

**Marine biotoxins in shellfish – okadaic acid and analogues<sup>1</sup>**  
**Scientific Opinion of the Panel on Contaminants in the Food chain**

(Question N<sup>o</sup> EFSA-Q-2006-065A)

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**SCIENTIFIC PANEL MEMBERS**

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**SUMMARY**

Okadaic acid (OA) and its analogues, the dinophysis toxins (DTX1, DTX2, and DTX3), together form the group of OA-toxins. These toxins are lipophilic and heat stable, are produced by dinoflagellates and can be found in various species of shellfish, mainly in filter-feeding bivalve molluscs such as oysters, mussels, scallops, and clams. While OA and DTX2 only differ by the position of one methyl group in the molecule, DTX1 has one additional methyl group and DTX3 represents a wide range of derivatives of OA, DTX1 and DTX2 esterified with saturated and unsaturated fatty acids.

OA-group toxins cause Diarrhoeic Shellfish Poisoning (DSP), which is characterized by symptoms such as diarrhoea, nausea, vomiting and abdominal pain. These symptoms may

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occur in humans shortly after consumption of contaminated bivalve molluscs such as mussels, scallops, oysters or clams. Inhibition of serine/threonine phosphoprotein phosphatases is assumed to constitute the mode of action of OA-group toxins.

The toxicological database for OA-group toxins is limited and comprises mostly studies on their acute toxicity. Based on LD<sub>50</sub> experiments following intraperitoneal injection in mice, the Panel established the following toxic equivalence factors (TEFs): OA = 1, DTX1 = 1, DTX2 = 0.6. For DTX3 the TEF values are equal to those of the corresponding unesterified toxins (OA, DTX1, and DTX2).

Pectenotoxins frequently co-occur with OA-group toxins and are currently included in the regulatory limit for OA group toxins but they do not share the same mechanism of action as OA-group toxins. Therefore their toxicity should not be expressed as OA-equivalents and they should not be included in the regulatory limit for the group of OA toxins.

No long-term toxicity/carcinogenicity experiments have been reported for OA-group toxins, but OA is identified as a tumour promoter in rodents. OA has shown some evidence for genotoxicity in non-standard *in vitro* assays. This includes some evidence for unspecific DNA-adduct formation in mammalian cell lines. However, the data are difficult to interpret, and the Panel noted that these effects may be related to the cytotoxicity of OA in these assays. For DTX2 and DTX3 no genotoxicity data are available. The Panel concluded that OA appears to be not mutagenic *per se*, but induces changes at the chromosome level and is aneugenic *in vitro*. The Panel noted that these effects may be related to cytotoxicity of OA.

The data on the chronic effects of OA in animals or humans were insufficient for a tolerable daily intake (TDI) to be established. In view of the acute toxicity of OA-group toxins, the Panel decided to establish an acute reference dose (ARfD) based on the available human data. Taking into account the uncertainties in the estimated exposure in the various human case reports, the Panel concluded that a lowest-observed-adverse-effect-level (LOAEL) for human illness is in the region of 50 µg OA equivalents/person, this approximates to 0.8 µg OA equivalents/kg bodyweight (b.w.) for adults. An uncertainty factor of three was applied to extrapolate this LOAEL to a no-observed-adverse-effect-level (NOAEL) which resulted in an ARfD of 0.3 µg OA equivalents/kg b.w. The Panel considered it not necessary to apply an additional uncertainty factor for the variation among humans as the data are based on observations in a rather large number of affected shellfish consumers, originating from various countries, and considered to comprise the most sensitive individuals.

In order to protect against the acute effects of OA-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 EU, were limited, therefore EFSA requested the Member States to provide information on consumption of

relevant shellfish species. Based on data provided by five Member States, the 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 a 400 g portion of shellfish meat containing OA-group toxins at the current EU limit of 160 µg OA equivalents/kg shellfish meat would result in a dietary exposure of 64 µg toxin. For a 60 kg adult this is equivalent to approximately 1 µg/kg b.w. This figure exceeds the ARfD by approximately 3-fold and is in the region of the LOAEL as derived from the human case studies. Therefore, this intake would be expected to exert effects in susceptible consumers. Based on the consumption and occurrence data, there is an approximately 20% chance of exceeding the ARfD of 0.3 µg OA equivalents/kg b.w. when consuming shellfish currently available on the European market. Thus DSP occurs under the current legislation and the prescribed reference methods for control. The 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 18 µg toxin, i.e. 45 µg OA equivalents/kg shellfish meat.

The mouse and the rat bioassay are the officially prescribed reference methods in the EU for the detection of OA-group toxins. The Panel concluded that both methods have shortcomings that make them inappropriate for assessing the current EU limit. The mammalian assays have limited capability to detect OA-group toxins at the current EU regulatory level of 160 µg OA equivalents/kg shellfish meat, and are not capable of detecting OA-group toxins below this level. In addition, the MBA are not able to detect DTX3.

The current EU legislation permits the replacement of the bioassays, provided that the alternative methods have been validated according to an internationally recognised protocol. The phosphoprotein-phosphatase assays and liquid chromatograph-mass spectrometry (LC-MS) based methods have the greatest potential to replace the mammalian assays, and to detect levels of OA-group toxins below the current EU regulatory limit. The 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, Okadaic acid, DTX1, DTX2, DTX3, shellfish, bivalve molluscs, mammalian biotests, acute reference dose, portion size, methods of analysis, human health, risk assessment.