

Marine biotoxins in shellfish – Yessotoxin group¹
Scientific Opinion of the Panel on Contaminants in the Food chain
(Question No EFSA-Q-2006-065D)

Adopted on 2 December 2008

SUMMARY

Yessotoxin group toxins (YTXs) have been detected in filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams from various parts of the world. They are primarily produced by the marine dinoflagellate *Protoceratium reticulatum*. YTXs are polyether compounds, consisting of 11 contiguously transfused ether rings, an unsaturated side chain, and two sulphate esters. More than 90 YTXs are known, but only a few dozens have been fully identified. The most important YTXs are, 1a-homoYTX, 45-hydroxyYTX, and 45-hydroxy-1a-homoYTX. YTXs seem to be heat stable in shellfish at temperatures relevant for cooking.

The toxicological database for YTX-group toxins is limited and comprises mostly studies on their acute toxicity in mice. There are no reports on adverse effects in humans associated with YTXs. The following toxic equivalence factors (TEF) have been applied in some countries: YTX = 1, 1a-homoYTX = 1, 45-hydroxyYTX = 1 and 45-hydroxy-1a-homoYTX = 0.5. Because the available data (lethality of very few mice following intraperitoneal (*i.p.*) injection are not sufficient to establish robust TEF values, the Panel on Contaminants in the Food Chain (CONTAM Panel) used these TEF values as an interim measure in order to provide a best estimate of the toxicity of YTX-group toxins.

No data on the chronic effects of YTXs in animals were available, so the CONTAM Panel could not establish a tolerable daily intake (TDI). In view of the acute toxicity of YTX-group toxins, the CONTAM Panel decided to establish an acute reference dose (ARfD) based on the available animal data on acute toxicity, due to the lack of observations in humans.

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Regarding the acute toxicity the mechanism of action of YTXs has not been determined with certainty, and the molecular processes underlying their toxicity are presently undetermined. The CONTAM Panel noted that four major molecular processes have been implicated in the mechanism of action of YTXs, comprising the modulation of calcium movements among different cellular compartments, the modulation of cellular adenosine 3',5'-cyclic monophosphate (cAMP) levels, the alteration of protein disposal, and apoptosis.

In a series of acute toxicity studies following oral administration no lethality and no clinical signs of toxicity were observed. This indicates that YTXs are far less toxic when given by the oral route than following *i.p.* administration for which LD₅₀ values in the range of 100 to 500 µg/kg body weight (b.w.) were observed.

Following oral administration cardiotoxicity was observed by the use of light microscopy down to a single dose of 7.5 mg/kg b.w. with a no effect level of 5 mg/kg b.w. The CONTAM Panel noted that ultrastructural changes in the myocardium have inconsistently been reported below this dose level. It also noted that these changes may be reversible, and that they were not accompanied by leakage of enzymes to serum. There were no indications of myocardial damage as identified by light microscopy. Therefore, in its derivation of an ARfD the CONTAM Panel decided to use the dose of 5 mg/kg b.w. *p.o.* as the most robust no-observed-adverse-effect level (NOAEL) for acute cardiotoxicity caused by YTXs as identified by light microscopy. However, because it is uncertain whether the ultrastructural changes should be considered as adverse or not, the CONTAM Panel decided to apply a factor of 2 in addition to the default uncertainty factor of 100 to establish an ARfD of 25 µg YTX equivalents/kg b.w.

In order to protect against the acute effects of YTX-group toxins, it is important to use a high portion size rather than a long-term average consumption in the health risk assessment of shellfish consumption. Consumption data for shellfish species across the European Union (EU) were limited, therefore the European Food Safety Authority (EFSA) requested the Member States to provide information on consumption of relevant shellfish species. Based on data provided by five Member States, the CONTAM Panel identified 400 g of shellfish meat as a large portion size to be used in the acute risk assessment of marine biotoxins.

It was noted that consumption of a 400 g portion of shellfish meat containing YTX-group toxins at the current EU limit of 1 mg YTX equivalents/kg shellfish meat would result in an intake of 400 µg YTXs (equivalent to 6.7 µg/kg b.w. in a 60 kg adult). This intake is below the ARfD of 25 µg YTX equivalents/kg b.w. (corresponding to 1500 µg YTX equivalents per portion for a 60 kg adult) and consequently does not pose any health risk.

For the deterministic and probabilistic estimate of the exposure to YTXs the CONTAM Panel could only use the occurrence data from Norway and Italy (North Adriatic Sea). Consumption of a 400 g portion of shellfish meat containing YTXs at 315 or 799 µg YTX equivalents/kg shellfish meat, corresponding to the 95th percentile of the concentration in Norway and Italy (North Adriatic Sea) respectively, would result in an intake of 126 or 320 µg YTXs (corresponding to

2.1 or 5.3 µg YTX equivalents/kg b.w. for a 60 kg adult). For both countries this estimated high intake is well below the ARfD of 25 µg YTX equivalents/kg b.w., indicating that there is no acute health risk with respect to consumption of shellfish containing the current levels of YTXs found on the market.

The liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) results show that none of the samples from the Norwegian and Italian data set that tested negative in the mouse bioassay (MBA), exceeded a value of 3.75 mg YTX equivalents/kg shellfish meat. Therefore the CONTAM Panel concluded that a 60 kg person, consuming a portion of 400 g of shellfish currently present on the market, would not exceed an ARfD of 25 µg/kg b.w.

The CONTAM Panel noted that, even taking into consideration all reported YTX occurrence data, thus both the MBA negative and MBA positive results, and thereby disregarding the current EU regulatory system, consumers of shellfish in Norway would not exceed the ARfD when consuming a 400 g portion of shellfish meat. In Italy (North Adriatic Sea), the ARfD would be exceeded under these circumstances by 2.9% of the consumers.

The CONTAM Panel concluded that in order for a 60 kg adult to avoid exceeding a dose of 1500 µg YTX equivalents, corresponding to the ARfD of 25 µg YTX equivalents/kg b.w., a 400 g portion of shellfish should not contain more than 3.75 mg YTX eq./kg shellfish meat. This level is above the current EU limit value of YTXs of 1 mg/kg shellfish flesh.

The MBA is the officially prescribed reference method in the EU for the detection of YTX-group toxins. The CONTAM Panel noted that the method has shortcomings e.g. it is not specific, not quantitative and has a high uncertainty at the level of the current regulatory limit.

The current EU legislation permits the replacement of the MBA, provided that the alternative methods have been validated according to an internationally recognised protocol. At this time however, none of the methods for the determination of toxins from the YTX-group have been validated by interlaboratory studies. The evidence available at this moment suggests that LC-MS/MS based methods have the greatest potential to replace the MBA. These methods also have the possibility for multi-toxin group detection/quantification. The CONTAM Panel noted that, whilst application of single laboratory validation according to recognised international guidelines to demonstrate their fitness-for-purpose can be an impetus for implementation of alternative instrumental analyses of marine biotoxins for regulatory purposes, method performance criteria should be stipulated where possible and validation by interlaboratory trials should be the long-term objective.

Keywords: Marine biotoxins, yessotoxin (YTX), 45-hydroxyYTX, 1a-homoYTX, 45-hydroxy-1a-homoYTX, shellfish, bivalve molluscs, mouse bioassay (MBA), acute reference dose (ARfD), portion size, methods of analysis, human health, risk assessment