  EC – DG SANTE  The Netherlands | [date]  [date]  [date] |
| **Follow up and comment received** | |

**Background**

The TTX is a potent neurotoxin that is found in organs of a variety of marine species and also in some terrestrial species [1] and is responsible for the highest fatality rate of all marine intoxications [2]. Over 20 species of puffer fish or *fugu* have been found to harbor the toxin especially in the liver, ovaries and skin. Besides puffer fish, other species known to harbor TTX include: gastropods, newts, crabs, frogs, sea slugs, star fishes, 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 bacteria species, such as *Vibrio*, *Bacillus*, *Aeromonas*, *Shewanella*, *Alteromonas* and *Pseudomonas*, which naturally inhabit the gut of the animals [1, 3]. A correlation between TTX occurrence in shellfish and the prevalence of *Prorocentrum minutum* in seawaters has been noted [4] and TTX-like compounds were also found in *P.* *minutum* cultures [5].

This toxin is a sodium channel blocker. It binds to the sodium channels of the excitable tissues of the victim (muscles and nerves) and immobilizes these tissues [1]. In humans, the onset and severity of the symptoms of TTX poisoning after ingestion is dose dependent. Initial symptoms include tingling (paresthesias) 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 was recently found in puffer fish [6, 7, 8] and gastropods [7, 9, 10] collected from European countries. Detection of TTX in European bivalve molluscs has been firstly reported by the United Kingdom in 2014 for shellfish in England harvested in 2013 and 2014 at a maximum level of 137 µg TTX eq/kg [11], then by Greece in 2015 for samples obtained in 2012 at a maximum level of 223 TTX µg 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]. A number of 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 waters 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 the presence of TTX in temperate waters and climate change with warming of the oceans. This study suggests a possible link between the presence of TTX in temperate zone and the presence of algal bloom of *Prorocentrum minimum*, microalgae that produce TTX. It also shows that TTX were already present in Greek shellfish since 2006, in concentrations ranging between 61,0 and 197,7 TTX µg eq/kg. To our knowledge, this is the earliest reported detection of TTXs in European bivalve shellfish [4].

Live bivalve molluscs are bio-filters and bio-accumulators. So they are able to accumulate toxins present in waters and transmit them to consumers.

These days, the first and unique European case of TTX intoxication happened in 2007 on 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 symptom and consequence [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 RIKILT (Institute for Food Safety in the Netherlands) 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 ranging from 13,7-124,1 µg TTX equivalent/kg shellfish meat were detected [12]. The Netherlands Food and Consumer Product Safety Authority (NVWA) considers it likely that TTX, even in low concentrations, may constitute a risk when live bivalve molluscs containing TTX are consumed. The advisory research (NVWA/BuRO /2016/79) of the Office for Risk Assessment (BuRO) has confirmed this and has also adviced the NVWA to act from a precautionary principle and to 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. BuRO therefore assumes that any presence of TTX in live bivalve molluscs may pose health risks if they are consumed [14].

So, the Netherlands have issued on April 18, 2016 a national draft regulation concerning the Tetrodotoxin (TTX) in live bivalve molluscs [14]. This project states that the NVWA has to adopt in force in 2016 [14]:

* To set up monitoring of Dutch shellfish production areas for TTX ;
* Against the placing on the market of live bivalve molluscs containing a concentration equal to or greater than 20 µg TTX equivalent/kg shellfish meat ; this concentration is corresponding to the limit of detection of the TTX by the method "LC - MS" (Dutch method) [15].

In their motion of May 3, 2016, to members of the Working Group “Live bivalve molluscs”, the Netherlands wish to extend to the European Member States the measures taken by the NVWA. These measures are overly stringent but not based on practical experience on illness. If this will become EU rules, Dutch trading companies can ask the producers of the States exporting to the Netherlands to certify the absence of TTX in their oysters and their mussels prior to any purchase. This certificate must be provided by a recognized laboratory according to the method "LC - MS" whereas the analysis is not validated in Europe.

Toxicological data on TTX is limited, making it difficult to identify the risks and to propose regulatory threshold. Further research on TTX is required to take evidence based effective restrictive measures, of course for the health of the consumers but also to the economic consequences of these “unfounded epidemic”.

With the support of NVWA, DG HEALTH of the European Commission asked on June 15, 2016 the European food safety authority (EFSA) to prepare a scientific opinion on:

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

EFSA accepted this request on July 26, 2016 and provide 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].

1,677 samples of bivalves collected between 2006 and 2016 were submitted to EFSA by The United Kingdom, Greece and the Netherlands, including mussels, oysters, cockles, clams, scallops and razor clams. In 92 % of the samples, by using the LC-MS method, TTX was not detected or quantified, with limits of detection ranging from 0,5 to 5 µg TTX equivalent/kg shellfish meat and limits of quantification ranging from 1 to 25 μg/kg. TTX analogues were detected in about 5 % of the samples [17, Part 3.3].

Based on a large portion size of shellfish meat (400g), an adult body weight of 70 kg and a group ARfD (Acute Reference Dose) of 0,25 μg TTX equivalent/kg 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 its equivalent toxic amount of its analogues per kg 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] gives further information on the regular used Uncertainty Factors (UF). The regular procedure to define an ARfD would be to select the NOAEL (No Observed Adverse Effect Level) 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 the 2nd, 2017 [19] with Dutch and Belgium experts was concluded that the information of this in vitro study results in a reduction of uncertainty of 2,5, resulting in safe level of 2,5 \* 44 = 110 µg TTX equivalent/kg shellfish meat.

The EFSA CONTAM Panel concluded that chemical-analytical methods, in particular LC-MS/MS (liquid chromatography with tandem mass spectroscopy), 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 shellfish meat. Although validated within laboratories, EFSA CONTAM Panel suggests that LC-MS/MS methods require further validation in interlaboratory trials [17]. At the present time, the validation of the method by the national reference laboratories [20] is in course [21].

The EFSA CONTAM Panel recommends to obtain more occurrence data on TTX and its analogues in edible parts of marine bivalves and gastropods from different EU waters in order 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 used the 44 µg TTX equivalent/kg shellfish meat as a standard for measures to close production areas. They follow the standard from the advice of EFSA, instead of the level of 20 µg TTX equivalent/kg shellfish meat firstly chosen [22]. During 2017 summer, there was no TTX crisis in Holland. The Netherlands currently continue to follow their national regulation concerning the TTX but it applies only to the Dutch products and not to all European products [21].

In 2018, in order to accomplish the ESFA recommendation, Leao and al. [2] investigated the possible presence of TTX in different bivalves molluscs from the Galician Rias, in the Atlantic west coast of Spain, in which the production of bivalves, and, in particular, mussels, is the most representative in the EU [2]. They also studied the determination of 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 the samples were TTX was detected; nevertheless, this presence was also observed in other *Vibrios* isolated from samples where TTX was not detected. Therefore, the association of this gene to the production of TTX is not clear and needs further investigation. Only two samples (on 1279 samples) from infaunal areas (cockle and oyster) showed TTX toxicity response, their concentration levels of TTX were significantly lower than those recommended by 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 UK. 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 in course to obtain more occurrence data [2].

**AAC and MAC opinion:**

1. AAC and MAC reaffirm that the health of consumers of its products remains a major priority ;
2. AAC and MAC underline the threat to intra-Community trade and to the image of shellfish in general in connection with an unfounded "epidemic" ;
3. AAC and MAC underline the need for more - evidence based - information on TTX toxicity (toxicokinetics, oral toxicity, chronic effects, combination with STX) to reduce the uncertainty factors on a responsible way ;
4. AAC and MAC stress the need for more occurrence data on TTX from different EU waters to provide a more reliable exposure assessment and the need for studies to the source and accumulation ;
5. AAC and MAC welcome the ongoing definition of a validated European reference method between the Member States with regard to the introduction;
6. AAC and MAC request to conduct a risk assessment when sufficient – evidence based - data on TTX toxicity, source and accumulation are available.

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