ANSES Opinion
Request No 2021-SA-0212
Related Request Nos 2007-SA-0227 and 2007-SA-0303

The Director General

Maisons-Alfort, 31 July 2023

REVISED OPINION[1] of the French Agency for Food, Environmental and Occupational Health & Safety

on the risks to human health associated with the proliferation of *Ostreopsis* spp. on the Basque coast

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 31 July 2023 shall prevail.

On 3 December 2021, ANSES received a formal request from the Directorate General for Health (DGS) and the Directorate General for Food (DGAL) to undertake an expert appraisal initially entitled: "*Request for an opinion on the risks associated with the proliferation of Ostreopsis spp. along the entire French coastline*."

1. BACKGROUND AND PURPOSE OF THE REQUEST

1.1. Background

In recent decades, several species of dinoflagellates of the genus *Ostreopsis* have been identified in the marine waters of European countries such as Albania, Croatia, Cyprus, Spain, France, Greece, Italy, Monaco and Portugal (Ciminiello *et al.*, 2006; Aligizaki *et al*., 2008; Amzil *et al.*, 2012; Del Favero *et al*., 2012; Funari, Manganelli and Testai, 2015; Accoroni and Totti, 2016; Fraga *et al*., 2017; dos Santos *et al*., 2009). When the cells or toxins produced by these microalgae are present in seawater, aerosols or certain seafood products, they can cause

[1] Cancels and replaces the Opinion of 12 May 2023

poisoning in humans (Pelin *et al*., 2016-; Walsh, 2017; Patocka *et al.*, 2018). The main route of human exposure is inhalation of aerosols, although it is not yet known whether the agents responsible for poisoning are *Ostreopsis* cells, cell debris, known toxins produced by *Ostreopsis*, or other as yet unidentified compounds. Other routes of exposure (dermal contact, eye contact, ingestion of contaminated water or seafood) are also possible (Paradis and Labadie, 2022).

Poisoning is manifested through various signs and symptoms (occurring within 48 hours of exposure):

- Neurosensory and neurological: paraesthesia (tingling sensation), dysaesthesia (burning sensation) and headaches;
- Respiratory, ENT region and stomatological: dysgeusia with a metallic taste, rhinorrhoea, cough, respiratory discomfort;
- Dermal: suggestive of urticaria;
- Cardiac: tachycardia, high blood pressure;
- Digestive: nausea, vomiting, diarrhoea;
- Systemic: fever;
- Locomotor: myalgia and arthralgia (muscle and joint pain).

People experience them in a variety of situations:

- Work or recreational activities in the sea (bathers, lifeguards, professional fishers, surfers, etc.);
- Work or recreational activities on or near the beach (holidaymakers, paramedics, beach cleaners, restaurant workers, etc.);
- Stays or residence in the immediate vicinity of the seashore;
- Consumption of contaminated seafood products.

In France, *Ostreopsis* has been identified repeatedly on the Mediterranean coast for around 15 years now (Mangialajo *et al.*, 2011; Gémin *et al.*, 2020a), whereas on the French and Spanish Basque coasts its presence is much more recent (Amzil *et al.,* 2021; Drouet, 2021; Chomérat, 2022). Two species have been identified on the Basque coast: *O*. cf. *siamensis* and *O*. cf. *ovata* (Amzil *et al.,* 2021). While the first, *O*. cf. *siamensis*, has been observed on this coast since 2018, the second, *O*. cf. *ovata*, was only seen in this part of the Bay of Biscay for the first time in 2021.

The epidemiological report showed that 674 people developed signs and symptoms associated with *Ostreopsis* aggregates floating on the sea surface, hereafter referred to as “sea flowers” (translation of French term “fleurs d’eau”) on the French Basque coast during the summer of 2021 (Paradis et Labadie 2022). This report also showed that they were not clinically different from the effects observed during *Ostreopsis* cf. *ovata* bloom episodes around the Mediterranean.

1.2. Purpose of the request

In their formal request letter (see Annex 2), the DGS and DGAL asked ANSES, as part of this expert appraisal, to update knowledge about *Ostreopsis* that had been reported in the Agency's opinions from 2007 and 2008 (ANSES, 2007 and 2008), and draw up specific

recommendations for the Atlantic coast. The Agency was also asked, if appropriate, to update the specific recommendations for the Mediterranean coast established in 2007-2008. This formal request relates to the health risks associated with the various routes of exposure: by inhalation (exposure to aerosols), by ingestion (consumption of contaminated seafood, or water swallowed during aquatic activities) or following dermal or eye contact, while taking account of the particularities associated with protecting the affected workers.

As part of the expert appraisal contract drawn up on 20 December 2021, the Agency redefined the scope of the formal request, reformulated the questions and changed the title to focus on the risks associated with the presence of *Ostreopsis* on the Basque coast.

The expert appraisal was carried out according to two themes, the first relating to the review of knowledge and the second to the drafting of recommendations, detailed as follows:

1- State of knowledge:

- Description of species of the genus *Ostreopsis*;
- Identification and description of the associated toxins;
- Ecology of the genus *Ostreopsis* (conditions favourable to *Ostreopsis* blooms and conditions associated with toxin production);
- Presence of *Ostreopsis* on the coasts of metropolitan France and, if possible, the French overseas territories (mapping using data from the REPHY monitoring network[2] and/or from research projects);
- Measurement of the genus *Ostreopsis* and its associated toxins in water, air, shellfish and on macroalgae (description of sampling and analysis methods, including rapid detection methods);
- Routes of exposure – populations exposed to *Ostreopsis* cells and their associated toxins, and sensitive populations;
- Description of international programmes on monitoring, standards or regulations relating to *Ostreopsis* in water, air and shellfish;
- Description of the monitoring programme in the Mediterranean;
- Data on toxicity according to the toxins identified and the routes of exposure (answers to the question: is human poisoning related to the toxins and/or the cells?);
- Analysis of epidemiological data from poison control and monitoring centres (CAP-TVs) (summer 2021 episode and previous episodes on the Mediterranean coast).

2- Recommendations based on the review of the current state of knowledge:

- Proposed acute and subchronic toxicological values for the reference toxin by the oral/respiratory/dermal routes;
- Proposed concentration threshold that should not be exceeded for *Ostreopsis* and/or toxins in water or on macroalgae and/or in the air and/or in shellfish. Does the threshold differ depending on the species of the genus *Ostreopsis*?
- Proposed additional measures/investigations if the threshold is exceeded;
- Recommendations for the sampling strategy and suitable methods for sampling and analysing cells and/or the associated toxins depending on the matrix (water, macroalgae, seafood, sea spray);

[2] network for observing and monitoring phytoplankton and hydrology in coastal waters

- Recommendations for monitoring *Ostreopsis* on the Basque coast (sampling points, type of matrix (water, macroalgae, other), frequency, etc.). Is it possible to identify high-risk areas/periods on the Basque/Atlantic coast?
- Answers to the question: what are the risks for the various users of the sea according to the different routes of exposure (food, aquatic activities, sea spray)?
- Specific recommendations for professionals and sensitive populations (asthmatics, etc.).

The expert appraisal report from the working group (WG), dated June 2023, sets out all of the work it conducted. This opinion summarises the findings, in particular to help with implementing *Ostreopsis* monitoring on the Basque coast with effect from the 2023 summer season. Therefore, in addition to the review of knowledge, Section 3 presents the main findings of the expert appraisal supporting the recommendations for monitoring *Ostreopsis* on the Basque coast:

- Proposed monitoring strategy for *Ostreopsis;*
- Proposed guideline value for *Ostreopsis* toxins in shellfish;
- Recommendations for limiting exposure of professionals and local residents;
- Proposed decision-support tool for managing *Ostreopsis* “sea flowers”.

Lastly, the list of research needs is presented in Annex 3.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)".

2.1. Procedure: means implemented and organisation

The expert appraisal falls within the sphere of competence of the Expert Committees (CESs) on Water, “Assessment of physico-chemical risks in food” (ERCA) and “Health reference values” (VSR).

ANSES entrusted examination of this request to the *Ostreopsis* Working Group (WG), set up following a public call for applications and reporting to the CES on “Water”.

The methodological and scientific aspects of the *Ostreopsis* WG's expert appraisal work were regularly submitted to the CES on “Water” on 10 May, 7 June and 6 December 2022 and on 7 March and 4 April 2023, as well as to the CES ERCA on 11 January, 12 May and 2 June 2022 and on 13 January, 7 February and 14 March 2023. The CES VSR was asked to establish two toxicity reference values (TRVs) for palytoxin, respectively for acute and repeated oral exposure. Its work was presented on 19 January 2023 and validated on 9 March 2023. It was detailed in a specific opinion published by the Agency in June 2023 (ANSES, 2023).

The collective expert appraisal report and the "Analysis and conclusions" section produced by the experts of the *Ostreopsis* Working Group take into account the comments and additional information provided by the members of the CES on “Water” and the CES ERCA. This opinion's sections on water and aerosols were adopted by the CES on “Water” on 4 April 2023, while the sections on seafood products were adopted by the CES ERCA on 14 March 2023.

This opinion, which is based on the expert appraisal report and the conclusions of the *Ostreopsis* WG, was adopted by the CES on "Water" at its meeting on 4 April 2023.

This revision is the result of editorial changes to assist publication of the report, which has now been finalised, and a change in the counting of cases. Annex 4 to this opinion sets out the changes made during this revision.

2.2. Prevention of risks of conflicts of interest

The results were therefore produced by a group of experts with complementary skills.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the website: https://dpi.sante.gouv.fr/.

2.3. Expert appraisal method

To answer the questions in the formal request, the *Ostreopsis* WG based its work on an in-depth literature review (scoping review). Hearings were also held with local stakeholders (scientists, managers, doctors).

3. ANALYSIS AND CONCLUSIONS OF THE *OSTREOPSIS* WG AND THE CES ON "WATER"

3.1. Review of knowledge: summary of the analysis of the literature review

3.1.1. Description

The unicellular organisms of the genus *Ostreopsis* are microalgae of the class Dinophyceae, measuring around 40 to 100 µm long and possessing two flagella. They are shaped like a more or less flattened lens and the genus is easily recognised with an optical microscope.

Twelve species of the genus *Ostreopsis* have been described to date. Studies are complicated by morphological similarities between certain species, combined with morphological variability within the same species, making genetic identification essential.
Molecular biology studies suggest the presence of other species (known as cryptic species). The term "cf." (*confer* in Latin), used extensively throughout the text for *Ostreopsis* cf. *ovata* and *Ostreopsis* cf. *siamensis*, indicates that these species resemble *Ostreopsis ovata* and *Ostreopsis siamensis* morphologically, but that molecular biological data are lacking or too fragmentary to confirm that they are indeed these species.

3.1.2. Biology and ecology of the genus *Ostreopsis*

Species of the genus *Ostreopsis* grow as biofilms on biotic substrates (macrophytes) or abiotic substrates (rocks/pebbles), generally in shallow waters that are relatively sheltered from winds and currents (Mangialajo *et al.*, 2011). Production of a mucilaginous matrix (mucus) enables cell aggregation and biofilm formation (Honsell *et al*., 2011; Escalera *et al*., 2014). When environmental conditions are favourable to cell growth, *O.* cf. *ovata* proliferates, with cellular

abundance that can exceed one million cells per gram of fresh weight of macroalgae (Mangialajo *et al.,* 2008; hearing with Ifremer 2022). Various biological or physical processes, such as swell and currents, can cause cells or cell aggregates to detach from the substrate and disperse into the water column. This is known as the planktonic phase of these microalgae. Aggregates can then accumulate on the surface to form “sea flowers” (translation of French term “fleurs d’eau”) visible to the naked eye (Mangialajo *et al*., 2011; Pavaux *et al*., 2021).

Ostreopsis had long been considered a genus that only grows in tropical and subtropical areas. However, since the early 2000s, *Ostreopsis* has been observed with increasing frequency in temperate zones such as the Mediterranean. Since it was first identified in France, in Villefranche-sur-Mer in 1972 (Taylor, 1979), the presence of the genus *Ostreopsis* has been reported throughout the Mediterranean (Tognetto *et al*., 1995; Vila *et al*., 2001; Aligizaki and Nikolaidis, 2006; Turki *et al*., 2006; Mangialajo *et al*., 2008; Accoroni *et al*., 2011; Illoul *et al*., 2012; Amzil *et al*., 2012; Accoroni *et al*., 2016). *Ostreopsis* cf. *ovata* is the most widespread species in the Mediterranean, particularly in the north-western part, while *O*. cf. *siamensis* and *O. fattorussoi* have mainly been observed in the western (Spain, Tunisia, Italy) and eastern (Cyprus, Lebanon) Mediterranean, respectively (Battocchi *et al*., 2010; Accoroni and Totti, 2016; Accoroni *et al*., 2016).

In recent years, the French and Spanish Basque coasts have become a new area for the development of *Ostreopsis* (Amorim *et al*., 2010; Laza-Martinez *et al*., 2011; Drouet, 2021; Chomerat, 2022). The species found in these areas are *O.* cf. *ovata* and *O.* cf. *siamensis*. This new habitat for *Ostreopsis* shows that this genus is capable of adapting to environmental conditions other than those found around the Mediterranean. The waters of the Aquitaine coastline are colder, less saline and have a strong hydrodynamic effect, caused partly by the tides. In principle, these characteristics are less favourable to the development of *O.* cf. *ovata* than the warmer, more stratified waters of the Mediterranean. On the other hand, the abundance of macroalgae on rocky substrates on the Basque coast is favourable to the development of *Ostreopsis* biofilms.

3.1.3. Toxins produced

Some species of *Ostreopsis* produce compounds that can threaten human health and marine fauna and flora. The toxins produced by *Ostreopsis* species are mainly isobaric palytoxin (isob-PLTX)[3], ovatoxins (OVTX-a to -i), ostreocins (OSTs) and mascarenotoxins. These compounds are identified in this document as "PLTX group toxins" because they all have a similar chemical structure to that of PLTX. Historically, PLTX was identified in a tropical soft coral from Hawaii (*Palythoa toxica*). It was responsible for human poisonings that developed following the handling of several genera of soft coral in aquariums (Calon *et al*., 2019). It should be noted that microalgae of the genus *Ostreopsis* can produce isob-PLTX but not PLTX.

The toxin profiles of *O*. cf. *ovata* strains isolated in the Mediterranean have revealed the presence of several ovatoxin (OVTX) analogues (Ciminiello *et al.* 2010; Tartaglione *et al.,* 2017). These same toxins were also detected in a sample taken during the August 2021 *Ostreopsis* bloomepisode on the Basque coast, and in *O.* cf. *ovata* cells cultured following the isolation of strains from the same sample (Amzil *et al*., 2021, Chomérat *et al*., 2022). However, no PLTX group toxins were found in *O*. cf. *siamensis* cells isolated from the same sample, confirming the results of previous studies conducted on several *O*. cf. *siamensis* strains from

[3] This is an isomer of PLTX, with the same empirical formula but a different structural formula.

the Mediterranean (Sicily, Italy) and the Atlantic (Sines and Cascais, Portugal) (Ciminiello *et al.*, 2013). No OSTs or mascarenotoxins have been found in strains of *O*. cf. *siamensis* or *O*. cf. *ovata* isolated from the Mediterranean and Basque coasts (Ciminiello *et al.*, 2006; Tartaglione *et al.,* 2017; Chomérat *et al*., 2022).

Moreover, certain strains of *Ostreopsis* produce compounds that do not belong to the PLTX group toxins, yet are potentially toxic to humans and to marine fauna and flora. This is particularly true of Korean and Mediterranean strains of *O.* cf. *ovata*, which can produce ostreols, ostreotoxins, liguriatoxins and rivieratoxins (the structures of these last two families of compounds are still not known).

To date, OVTXs are the only identified PLTX group toxins produced by *Ostreopsis* species proliferating on the Basque coast.

3.1.4. Toxicity

Studies on the toxins produced by *Ostreopsis* (PLTX group toxins and other toxins) are very fragmentary. Due to the availability of several *in vivo* studies with PLTX and given the high structural similarity between PLTX and PLTX group toxins, the *Ostreopsis* WG chose to use PLTX as a representative compound (proxy) of PLTX group toxins.

The studies available on PLTX mainly concern oral exposure; less work has been carried out on inhalation and dermal contact.

Palytoxin

There are no data available for establishing toxicokinetic parameters for PLTX. **The acute toxicity** of PLTX has been studied in numerous animal species (mainly rodents, but also lagomorphs, dogs and monkeys), via several routes of administration. They show that animal mortality after gavage occurs at doses up to 15,000 times higher than administration by inhalation or injection (intravenous, intraperitoneal, intramuscular) (Boente-Juncal *et al*., 2020a; Poli *et al*., 2018; Tubaro *et al*., 2011; Sosa *et al*., 2009).

The onset kinetics, type and intensity of the signs and symptoms may differ depending on the route of administration being considered. In the acute oral toxicity studies cited above, rapid mortality (within a few hours) was observed at the highest doses. It followed neurotoxic effects with muscular paralysis and respiratory distress. For lower doses that did not cause rapid mortality, lesions were observed at contact sites depending on the route of exposure (nasal cavity, lung, skin, stomach, intestine), as well as at systemic level (heart, liver, kidneys, lymphoid tissues, salivary glands), indicating a very broad distribution of the toxin within the body. These multiple effects are most likely explained by the fact that PLTX acts on a transmembrane transport protein (Na/K-ATPase) (Takeuchi *et al*., 2009; Scheiner-Bobis *et al*., 2002) that is found in all cell types (Mobasheri *et al.*, 2000).

Repeated oral toxicity studies over 7 or 28 days showed that while organs such as the lungs, heart and spleen were affected at the highest doses, only the stomach, intestines and liver were affected at lower doses (Del Favero *et al*., 2013; Boente-Juncal *et al*., 2020b).

Given the lack of data on human and animal poisoning (Wiles *et al.*, 1974; Fujiki *et al*., 1986; Poli *et al*., 2018), **it was not possible to propose a TRV for short-term exposure to PLTX by inhalation or dermal contact**.

Symptoms observed following acute oral exposure of mice to PLTX (by gavage) include piloerection, lethargy, ataxia, abdominal pain and dyspnoea. **An acute oral TRV of 0.08 µg**

PLTX.kg^{-1} bw was developed by the CES VSR, based on a NOAEL[4] derived from the study by Boente-Juncal *et al*. (2020a). **This value is associated with a moderate-low confidence rating[5] (ANSES, 2023).**

Ovatoxins

Unlike PLTX toxicity studies, OVTX toxicity studies are rare due to the difficulty of obtaining pure toxins (Gémin, 2020b).

Just one *in vivo* acute toxicity study has been conducted with OVTX-a (Poli *et al*., 2018). It showed that exposure of rats by inhalation led to changes in the nasal cavity as well as lesions in the lungs, heart, liver and kidneys, while little or no effect was observed in the muscles and gastrointestinal tract. In view of the data available, the doses that induce mortality and the symptoms and lesions observed appear to be very similar between PLTX and OVTX-a. **All these results obtained from *in vivo* and *in vitro* studies suggest that** the cellular mechanisms of action of PLTX and OVTX-a are very similar.

Other identified toxins

Only acute toxicity data are available for OST-D by various routes of administration. The effects reported are fairly similar to those of PLTX.

Data on other known toxins are very fragmentary, with most studies being *in vitro*.

For these reasons, it was **not possible to establish TRVs for all these other toxins**.

Given the similarity of the effects induced by PLTX, OVTXs or OST-D in animals, the *Ostreopsis* WG believes that the acute TRV developed for oral exposure to PLTX applies to the unweighted sum of the exposure doses (assuming toxic equivalency relative to body weight).

3.1.5. Contamination of environmental media by *Ostreopsis* toxins

This section presents the current state of knowledge about the contamination of water, aerosols and seafood by toxins produced during *Ostreopsis* bloomepisodes.

As a reminder, it has been shown that the *Ostreopsis* cf. *ovata* strains present on the Basque coast during the summers of 2021 and 2022 produce OVTXs.

Water

Contamination of seawater by *Ostreopsis* toxins is not well documented. Laboratory experiments have shown that OVTXs produced by *Ostreopsis* cells (intracellular toxins) can also be found in water or mucus (extracellular toxins). This distribution of OVTXs in two fractions (intracellular and extracellular) has an impact on the bioavailability of the toxins and therefore on the potential exposure levels of the population. The intracellular concentration of

[4] The NOAEL (no observed adverse effect level) is the highest dose of a chemical that produces no observable adverse effects in a toxicity study.

[5] ANSES assigns an overall confidence level to each TRV, taking account of the type and quality of the data, the critical effect and its mode of action, the choice of key study and choice of critical dose. For each criterion, a high, moderate or low confidence level must be set, and by combining the levels for each criterion, an overall confidence level is determined by expert judgement (ANSES, 2017). There are five levels of confidence corresponding to five colours: **high (dark green), moderate-high (light green), moderate (yellow), moderate-low (orange) and low (red**).

OVTX varies greatly from one geographical area to another, with a minimum of around 10^{-3} pg.cell^{-1} on the Atlantic coast (Morocco, Basque Country) and a maximum of 75 pg.cell^{-1} on the Mediterranean coast (Adriatic Sea) (Alkhatib *et al.*, 2022; Chomerat *et al.*, 2022). Various biotic and abiotic factors and processes, such as the nature of the substrate, the growth phase, the presence of competitors, nutrient availability and/or temperature, may contribute to this variability.

Measurement of extracellular OVTX concentrations is limited by the lack of an optimised method for extracting these toxins from seawater. No data are currently available on the concentration of OVTX in the water column or in mucus in the natural environment. For the same reasons, no data on contamination of water or mucus by the other toxins produced by *Ostreopsis* are available to date.

The *Ostreopsis* WG believes that in the absence of sufficient data, it is not possible to rule out the risk of exposure through ingestion of or dermal and eye contact with water contaminated by PLTX group toxins, in the event of *Ostreopsis* blooms episodes.

Aerosols

The nature of the aerosolised compounds that are toxic to human populations has not yet been clearly identified. The toxicity of aerosols produced during *Ostreopsis* “sea flowers” episodes could result from them containing PLTX group toxins, the other toxins mentioned above, *Ostreopsis* cells or cell debris, or other as yet unknown compounds.

The only data on aerosol contamination by OVTXs currently available are fragmentary. Some field and laboratory studies show that OVTXs can be aerosolised. Aerosol enrichment in toxic material seems to be partly dependent on the cellular abundance of *Ostreopsis* in the surface layer of the water column, but above all on meteorological conditions (wind speed). However, it is not possible to determine from the available data whether the OVTXs transferred from the water column are dissolved or associated with *Ostreopsis* cell debris.

Despite the lack of sufficient knowledge about the type of toxic compounds and the processes responsible for their transfer to the atmosphere, the *Ostreopsis* WG experts confirm that the main route of human exposure is inhalation, although they are not currently in a position to characterise and assess the risk associated with *Ostreopsis* blooms by this route of exposure.

Seafood products

Contamination of seafood products by PLTX group toxins produced by *Ostreopsis* has been observed in many countries, particularly around the Mediterranean. Among the contaminated organisms, herbivores generally have the highest concentrations of toxins, probably because they graze directly on the macroalgae on which the *Ostreopsis* cells proliferate. Most of the toxins are found in the digestive glands of mussels and the digestive tracts of other organisms (sea urchins, cephalopods, fish), with a dominance of OVTX (at least 90%) and a small proportion of (isob-)PLTX (around 10%). Although OVTX profiles can vary according to the organism, the few data available indicate that OVTX-a is the main toxin, accounting for around 70% of the toxin profile, followed by OVTX-b (20-30%), then OVTX-c and -e toxins (0-10%). The accumulation of PLTX group toxins in sea urchins and mussels is often correlated with the *in situ* cellular abundance of *O.* cf. *ovata*. Although (1) the harvesting of sea urchins is prohibited in the Mediterranean during the summer, and (2) the edible parts of sea urchins are

a priori the gonads, a health concern cannot be ruled out for people who are ill-informed or who consume all or part of the contents of these animals' digestive tracts.

One of the major limitations of this review of knowledge about the occurrence and quantification of toxins in seafood is the poor quality of the data available. In many of the studies, the analytical techniques used do not allow unequivocal identification or robust quantification (reproducibility and trueness) of PLTX group toxins, especially since only one analytical standard for PLTX isolated from the coral *Palythoa* is commercially available to date. Furthermore, because the units of measurement for seafood products are not harmonised, it is often difficult to compare data from different studies. Data quality is an essential prerequisite for risk assessment.

Rare cases of human poisoning have been associated with the consumption of seafood (crabs, fish) contaminated by *Ostreopsis* in the Philippines and Madagascar. However, the analytical methods used (bioassays) were unable to attribute them with certainty to the presence of PLTX group toxins produced by *Ostreopsis*.

The *Ostreopsis* WG believes that in the absence of sufficient data, it is not possible to rule out the risk of oral exposure from the consumption of seafood contaminated by PLTX group toxins, especially for organisms consumed uneviscerated.

3.2. Recommendations for monitoring *Ostreopsis* and its toxins, and for preventing the hazards associated with its blooms

Given the lack of certainty that the OVTXs produced by *O*. cf. *ovata* were responsible for the human poisoning cases that occurred during the *Ostreopsis* bloom episodes on the Basque coast over the last two summers, the *Ostreopsis* WG recommends limiting the monitoring of *Ostreopsis* blooms to monitoring of this microalgae's cells, and not combining this for the time being with monitoring of the toxins produced by *Ostreopsis* in the water or in aerosols. This monitoring should be quantitative for the benthic and planktonic compartments and qualitative only for “sea flowers”.

Nevertheless, recommendations have been made for preventing exposure to the toxins produced by *Ostreopsis*, taking all routes of exposure into account.

All the recommendations have been drawn up on the basis of observations, studies and experience acquired around the Mediterranean over the last 15 years or so.

3.2.1. Monitoring of *Ostreopsis* in the water along the Basque coastline

General strategy for monitoring *Ostreopsis*

One of the inherent difficulties in detecting *Ostreopsis* cells is the heterogeneity of their distribution along the coastline, as well as their spatial and temporal distribution in the benthic and planktonic compartments. This lack of uniformity means that the protocols for sampling *Ostreopsis* cells in the various compartments need to be optimised in order to quantify these cells and recommend suitable health management measures.

Observations and environmental or health monitoring have shown that blooms generally occur on the same sites from one year to the next. **The *Ostreopsis* WG therefore recommends that quality monitoring preferably be carried out at sites already affected by *Ostreopsis* blooms on the Basque coast, whether these are beaches already subject to bathing**

water quality monitoring, or water sports sites that are very popular in this region throughout the year.

Given that the DNA of *Ostreopsis* cf. *siamensis* has been detected in the environment at various points along the French Atlantic coast (Drouet, 2021) and that *O*. cf. *siamensis* and *O*. cf. *ovata* share the same ecological niche, the appearance of new sea flower sites cannot be ruled out, which should lead to the monitoring zone being extended to the entire Nouvelle-Aquitaine coastline in the coming years.

In addition to quality monitoring of bathing water and at water sports sites, the occurrence of any of the situations listed below, within or outside the areas concerned by this monitoring, should lead the Regional Health Agency (ARS) to take samples to confirm the presence of *Ostreopsis* in the water and on macrophytes:

- visual observation of “sea flowers”;
- reported cases of human poisoning and/or a metallic taste in the mouth, without the person necessarily having come into contact with water.

Implementation period for *Ostreopsis* monitoring

In the Mediterranean, it is currently recommended that *Ostreopsis* monitoring take place during the usual bathing water quality monitoring period, i.e. 15 June to 15 September. However, the *Ostreopsis* WG recommends that earlier monitoring be carried out along the Basque coast, depending on weather conditions. In the Mediterranean, it has been found that the date on which *Ostreopsis* blooms begin depends on the sum of anomalies in sea surface temperatures in springtime (the warmer the spring, the warmer the surface water temperature and the earlier the *Ostreopsis* blooms – Drouet *et al*., 2022).

Pending the availability of similar data for the Basque coast, the *Ostreopsis* WG recommends beginning water quality monitoring for *Ostreopsis* from 15 May. The sampling frequency should be adapted according to the abundance of *Ostreopsis* measured, following the flow chart (see Section 3.5) proposed by the WG.

***Ostreopsis* sampling strategy for water quality monitoring**

Given that *Ostreopsis* blooms initially develop in the benthic compartment before entering the water column, **the *Ostreopsis* WG recommends monitoring *Ostreopsis* abundance on macrophytes, in addition to the assessment of *Ostreopsis* abundance in water carried out during bathing water quality monitoring**.

Moreover, major diurnal variations in cellular abundance have been demonstrated around the Mediterranean, both in the benthic and planktonic compartments and at the surface of the water column. Cellular abundance is highest in the benthic compartment in the morning, and in the planktonic compartment and the “sea flowers” in the late afternoon. In 2022, similar variations were observed on the Basque coast. However, **pending confirmation of the same "water column-benthic substrate" dynamics on the Basque coast, the *Ostreopsis* WG recommends prioritising sampling in the late afternoon, in order to optimise the estimation of plankton abundance**. If a similar vertical dynamic is confirmed, the *Ostreopsis* WG recommends, as far as possible, that samples be taken from both compartments (planktonic and benthic) during the time periods corresponding to maximum cellular

abundance (in the Mediterranean: 8am to 10am for the benthic compartment, and 4pm to 6pm for the planktonic compartment).

Sampling and storage of planktonic *Ostreopsis* cells and "sea flowers"

In the absence of a standardised method for sampling *Ostreopsis* cells in the planktonic compartment, and with a view to harmonising sampling practices, the *Ostreopsis* WG recommends taking a composite sample in the bathing area and at sites used for water-based activities. For an area with a depth of about one metre, this sample corresponds to a mixture of water samples taken at three points, evenly distributed throughout the area being monitored. At each sampling point, samples must be taken between the surface and a depth of 20 to 50 cm. The three samples should then be mixed, in equal volumes, in a bucket or other similar container (previously rinsed three times with water from the environment) from which the final sample to be analysed will be taken (just after mixing). This sample will be used to identify and count *Ostreopsis* cells.

To optimise and harmonise sampling practices, the *Ostreopsis* WG recommends the use of plastic bottles with a minimum capacity of 250 mL to collect the samples intended for identifying and counting *Ostreopsis* cells. They should be two-thirds full to ensure thorough mixing by shaking. The sample should then be fixed with acid Lugol (1% volume/volume) within one hour of collection, and stored in an isothermal container in the dark at room temperature until analysis.

"Sea flowers" can accumulate at varying distances from areas of benthic *Ostreopsis* production, depending on the direction and speed of currents and winds. They should therefore be sought preferentially in areas where waste or planktonic marine organisms (such as jellyfish) are often found in large quantities, under the effect of these same winds and currents. No method has so far been developed to estimate the surface area impacted, or the number and size of aggregates. If "sea flowers" are found, even if they are a long way from a bathing site, water sports activities site or shellfish-farming areas, the *Ostreopsis* WG recommends sampling these aggregates with the surrounding water using a plastic bag or a wide-mouthed plastic bottle to facilitate collection. The sample should be fixed with acid Lugol (1% v/v) and then stored in an isothermal container in the dark at room temperature pending analysis.

Collection and storage of benthic *Ostreopsis* cells

As with the planktonic compartment, there is no standardised method for collecting *Ostreopsis* cells from benthic substrates, nor is there any approach for estimating the extent of the area covered by *Ostreopsis* biofilms on the various substrates at the sites being monitored. However, the most commonly used method of counting benthic *Ostreopsis* cells involves counting the dinoflagellates present on the macrophytes and relating them to a unit of fresh weight of sampled macrophytes.

As with the sampling of cells in the planktonic compartment, the *Ostreopsis* WG recommends taking three samples of *Ostreopsis* cells from substrates located at a depth of 50 cm and evenly distributed throughout the area subject to quality monitoring. The macrophytes should be sampled with the surrounding seawater in a 250-mL plastic bottle, bearing in mind that it is recommended to collect between 5 and 10 g (wet mass) of macrophytes per sample. The samples (water + macrophyte) should be fixed in situ with acid Lugol (1% volume/volume) and stored in an isothermal container in the dark at room temperature. *Ostreopsis* cells should be

counted on each of the three samples, and the highest abundance value should be used for quality monitoring.

The *Ostreopsis* WG recommends that the results from the identification and counting of *Ostreopsis* cells in the collected samples be reported within a maximum of 48 hours to facilitate early management of "sea flowers".

Identification and enumeration of *Ostreopsis* cells

The *Ostreopsis* WG recommends carrying out *Ostreopsis* cell counts in samples taken from the benthic and planktonic compartments using the Utermöhl method, with an inverted microscope, or using calibrated 1 mL slides (e.g. Sedgewick Rafter counting slides) when the first method is no longer suitable due to high cellular abundance. Observation on calibrated 1 mL slides is generally through an upright microscope, but an inverted microscope can also be used. For both methods, a minimum of 200 to 400 *Ostreopsis* cells should be enumerated for this count to have a confidence interval of 14% and 10%, respectively.

The genus *Ostreopsis* can be recognised using light microscopy, from the lens shape of the cells. However, this method is unable to differentiate between the species *O*. cf. *ovata* and *O*. cf. *siamensis*.

Given the lack of a reliable and rapid method for distinguishing between *O*. cf. *ovata* and *O.* cf. *siamensis*, the *Ostreopsis* WG recommends limiting identification to the genus level of *Ostreopsis* cells in the various compartments (benthic, planktonic, "sea flowers").

Identifying *Ostreopsis* cells at the genus level and counting them are complicated steps requiring qualified personnel. The *Ostreopsis* WG therefore recommends that laboratory operators be trained to easily identify and count *Ostreopsis* cells.

3.2.2. Monitoring of *Ostreopsis* in aerosols

Given the lack of sufficient knowledge about the type of toxic compounds and the processes responsible for their transfer to the atmosphere, the *Ostreopsis* WG experts do not recommend introducing quality monitoring of aerosols for the time being.

3.2.3. Monitoring of seafood products

Faced with the risk of new sites being colonised by *Ostreopsis* on the Nouvelle-Aquitaine coastline, the *Ostreopsis* WG recommends without delay selecting one or more shellfish-farming and shellfish-harvesting sites on the Atlantic coast where contamination could potentially occur, to be included in the network for monitoring the emergence of marine biotoxins in shellfish (EMERGTOX).

In addition, the *Ostreopsis* WG recommends implementing specifications at national level describing the strategy for sampling shellfish in shellfish-farming areas affected by an *Ostreopsis* bloom, as well as the analytical approach to be used to identify and quantify the toxins involved. This will provide a decision-support tool for managers, as is the case for toxins that are already covered by a recommendation (REPHYTOX prescriptions).

In addition to monitoring bivalve molluscs, it will be necessary to assess the contamination of other seafood products collected in an area affected by an *Ostreopsis* bloom (cephalopods, crustaceans, gastropods, sea urchins, fish, seaweed intended for human consumption). The

possibility of using sea urchins (gonads and remaining flesh) as sentinel organisms for detecting contamination of seafood products by PLTX group toxins should be studied.

3.2.4. Proposed guideline value for toxins produced by *Ostreopsis*

Given the lack of knowledge about the compound(s) responsible for human poisonings reported during *Ostreopsis* bloom episodes on the Basque coast, the *Ostreopsis* WG is not proposing a guideline value for the toxins produced by *Ostreopsis* in water or aerosols.

For shellfish, based on the acute oral TRV of 0.08 µg PLTX.kg^{-1} bw (ANSES, 2023), a default serving size of 400 g of bivalve mollusc meat (EFSA, 2010) and a default body weight of 70 kg, the *Ostreopsis* WG and the CES ERCA recommend **a guideline value of 15 µg eq. PLTX.kg^{-1} of shellfish meat** (whole body or in any edible part separately), **for the sum of PLTX + OVTX + OST-D** (unweighted mass sum).

If this guideline value is exceeded in shellfish, the *Ostreopsis* WG recommends:

- avoiding consumption of shellfish from the area affected by the bloom (both professional and recreational harvesting);
- acquiring additional data on shellfish contamination in the area affected by the bloom (for the species in which the values were exceeded and for other species harvested and/or consumed in the area).

For seafood products other than shellfish collected in an area impacted by an *Ostreopsis* bloom (cephalopods, crustaceans, sea urchins, fish, seaweed intended for human consumption) and given the current state of knowledge, the *Ostreopsis* WG and the CES ERCA are not recommending a guideline value for PLTX group toxins. The *Ostreopsis* WG draws attention to the need to acquire contamination data in France in order to determine whether a guideline value should be proposed in the future. In addition, during *Ostreopsis* bloom episodes, **the *Ostreopsis* WG recommends that fish caught in the area affected by the bloom be systematically eviscerated before consumption and before freezing**. **Small fish should not be eaten whole.**

For sea urchins, PLTX group toxins are also found preferentially in the viscera, which are not normally consumed. The *Ostreopsis* WG recommends informing consumers of sea urchins that they should not eat the viscera and should comply with the summer fishing ban when it is in place.

Lastly, in areas affected by an *Ostreopsis* bloom, the *Ostreopsis* WG recommends not harvesting macroalgae for the duration of the episode.

3.3. Recommendations for toxin detection and quantification

There are numerous protocols in the literature for the extraction and analysis of PLTX group toxins, but none are standardised, whether in microalgae cells, seawater, aerosols or marine organisms. The analytical approaches used are chemical or biological. The most suitable methods for analysing the toxins produced by *Ostreopsis*, depending on their performance and specificity, are LC-MS/MS[6] and bioassays: immunological tests (ELISA) or cellular (Neuro-2a

[6] Liquid chromatography coupled with tandem mass spectrometry

and haemolytic) tests. If the targeted LC-MS/MS can determine the toxin profile of the extract analysed, it could be used in conjunction with one of the three bioassays, since they provide insights into the diversity of toxins present, even in the absence of standards. The results are then expressed in eq. PLTX.

For toxins that do not belong to the PLTX group, the *Ostreopsis* WG has not identified an analytical method that can be used at present.

For shellfish, **the *Ostreopsis* WG recommends continuing to optimise targeted chemical analysis methods such as LC-MS/MS coupled with detection by molecular absorption spectrometry** (in the ultraviolet, UV) by including all the PLTX group toxins in addition to PLTX and, in the absence of a standard, focusing on their molecular masses and UV absorption maxima. **The method developed should be sensitive enough to be consistent with the proposed guideline value for shellfish (15 µg eq. PLTX.kg^{-1}).**

The *Ostreopsis* WG also recommends developing a bioassay to screen for PLTX group toxins not covered by targeted LC-MS/MS analysis. As soon as standards for the PLTX group toxins become available, this pair of approaches will need to be validated before they can be considered for standardisation.

In the event of a discrepancy between the results of this pair of approaches (underestimation by the targeted chemical analysis), the *Ostreopsis* WG recommends subsequently using non-targeted analysis by high-resolution mass spectrometry (LC-HRMS) in order to acquire physico-chemical information on any new metabolites not detected by the targeted analysis but taken into account by the bioassay (empirical formula, spectral data, etc.).

3.4. Recommendations for limiting exposure of professionals and local residents on the Basque coast

Professionals – whether they are lifeguards, staff supervising leisure activities, people tasked with sampling (hereinafter referred to as samplers) or shopkeepers and restaurant workers operating along the beach – are particularly affected by the presence of *Ostreopsis* blooms. An analysis of cases of poisoning among professionals on the Basque coast during the summers of 2021 and 2022 suggested that signs and symptoms became more aggravated and longer-lasting from one year to the next. In Spain and France, cases of poisoning have also been reported among local residents exposed to aerosols during *Ostreopsis* bloom episodes (Berdalet *et al*., 2022).

The *Ostreopsis* WG stresses the importance of disseminating information to professionals, starting from the 2023 bathing season, about the risks associated with *Ostreopsis* blooms, and recommending specific, appropriate preventive measures (e.g. frequent hand washing after sampling, showering, cleaning and covering wounds, etc.). The WG also recommends specific medical monitoring for acute or repeated exposure among local residents and professionals (whether permanent or seasonal staff) working on or in the immediate vicinity of sites affected by *Ostreopsis* blooms. Self-employed workers can request a medical consultation from their health insurance fund. In addition, a seasonal review of poisoning cases among people living near sites affected by *Ostreopsis* blooms would provide more insights into their exposure.

Furthermore, in order to draw up an action and prevention plan for professionals with regard to *Ostreopsis* exposure, the *Ostreopsis* WG also recommends carrying out a risk assessment for each potentially exposed job (obligation defined by Article L4121-2 of the French Labour

Code). For the risk specifically associated with the inhalation of aerosols, the daily exposure of professionals stationed on or near a contaminated beach could be monitored with the sampling methods used to collect aerosols containing brevetoxins (ANSES, 2021). These data could eventually be used to define toxicity thresholds for aerosols produced during *Ostreopsis* blooms.

Lastly, given the possibility of *Ostreopsis* blooms spreading to shellfish-farming areas on the Nouvelle-Aquitaine coast, the *Ostreopsis* WG recommends immediately communicating information messages on the risks associated with *Ostreopsis* blooms to professionals in the shellfish-farming sector (fishers, workers at shellfish-farming or fishing sites), as well as to seaweed farmers.

Pending the results of the studies advocated above, and in order to limit the exposure of professionals, the *Ostreopsis* WG points out that according to Article L. 230-2 of the French Labour Code,"*the employer shall take the necessary measures to ensure the safety and protect the health of workers in the establishment, including temporary workers*". To this end, the *Ostreopsis* WG proposes introducing specific preventive measures and making specific personal protective equipment available to workers, depending on the tasks to be carried out. In particular, samplers are recommended to wear gloves, appropriate footwear and a diving suit. The *Ostreopsis* WG also recommends that protective equipment be rinsed with fresh water after the sampling session, and that samplers take a shower with soap and fresh water after taking samples.

In addition, the *Ostreopsis* WG **recommends adapting working hours for jobs most exposed to aerosols, as soon as an *Ostreopsis* bloom is reported**, **in order to limit the duration of exposure**.

Furthermore, in areas affected by *Ostreopsis* blooms, the *Ostreopsis* WG recommends that occupational health physicians be made aware of the risks associated with acute or repeated exposure to *Ostreopsis*.

3.5. Decision-support tool for managing *Ostreopsis* blooms on the Basque coast

To help local authorities affected by *Ostreopsis* blooms, the *Ostreopsis* WG is proposing a surveillance and quality monitoring strategy (Figure 1) based on collaboration between site managers and the regional health agencies (ARSs), **applicable to sites currently subject to bathing water quality monitoring and water sports sites that meet the conditions below**.

Firstly, the *Ostreopsis* WG recommends that quality monitoring be carried out on sites known to have *Ostreopsis* blooms. This monitoring should be carried out at least during the summer period (15 May to 15 September) and be based on sampling of water, macrophytes and “sea flowers”, if present.

Alongside this quality monitoring, even outside the summer period, any case of *Ostreopsis* poisoning reported by a medical facility should trigger sampling of water, macrophytes and “sea flowers”, if present, for analysis.

The *Ostreopsis* WG recommends daily visual monitoring of the site from late spring onwards, in order to detect the presence of “sea flowers” or changes in water colour in real time. Any

metallic taste in the mouth noted during this monitoring will also be a potential indicator of the presence of an *Ostreopsis* bloom. These phenomena should trigger sampling of water and macrophytes for analysis. This sensory monitoring could be carried out by professionals working on the coast or as part of organised participatory monitoring programmes.

If *Ostreopsis* is present in at least one of the compartments (benthic or planktonic), a cell count should be carried out. The results of the *Ostreopsis* cell count determine three different alert levels. The cellular abundance thresholds chosen to define these alert levels (for the planktonic compartment) are the same as those currently applied on the Mediterranean coast, whether in France, Italy or Spain.

Alert 1 is triggered when the abundance of *Ostreopsis* cells is less than 30,000 cells.L^{-1} in water and less than 200,000 cells.g^{-1} fresh weight (FW) on macrophytes, and/or when a case of human poisoning is reported. With this alert level, activities can continue as normal. Samples are taken twice a month as part of bathing water quality monitoring. However, if the thresholds of 10,000 cells.L^{-1} in water or 20,000 cells.g^{-1} FW on macrophytes are exceeded (while being less than 30,000 cells.L^{-1} and 200,000 cells.g^{-1}, respectively), it is advisable to inform the site manager of a risk of blooms and repeat the sampling at a weekly frequency for new counts.

Alert 2 is triggered when the abundance of *Ostreopsis* cells is between 30,000 and 100,000 cells.L^{-1} in water or between 200,000 and 500,000 cells.g^{-1} FW on macrophytes, and/or when between two and nine human poisoning cases are reported on the same site over a 48-hour period. This alert level leads to implementation of the following management measures:

- The public is informed of the hazard, with particular emphasis on at-risk populations (people with a history of respiratory problems);
- Bathing and/or leisure activities on or near the contaminated site are not recommended for at-risk populations;
- The work stations of professionals working on or near the beach must be adapted in line with occupational health recommendations.

At this alert level, water and macrophyte samples must be taken twice weekly to monitor the bloom dynamics.

Alert 3 is triggered when the abundance of *Ostreopsis* cells exceeds 100,000 cells.L^{-1} in water or 500,000 cells.g^{-1} FW on macrophytes, and/or when 10 or more human poisoning cases are reported on the same site over a 48-hour period. In addition to the recommendations made under alert level 2 in terms of informing and protecting the public and professionals, alert level 3 leads to the closure of the beach and a ban on leisure activities on or near the beach where *Ostreopsis* blooms have been observed.

At this alert level, daily sampling of water and macrophytes are used to monitor development of the bloom, and the beach can be reopened and water-based activities resumed as soon as *Ostreopsis* cellular abundance is below the thresholds set for Alert 2.

The prevention messages to be put in place from Alert 2 onwards should recommend taking a shower in fresh water after bathing or any other water-based activity, and seeking a medical consultation if suspicious clinical signs appear following exposure to seawater or aerosols. These messages should encourage at-risk populations (people with a history of respiratory problems) to seek advice from a healthcare professional and limit their potential exposure to *Ostreopsis* by avoiding the affected areas.

Regarding the Nouvelle-Aquitaine coastline, the *Ostreopsis* WG also recommends continuing the case reporting procedure established by the Bordeaux poison control and monitoring centre (CAP-TV) and the Nouvelle-Aquitaine regional health authority (ARS) for the 2022 season. Lifeguard stations could also be involved in collecting reports of poisoning, according to arrangements to be defined with the Bordeaux CAP-TV.

The *Ostreopsis* WG reiterates that the cellular abundance thresholds chosen to define the alert levels are the same as those currently applied on the Mediterranean coast for the planktonic compartment, whether in France, Italy or Spain. The alert thresholds for the abundance of *Ostreopsis* cells in the benthic compartment were defined on the basis of feedback from monitoring carried out on the Mediterranean coast. They can be reassessed for the Basque coast at the end of the coming summer seasons.

Lastly, the *Ostreopsis* WG recommends collecting meteorological and oceanographic data (on temperature, wind speed, hydrodynamics, etc.) with effect from the 2023 summer season, to help optimise the monitoring strategy in subsequent seasons.

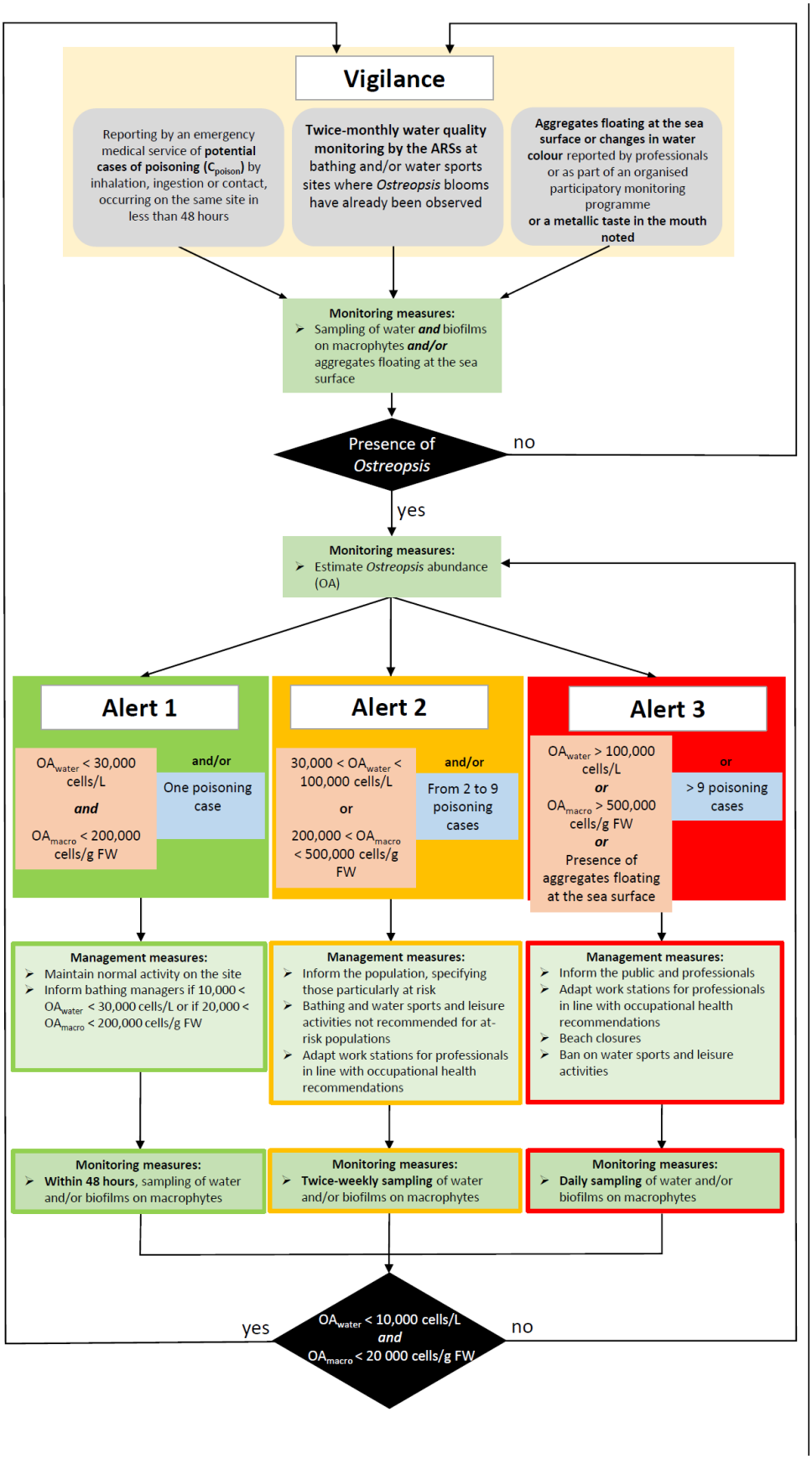

Figure 1: Decision tree for surveillance and quality monitoring of bathing sites and water sports activity sites affected by *Ostreopsis* blooms

3.6. Research needs

Knowledge about the genus *Ostreopsis* in temperate zones is still very fragmentary, due to the relatively recent expansion of its range in these zones. There is therefore a great need for research into the diversity, biology, physiology and ecology of this genus, as well as into the diversity of the toxins, their structures and their toxicity by different routes of exposure. The health impacts on populations also call for significant research into the short-, medium- and long-term effects of these compounds, according to the different routes of exposure. Details of the research needs expressed by the *Ostreopsis* WG are provided in Annex 3.

4. AGENCY'S CONCLUSIONS

In France, the presence of marine microalgae of the genus *Ostreopsis* has been identified repeatedly on the Mediterranean coast for several years now. Because of changing climate conditions, the areas in which *Ostreopsis* is found are expanding. During the summers of 2021 and 2022, major *Ostreopsis* bloom episodes were reported on the Basque coast. These microalgae can cause poisoning in humans when people are exposed to the cells or to the toxins they produce. The epidemiological report produced by the Bordeaux CAP-TV in 2021 and 2022 on 777 people poisoned during the Basque episodes showed that the main route of exposure to *Ostreopsis* was inhalation of contaminated marine aerosols, although other routes are possible (dermal contact, eye contact, ingestion of water). With regard to the dietary route, rare cases of human poisoning have been reported in the literature related to the consumption of seafood contaminated with palytoxin group toxins, but the link remains suspected and unproven to date.

The French Agency for Food, Environmental and Occupational Health & Safety endorses the experts' conclusions.

The literature review conducted as part of this expert appraisal revealed that knowledge about the genus *Ostreopsis* (diversity, biology, ecology, toxins produced) is still too fragmentary to characterise the hazard and risk to human health. It is not yet possible to determine whether the reported health effects are attributable to *Ostreopsis* cells, cell debris, the toxins produced or other compounds. The Agency therefore stresses the need to continue research on *Ostreopsis* cells and in particular to determine the toxic compounds in water, aerosols and seafood responsible for the symptoms observed in humans. In addition, the Agency encourages studies that will shed light on the vertical migration mechanism of *Ostreopsis* cells, which occurs during the day between benthic substrates and the water column. To do this, scientists will be able to use the ocean-climate database built by Ifremer and the Basque Coast Scientific Interest Group (GIS).

Pending the results of this work, and in order to support the health authorities and local authorities affected by *Ostreopsis* blooms, the Agency is proposing a graduated strategy for monitoring, official control and management of sites used for bathing and water sports activities. This strategy, based on collaboration between site managers and the regional health agencies (ARSs), is summarised in the form of a decision tree presented in this opinion (see

Figure 1, page 19). To support deployment of this strategy, the Agency is also underlining the experts' recommendations concerning the development and standardisation of analytical detection and monitoring methods.

Structured around three levels of alert for graduated monitoring and management measures at the sites affected by *Ostreopsis* blooms, this strategy draws on the scientific and management knowledge acquired around the Mediterranean over the last 15 years or so. However, as the topography, hydrodynamics and meteorological conditions of the Nouvelle-Aquitaine coast differ from those of the Mediterranean, the Agency stresses that the proposed management measures will need to be reassessed depending on the results of the monitoring data that will be collected over the next two bathing seasons, and adjusted as necessary.

In addition, analysis of the poisoning cases reported on the Basque coast showed that professionals are particularly affected by *Ostreopsis* blooms; the severity of their symptoms is no different to those developed by the general population, but these symptoms appear to be more numerous and of longer duration, even outside the bloom period. The Agency stresses the importance of disseminating messages to the professionals concerned on prevention of the risks associated with *Ostreopsis* blooms, adapting working hours, and setting up specific medical monitoring for these populations, to be defined jointly by occupational health services, with the support of poison control and monitoring centres in conjunction with employers.

Lastly, the Agency believes that given the emergence of these *Ostreopsis* bloom phenomena on the Basque coast and with a view to adapting preventive measures, it would be worth documenting the level of perception of the hazards associated with *Ostreopsis* blooms by the different categories of sea and beach users, as well as the understanding (and/or acceptability) of the measures taken to protect people from these hazards.

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Ostreopsis, *dinoflagellates, emerging toxins, marine biotoxins, shellfish, bathing water, seawater*

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ANNEX 1

Presentation of the participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

Working Group

Chair

Mr Rodolphe LEMÉE – Professor at Sorbonne University, Director of the Villefranche Oceanography Laboratory (LOV, UMR 7093 CNRS-Sorbonne University) – Expertise in toxic and harmful microalgae, *Ostreopsis*

Vice-Chair

Mr Jean-François HUMBERT – Research Director, Institute of Ecology and Environmental Sciences of Paris (iEES), National Research Institute for Agriculture, Food and the Environment (INRAE), Paris – Expertise in water microbiology, cyanobacteria, microbial ecology

Members

Mr Eric ABADIE – Researcher, Ifremer/BIODIVENV, Le Robert, Martinique – Expertise in dinoflagellate ecophysiology and ecology, marine biotoxins, phytoplankton and phycotoxin monitoring and observation network

Mr Zouher AMZIL – Researcher, Ifremer, Nantes – Expertise in toxic and harmful microalgae, chemistry of marine phycotoxins & cyanotoxins – National coordinator of the network for monitoring the emergence of marine biotoxins in shellfish (EMERGTOX)

Ms Elisa BERDALET – Researcher at the ICM-CSIC (Institute for Marine Science, Spanish National Research Council), Doctor, Barcelona, Spain – Expertise in toxic and harmful microalgae, *Ostreopsis*

Mr Ronel BIRÉ – Project leader at ANSES's Maisons-Alfort Laboratory for Food Safety, Pesticides and Marine Biotoxins Unit (UPBM) – Expertise in the analytical chemistry of marine biotoxins

Ms Marie-Yasmine DECHRAOUI BOTTEIN – University Professor, University of Côte d'Azur, ECOSEAS laboratory (Ecology and Conservation Science for Sustainable Seas) – Expertise in marine biotoxin toxicology, analytical methods, ecotoxicology

Mr Luc DE HARO – Clinical Toxicologist, Hospital Practitioner, Sainte Marguerite Hospital, Marseille poison control centre – Expertise in the clinical toxicology of marine biotoxins

Mr Nicolas DELCOURT – Lecturer, Hospital Practitioner, Poison Control and Monitoring Centre, Purpan University Hospital, University of Toulouse – Expertise in biochemistry and clinical toxicology, neurobiology

Ms Valérie FESSARD – Head of Unit, ANSES Fougères Laboratory – Expertise in toxicology

Mr Jean-Philippe JAEG – Lecturer, National Veterinary School of Toulouse – Expertise in toxicology, veterinary clinical toxicology and food chemical safety

Mr César MATTEI – Lecturer, University of Angers – Expertise in toxin toxicology, nervous system, receptors, neurotoxicity, ion channels

Ms Anne OPPLIGER – Head of Research at the Lausanne University Centre for General Medicine and Public Health (Unisanté) – Expertise in aerosols

Ms Anne-Sophie PAVAUX – Post-doctoral Researcher at the Process and Materials Engineering Laboratory (LGPM) at CentraleSupélec – Expertise in marine chemical ecology, *Ostreopsis*

Ms Eva TERNON – Post-doctoral Researcher until October 2022 at the Villefranche Oceanography Laboratory (LOV, UMR 7093 CNRS-Sorbonne University) – Expertise in *Ostreopsis* chemistry (particulate, dissolved, aerosols) and chemical ecology

- **Expert Committee (CES) on Water**

Chair

Mr Gilles BORNERT – Head of Department, Armed Forces Veterinary Group of Rennes – Microbiology, regulations, degraded situations, water defence

Vice-Chair

Mr Jean-François HUMBERT – Research Director – Institute of Ecology and Environmental Sciences of Paris (iEES), National Research Institute for Agriculture, Food and the Environment (INRAE), Paris – Water microbiology including cyanobacteria, microbial ecology

Ms Anne TOGOLA – Research Project Manager, French Bureau of Geological and Mining Research (BRGM) – Organic micropollutants, analytical chemistry, groundwater

Members

Mr Jean BARON – Research Engineer/Department Manager, Eau de Paris – Materials in contact with water, water treatment products and processes (treatment systems), corrosion

Mr Jean-Luc BOUDENNE – Professor, Aix-Marseille University, Environmental Chemistry Laboratory – Water metrology, chemistry and water quality

Mr Nicolas CIMETIÈRE – Lecturer, National School for Chemistry, Rennes (ENSCR) – Water analysis and treatment (DW, organic micropollutants)

Mr Bruno COULOMB – Lecturer, University of Aix-Marseille, Environmental Chemistry Laboratory – Chemical contaminants, analysis methods, fate of contaminants

Mr Christophe DAGOT – Professor/Department Director, University of Limoges, UMR Inserm 1092, RESINFIT – Antimicrobial resistance (integrons, process engineering), effluent quality (antibiotics and resistant bacteria)

Ms Sabine DENOOZ – Process and Water Quality Expert, Wallonia Water Company (SWDE) – Water treatment products and processes (DW), water safety management plans (PGSSE), technical expertise

Ms Isabelle DUBLINEAU – Project Officer for the Director of Human Radiological Protection / Doctor authorised to supervise research, IRSN, Fontenay-aux-Roses – Toxicology, radioelements

Mr Frédéric FEDER – Director of the "Recycling and Risk" Unit, French Agricultural Research Centre for International Development (CIRAD) – Geochemistry, water/soil/plant contaminant transfer, environmental risk assessment, water, soil and plant analyses, reuse of treated wastewater

Mr Matthieu FOURNIER – Lecturer, authorised to supervise research (HDR) in Geosciences, Rouen Normandy University – Hydrogeology, hydrology, DW, transfer and fate of micro-organisms in the environment, modelling, health risks

Mr Stéphane GARNAUD-CORBEL – Research Officer for "Water, biodiversity and urban development", French Biodiversity Agency (OFB) – Sanitation, integrated rainwater management, sludge treatment, use of non-conventional water sources

Ms Nathalie GARREC – Research and Expertise Engineer, French Scientific and Technical Centre for Building (CSTB) – Water microbiology, opportunistic pathogens, effectiveness of biocides

Mr Johnny GASPÉRI – Researcher, Gustave Eiffel University – Organic micropollutants, urban water, surface water, wastewater treatment

Mr Julio GONÇALVÈS – Professor, European Centre for Research and Teaching in Environmental Geoscience (Cerege), Aix en Provence – Hydrogeology, water resources, transfer of contaminants in groundwater, modelling, recharge

Mr Jean-Louis GONZALEZ – Researcher authorised to supervise research, French Research Institute for Exploitation of the Sea (Ifremer) – Marine environment, chemical contaminants, speciation, modelling, passive sampling

Mr Olivier HORNER – Professor, *École Polytechnique Féminine*, Paris – Water chemistry, water treatment

Mr Michel JOYEUX – Retired, Doctor of Medicine, Doctor of Science – Medicine, toxicology, quantitative health risk assessment, hazard analysis methods, water chemistry, DW treatment products and processes, environmental health

Mr Jérôme LABANOWSKI – CNRS Research Officer, University of Poitiers, UMR CNRS 7285 IC2MP, National Engineering School (ENSI) Poitiers – Effluent quality, river biofilms, sediments, fate of effluent-river contaminants

Ms Sophie LARDY-FONTAN – Director of the Nancy Laboratory for Hydrology – Metrology, analytical chemistry, micropollutants, ultratrace elements, quality assurance/quality control (QA/QC)

Ms Françoise LUCAS – Professor, University of Paris-Est Créteil – Virology, microbial ecology, indicators of faecal contamination, bacteriophages, mycobacteria, enteric viruses, wastewater and rainwater

Mr Christophe MECHOUK – Head of the "Studies and Construction" Division, Water Department of the City of Lausanne – Water engineering (drinking water, wastewater, process water, swimming pools), water treatment (processes), physical chemistry and microbiology of water, micropollutants

Mr Laurent MOULIN – Head of Research and Development Department, *Eau de Paris* – microbiology, virology, disinfection treatments, amoebae

Mr Damien MOULY – Epidemiologist, Unit Manager, in charge of monitoring waterborne outbreaks, *Santé Publique France* – Infectious risks, chemical risks, PGSSE, epidemiology, health risk assessment, exposure assessment, monitoring, alerts

Ms Fabienne PETIT – Professor, University of Rouen – Microbial ecology

Ms Catherine QUIBLIER – Professor, Paris Cité University, French Natural History Museum – Ecology of aquatic environments, ecology and toxicity of planktonic and benthic cyanobacteria, monitoring

Ms Pauline ROUSSEAU-GUEUTIN – Lecturer, French School of Public Health (EHESP) – Hydrogeology, hydrology, contaminant transfer, catchment protection areas, PGSSE

Ms Marie-Pierre SAUVANT-ROCHAT – Professor, Clermont-Auvergne University / School of Pharmacy – Public health and the environment, epidemiology, health risk assessment

Ms Michèle TREMBLAY – Doctor of Medicine specialising in community health / Medical advisor for occupational health and infectious diseases, Retired – Occupational health, microbiology of water

- **CES on Assessment of physical-chemical risks in food (CES ERCA 2022-2026)**

Chair

Mr Bruno LE BIZEC – University Professor – Expertise in analytical chemistry and risk assessment

Vice-Chair

Ms Marie-Louise SCIPPO – University Professor – Expertise in analytical chemistry and risk assessment

Members

Mr Claude ATGIE – University Professor – Expertise in toxicology

Mr Pierre-Marie BADOT – University Professor – Expertise in contaminant transfer

Ms Marie-Yasmine BOTTEIN – Researcher in Environmental Toxicology – Expertise in marine biotoxins

Ms Rachida CHEKRI – Laboratory Manager – Expertise in analytical chemistry

Mr Nicolas DELCOURT – University Lecturer, Hospital Pharmacist – Expertise in clinical toxicology

Ms Christine DEMEILLIERS – University Lecturer – Expertise in toxicology

Ms Virginie DESVIGNES – Research Engineer – Expertise in exposure and risk assessment

Mr Erwan ENGEL – Research Director – Expertise in analytical chemistry

Mr Gautier EPPE – University Professor – Expertise in analytical chemistry

Ms Anne-Sophie FICHEUX – Research engineer – Expertise in toxicology

Mr Eric HOUDEAU – Research Director – Expertise in toxicology

Mr Jean-Philippe JAEG – Lecturer – Expertise in toxicology and animal nutrition

Ms Emilie LANCE – University Lecturer – Expertise in ecotoxicology and cyanotoxins

Mr Olivier LAPREVOTE – University Professor and Hospital Practitioner – Expertise in toxicology

Mr Michel LAURENTIE – Research Director – Expertise in pharmacokinetics

Mr Ludovic LE HEGARAT – Deputy Head of Unit – Expertise in toxicology

Mr Jean-Charles LEBLANC – Head of Unit – Expertise in exposure and risk assessment

Mr Nicolas LOISEAU – Research Officer – Expertise in biochemistry

Mr David MAKOWSKI – Research Director – Expertise in statistics and modelling

Ms Francesca MANCINI – Research Officer – Expertise in epidemiology

Mr Eric MARCHIONI – University Professor – Expertise in analytical chemistry

Mr Jean-François MASFARAUD – University Lecturer – Expertise in contaminant transfer

Ms Mathilde MUNIER – Hospital Researcher – Expertise in toxicology

Ms Isabelle OSWALD – Research Director – Expertise in toxicology

Ms Anne PLATEL – University Lecturer – Expertise in toxicology

Mr Yann SIVRY – University Lecturer – Expertise in contaminant transfer

Ms Paule VASSEUR – Professor Emeritus – Expertise in toxicology

ANSES PARTICIPATION

Coordination and scientific contributions

Ms Carole CATASTINI – Scientific Expert Appraisal Coordinator, Water Risk Assessment Unit, Risk Assessment Department

Ms Nathalie ARNICH – Deputy Head of the Food Risk Assessment Unit, Risk Assessment Department

Ms Estelle CHECLAIR-WESTERBERG – Scientific Expert Appraisal Coordinator, Water Risk Assessment Unit, Risk Assessment Department

Ms Éléonore NEY – Head of the Water Risk Assessment Unit, Risk Assessment Department

Administrative secretariat

Ms Françoise LOURENCO – Expert Appraisal Support Department, Risk Assessment Department

HEARINGS WITH EXTERNAL PERSONS

Basque Coast GIS

Ms Aurélie BOCQUET-ESCOURROU – Scientific Coordinator of the Basque Coast GIS

Nouvelle-Aquitaine ARS

Thomas MARGUERON – Head of the Public Health and Environmental Health Unit

Ifremer

Ms Elvire ANTAJAN- Researcher in Zooplankton Ecology, Arcachon Environment & Resources Laboratory

Nouvelle-Aquitaine Poison Control and Monitoring Centre

Ms Magalie LABADIE – Doctor, Head of Department

Mr Camille PARADIS – Pharmacist

ANNEX 2

GOUVERNEMENT
Liberté
Égalité
Fraternité

2021-SA-0212

Ministère de l'Agriculture et de l'Alimentation
Direction générale de l'alimentation

Ministère des Solidarités et de la Santé
Direction générale de la santé

Nos réf. : D-21-029006

Paris, le - 3 DEC. 2021

Le directeur général de l'alimentation
Le directeur général de la santé

à

Monsieur le Directeur Général
de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

4 rue Pierre et Marie Curie
94701 Maisons-Alfort Cedex

Objet : Demande d'avis de l'Anses relatif aux risques liés aux efflorescences d'Ostreopsis spp. sur l'ensemble du littoral français

Durant le mois d'août 2021, plus de 600 personnes ont déclaré des symptômes d'intoxication après avoir fréquenté des plages de la côte basque. Ces intoxications ont été mises en relation avec une efflorescence d'un dinoflagellé phytoplanctonique du genre *Ostreopsis*. Cette microalgue benthique avait déjà été signalée dès septembre 2020 sur la côte basque.

A la suite d'un épisode d'efflorescence d'*Osteopsis* spp. dans l'eau de mer, l'exposition par voie respiratoire (contact avec les embruns lors de promenade, de surveillance des plages...), par contact cutané avec de l'eau de mer (baignade, activité nautique...) ou par ingestion (notamment par la consommation de produits de la mer contaminés par les toxines d'*Ostreopsis* spp.) est susceptible de provoquer des manifestations cliniques polymorphes telles que : toux, rhinorrhée, irritations de la sphère ORL et des yeux, céphalées, fièvre, difficultés respiratoires, nausées, vomissements, diarrhées, douleurs abdominales, myalgies, rougeurs, démangeaisons ou saignements de nez. Lors de ce dernier épisode, certains cas graves d'intoxications ont nécessité des hospitalisations.

L'augmentation brutale du nombre de cas enregistrés par le Centre antipoison et de toxicovigilance (CAP-TV) de Bordeaux en août 2021 a conduit à prendre des mesures de gestion immédiates sur les plages de la côte basque, alors même que l'espèce exacte d'*Ostreopsis* et la (les) toxine(s) incriminée(s) n'étaient pas encore identifiées : fermeture temporaire de plages (pendant quelques jours), information du public (notamment par le biais d'affichage et de communiqués de presse), sensibilisation et mobilisation du corps médical pour identifier les cas.

251 rue de Vaugirard – 75732 Paris cedex 15 – Tél. 01 49 55 49 55 – www.agriculture.gouv.fr
14 avenue Duquesne – 75350 Paris 07 SP - Tél. 01 40 56 60 00 - www.social-sante.gouv.fr

Le traitement de vos données est nécessaire à la gestion de votre demande et entre dans le cadre des missions confiées aux ministères sociaux.
Conformément au règlement général sur la protection des données (RGPD), vous pouvez exercer vos droits à l'adresse dgs-rgpd@sante.gouv.fr ou par voie postale.
Pour en savoir plus : https://solidarites-sante.gouv.fr/ministere/article/donnees-personnelles-et-cookies

Une efflorescence d'*Ostreopsis* de moindre amplitude avait déjà été signalée pendant l'été 2020 sur la côte basque. Aucun cas humain n'avait été rapporté. Il est donc possible que le phénomène émergent devienne récurrent et puisse s'amplifier. Par conséquent, l'expertise de l'Anses est rendue nécessaire par l'émergence de cette problématique sur la côte atlantique, alors qu'elle était jusque-là rencontrée sur le seul littoral méditerranéen, et par l'importance du nombre de cas signalés (le plus important de l'ensemble des épisodes rencontrés en France métropolitaine).

L'objectif est, d'une part, la mise à jour des données relatives aux microalgues du genre *Ostreopsis* et, d'autre part, l'établissement de recommandations spécifiques à la côte atlantique ainsi que, si cela s'avère opportun, la mise à jour des recommandations spécifiques à la côte méditerranéenne établies en 2007-2008. Enfin, cette saisine porte sur les risques sanitaires liés aux diverses voies d'exposition : par l'air (exposition aux embruns), par la consommation de produits de la mer contaminés et par les activités aquatiques, tout en prenant en considération les particularités liées à la protection des travailleurs concernés.

Contexte :

Les dinoflagellés du genre *Ostreopsis* sont connus en zones côtières tropicales pour être à l'origine d'intoxications alimentaires (souvent mortelles) suite à l'accumulation de la palytoxine et /ou de ses analogues (palytoxines-like) dans des crabes, des oursins ou des poissons. Probablement en lien avec le changement climatique, *Ostreopsis* spp. est de plus en plus présent en Méditerranée nord occidentale (Italie, Espagne, Grèce).

Avant septembre 2020, la présence de micro-algues du genre *Ostreopsis* n'était connue en métropole que sur la côte méditerranéenne. En 2006, année de la première prolifération d'*Ostreopsis sp* en Méditerranée, les signalements de cas d'irritation cutanéo-muqueuse chez des plongeurs avaient conduit à réaliser des analyses d'eau, révélant la présence *d'Ostreopsis ovata*. Cette espèce, alors émergente en Méditerranée, est capable de produire une toxine appartenant à la famille des « palytoxine-like ».

La palytoxine est une toxine très puissante produite par des coraux mous et par certaines espèces d'*Ostreopsis*. D'autres toxines analogues à la palytoxine mais moins toxiques sont également produites par des espèces d'*Ostreopsis*. Les « palytoxines-like » regroupent ainsi 8 analogues : la palytoxine au sens strict (*a priori* la plus toxique), l'ostreocine-D, l'ovatoxine-A, l'homopalytoxine, la bishomopalytoxine, la neopalytoxine, la deopalytoxine et la 42-hydroxypalytoxine.

Par ailleurs, les données acquises, durant les blooms estivaux d'*Ostreopsis* cf. *ovata* de 2008 et 2009, montrent qu'il existe un danger réel d'intoxication humaine suite à la mise en évidence d'une bio-accumulation des PLT-like (palytoxine + ovatoxine-a) aussi bien dans les bivalves filtreurs (moules immergées) que dans les échinodermes herbivores (oursins). La teneur totale en PLT-like accumulée dans les oursins a atteint 450 µg eq PLT/kg de chair totale (été 2008). Concernant les moules, la teneur maximale était de 230 µg éq PLT/kg (été 2009) pour un maximum de 360 µg trouvé dans les oursins durant la même période au niveau du même site.

La palytoxine s'accumulant potentiellement dans les produits de la mer, l'Agence française de sécurité sanitaire des aliments (devenue Anses) avait été saisie pour établir un bilan des connaissances réparti dans deux avis scientifiques et techniques :

- . L'avis scientifique et technique du 22/08/2007 (saisine n°2007-SA-0227[1]) apporte des bilans de connaissance relatives :
    - aux toxines produites par les microalgues du genre *Ostreopsis* (dont *ovata* et *siamensis*) ;
    - à la possibilité de transfert dans la chaîne alimentaire des toxines produites par les microalgues du genre *Ostreopsis* ;
    - aux méthodes d'analyse, biologiques et physicochimimiques, utilisables en routine ou en développement (recherche) ;
    - aux seuils de toxicité connus, et l'Afssa avait conclu qu'il n'en existait pas pour la palytoxine et les palytoxine-like.

[1] https://www.anses.fr/fr/system/files/RCCP2007sa0227.pdf

- L'avis du 11/07/2008 (saisine n°2007-SA-0303[2] liée à la saisine précédente) apporte les informations complémentaires relatives :
    - à des pistes d'amélioration du dispositif de surveillance d'*Ostreopsis* spp dans le milieu marin et dans les aliments ;
    - aux études nécessaires pour mieux caractériser le risque alimentaire.

Par ailleurs, l'Autorité européenne de sécurité des aliments (EFSA) a rendu en 2009 un avis sur les toxines de la famille des « palytoxine-like »[3]. L'avis souligne le manque de données de toxicité, mais propose une dose de toxicité aiguë (ARfD) de 0,2 µg/kg de poids corporel, ce qui correspond à un seuil dans l'aliment à ne pas dépasser de 30 µg/kg (somme de la palytoxine et de l'ostréocine-D).

A la lumière des avis de l'Afssa sus-visés et afin de prévenir la survenue de cas d'intoxication liés à la présence d'*Ostreopsis ovata* en Méditerranée, une note de service DGS[4] a été diffusée courant septembre 2010. Cette instruction propose des modalités de surveillance, de gestion des efflorescences et de communication. Toutefois, si elle cible l'ensemble des espèces du groupe *Ostreopsis*, elle ne porte que sur la problématique rencontrée en Méditerranée, rendant difficile son application lors de l'épidémie survenue sur la côte basque en août dernier. Une mise à jour de celle-ci pourrait donc s'avérer nécessaire, impliquant une actualisation des connaissances scientifiques relatives aux *Ostreopsis* spp et à leurs toxines.

Portée de la saisine :

Conformément à l'article R. 1313-1 du code de la santé publique, nous sollicitons donc l'Anses pour répondre aux questions suivantes :

- Quelles sont les nouvelles données scientifiques publiées depuis les précédents avis, relatives à la fois aux microalgues du genre *Ostreopsis*, et également aux toxines qu'elles produisent ? Quels sont les niveaux de toxicité connus pour les différentes toxines produites ? Quelles sont les conditions favorables pour que les microalgues du genre *Ostreopsis* libèrent des toxines ? L'intoxication humaine est-elle liée à une exposition directe aux toxines, ou survient-elle lors de l'exposition aux microalgues qui produisent ensuite les toxines ? Quels sont les risques pour les différents usagers de la mer selon les différentes voies d'exposition (alimentaire, activités aquatiques, embruns) ?
- Quelles sont les conditions environnementales favorables au développement des différentes espèces du genre *Ostreopsis* ? Quelle est la répartition et la dynamique des populations d'*Ostreopsis* sur les côtes françaises ? Est-il envisageable à court ou moyen terme d'établir une cartographie du risque d'efflorescence d'*Ostreopsis* spp. sur les côtes métropolitaines ? Un plan exploratoire est-il nécessaire pour établir cette cartographie ? Si oui, quelles devraient en être les lignes directrices ?
- Sur la base de l'expérience acquise en Méditerranée, quelles sont les préconisations en matière de méthodologie de prélèvement et d'analyse pour surveiller l'apparition d'*Ostreopsis* sur la côte atlantique ? Dans quelles conditions la recherche du paramètre « *Ostreopsis* spp. » doit-elle être intégrée au contrôle sanitaire des eaux de baignade ?
- Quel est le seuil de concentration d'*Ostreopsis* dans l'eau / de toxines dans l'air ou les aliments devant déclencher une alerte sur la côte atlantique ? Quelles sont les recommandations en matière de mesures de gestion en cas de dépassement de ces seuils ? Existe-t-il des espèces indicatrices, plus aisément identifiables en routine, susceptibles d'alerter sur la présence de microalgues du genre *Ostreopsis* ?
- Existe-t-il dans d'autres pays des méthodes de détection rapides qui pourraient être mises en œuvre pour identifier la toxine en cause lors d'une épidémie ?

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[2] https://www.anses.fr/fr/system/files/RCCP2007sa0303.pdf
[3] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.1393
[4] Note de service DGS/EA3/EA4 n° 2010-238 du 30 juin 2010 relative à la surveillance sanitaire et environnementale et aux modalités de gestion des risques sanitaires pour la saison balnéaire 2010, liés à la présence de la microalgue toxique *Ostreopsis* spp. dans les eaux de baignade en méditerranée et à la contamination par ses toxiques des produits de la mer issus de la pêche de loisir : https://solidarites-sante.gouv.fr/fichiers/bo/2010/10-08/ste_20100008_0100_0161.pdf

- En matière de sécurité des travailleurs, quelles sont les recommandations spécifiques pour éviter une exposition chronique en cas d'efflorescence ?

Délai souhaité :

Afin d'anticiper au mieux les risques potentiels d'intoxication au cours de l'été 2022 et en l'absence de recommandations existantes pour la côte atlantique, une réponse est souhaitée au plus tard le 1/04/2022. L'agence demeure libre de répartir les différentes questions sous plusieurs numéros de saisine, et pourra en particulier rendre son avis sur la mise à jour des recommandations pour la côte méditerranéenne dans un second temps.

Nos services se tiennent à votre disposition pour vous apporter toute information complémentaire.

Nous vous remercions de bien vouloir accuser réception de la présente demande en nous précisant le ou les comités d'experts spécialisés qui sont saisis du dossier.

Le directeur général
de l'alimentation

Bruno FERREIRA

BRUNO FERREIRA ID

Signature numérique de BRUNO FERREIRA ID
Date : 2021.12.03 09:11:27 +01'00'

Le directeur général
de la santé

Jérôme SALOMON

ANNEX 3 – LIST OF RESEARCH NEEDS

RESEARCH ON THE DIVERSITY, DISTRIBUTION, ECOLOGY AND MONITORING OF *OSTREOPSIS*

Improve knowledge about the diversity and biogeography of *Ostreopsis*

Some species of the genus *Ostreopsis* have not yet been described morphologically or genetically, while others have an uncertain taxonomic position. It is important to improve knowledge about the description of these taxa and their phylogeny, both in temperate and tropical zones. These studies will make it possible to better document the geographical range of the various species and how they may evolve in the future.

In metropolitan France, it is particularly important to:

- clarify the taxonomic status of *Ostreopsis* cf. *ovata* and *Ostreopsis* cf. *siamensis;*
- monitor changes in their range along the Atlantic coast at sentinel sites;
- confirm or rule out the absence of *O*. cf. *siamensis* on the French Mediterranean coast.

Improve knowledge about the ecology of *Ostreopsis*

It is also necessary to improve knowledge about:

- the factors and processes (climate change and other anthropogenic pressures) favouring *Ostreopsis* blooms in temperate zones;
- the spatial and temporal dynamics of the development of planktonic *Ostreopsis* cells and biofilms on substrates depending on abiotic and biotic factors and processes; regarding biofilms, particular attention should be paid to the phanerogams found on the Nouvelle-Aquitaine coast;
- the factors and processes that lead to the detachment of biofilms from their substrates;
- the production, composition and role of mucus in the proliferation of the two species of *Ostreopsis* found in France;
- the determinism of the vertical migrations of *Ostreopsis* cells that occur during the day between benthic substrates and the water column, using for example experimental approaches in micro- and mesocosms;
- competition between *O*. cf. *ovata* and *O*. cf. *siamensis* depending on environmental conditions, in order to predict which species will be favoured on the affected sites;
- the development of predictive models for *Ostreopsis* population dynamics.

Develop new strategies and tools for monitoring *Ostreopsis*

New approaches need to be developed and standardised to improve the monitoring of *Ostreopsis* blooms. For example, it would be worthwhile to work on:

- development and validation of a methodology for estimating (i) the coverage (percentage cover) of substrates by *Ostreopsis* biofilms at the scale of the sites studied, and (ii) the extent and density of *Ostreopsis* “sea flowers”;
- development and validation of molecular tools (e.g. quantitative PCR) for rapidly estimating the abundance of different *Ostreopsis* species in the planktonic and benthic compartments and at the surface of the water column (“sea flowers”);

- validation of sampling methods using natural or artificial substrates, and cell counting methods (e.g. cytometry);
- selection of the most effective method for sampling aerosols containing *Ostreopsis*;
- development of citizen science approaches involving professionals (fishers, beach attendants, lifeguards, etc.) and the general public, to monitor benthic populations and *Ostreopsis* "sea flowers".

RESEARCH ON THE TOXINS PRODUCED BY *OSTREOPSIS* AND THEIR IMPACT

Improve knowledge about the diversity and regulation of toxin production by *Ostreopsis*

To gain a better understanding of the toxic potential of *Ostreopsis*, it is necessary to:

- continue identifying and characterising all the toxins produced by species of the *Ostreopsis* genus and describe their biosynthesis pathways;
- determine whether there are genotypes in *Ostreopsis* populations that produce toxins and others that do not because they lack the genes for their biosynthesis. If both types of genotypes coexist in populations, it will be necessary to study the variations in their proportions and the factors and processes that influence these variations;
- determine the biotic and abiotic factors and processes that regulate the production and secretion of toxins by different *Ostreopsis* species.

Improve knowledge about toxin behaviour and transfer processes

- study the fate of toxins (stability, degradation, diffusion, transfer) in the various abiotic compartments (water, air and sediment, bearing in mind that there are currently no data on their possible presence in the latter compartment);
- study the potential transfer and bioavailability of these toxins in food webs;
- confirm the presence of extracellular toxins adsorbed to mucus (Ciminiello *et al.* 2006).

Develop new strategies and new tools for monitoring *Ostreopsis* toxins

To improve toxin monitoring and thus limit the exposure of human populations to these substances, studies should be carried out on:

- the development of rapid monitoring methods (e.g. ELISA) for PLTX group toxins at coastal sites favourable to *Ostreopsis* blooms;
- improvements in passive sampling techniques (e.g. SPATT) that can concentrate *in situ* toxins found at low concentrations in seawater, in order to obtain data on extracellular toxins in the natural environment;
- the development and validation of equipment and methods for trapping and then quantifying *Ostreopsis* toxins present in aerosols;
- screening for biomarkers, bioindicators or sentinel species that could enable early detection of *Ostreopsis* and/or its toxins.

Improve and develop methods for analysis in different compartments (microalgae, water, aerosols, seafood)

For all compartments, it is necessary to:

- optimise protocols for purifying PLTX group toxins;
- develop certified reference materials such as high-purity standards and matrix materials with known concentrations of PLTX group toxins;
- optimise, validate and standardise analytical methods for identifying and quantifying *Ostreopsis* toxins in different matrices (water, air, seafood);
- develop methods for detecting toxic compounds in real time.

Improve knowledge about toxins in aerosols and seafood products

For aerosols in particular, it will be necessary to:

- characterise the physico-chemical parameters of aerosols (concentration, particle size, chemical composition);
- study the factors (biotic and abiotic) that encourage aerosolisation of the toxic compounds;
- study the chemodiversity of aerosols, in particular the toxic compounds, and identify the compounds responsible for the symptoms observed;
- develop models of marine aerosol transport depending on various physical and biological parameters.

For the seafood products consumed, it is necessary to:

- develop high-volume culture processes to obtain the *Ostreopsis* cell biomass needed to study experimentally the kinetics of seafood contamination and decontamination, organotropism and the products of metabolism;
- study the link between seafood contamination and the dynamics of *Ostreopsis* blooms in the natural environment;
- determine the cause of the mortality of certain marine organisms associated with *Ostreopsis* blooms in the Mediterranean and on the Atlantic coast;
- acquire data on the contamination of seafood products by PLTX group toxins using robust methods and LOD/LOQs in line with the guideline value, in order to be able to estimate dietary exposure and characterise the risk;
- study the effects of cooking on PLTX group toxins in seafood products;
- conduct ecotoxicology studies to assess the impact of *Ostreopsis* blooms on marine organisms.

Improve knowledge about the toxicity of toxins produced by *Ostreopsis*

Toxin toxicity studies are limited by the availability of these compounds. The first priority will therefore be to purify these compounds and carry out *in silico* and/or *in vitro* studies requiring little or no materials. Further studies with PLTX, which is commercially available and used as a proxy, will enable the results to be extrapolated to other PLTX group toxins. For *Ostreopsis* toxins that are not in the PLTX group, no compounds have been identified that could be used as proxies.

In order to be able to carry out regulatory toxicity tests (mainly on animals), it is above all necessary to have sufficient quantities of purified toxins. Their very restricted availability will limit the studies that can be carried out.

The toxicity tests (if possible in accordance with OECD guidelines) required to conduct a health risk assessment concern:

- acute and repeated toxicity by inhalation;
- acute oral toxicity for OVTX-a and OST-D;
- repeated oral toxicity:
- eye and skin irritation and skin sensitisation using *in vitro* tests;
- *in vitro* genotoxicity and then *in vivo* if necessary;
- carcinogenesis with, for example, cell transformation assays for the tumour-promoting aspect;
- reprotoxicity;
- immunotoxicity;
- toxicity to development and neurodevelopment.

In order to determine the toxins' mechanisms of action, it is important to:

- determine the action of PLTX group toxins on Na/K-ATPase and compare their affinity with that of PLTX;
- determine the action of PLTX group toxins on other H^+-ATPase-type pumps;
- assess the toxic equivalency factors (TEFs) of PLTX group toxins;
- carry out additional *in vitro* and/or *in vivo* studies to determine the molecular targets and mechanisms of action of all the toxins produced by *Ostreopsis*, in order to predict the expected diseases according to the different routes of exposure.

In order to study the toxins' kinetics, it is necessary to:

- identify the metabolites formed and the enzymes involved, particularly in the liver, and assess the toxicity of the main metabolites;
- determine the kinetic parameters needed to build toxicokinetic models according to the different routes of exposure;
- assess the impact of digestion on toxin accessibility using *in vitro* digestion systems, which can also incorporate the microbiota, in particular to investigate the reasons for PLTX's lower oral toxicity compared with inhalation.

RESEARCH ON EXPOSURE AND THE EFFECTS OF *OSTREOPSIS* ON POPULATIONS

It will be necessary to:

- carry out studies on the severity and inter-annual variability of symptoms among professionals and local residents, which will make it possible to detect any cumulative effects or sensitisation of these populations;
- screen for biomarkers of exposure and effects of compounds produced by *Ostreopsis* in humans.

ANNEX 4 – CHANGES TO THE VERSION OF THE OPINION DATED 12 MAY 2023

Page number	Change made
2	The sentence: "The epidemiological report showed that almost 900 people have developed signs and symptoms associated with *Ostreopsis* blooms on the French Basque coast since 2020 (Paradis and Labadie 2022)." has been replaced by: "The epidemiological report showed that **674** people developed signs and symptoms associated with *Ostreopsis* blooms on the French Basque coast during the summer of 2021 (Paradis et Labadie 2022)."
4	The footnote indicating that the collective expert appraisal report would be published at a later date has been deleted. The words "dated June 2023" have been added.
4	The sentence: "This work was detailed in a specific opinion currently being finalised by the Agency (ANSES, 2023)." has been replaced by: "It was detailed in a specific opinion published by the Agency in June 2023 (ANSES, 2023)."
4	The sentence: The collective expert appraisal report and opinion produced by the *Ostreopsis* Working Group take into account the comments and additional information provided by the members of the CES on Water and the CES ERCA. has been replaced by: "The collective expert appraisal report and the "Analysis and conclusions" section produced by the experts of the *Ostreopsis* Working Group take into account the comments and additional information provided by the members of the CES on “Water” and the CES ERCA."
5	The sentence: "This revision is the result of editorial changes to assist publication of the report, which has now been finalised, and a change in the counting of cases. Annex 4 to this opinion sets out the changes made during this revision." has been added.
5	The sentence: "The report will be published after this opinion." has been deleted.
20	The sentence: "The epidemiological report produced by the Bordeaux CAP-TV on almost 900 people poisoned during the Basque episodes showed that

	the main route of exposure to *Ostreopsis* was inhalation of contaminated marine aerosols, although other routes are possible (dermal contact, eye contact, ingestion of water)." has been replaced by: "The epidemiological report produced by the Bordeaux CAP-TV in 2021 and 2022 on 777 people poisoned during the Basque episodes showed that the main route of exposure to *Ostreopsis* was inhalation of contaminated marine aerosols, although other routes are possible (dermal contact, eye contact, ingestion of water)."