Marine biotoxins in shellfish – Azaspiracid group[1]

Scientific Opinion of the Panel on Contaminants in the Food chain

(Question N° EFSA-Q-2006-065B)

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PANEL MEMBERS

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SUMMARY

Azaspiracids (AZAs) are a group of shellfish toxins causing AZA poisoning (AZP) which is characterized by symptoms such as nausea, vomiting, diarrhoea and stomach cramps. Approximately 20 different analogues have been identified, of which AZA1, AZA2 and AZA3 are the most important ones based on occurrence and toxicity. AZAs can be found in various species of filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams. Monitoring of AZAs in shellfish in Ireland has shown that mussels are the most affected species for this group of toxins. Only recently has the dinoflagellate that produces the AZA toxins been isolated. AZAs are nitrogen-containing polyether toxins comprising a unique spiral ring assembly, a heterocyclic amine (piperidine) and an aliphatic carboxylic acid moiety. AZAs in shellfish are not decomposed at temperatures relevant for cooking.

The toxicological database for AZAs is limited and comprises mostly studies on their acute toxicity. The following toxic equivalence factors (TEF) have been applied in some countries: AZA1 = 1, AZA2 = 1.8 and AZA3 = 1.4. Because the available data (lethality of very few mice

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following intraperitoneal (*i.p.*) administration) are not sufficient to establish robust TEF values, the Panel on contaminants in the food chain (CONTAM Panel) adopted these TEF values as an interim measure in order to provide a best estimate of the toxicity of AZAs.

Only a few limited repeated-dose toxicity studies of longer duration (maximum duration 1 year) were available for AZA1. Pathological changes were observed in multiple organs; lungs, stomach, small intestine and liver. Occasionally lung tumours were observed. Because these tumours were only observed at doses causing severe toxicity, the CONTAM Panel considered this observation of limited relevance. No data on genotoxicity have been reported for AZAs.

The data on the chronic effects of AZAs in animals or humans were insufficient for a tolerable daily intake (TDI) to be established. In view of the acute toxicity of AZAs, the CONTAM Panel decided to establish an acute reference dose (ARfD) based on the available human data.

There were only data available from one incident of human poisoning involving AZAs that could be used for the derivation of an ARfD. The CONTAM Panel concluded that the most probable estimate of a lowest-observed-adverse-effect-level (LOAEL) resulting in AZA poisoning was 113 µg AZA1 equivalents per person (1.9 µg AZA1 equivalents/kg body weight (b.w.) for a 60 kg adult). Uncertainty factors were required to extrapolate from the LOAEL to a no-observed-adverse-effect level (NOAEL), and for variability within the human population. Because the effects considered were mild and reversible a factor of three was applied for the extrapolation of the LOAEL to NOAEL. The CONTAM Panel decided that the usual factor of 10 for human variability was not required because the reported incident was expected to have occurred in sensitive, rather than average, individuals. However, an additional factor of three was applied because the available data related to a small number of individuals from a single incident. Consequently the Panel established an ARfD of 0.2 µg AZA1 equivalents/kg b.w.

In order to protect against the acute effects of AZAs, 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 the high 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 AZAs at the current EU limit of 160 µg AZA1 equivalents/kg shellfish meat would result in a dietary exposure of 64 µg AZA1 equivalents For a 60 kg adult this is approximately 1 µg AZA1 equivalents/kg b.w. This figure is 5-fold higher than the ARfD established by the CONTAM Panel. Therefore, it cannot be excluded that this intake could exert effects in susceptible consumers. Based on the consumption and occurrence data, there is an approximately 4% chance for 60 kg adults of exceeding the ARfD of 0.2 µg AZA1 equivalents/kg b.w. when consuming

shellfish currently available on the European market. The CONTAM Panel concluded that in order for a 60 kg adult to not exceed the ARfD, a 400 g portion of shellfish should not contain more than 12 µg AZA1 equivalents, i.e. 30 µg AZA1 equivalents/kg shellfish meat.

The mouse and the rat bioassay are the officially prescribed reference methods in the EU for the detection of AZAs. The CONTAM Panel noted that both methods have shortcomings e.g. they are not specific and not quantitative, and that method performance characteristics for AZAs have not been established for the mammalian assays. Based on limited data on acute *i.p.* toxicity in mice, it is not clear whether the mouse bioassay (MBA) can detect levels at the current EU regulatory level of 160 µg AZA1 equivalents/kg shellfish meat.

The current EU legislation permits the replacement of the bioassays, provided that the alternative methods have been validated according to an internationally recognised protocol. At this point however, none of the methods for the determination of toxins from the AZA group have been validated by interlaboratory studies. The evidence available at this moment suggests that liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) based methods have the greatest potential to replace the mammalian assays. Moreover, they are able to detect AZAs at concentrations well below the current regulatory limit of 160 µg AZA1 equivalents/kg shellfish meat. The LC-MS/MS based methods also have the possibility for multi-toxin group detection/quantification. The CONTAM Panel noted that, while 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, azaspiracids, AZA1, AZA2, AZA3, AZA4, AZA5, shellfish, bivalve molluscs, mammalian bioassays, acute reference dose, portion size, methods of analysis, human health, risk assessment.