



## OPINION

### of the French Food Safety Agency on the proposal to implement multi-criteria management of shellfish farming areas in the framework of testing for lipophilic phytotoxins

THE DIRECTOR-GENERAL

#### 1. SUMMARY OF THE REQUEST

The French Food Safety Agency (AFSSA) was requested by the French Directorate General for Food (DGAI) and the French Directorate General for Health (DGS) on 17 April 2009 to provide scientific and technical support to the proposal to implement multi-criteria management of shellfish farming areas in the framework of testing for lipophilic phytotoxins.

#### 2. CONTEXT AND ISSUES RAISED

The French regional shellfish farming association (SRC) for Arcachon, Aquitaine, requested that the French Ministry for Agriculture and Fisheries revise the management structures in accordance with the results of tests for lipophilic phytotoxins in shellfish by proposing a protocol combining a mouse bioassay with a five- and 24-hour observation period, chemical analysis and counts of the toxin-producing algae in the environment.

This protocol has been prepared at the request of the French Minister for Agriculture and Fisheries in view of submission to the European Commission. It makes it possible to compare the current management structures in various scenarios to the structures hoped for by the professionals and those proposed by the managers.

In this context AFSSA has been requested to:

- Assess if, in each of the situations analysed, the management proposals meet Community requirements, namely to provide a level of public health protection comparable to the single mouse bioassay at 24 hours; and
- Identify those cases that offer a level of protection that is below requirements but could still be deemed acceptable on the condition that they are combined with accompanying measures or consumption guidelines in order to provide a level of consumer protection meeting Community requirements.

#### 3. METHOD OF EXPERT ASSESSMENT

An emergency collective expert assessment group (GECU), which was created at the instigation of the Director-General of the French Food Safety Agency in consultation with the Chairman of the scientific panel “Chemical and physical residues and contaminants”, was asked to perform this expert assessment.

The “Phycotoxins” GECU comprises:

- experts from the scientific panel “Chemical and physical residues and contaminants”, including the Chairman of the panel;
- an expert from Ifremer<sup>1</sup>, Environment, Microbiology and Phycotoxins Department;
- experts from the Toxin Characterisation Unit (CAT) of the laboratory for studies and research on the quality of foods and food processes (LERQAP), French national reference laboratory (LNR) for marine biotoxins; and
- an expert from the Department for the Evaluation of Nutritional and Health Risks (DERNS).

Following consultation of the “Phycotoxins” GECU, which met on 24 April 2009, AFSSA is issuing the following opinion.

<sup>1</sup> Ifremer: French Research Institute for Exploitation of the Sea

## 4. ARGUMENTATION

### 4.1 Summary of the conclusions of the AFSSA opinion dated 16 September 2008 and the regulatory requirements

Regulation (EC) no. 2074/2005 (replacing Decision 2002/225/EC) specifies that a mouse bioassay must be considered as positive if two in three mice die within 24 hours (irrespective of the time of death between 0 and 24 hours).

In its opinion dated 16 September 2008 (on request 2008-SA-0268) AFSSA comes to the conclusion that the 24-hour period of observation in the mouse bioassay is absolutely necessary in order to detect a limit of 160 µg eq OA/kg, DTX3 and azaspiracids and therefore ensure consumer safety as all these toxins have been linked to diarrhetic shellfish poisoning in humans. It is to be recalled that injecting each mouse with the equivalent of 25 g of shellfish meat (i.e. the equivalent of 5 g of digestive gland, the organ in which almost all lipophilic toxins are concentrated) makes it possible to test the safety limit of 160 µg eq OA/kg of shellfish flesh. Each mouse weighing 20 g responds to 4 µg eq OA (corresponding to the LD50 of 192 µg of OA/kg of mouse).

Works by Vale and Sampayo (1996) and Vieytes *et al.* (1997) have shown that mouse mortality at five hours is only observed at concentrations equal to or higher than 400 µg/kg of flesh for okadaic acid (i.e. over twice the limit value of 160 µg/kg).

### 4.2 Analysis of the proposed stratification

#### **Concerning the stratification in seven cases**

The multi-criteria management protocol put forward (included in the annex) is based on seven cases, each defined by the number of dead mice (zero, one, two or three dead mice out of three mice tested) and their time of death (no death, death within five hours or death between five and 24 hours).

AFSSA considers that there is no new scientific data to justify a stratification based on a change to the mouse bioassay parameters without compromising the reliability of the current system.

#### **Concerning the stratification in each case**

Each of these seven cases is broken down into nine to twelve situations (identified from A to L) in accordance with the following criteria:

- presence/absence of toxinogenic phytoplankton of the *Dinophysis*<sup>2</sup> genus only,
- results from the mouse bioassay, taking into account the time of death of each mouse,
- results from chemical analysis, considering:
  - both quantifiable and non-quantifiable regulated toxins<sup>3</sup>, and
  - an additional category called “others”. It is stressed that this category includes interferences in the literal sense<sup>4</sup>, but primarily all toxins that are currently under evaluation by the European Food Safety Authority (EFSA) (e.g. spirolides and gymnodimines).

It is important to specify that the non-detection of *Dinophysis* in water samples does not guarantee its absence in the environment. There is no guarantee that the water sample is taken from the *Dinophysis* bloom (given the heterogeneous distribution of plankton cells in the water column).

AFSSA considers that a management decision based on the detection of *Dinophysis* or on results of the chemical analysis taken in isolation does not guarantee consumer safety.

<sup>2</sup> *Dinophysis* is the genus producing okadaic acid and dinophysistoxins as well as pectenotoxins. It should be noted that the mouse bioassay also aims to detect other regulated lipophilic toxins, namely azaspiracids and yessotoxins, which are produced by other genera for which testing is not included in routine monitoring.

<sup>3</sup> For interpretation, the LNR has only taken into account okadaic acid and dinophysistoxins (OA/DTXs) as regulated quantifiable phycotoxins. Other regulated toxins such as azaspiracids (AZAs), pectenotoxins (PTXs) and yessotoxins (YTXs) have been considered as non-quantifiable (analytical method approved for OA/DTXs only).

<sup>4</sup> Compounds toxic for mice by intraperitoneal injection but proven to be harmless to humans by oral route (as is the case for free fatty acids).

### Conclusion concerning stratification

In order to maintain the consistency of the current system, cases one to four must be processed in the same way (positive result<sup>5</sup> of the bioassay), as must cases five to seven (negative result<sup>6</sup> of the bioassay) as shown in table 1 below.

**Table 1: Grouping of the cases proposed in the protocol according to interpretation of the mouse bioassay in the current system**

	Case no. 1 and 2		Case no. 3 and 4		Case no. 5 and 6		Case no. 7
<b>Mortality detailed in the mouse bioassay</b>	2-3 <5h	1<5h	1<5h	0<5h	1<5h	0<5h	3>24h
	1-0 <24h	2<24h	1<24h	2<24h	0 <24h	1<24h	
			1>24h	1>24h	2>24h	2>24h	
<b>Overall mortality in the mouse bioassay</b>	3/3<24h		2/3<24h		1/3<24h		0/3<24h
<b>Regulatory result</b>	Positive		Positive		Negative		Negative

The situations corresponding to a change in management decision from closure to proposed non-closure are listed below and analysed on a case-by-case basis.

Situations that do not entail a change of management have not been analysed.

### 4.3 Case analysis

#### **Cases one to four: positive mouse bioassay (2/3 or 3/3 mouse mortality)**

For situations A to K a positive mouse bioassay result is associated with the following additional criteria:

- detection of *Dinophysis*;
- and/or detection of quantifiable or non-quantifiable regulated toxins by chemical analysis;
- and/or detection of non-regulated toxins by chemical analysis.

*The current management system makes provision for the closure of the shellfish farming area in all these cases.*

*The professionals make provision for several non-closure situations (corresponding to cases where *Dinophysis* has not been detected).*

*The managers do not make provision for a change in the management decision.*

According to AFSSA the mouse bioassay must remain the decisive tool for all those situations. The changes proposed in this management protocol would lower the level of consumer protection.

For situation L a positive mouse bioassay result is associated with the following three criteria:

- non-detection of *Dinophysis*;
- non-detection of quantifiable or non-quantifiable regulated toxins by chemical analysis;
- non-detection of non-regulated toxins by chemical analysis.

Situation L corresponds to an atypical toxicity situation<sup>7</sup>.

*The current management system makes provision for closure of the shellfish farming area in all these cases.*

*The professionals make provision for non-closure in all these cases.*

*The managers make provision to move from management of closure to non-closure in two cases (one mouse dead within five hours and one dead between five and 24 hours, two or three mice dead between five and 24 hours).*

<sup>5</sup> Positive result: 2/3 or 3/3 mice dead within 24 hours.

<sup>6</sup> Negative result: 0/3 or 1/3 mice dead within 24 hours.

<sup>7</sup> This is an episode of shellfish contamination detected by the official test of toxic response in the mouse after intra-peritoneal (IP) injection of an extract of the digestive glands, but whose source cannot be explained by the presence of known toxins generally sought (by the LC/MS method of analysis) or by a quantity of known toxin(s) sufficient to explain the positive result of the mouse bioassay, and which cannot be linked to the presence of known toxinogenic phytoplankton in the environment.

AFSSA considers that without any data on the source of atypical toxicities, a risk for the consumer cannot be ruled out. Pending a response based on research to identify the agent(s) causing the death of the mice, a relaxation of the management protocol (non-closure) cannot allow for a level of public health protection comparable to the single bioassay at 24 hours.

**Cases five to seven: negative mouse bioassay (0/3 or 1/3 mouse mortality)**

For situations A to D a negative mouse bioassay result is associated with the presence of regulated toxins above the safety limit value.

*The current management system makes provision for non-closure of the shellfish farming area in all these cases.*

*The professionals and managers make provision for management based on the results of the chemical analysis.*

*The professionals make provision for closure only when the amount of toxins is above the regulatory limit values and Dinophysis is present.*

*The managers make provision for closure in all cases in which the amount of toxins is above the regulatory limit values.*

Firstly, it should be stressed that of the 300 samples to undergo joint mouse bioassay and chemical analysis nationwide in 2008, such non-concordance situations arose in only 2% of cases.

AFSSA considers that these non-concordance situations must be the subject of discussions between the LNR and the laboratory in question in order to determine the source of anomalies in the results. In the meantime, the area must be considered to present a risk to consumer safety.

## 5. CONCLUSIONS

Following analysis of proposals from professionals or managers on changes to the management of shellfish farming areas, AFSSA considers that these proposals do not make it possible to ensure a level of consumer health protection comparable to that achieved with the current system.

In the current state of knowledge, no accompanying measure can be proposed in the short term that would guarantee the same level of consumer health protection should the commercialisation of shellfish from a production area with a positive mouse bioassay be maintained.

## 6. KEYWORDS.

Lipophilic phycotoxins, marine biotoxins, multi-criteria management, atypical toxicity.

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