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# Round Table 1 of the International Conference of *Ostreopsis* development: Secondary metabolites and toxicity of *Ostreopsis*

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The roundtable gathered the following speakers: Takeshi Yasumoto, Aurelia Tubaro, Ernesto Fattorusso, Ronel Biré, Pilar Riobó, and was moderated and synthesized by Gian Paolo Rossini.

The meeting organization of ICOD provided a list of nine questions to be addressed by the speakers during the roundtable. These questions have been approached sequentially, as reported below.

## 1) What are the different secondary metabolites in *Ostreopsis* species?

In a first instance, the term secondary metabolite has been intended to mean palytoxin-group compounds. Twelve distinct palytoxin-group compounds have been distinguished so far. These include palytoxin and ostreocins, ovatoxins, and mascarenotoxins. The pathway of palytoxin biosynthesis has not been fully characterized, and it is uncertain whether some of detected compounds might represent metabolic intermediates.

The possible existence of toxic secondary metabolites other than palytoxin-group compounds in *Ostreopsis* species has been raised and considered, in the light of the many algal strains which have been cultured in different labs. It was acknowledged that the existence of new toxins, including substances other than palytoxin-group compounds, may not be excluded at the moment. The search for new toxic compounds should continue, by the use of multiple means for the detection of noxious effects, including animal systems and batteries of *in vitro* tests.

## 2) Do we know exactly the structure and the toxicity of all those different secondary metabolites?

The compounds differ in the position and characteristics of chemical groups. The structures of some compounds have not been fully characterized. Small structural changes have been shown to affect cytotoxicity, but the toxicity of analogues isolated so far has not been completed in animal systems yet.

## 3) Do we find the same toxins (and the same concentrations of toxins) in each *Ostreopsis* species? Do we also observe difference in toxin content between strains of the same species?

Palytoxin is found primarily in *Palythoa* spp., ostreocins are found primarily in *Ostreopsis siamensis,* ovatoxins are found primarily in *Ostreopsis ovata*, and mascarenotoxins are found primarily in *Ostreopsis mascarenensis*. The

possibility that compounds are synthesized in one species (microalga) and accumulated in others (for instance, *Palythoa*), as well as the possible bacterial origin of palytoxin-group compounds remain to be fully clarified. Concentrations of different analogues in the same organism vary. For instance, *Ostreopsis ovata* isolated from Mediterranean coasts contains high levels of ovatoxin a, and other ovatoxins, whereas palytoxin is found only at much lower levels.

The toxin profiles of environmental samples of *Ostreopsis ovata* vary in different years and coastal areas.

**4) Is the allergic reaction due to thecal proteins and/or secondary metabolites?**

The allergic nature of effects recorded in some individuals which sought medical care at hospitals during the Genoa episode in 2005 remains uncertain. Analyses of aerosol during *Ostreopsis* blooms in Llavaneres (Spain) did not lead to detection of palytoxin-group compounds, although the presence of *Ostreopsis* was detected by PCR technique. Non-steroidal anti-inflammatory drugs have been used as a pharmacology support during toxic episodes. In the lack of additional information the term "irritative" reaction is proposed to indicate the recorded symptoms.

Existing data are not sufficient to establish whether palytoxin-group compounds or *Ostreopsis* algae/remnants represent the primary cause of recorded reactions.

**5) What are the official organizations that are in charge of poisoning potentially linked to *Ostreopsis*?**

In the EU, Regional authorities are often in charge of risk management.

Different routes of exposures may potentially be responsible for health problems (ingestion of seafood, possible inhalation of contaminated aerosol, direct skin contact in the waters containing toxic *Ostreopsis*), relating to areas of intervention which could be under the responsibility of different administrations/ organisms/institutions. Risk managers might consider the opportunity to coordinate and harmonize interventions, and develop alert systems for the dissemination of information regarding crisis involving palytoxin-group compounds, tailored for different kinds of possible exposures.

The speakers agree about the importance that risk assessment and risk management be tasks of separate bodies of experts.

**6) In which species and organs components are *Ostreopsis* secondary metabolites found? Do *Ostreopsis* metabolites transfer (or accumulate?) in food web?**

Palytoxin-group compounds are part of food chains, and can be accumulated in the flesh of some organisms (for instance, crabs). The presence of palytoxin group compounds in fish flesh is known only in a limited number of species (trigger fish, file fish). High concentrations of ovatoxins have been detected in mussels, in the digestive tube of sea urchins and of several fish. A detailed knowledge of distribution of palytoxin-group compounds in different organisms, and the tissue distribution of palytoxin-group compounds within the same organism, is lacking.

**7) What are the EU thresholds concerning the *Ostreopsis* secondary metabolites in food? What about other temperate areas?**

Good Practice Review (GPR) outlined the process of risk assessment at the basis of EFSA's opinion on palytoxin-group toxins. Based on the fact that there were no reliable quantitative data on acute toxicity of palytoxin-group toxins in humans, and the absence of long term toxicity studies, an acute reference dose (ARfD) using the lowest-observed adverse-effect-level (LOAEL) for oral toxicity (gavage) in mice was established in the EFSA's opinion. Uncertainty factors were used to calculate this ARfD. The human exposure was referred to shellfish consumption, based on assessments of human consumption in some EU Countries. The existing threshold (30 μg/kg shellfish meat) refers to the sum of palytoxin and ostreocin d, whose oral toxicity in mice have been already reported in peer-reviewed journals. The proposed ARfD could not take into account palytoxin-group compounds whose oral toxicity had not been established at that time.

The speakers remarked that the assessment was based on ingestion of contaminated shellfish without taking into account other seafood, and the opportunity that a threshold for ovatoxin is established. It was also outlined that the 400 g portion that has been taken into consideration in the EFSA risk assessment may not be relevant for all fishery products.

**8) Can we make recommendations to national and international Food Safety Agencies concerning seafood risk associated to *Ostreopsis* development?**

A threshold for ovatoxins, particularly ovatoxin a, representing the major toxin found in Mediterranean waters, should be considered.

The specific features of different seafood (for instance, mussels, sea urchins, or even fishes), whenever contaminated by palytoxin-group compounds, should be characterized. Likewise, the tissue/organ distribution of palytoxin-group compounds in different organisms should be ascertained. The issues related to human exposure, when eating different seafood, should be considered.

Data on repeated exposure (oral) should be gathered.

The differences in toxin profiles found in natural materials should be considered when establishing thresholds.

Different methods for the detection of palytoxin-group compounds in natural matrices have been considered. It has been recognized that in the past mouse bioassays have played an important role in the detection of toxins in contaminated seafood, and might contribute to identification of new toxins in the future. Furthermore, the advantages of instrumental methods for accurate, quantitative measurements of palytoxins in naturally contaminated materials have been highlighted.

The haemolytic assay can be used to detect palytoxin-group compounds for screening purposes, if appropriate controls are included. ELISA and sensor-based methods are under development by Aurelia Tubaro's group.

The importance of developing alternative biological methods, including *in vitro* functional and other bio-molecular assays, for specific and quantitative detection of new toxic compounds contaminating natural matrices relevant for human health, was stressed.

The availability of appropriate standards represents an absolute need for any kind of analytical assay.

The use of toxin equivalents (TE) is useful for monitoring seafood contaminations, and a system for defining TE of palytoxin-group compounds must be identified in different test systems.

**9) What are the research priorities in the near future concerning the toxicity of *Ostreopsis* metabolites?**

Several points were mentioned:

- Full characterization of the structures of already identified analogues. Identification of other analogues.
- Obtain pure standards of palytoxin-group compounds for toxicology and analytical purposes.
- Further develop instrumental methods for the detection of palytoxin-group compounds.
- Include major ovatoxins in the establishment of thresholds identify the reference compound of the group and define the system for the quantification of toxin equivalents.
- Further develop *in vitro* functional and other bio-molecular assays for specific and quantitative detection of palytoxin-group compounds contaminating natural matrices.
- Further clarify whether palytoxin-group compounds and/or remnants of *Ostreopsis* algae are present in the aerosol during algal blooms.
- Further clarify the actual biological origin of palytoxin-group compounds, and whether they might originate from bacteria contaminating *Ostreopsis* and *Palythoa* spp.
- Further characterize the edible seafood species that can be naturally contaminated by palytoxin-group compounds. Ascertain the distribution of palytoxin-group compounds in body districts of different organisms.
- Better characterization of human consumption of seafood found contaminated by palytoxin-group compounds. Evaluate the portion size for sea urchins, fish, and other relevant species.
- Establishing the toxicity of ovatoxin a and other palytoxin-group compounds in animal systems by different routes of exposure (oral, inhalation, cutaneous) is urgent.
- The characterization of toxicity of palytoxin-group compounds after repeated oral ingestion should be obtained.
- The toxicity of palytoxin-group compounds when present in association with other toxins should be studied at molecular level and *in vivo*.
- Develop a portfolio of alternative, cellular-based methods for identification of new toxins, in keeping with existing EC directives and the recent report "*Toxicity testing in the 21st century – A vision and a strategy*", issued by the US National Research Council.