

ANNEX III

RECOGNISED TESTING METHODS FOR DETECTING MARINE BIOTOXINS

The following analytical methods shall be used by the competent authorities to check compliance with the limits laid down in Chapter V(2) of Section VII of Annex III to Regulation (EC) No 853/2004 and, where appropriate, by food business operators.

In accordance with Article 7(2) and (3) of Council Directive 86/609/EEC⁽¹⁾, elements of replacement, refinement and reduction must be taken into account when biological methods are used.

CHAPTER I

PARALYTIC SHELLFISH POISON (PSP) DETECTION METHOD

1. The paralytic shellfish poison (PSP) content of edible parts of molluscs (the whole body or any part edible separately) must be detected in accordance with the biological testing method or any other internationally recognised method. The biological testing method may be carried out in association, if necessary, with another method for detecting Saxitoxin and any of its analogues for which standards are available.
2. If the results are challenged, the reference method shall be the biological method.

CHAPTER II

AMNESIC SHELLFISH POISON (ASP) DETECTION METHOD

The total content of amnesic shellfish poison (ASP) of edible parts of molluscs (the entire body or any part edible separately) must be detected using the high-performance liquid chromatography (HPLC) method or any other recognised method.

If the results are challenged, the reference method shall be the HPLC method.

CHAPTER III

LIPOPHILIC TOXIN DETECTION METHODS

- A. Biological methods
 1. 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 used 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. 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.
 2. A single mouse bioassay involving acetone extraction may be used to detect okadaic acid, dinophysistoxins, 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. Azaspiracid detection at

regulatory levels by means of this procedure shall involve the use of the whole body as the test portion.

3. 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.
 4. 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.
 5. 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.
- B. Alternative detection methods
1. A series of methods, such as high-performance liquid chromatography (HPLC) with fluorimetric detection, liquid chromatography (LC), mass spectrometry (MS), immunoassays and functional assays, such as the phosphatase inhibition assay, shall be used as alternatives or supplementary 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: a hydrolysis step may be required 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.
 2. If new analogues of public health significance are discovered, they should be included in the analysis. Standards must be available before chemical analysis is possible. Total toxicity shall be calculated using conversion factors based on the toxicity data available for each toxin.
 3. The performance characteristics of these methods shall be defined after validation following an internationally agreed protocol.
 4. Biological methods shall be replaced by alternative detection methods as soon as reference materials for detecting the toxins prescribed in Chapter V of Section VI of Annex III to Regulation (EC) No 853/2004 are readily available, the methods have been validated and this Chapter has been amended accordingly.

(1) OJ L 358, 18.12.1986, p. 1.