COMMISSION DECISION

of 15 March 2002

laying down detailed rules for the implementation of Council Directive 91/492/EEC as regards the maximum levels and the methods of analysis of certain marine biotoxins in bivalve molluscs, echinoderms, tunicates and marine gastropods

(notified under document number C(2002) 1001)

(Text with EEA relevance)

(2002/225/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs [1], as last amended by Directive 97/79/EC [2], and in particular Chapter V, paragraphs 3 and 5, of the Annex thereto,

Whereas:

(1) Chapter V, point 7, of the Annex to Directive 91/492/EEC provides that the customary biological testing methods must not give a positive result to the presence of diarrhetic shellfish poisoning (DSP) in the edible parts of molluscs (the whole body or any part edible separately).

(2) It has been scientifically proven that certain marine biotoxins such as those of the diarrhetic shellfish poisoning (DSP) complex (okadaic acid (OA) and dinophysistoxins (DTXs)) and also yessotoxins (YTXs), pectenotoxins (PTXs) and azaspiracids (AZAs), pose a serious hazard to human health when present above certain limits in bivalve molluscs, echinoderms, tunicates or marine gastropods.

(3) In the light of recent scientific studies it is now possible to establish maximum levels and methods of analysis for those biotoxins.

(4) Maximum levels and methods of analysis should be harmonised and be implemented by the Member States in order to protect human health.

(5) In addition to biological testing methods, alternative detection methods such as chemical methods and *in vitro* assays should be accepted if it is demonstrated that the performance of the chosen methods is not less effective than the performance of the biological method and that their implementation provides an equivalent level of public health protection.

(6) The proposed maximum levels are based on provisional data and should be re-evaluated when new scientific evidence becomes available.

(7) The measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

This Decision lays down the maximum levels for the marine biotoxins of the diarrhetic shellfish poisoning (DSP) complex (okadaic acid and dinophysistoxins), yessotoxins, pectenotoxins and azaspiracids and the methods of analysis to be used for their detection. It applies to bivalve molluscs, echinoderms, tunicates and marine gastropods that are intended for immediate human consumption or for further processing before consumption.

Article 2

The maximum level of okadaic acid, dinophysistoxins and pectenotoxins together in the animals referred to in Article 1 (the whole body or any part edible separately) shall be 160 µg of okadaic acid equivalents/kg. The methods of analysis are set out in the Annex.

Article 3

The maximum level of yessotoxins in the animals referred to in Article 1 (the whole body or any part edible separately) shall be 1 mg of yessotoxin equivalent/kg. The methods of analysis are set out in the Annex.

Article 4

The maximum level of Azaspiracids in the animals referred to in Article 1 (the whole body or any part edible separately) shall be 160 µg of azaspiracid equivalents/kg. The methods of analysis are set out in the Annex.

[1] OJ L 268, 24.9.1991, p. 1.
[2] OJ L 24, 30.1.1998, p. 31.

Article 5

When the results of the analyses performed demonstrate discrepancies between the different methods, the mouse bioassay should be considered as the reference method.

Article 6

This Decision is addressed to the Member States.

Done at Brussels, 15 March 2002.

For the Commission

David BYRNE

Member of the Commission

ANNEX

Detection methods

Biological methods

A series of mouse bioassay procedures, differing in the test portion (hepatopancreas or whole body) and in the solvents used for the extraction and purification steps, can be used for detection of the toxins mentioned in Article 1. Sensitivity and selectivity depend on the choice of the solvents used for the extraction and purification steps and this should be taken into account when making a decision on the method to be used, in order to cover the full range of toxins.

A single mouse bioassay involving acetone extraction can be used to detect okadaic acid, dinophysistoxins, pectenotoxins and yessotoxins. This assay may be complemented if necessary with liquid/liquid partition steps with ethyl acetate/water or dichloromethane/water to remove potential interferences. Azaspiracids detection at the regulatory levels by means of this procedure requires the use of the whole body as the test portion.

Three mice should be used for each test. The death of two out of three mice within 24 hours after inoculation into each of them of an extract equivalent to 5 g of hepatopancreas or 25 g whole body should be considered as a positive result for the presence of one or more of the toxins mentioned in Article 1 at levels above those established in Article 2, 3 and 4.

A mouse bioassay with acetone extraction followed by liquid/liquid partition with diethylether can be used to detect okadaic acid, dinophysistoxins, pectenotoxins and azaspiracids but it cannot be used to detect yessotoxins as losses of these toxins may take place during the partition step. Three mice should be used for each test. The death of two out of three mice within 24 hours after inoculation into each of them of an extract equivalent to 5 g of hepatopancreas or 25 g whole body should be considered as a positive result for the presence of okadaic acid, dinophysistoxins, pectenotoxins and azaspiracids at levels above those established in Article 2 and 4.

The rat bioassay can detect okadaic acid, dinophysistoxins and azaspiracids. Three rats should be used for each test. A diarrhetic response in any of the three rats is considered a positive result for the presence of okadaic acid, dinophysistoxins and azaspiracids at levels above those mentioned in Article 2 and 4.

Alternative detection methods

A series of methods such as high performance liquid chromatography (HPLC) with fluorimetric detection, liquid chromatography (LC)-mass spectrometry (MS), inmunoassays and functional assays such as the phosphatase inhibition assay can be used as alternative or complementary methods to the biological testing methods, provided that either alone or combined they can detect at least the following analogues, that they are not less effective than the biological methods and that their implementation provides an equivalent level of public health protection:

— okadaic acid and dinophysistoxins: an hydrolysis step may be required in order to detect the presence of DTX3,

— pectenotoxins: PTX1 and PTX2,

— yessotoxins: YTX, 45 OH YTX, homo YTX, and 45 OH homo YTX,

— azaspiracids: AZA1, AZA2 and AZA3.

If new analogues of public health significance are discovered they should be included in the analysis. Standards will have to be available before chemical analysis will be possible. Total toxicity will be calculated using conversion factors based on the toxicity data available for each toxin.

The performance characteristics of these methods should be defined after validation following an internationally agreed protocol.