
Changes to legislation: There are currently no known outstanding effects for the
Commission Regulation (EU) No 15/2011, ANNEX. (See end of Document for details)

ANNEX

In Annex III to Regulation (EC) No 2074/2005, Chapter III is replaced by the following:

CHAPTER III

LIPOPHILIC TOXIN DETECTION METHODS

A. **Chemical methodology**

(1) The EU-RL LC-MS/MS method shall be the reference method for the detection of marine toxins as referred to in Chapter V(2)(c), (d) and (e) of Section VII of Annex III, to Regulation (EC) No 853/2004. This method shall determine at least the following compounds:

- okadaic acid : OA, DTX1, DTX2, DTX3 including their esters,
group toxins
- pectenotoxins : PTX1 and PTX2,
group toxins
- yessotoxins : YTX, 45 OH YTX, homo YTX, and 45 OH homo YTX,
group toxins
- azaspiracids : AZA1, AZA2 and AZA3.
group toxins

(2) Total toxicity equivalence shall be calculated using toxicity equivalent factors (TEFs) as recommended by EFSA.

(3) If new analogues of public health significance are discovered, they should be included in the analysis. Total toxicity equivalence shall be calculated using toxicity equivalent factors (TEFs) as recommended by EFSA.

(4) Other methods, such as liquid chromatography (LC) mass spectrometry (MS) method, high-performance liquid chromatography (HPLC) with appropriate detection, immunoassays and functional assays, such as the phosphatase inhibition assay, can be used as alternatives or supplementary to the EU-RL LC-MS/MS method, provided that:

- (a) either alone or combined they can detect at least the analogues as identified in point A(1) of this Chapter; more appropriate criteria shall be defined when necessary;
- (b) they fulfil the method performance criteria stipulated by the EU-RL. Such methods should be intra-laboratory validated and successfully tested under a recognised proficiency test scheme. The EU-RL shall support activities toward inter-laboratory validation of the technique to allow for formal standardisation;
- (c) their implementation provides an equivalent level of public health protection.

B. **Biological methods**

(1) To allow Member States to adapt their methods to the LC-MS/MS method as defined in point A(1) of this Chapter, a series of mouse bioassay procedures, differing in the test portion (hepatopancreas or whole body) and in the solvents used for extraction and purification, may be still used until 31 December 2014 for detecting marine toxins as referred to in Chapter V(2)(c), (d) and (e) of Section VII of Annex III to Regulation (EC) No 853/2004.

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- (2) Sensitivity and selectivity depend on the choice of solvents used for extraction and purification and this should be taken into account when a decision is made on the method to be used in order to cover the full range of toxins.
 - (3) A single mouse bioassay involving acetone extraction may be used to detect okadaic acid, dinophysistoxins, azaspiracids, pectenotoxins and yessotoxins. This assay may be supplemented, if necessary, with liquid/liquid partition steps with ethyl acetate/water or dichloromethane/water to remove potential interferences.
 - (4) Three mice shall be used for each test. Where two out of three mice die within 24 hours of inoculation with an extract equivalent to 5 g hepatopancreas or 25 g whole body, this shall be considered a positive result for the presence of one or more toxins as referred to in Chapter V(2)(c), (d) and (e) of Section VII of Annex III to Regulation (EC) No 853/2004 at levels above those laid down.
 - (5) A mouse bioassay with acetone extraction followed by liquid/liquid partition with diethylether may 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 shall be used for each test. Where two out of three mice die within 24 hours of inoculation with an extract equivalent to 5 g hepatopancreas or 25 g whole body, this shall be considered a positive result for the presence of okadaic acid, dinophysistoxins, pectenotoxins and azaspiracids at levels above those laid down in Chapter V(2)(c) and (e) of Section VII of Annex III to Regulation (EC) No 853/2004.
 - (6) A rat bioassay may be used to detect okadaic acid, dinophysistoxins and azaspiracids. Three rats shall be used for each test. A diarrhetic response in any of the three rats shall be considered a positive result for the presence of okadaic acid, dinophysistoxins and azaspiracids at levels above those laid down in Chapter V(2)(c) and (e) of Section VII of Annex III to Regulation (EC) No 853/2004.
- C. After the period established in point B(1) of this Chapter, the mouse bioassay shall be used only during the periodic monitoring of production areas and relaying areas for detecting new or unknown marine toxins on the basis of the national control programmes elaborated by the Member States.

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