

Marine biotoxins in shellfish – Saxitoxin group

Scientific opinion of the Panel on Contaminants in the Food Chain

(Question No EFSA-Q-2006-065E)

Adopted on 25 March 2009

SUMMARY

Saxitoxin (STX)-group toxins are a group of closely related tetrahydropurines and have been detected in filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams from various parts of the world. They are mainly produced by dinoflagellates belonging to the genus *Alexandrium*: e.g. *Alexandrium tamarensis*, *A. minutum* (syn. *A. excavata*), *A. catenella*, *A. fraterculus*, *A. fundyense* and *A. cohorticula*. STX-group toxins cause paralytic shellfish poisoning (PSP) in humans, characterised by symptoms varying from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis. In fatal cases respiratory arrest occurs 2 to 12 hours following consumption of shellfish contaminated with STX-group toxins. More than 30 different STX analogues have been identified of which STX, NeoSTX, GTX1 and dc-STX seem to be the most toxic ones.

The toxicological database for STX-group toxins is limited and comprises mostly studies on their acute toxicity following intraperitoneal administration. For monitoring purposes using high performance liquid chromatography (HPLC) techniques toxicity equivalency factors (TEFs) have been applied to express the detected analogues as STX equivalents. Until better information is available the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) proposes the following TEFs based on acute *i.p.* toxicity in mice: STX = 1, NeoSTX = 1, GTX1 = 1, GTX2 = 0.4, GTX3 = 0.6, GTX4 = 0.7, GTX5 = 0.1, GTX6 = 0.1, C2 = 0.1, C4 = 0.1, dc-STX = 1, dc-NeoSTX = 0.4, dc GTX2 = 0.2, GTX3 = 0.4, and 11-hydroxy-STX = 0.3.

Based on available information it can be concluded that the binding of STX-group toxins to voltage-gated sodium channels and the consequent blockade of ion conductance through these channels is the major molecular mechanism of action of this group of toxins on nerves and muscles fibres.

No data on the chronic effects of STX-group toxins in animals or humans were available, so the CONTAM Panel could not establish a tolerable daily intake (TDI). In view of the acute toxicity of STX-group toxins, the CONTAM Panel decided to establish an acute reference dose (ARfD). From the available reports on intoxications in humans, comprising more than 500 individuals, a lowest-observed-adverse-effect-level (LOAEL) in the region of 1.5 µg STX equivalents/kg b.w. could be established. Because many individuals did not suffer adverse reactions at higher intakes it is expected that this LOAEL is close to the threshold for effects in sensitive individuals. Therefore the CONTAM Panel concluded that a factor of 3 was sufficient to move from this LOAEL to an estimated no-observed-adverse-effect level

(NOAEL) of 0.5 µg STX equivalents/kg b.w. No additional factor for variation among humans was deemed necessary because the data covered a large number of affected consumers, including sensitive individuals. Thus the CONTAM Panel established an acute reference dose (ARfD) of 0.5 µg STX equivalents/kg b.w.

In order to protect against the acute effects of STX-group toxins, it is important to use a large 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.

The CONTAM Panel noted that consumption of a 400 g portion of shellfish meat containing STX-group toxins at the current EU limit of 800 µg STX equivalents/kg¹ shellfish meat would result in an intake of 320 µg toxin (equivalent to 5.3 µg/kg b.w. in a 60 kg adult). This intake is considerably higher than the ARfD of 0.5 µg STX equivalents /kg b.w. (equivalent to 30 µg STX equivalents per portion for a 60 kg adult) and is a concern for health.

In order for a 60 kg adult to avoid exceeding the ARfD of 0.5 µg STX equivalents/kg b.w., a 400 g portion of shellfish should not contain more than 30 µg STX equivalents corresponding to 75 µg STX equivalents/kg shellfish meat.

Given the considerable differences in toxin profiles, different number of analogues determined and diverse limits of quantification of analytical methods applied in different European Countries, and the high number of non-quantifiable samples, the CONTAM Panel concluded that there were too many uncertainties for a reliable and representative estimation of dietary exposure to STX-group toxins for EU countries. In addition, the difference in acidic conditions used during the extraction step of the various methods could lead to differences in conversion of STX analogues with low toxicity (low TEF) into STX analogues with high toxicity (high TEF). Therefore the CONTAM Panel could not comment on the risks associated with consumption of shellfish that currently reach the market.

Water loss during household processing (cooking, steaming) of shellfish leads to leaching-out of STX-group toxins from the flesh into the cooking fluid. A reduction in the concentrations of STX-group toxins of about 40-65 % was observed for lobster hepatopancreas, indicating that more STX-group toxins are leached out during processing than would be expected due to water loss only. It was suggested that the levels of some analogues were more reduced than others due to their lesser adsorption in hepatopancreas matrix components. STX-group toxins are heat stable in shellfish at temperatures relevant for cooking and steaming (about 100°C). Commercial processing such as autoclaving at higher temperatures (115-120°C) may lead to a reduction in the concentration of STX-group toxins in shellfish flesh up to 90 %. This was partly attributed to leaching-out of STX-group toxins, partly to destruction at these high temperatures or to interconversion of STX analogues. The CONTAM Panel concluded, however, that the available information made it difficult to draw firm conclusions on possible interconversion or destruction occurring during commercial processing.

The mouse bioassay (MBA) and the Association of Official Analytical Chemists (AOAC) HPLC method (so-called Lawrence method) are officially prescribed methods in the EU for the detection of STX-group toxins. Both methods have been interlaboratory-validated

¹ In the Commission Regulation (EC) No 853/2004 a limit value for paralytic shellfish poison (PSP) of 800 micrograms per kilogram is given. In this opinion the CONTAM Panel adopted this figure as being expressed as µg STX equivalents/kg shellfish meat.

according to international protocols. They are capable to detect STX-group toxins at the current EU regulatory levels of 800µg STX equivalents/kg shellfish meat. The MBA has a limit of detection of approximately 370 µg STX equivalents/kg shellfish meat. The limit of quantification of the Lawrence method depends on toxin profiles, which may differ in practice. For individual toxins limits of quantification range from 10-80 µg STX equivalents for the different STX-analogues. Stringent reductions of the regulatory limit for STX-group toxins would make it necessary to modify the Lawrence method, so as to reduce its limits of quantification, subsequently followed by re-validation of the revised method, to establish new performance characteristics. In the MBA the extraction of STX-group toxins from shellfish meat is carried out by boiling with hydrochloric acid, whereas in the Lawrence method it is boiling with acetic acid. The CONTAM Panel noted that this difference in extraction conditions may lead to differences in toxin profiles detected and to different results when the analytical data are expressed in STX equivalents/kg. Other methods that have potential to determine STX-group toxins are receptor-based assays, antibody-based methods and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The biomolecular methods are merely suitable for screening purposes. LC-MS/MS has potential for confirmatory analyses. Neither of these methods has been formally validated yet in interlaboratory studies, following internationally recognised protocols, so their performance characteristics cannot be evaluated and compared with the official methods.

Key words: Marine biotoxins, saxitoxin (STX)-group toxins, shellfish, bivalve molluscs, mouse bioassay (MBA), acute reference dose, portion size, methods of analysis, human health, risk assessment.