Maisons-Alfort, 08 February 2008

STATEMENT

of the French Food Safety Agency (AFSSA) on the phycotoxin-related risk in shellfish other than live bivalve molluscs (gastropods, echinoderms and tunicates)

AFSSA (French Food Safety Agency) received a request from the DGAl (French Directorate General for Food) on 04 January 2007 for an opinion concerning evaluation of the phycotoxin-related risk in shellfish other than live bivalve molluscs (gastropods, echinoderms and tunicates). In agreement with the DGAl, this request was requalified as a request for scientific and technical support as a preliminary step in setting up the monitoring of such contaminations.

1. Background

EC regulation no. 853/2004 of 29 April 2004 setting specific rules for hygiene applicable to foodstuffs of animal origin, specifies in annex III section VII the specific requirements applicable to live bivalve molluscs. These conditions, except for those concerning microbiological purification, also apply to echinoderms, tunicates and live marine gastropods (referred to as “shellfish other than live bivalve molluscs”).

Among these conditions, an obligation exists for the competent authority to monitor levels of contamination by marine phycotoxins in all marketed shellfish to ensure that they comply with safety standards specified in annex III, section VII, chapter V of the aforementioned regulation (annex 1).

In this context, the Agency was asked to determine if gastropods, echinoderms and tunicates have the ability to accumulate regulated families of marine phycotoxins in quantities which can present a risk for human health.

2. Glossary/Terminology

Echinoderms: spiny, invertebrate marine animals, for example urchins (*Paracentrotus lividus*), holothuria, and starfish.

Marine Gastropods: invertebrate marine animals with a shell, for example whelks (also known as busycon whelks *Buccinum undatum*), winkles (*Littorina littorea*), abalone (*Haliotis tuberculata tuberculata*) and limpets (*Patella vulgata*).

Phycotoxins: toxins produced by marine phytoplankton, but also known as algal toxins or marine biotoxins.

Saxitoxin (STX): a toxin from the PSP family (paralytic shellfish poisonning), hyodrophilic type, including neo-saxitoxins (Neo-STX), decarbamoyl-saxitoxin (dcSTX), gonyautoxins (GTXs) and C-type toxins (C toxins). The quantity of different toxins in this family is expressed in saxitoxin-equivalents (eqSTX).

Tunicates: invertebrate marine animals, for example orange sheath tunicates (*Botrylloïdes violaceus*).

3. Introduction

The accumulation of phycotoxins in marine shellfish is a seasonal phenomenon which occurs in temperate latitudes and the majority of episodes of toxic contamination concerns filtering bivalve molluscs (oysters, mussels, scallops, etc). As a result of their method of feeding involving filtration of water with ingestion of microorganisms in suspension, bivalves can quickly be contaminated with phycotoxins, which are concentrated mainly in the digestive gland (also called the hepato-pancreas). Extensive documentation is available both on levels of contamination as well as on the process of accumulation and elimination of phycotoxins by bivalves with commercial utility.

Bivalve molluscs are the shellfish that are the most widely consumed, but data from the CALIPSO study, a French study on dietary consumption of seafood and contamination with trace elements, pollutants and omega 3 compounds (2006), showed that other "shellfish" are also eaten more or less regularly in France, depending on local custom and recreational fishing (table 1).

Table 1: Detailed list of shellfish consumption by high consumers of seafood (g/week expressed as fresh flesh), *sea gastropods, echinoderms, tunicates in bold* **(source: CALIPSO, 2006).**

	Mean (g/week)	P5	P50	P95
Winkle, *Littorina littorea*	4.2	0	0	25.0
Whelk, *Buccinum undatum*	15.4	0	0	75.0
Clam, *Venus mercenaria, Mercenaria mercenaria*	*0.2*	*0*	*0*	*0*
Cockle, *Cerastoderma sp*	*3.1*	*0*	*0*	*15.0*
Scallop, *Pecten maximus, P. jacobeus*	*39.3*	*0*	*25.0*	*156.3*
Razor shell clam, *Ensis sp*	*0.4*	*0*	*0*	*0*
Oyster, *Crasostera sp, Ostrea sp*	*34.4*	*0*	*18.0*	*144.0*
Mussel, *Mytilus spp*	*22.5*	*0*	*17.5*	*70.0*
Abalone, *Haliotis tuberculata*	0.6	0	0	0
Sea urchin, *Paracentrotus lividus,*	11.6	0	0	52.5
Carpet shell clam, *Tapes decussates, Ruditapes spp*	*2.8*	*0*	*0*	*12.3*
Limpet, *Patella sp*	1.0	0	0	0
Variegated scallop, *Chlamys varia, C. opertulis*	*14.7*	*0*	*0*	*56.3*
Warty clam, *Venus verrucosa*	*1.5*	*0*	*0*	*7.5*
Wedge shell, *Tellina sp, Donax trunculus*	*0.3*	*0*	*0*	*0*
Queen scallop, *Aequipecten opercularis*	*0.5*	*0*	*0*	*0*
Violet, Microcosmus *sabatieri*	1.0	0	0	0
Total Bivalve molluscs	***119.7***	***7.5***	***79.8***	***350.3***
Total Gastropods	**21.2**	**0**	**3.8**	**87.5**
Total Echinoderms	**11.6**	**0**	**0**	**52.5**
Total Tunicates	**1.0**	**0**	**0**	**0**

The literature concerning these "other shellfish" is much more limited and involves mainly PSP toxins, saxitoxin (STX) and its parent-compounds.

4. Gastropods and PSP toxins

The table in annex 2 contains an inventory of the highest levels of contamination with PSP toxins reported for gastropods, based on review performed by Shumway in 1995 and updated with data published since then.
PSP toxins in gastropods were detected in the 1960's with the mouse bioassay, in different species in North America and later in different countries with temperate and tropical climate zones. The maximum concentration of 5,629 µg eqSTX /100g of flesh is reported in *Argobuccinum sp* in Chile, followed by 3,337 µg eqSTX/100g in another whelk *Buccinum undatum* in the Gulf of Maine (USA). The range of levels of contamination in gastropods is vast and it is not possible to differentiate between species due to the large individual variability.

Human deaths following ingestion of gastropods contaminated by PSP toxins have been reported in the literature (Shumway, 1995). Some authors have concluded that such shellfish can present a risk for public health, and they recommend that they be included in a monitoring network (Shumway, 1995; Pitcher *et al*., 2001).
Moreover, in October 2007, using the rapid alert on foodstuffs and feed (RASFF) managed by the European Commission, Spain reported a health alert following coma in a person who ate gastropods (*Murex spp*.) from Portugal, contaminated with PSP toxins (151 µg STX/100g flesh, alert 2007.0752).

For other phycotoxins (ASP toxins (amnesic shellfish poisoning), DSP toxins (diarrhetic shellfish poisoning), the absence of data does not mean that gastropods are not contaminated but only that these toxins have not been sought in their tissues.

Few reports have been published recently on this specific topic but a few of them have attempted to expand on the local geographic distribution, tissue distribution and the process of contamination.

4.1. Geographic distribution and pathway of contamination

Contamination of carnivorous and/or necrophagous gastropods occurs naturally through the ingestion of food: they accumulate toxins when their food is contaminated.

This transfer was demonstrated in an experimental study conducted in Taiwan (Chen and Chou, 1998) in which a predator gastropod (*Babylonia areolata* Link) was fed with a bivalve (*Hiatula diphos*) experimentally contaminated with *Alexandrium minutum*. Two field studies confirmed these results.

1) In Chile, experimental contamination of two gastropods (*Concholepas concholepas* and *Argobuccinum ranelliformes*), predators of a given bivalve (*Aulacomya ater*) was carried out over a year at a specific site (Compagnon *et al*., 1998). During the follow-up period, a bloom of *Alexandrium catenella* took place from February to March. The two gastropods reached maximum levels of contamination 4-5 months after the peak of toxic bloom with the following levels:

- *Concholepas concholepas*: 9,164 µg eqSTX/100g in the digestive gland and 737 µg eqSTX/100g in the foot muscle;
- *Argobuccinum ranelliformes*: 14,057 µg eqSTX/100g in the digestive gland and 31 µg eqSTX/100g in the foot muscle.

However, it should be noted that contamination of one of the gastropods (*C. concholepas*) was observed well before the occurrence of algal bloom.

2) In Japan, monitoring of the carnivorous gastropod *Rapana venosa,* over almost two years, showed that it was contaminated in the spring, just after the bivalves on which it feeds (short-necked clam: *Tapes japonica*) reached maximum contamination levels. Only

the viscera were shown to be contaminated at levels of 3.3-4.2 MU[1]/g in May 2001 and 0.2 - 11.4 MU/g in April-June 2002 (Ito *et al*., 2004). It should be noted that this tropical gastropod *Rapana venosa* may be present in the bay of Quiberon (Morbihan, France) (La Vigie, No.26, 3-9, April 2001).

Although food-borne contamination appears to be confirmed for some carnivorous gastropods, it has not been for other gastropods whose diet is different, such as abalone.
A spatial description of contamination of *Haliotis midae* produced in South Africa (Pitcher *et al*., 2001) with samples collected in 5 aquatic farms, distributed along the coast from the northwest down to the southeast, revealed that contamination of abalone does not coincide with blooms of the dinoflagellate which produces PSP toxins (*Alexandrium catenella*).

The same phenomenon was observed in Spain (Galicia), where contamination of *Haliotis tuberculata* was demonstrated for the first time in 1991 (78 µg eqSTX/100g flesh). Since then, the extent of contamination has been the subject of studies which showed there was no relationship between the existence of PSP toxins in abalone and toxicbloom (Bravo *et al*., 1996, 1999).

It should be noted that PSP toxins in gastropods may not only be the passive result of contaminated food. These toxins, in fact, could play an ecological role in defence mechanisms, since a study showed the power of attraction of PSP toxins-contaminated food in gastropods (*Hwang et al*., 2007).

4.2. Distribution, profile, and variability of the toxin content

a) Tissue distribution

As in bivalve species, PSP toxin concentrations in the digestive gland of gastropods are generally much higher than those measured in the rest of the flesh, but with a high variability.

Starting in 1977, Yasumoto *et al.* reported high contamination in the viscera of the herbivorous gastropod *Turbo marmorata*. Similarly, in Taiwan, the carnivorous gastropod *Babylonia areolata,* experimentally fed with a bivalve contaminated by PSP toxins, concentrated the toxins in its digestive gland (Chen and Chou, 1998).

In Chile, two species of gastropods which were followed up for over a year showed great differences in the levels and distribution of phycotoxins (Compagnon *et al.,* 1998). In *Argobuccinum ranelliformis*, only the viscera were contaminated. The highest level of contamination reached at the end of the summer was 14,057 µg eqSTX/100g in the digestive gland, while in the foot muscle the level did not exceed 31 µg eqSTX/100g. In *Choncholepas concholepas*, the maximum concentration in the digestive gland measured in June was 9,164 µg eqSTX/100g while that of muscle was 737 µg eqSTX/100g.

In South Africa, *Pitcher et al.* (2001) conducted a study on the anatomical distribution of phycotoxins in abalone *Haliotis midae*. She confirmed both that viscera contain a high proportion of phycotoxins (although highly variable) and also that contamination in the foot muscle was not uniform depending on whether muscle tissue or epithelium was considered. The peri-podal fringe showed concentrations of 900 µg eqSTX/100g (SD of 400 µg eqSTX/100g for 12 subjects) while those of foot muscle and viscera were less than 200 µg eqSTX/100g. Moreover, another sample from 10 subjects showed that the gills can also be highly contaminated (1250 ± 400 µg eqSTX/100g).

In Spain, Bravo *et al*. (1999) also analysed the distribution of phycotoxins in different tissue compartments of abalone:

- epithelium of the foot: 10,500 ± 1,500 µg eqSTX/100g (9,500 dcSTX and 1,000 STX);

[1] A mouse unit [MU] is defined as the minimum amount of toxin needed to cause the death of an 18 to 22 g white mouse in 15 min upon intraperitoneal injection (AOAC, 1990).

- digestive tube: 28 ± 5 µg eqSTX /100g (12 dcSTX and 16 STX);
- foot muscle: 27 ± 6 µg eqSTX /100g (2 dcSTX and 25 STX).

Considering its mass compared to the rest of the body, the epithelium may contribute to 64% of total contamination of the subject. Since the ratio of the weight of epithelium to weight of muscle decreases with the length of the animal, this would explain a higher contamination of abalone, which are less than 65 mm in size.

b) Toxin profile

Physico-chemical analyses in gastropods have demonstrated a toxin profile that differs from that of algae contaminating bivalves.

In the study conducted in Chile, comparison of profiles involved total flesh of the bivalve prey and the digestive gland and muscle of the two predator gastropods. These data are listed in Table 2.

Table 2: Toxin composition (molar %) of three molluscs at time of their maximum contamination (from Compagnon *et al*., 1998).

		Maximal total contamination in µg eqSTX/100g	STX	dcSTX	Neo-STX	GTXs 1-5
Bivalve ***A. ater***	Flesh	13,259	3	2	10	85
Gastropod ***C. concholepas***	Digestive gland	6,183	12	3	1	84
	Muscle	1,068	65	5	30	0
Gastropod ***A. ranelliformes***	Digestive gland	4,732	38	2	0	60
	Muscle	12	65	22	0	12

It should be noted that total STX and Neo-STX (the two most toxic compounds) account for 95% and 87% respectively of toxic muscle content in the two gastropods. The qualitative profile of the digestive glands in the two predators appears similar to that of the bivalve, but the authors report that this profile changes during follow up. In fact, from October 1995 to November 1995, the phycotoxin profile of the digestive gland in *C. concholepas* showed a very simple profile before and after algal bloom, represented mainly by STX (a little Neo-STX and GTX 5 afterwards). Subsequently, from January 1996 to March 1996, the profile became complex because it showed GTXs in different proportions depending on the period.

In the study by Pitcher *et al.* (2001) in South Africa, phycotoxin contamination of *Haliotis midae* was comprised only of STX while mussels at the same site contained 56-57% Neo-STX, 41% STX; the remainder was divided between C1 and C2 toxins, for a total contamination of between 1,610 and 60,940 µg eqSTX/100g. The dinoflagellate harvested in the same area had a relatively complex profile but with low contamination of 1.75 pg eqSTX/cell distributed as follows:

- C1 and C2 N-sulfocarbamoxyl: 60% (less toxic);
- carbamate STX Neo-STX and GTX 3.4: 30-32 %;
- decarbamoxyl GTX 3: 8%.

A similar observation was reported by a Japanese team for the carnivorous gastropod *Rapana venosa* whose phycotoxin composition is simpler than that of the dinoflagellate *A. tamarense* but qualitatively similar to the bivalve on which it feeds (Ito *et al*., 2004). The major components (94% molar ratio) are comprised of GTX 2-4 (76%), of Neo-STX and STX (18%).

In Galicia, 80% of muscle contamination in *Haliotis tuberculata* is based on dcSTX, the remainder being of STX while the digestive gland contains the two toxins in approximately equal quantities (Bravo *et al*., 1999).

It should be noted that abalone from South Africa and Galicia do not have the same toxin profile.

In conclusion, these results show a trend towards retention of the most active phycotoxins in gastropods: STX, Neo-STX and dcSTX.

c) Variability and retention

All of the aforementioned studies reported a high individual variability in phycotoxin contamination. Bravo *et al*. (1999) did not observe a difference between the 7 sites on the Galician coast based on samples of 3 or 4 same-size individuals after evisceration of the animals.

In South Africa, individual variability was studied in total flesh of animals of the same size raised on the same site (Pitcher *et al*., 2001). Results are listed in Table 3.

Table 3: Individual variability in abalone in South Africa (Pitcher *et al*., 2001)

Place	Number of Individuals	Size (mm)	Maximal contamination µgSTXeq/100g	Multiplier factor
Farm A	10	30	77 -383	X 5
Farm A	?	?	63-1609	
Farm B	10	55-60	57-307	X 5
North Zone of the Cape	52	115-170	29-314	X 10

All decontamination tests set up concluded in the extremely slow elimination of the toxins, in particular those contained in muscle.

In Chile, muscle of one of the two predators (*C. concholepas*) showed contamination well before occurrence of algal bloom. The authors suggest the possibility of residual contamination due to previous algal bloom.

In Spain, a decontamination experiment was conducted for 3 months with 3 groups of contaminated abalone kept in fresh water and fed with 3 species of macrophytes. No decontamination was observed over the duration of the experiment, regardless of the feed given. Muscle was more highly contaminated than viscera: 220 ± 92 and 104 ± 68 eq STX/100g respectively. No relationship was demonstrated between contamination and composition of macroalgae in sampling stations and no PSP toxin was found in the macroalgae or the substrate.

Pitcher *et al*. (2001) observed extended retention of phycotoxins over a 7 month period and concluded that this was a characteristic of abalone. The variations observed may reflect only an individual variation.

5. Other organisms and other toxins

a) Other organisms

In echinoderms, only two articles (Ito *et al*., 2003 and Asakawa *et al*., 1997) reported the presence of PSP toxins in starfish (*Asterina pectinifera* and *Asterias amurensis*). The values range from "undetectable" to about 12 MU in total flesh.

A review of the literature focussing on *Paracentrotus lividus* over the last 20 years did not lead to identifying any publication involving a phycotoxin-related risk in the sea urchin, although the latter can be a healthy carrier. Its sensitivity to various neurotoxic pesticides has been reported (Pesando *et al*., 2003) as well as to toxins of *Caulerpa* (Pedrotti and Lemée, 1999).

Concerning tunicates, studies by Freitas *et al*., (1996) showed that *Phallusia nigra* contained saxitoxin and gonyautoxins (30 MU/100g of flesh).

Robertson *et al.* (2004) demonstrated the existence of PSP toxins in an octopus (*Octopus sp*). Maximal contamination was comprised solely of saxitoxin at a level of 246 µg STX/100g of tissue and was not linked to the existence of toxic phytoplankton.

In crustaceans, contamination associated with PSP and ASP toxins have been reported, in particular in *Carcinus maenas* (green crab) and *Cancer pagurus* (brown crab) which contained diarrhoea-causing phycotoxins and azaspiracids (Torgersen *et al*., 2008).

b) Other toxins

Tetrodotoxin (TTX) was described for the first time in a gastropod, *Charonia saulia* (Trumpet shell), in 1981 (by Narita *et al*., mentioned by Yu *et al*., 2004) This toxin is more commonly known as Fugu toxin, that is, of Pufferfish called Tetrodon fish of the genus Takifugu. Its presence has also been reported in crabs (*Atergatus floridus,* xanthid crab), marine gastropods of the genera *Zeuxis*, *Oliva* and *Nassarius* in China (Huang *et al*., 2008), sea urchins of the Caribbean (*Meoma ventricosa*) (Ritchie *et al*., 2000) and tunicates (Freitas *et al*., 1996).
The existence of tetrodotoxin in these species in France is not known, but this toxin can be produced by *Alexandrium* and contaminating bacteria called *Microbacterium arabinogalactanolyticum, Serratia marcescens* and *Vibrio alginolyticus* (Yu *et al*., 2004).

Palytoxin (PLT) is one of the most active non-protein toxins known to date in mammals. Furthermore, PLT produces very short survival times in mice bioassay for lipophilic phycotoxins, with no other associated plankton other than *Ostreopsis*. In humans, toxic effects and deaths have been observed after ingestion of various crabs or fish from the Indian Ocean and the Pacific Ocean (Molgo *et al.*, 1999; Onuma *et al.*, 1999). Recently, dinoflagellates of the genus *Ostreopsis* have also been implicated as sources of contamination, in particular *O. siamensis,* in Greece The bivalve shellfish studied in which PLT was found are as follows: *Mytilus galloprovincialis, Venus verrucosa* and *Modiolus barbatus*, in quantities less than the provisional limit set in 2005 by the European regulation at à 250 µg/kg (Aligizi *et al*., 2008).

Concerning microcystines, which are mostly fresh water toxins which can be found in Netpen liver disease in salmon, the breeding of sea urchins along with salmon could cause cross contamination, but no data exists on this issue (Cook and Kelly 2007). Fresh water gastropods are known to accumulate microcystines (White *et al*., 2006; Xie *et al*., 2007).

DSP toxins have been the subject of two studies on gastropods. One of them mentions trace amounts of okadaic acid (OA) in *Neverita didyma* in amounts of 3.2 µg OA/100g flesh (Zhen and Wang, 2005), while the other did not detect any toxins (Ito *et al*., 2004).
Crabs can also accumulate such toxins, in particular in their digestive gland, as shown in studies by Jorgensen *et al*. (2008) who fed crabs (*Carcinus maenas*) experimentally with contaminated mussels and observed a phycotoxin content ranging up to 503 µg OA/kg in the digestive gland versus only 12 µg/kg in the flesh, the latter level being much lower than the established safety level.

6. Conclusion

A review of the literature makes it possible to reach the conclusion that gastropods can accumulate PSP toxins; but no data is available concerning:

- the species of gastropods that are the most widely consumed in France such as the whelk *Buccinum undatum* and the winkle *Littorina littorea;*
- other families of regulated phycotoxins (ASP, DSP toxins).

Although the results do not involve a large number of species, it would appear that carnivorous gastropods may tend to store (or even accumulate) phycotoxins in the viscera while herbivorous gastropods would tend to accumulate them in the muscles of the foot.

Studies which simultaneously involve bivalves (prey) and carnivorous gastropods (predators) make it possible to conclude that bivalve contamination is much higher than gastropod contamination (by a factor of about 10).

But gastropods have several characteristics that should be taken into account:

1. retention time of PSP toxins is very long, some studies even finding an absence of decontamination;
2. contamination seems to be random, and does not coincide with bloom of the dinoflagellate PSP toxin producer (case of abalone);
3. in herbivorous gastropods, phycotoxin contamination is higher in the most commonly eaten part of the animal (foot muscle);

4. PSP toxins may be part of a system of defence in which gastropods would have a preference for ingesting contaminated food.

Furthermore, a case of poisoning by PSP toxins following ingestion of sea gastropods was reported in Spain.

Consequently, a study designed to determine the level of phycotoxin contamination of the most widely consumed gastropods present on the French coasts should be recommended, taking into account possible seasonal variations.

Concerning tunicates, data from the literature show that they can accumulate PSP toxins as a result of their filtration activity. A single study in the event of toxic bloom could be considered.

Lastly, concerning echinoderms, the only species of utility is the sea urchin. Although sensitivity of urchin eggs to toxins is well known, currently no data is available to determine their capacity for accumulation of phycotoxins.

7. Bibliographical references

Aligizaki K., Katikou P., Nikolaidis G., Panou A., 2008. First episode of shellfish contamination by palytoxin-like compounds from *Ostreopsis* species (Aegean Sea, Greece). *Toxicon*, in press.

Andersen R.J., Luu H.A.,Chen D.Z.X., Holmes C.F.B., Kent M.L., Le Blanc M., Taylor F.J.R., Williams D.E., 1993. Chemical and biological evidence links microcystins to salmon « Netpen liver disease". *Toxicon* **31**: 1315-1323.

AOAC - Association of Official Analytical Chemists - 1990. Paralytic shellfish poison. Biological method. Final action. *In*: *Official methods of analysis*.,15th Edition, Hellrich K. (ed), Arlington, sect. 959.08, 881-882.

Asakawa M., Nishimura F., Miyazawa K., Noguchi T., 1997. Occurrence of paralytic shellfish poison in the starfish Asterias amurensis in Kure Bay, Hiroshima Prefecture, Japan. *Toxicon* **35**(7): 1081-1087.

Bravo I., Cacho E., Franco J.M., Miguez A., Reyero M.I., Martinze A., 1996. Study of PSP toxicity in *Haliotis tuberculata* from the Galician coast. In: *Harmful and Toxic Algal Blooms*. Yasumoto T;, Oshima Y.and Fukuyo Y. (Eds) ICO of UNESCO, Senday, pp 421-424.

Bravo I., Reyero M.I., Cacho E., Franco J.M., 1999. Paralytic shellfish poisoning in *Haliotis tuberculata* from the Galician coast: geographical distribution, toxicity by lengths and parts of the mollusc. *Aquatic toxicology* (Amst) **46**: 79-85.

Bravo I., Franco J.M., Alonso A., Dietrich R., Molist P., 2001. Cytological study and immunohistochemical location of PSP toxins in foot skin of the ormer, *Haliotis tuberculata*, from Galicia coast (NW Spain). *Marine Biology* **138**: 709-715.

Chen C. and Chou H., 1998. Transmission of the paralytic shellfish poisoning toxins from dinoflagellate to gastropod. *Toxicon* **36(**3): 515-522.

Compagnon D., Lembeye G., Marcos N., Ruiz-Tagle N., Lagos N., 1998. Accumulation of paralytic shellfish poisoning toxins in the bivalve *Aulacomya ater* and two carnivorous gastropods *Concholepas concholepas* and *Argobuccinum ranelliformes* during an *Alexandrium catenella* bloom in Southern Chile. *Journal of Shellfish Research* **17**(1): 67-73.

Cook E.J and Kelly M.S., 2007. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with atlantic salmon *Salmo salar*. *Aquaculture* **273**, 573-585

Freitas J.C., Ogata T., Veit C.H., Kodama M., 1996 Occurrence of tetrodotoxin and paralytic shellfish toxins in *Phallusia nigra* (Tunicata, ascidiacea) from the Brazilian coast. *J. Venom. Anim. Toxins* **2**(1): 1-7.

Hwang P., Noguchi T., Hwang D., 2007. Paralytic shellfish poison as attractant for toxic snails. *Fisheries Science* **73**(1): 202-207.

Huang H.N., Lin J., Lin H.L., 2008. Identification and quantification of tetrodotoxin in the marine gastropod Nassarius by LC-MS. *Toxicon*, in press.

Ito K., Asakawa M., Beppu R., Takayama H., Miyazawa K. 2004. PSP-toxicification of the carnivorous gastropod *Rapana venosa* inhabiting the estuary of Nikoh River, Hiroshima bay, Hiroshima Prefecture, Japan. *Marine Pollution Bulletin* **48**: 116-1121.

Jorgensen K., Cold U., Fisher K., 2008. Accumulation and depuration of okadaic acid esters in the European green crab (*Carcinus maenas*) during a feeding study. *Toxicon*, in press.

Leblanc J. Ch., coordinateur. Août 2006. CALIPSO – Etude des consommations alimentaires de produits de la mer et imprégnation aux éléments traces, polluants et oméga 3.

Lin S., Tsai Y., Lin H., Hwang P., 1998. Paralytic toxins in Taiwanese starfish Astropecten scoparius. *Toxicon* **36(**5): 799-803.

Llewellyn L., Negri A., Robertson A., 2006. Parallytic shellfish toxins in tropical oceans. *Toxin Reviews* **25**:159-196.

Moore R.E. and Scheuer P.J., 1971. Palytoxin: a new marine toxin from a coelenterate. *Science* **172**: 495-498.

Molgo J., Benoit E., Legrand A.M., Kreger A.S., 1999. Bioactive agent involved in fish poisoning: an overview. *In*: Proc. 5th Indo-Pacific Fish Conf., Nouméa, New Caledonia, *Soc. Fr. Ichtyol*., Paris, 721-728.

Narita H, Noguchi T., Maruyama J., Ueda Y., HAashimoto K., Watanabe Y., Hida K. 1981. Occurrence of tetrodotoxin on a trumpet shellfish "boshubora" *Charonia sauliae* . *Nippon Suisan Gakkaishi* **47**: 934-941.

Onuma Y., Satake M., Ukena T., Roux J., Chanteau S., Rasolofonirina N., Ratsimaloto M., Naoki H., Yasumoto T., 1999. Identification of putative palytoxin as the cause of clupeotoxism. *Toxicon* **37**(1):55-65.

Pesando D., Huitorel P., Dolcini V., Anagelini C., Guidetti P., Falugi C., 2003. Biological targets of neurotoxic pesticides analysed by alterationof developmental events in the Mediterranean sea urchin *Paracentrotus lividus.* Marine environmental Research 55, 39-57.

Pedrotti M.L. and Lemee R., 1999. Effect of microalgae treated with natural toxins on the nutrition and development of filter-feeding sea-urchin larvae. *Marine environmental Research* **48**: 177-192.

Pitcher G.C., Franco J.M., Doucette G.J., Powell C.L., Mouton A., 2001. Paralytic shellfish poisoning in the abalone *Haliotis midae* on the west coast of South Africa. *Journal of shellfish research* **20**(2): 895-904.

Ritchie K.B., Nagelkerken I., James S., Smith G.W., 2000. Environmental microbiology: a tetrodotoxin-producing marine pathogen. *Nature* **404**(6676): 354.

Robertson A., Stirling D., Robillot C., Llewellyn L., Negri A., 2004. First report of saxitoxin in octopi. *Toxicon* **44**: 765-771.

Sekiguchi K., Sato S., Kaga S., Ogata T., Kodama M., 2001. Accumulation of paralytic shellfish poisoning toxins in bivalves and an ascodian fed on *Alexandrium tamarense* cells. *Fisheries science* **67**: 301-305.

Shumway S.E. 1995. Phycotoxin-Related Shellfish Poisoning: Bivalve molluscs are not the only vectors. Reviews in *Fisheries Science* **3**:1-31.

Togersen T., Bremnes N.B., Rundberget T., Aune T., 2008. Structural confirmation and occurence of azaspiracids in Scandinavian brown crabs (*Cancer pagurus*).*Toxicon* **51:** 93-101.

White S.H., Duivenvoorden L.J., Fabbro L.D., Eaglesham G.K., 2006. Influence of intracellular toxin concentrations on cylindrospermopsinnbioaccumulation in a freshwater gastropod (*Melanoides tuberculata*). Toxicon **47**(5):497-509.

Wiles J.S., Vick J.A., Christensen M.K., 1974. Toxicological evaluation of palytoxin in several animal species. *Toxicon* **12**: 427-433.

Wu J.Y., Zhen L., Wang J.H., 2005. Contamination of shellfish from Shanghai seafood markets with paralytic shellfish poisoning and diarrhetic shellfish poisoning toxins determined by mouse bioassay and HPLC. *Food Additives and Contaminants* **22**(7): 647-651.

Xie L., Yokoyama A., Nakamura K., Park H., 2007. Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan. *Toxicon* **49**: 646-652.

Yasumoto T. and Kotaki Y., 1977. Occurrence of Saxitoxin in a Green Turban Shell. *Bulletin of the Japanese Society of Scientific Fisheries* **43**(2): 207-211.

Yotsu-Yamashita M., Mebs D., Kwet A., Schneider M., 2007. Tetrodotoxin and its analogue 6-*epi*tetrodotoxin in newts (*Triturus* spp; Urodela, Salamandridae) from Southern germany. *Toxicon* **50:** 306-309.

Yu C-F., Yu P.H-F., Chan P-L., Yan Q., Wong P-K., 2004. Two novel species of tetrodotoxin-producing bacteria isolated from toxic marine puffer fishes. *Toxicon* **44**: 641-647.

8. Key words

Phycotoxins, marine biotoxins, gastropods, echinoderms, tunicates.

Annex 1
Limits for public health set by EC regulation no. 853/2004, Annex III, section VII, chapter V

Phycotoxin family subjected to testing	Limits
PSP	800 µg equivalent saxitoxin/kg of flesh
ASP	20 mg domoic acid/kg of flesh
Okadaic acid + Dinophysistoxins + Pectenotoxins	160 µg equivalent okadaic acid/kg of flesh
Yessotoxins	1 mg equivalent yessotoxin/kg of flesh
Azaspiracids	160 µg equivalent azaspiracids/kg

Annex 2:
Maximal concentrations of PSP toxins in echinoderms, gastropods and tunicates

Name Species /genus	Location	Source	Level of contamination	References
Echinoderms				
Asterina pectinifera	Taiwan Japan	? *A. tamarense ?*	225 µg eqSTX/100g 12,5 MU/g of total flesh (11 in the tegument and 3.9 in the viscera)	Lin *et al*.,1998 Ito *et al*, 2003
Astropecten scoparius	Taiwan	?	640 µg STX/100g	Lin *et al*., 1998
Asterias amurensis	Japan	?	8 MU.g of total flesh (28.7 MU/g in viscera)	Asakawa *et al*., 1997
Gastropods				
Babylonia aréolata carnivore	Taiwan	?	246 µg STX/100d edible flesh (experimental contamination)	Chen and Chou , 1998
Oliva vidua fulminans	Malaysia	*Pyrodinium bahamense*	454 µg eqSTX/100g	Shumway, 1995
Rapana venosa	Japan	*A. tamarense*	Max 4.2 MU/g viscera – 224 MU /specimen followed up annually)	Ito *at al*., 2004
Neverita didyma	China (Shanghai)	?	0.2 µg eqSTX/100g	Wu *et al*., 2005
Concholepas concholepas	Chile	?	Mean: 568 = 143 µg eqSTX/100g in foot muscle	Compagnon *et al*., 1998
Argobuccinum ranelliformi *Argobuccinum sp*	Chile Chile	 *A. catenella*	Max: 31 µg eqSTX/100g in foot muscle 5,629 µg eqSTX/100g viscera – 92 µg eqSTX/10g muscle	Compagnon *et al*., 1998 Shumway, 1995
Neptunea sp	USA (Alaska)	*A. catenella*	200-250 µg eqSTX/100g	Shumway,1995
Thais/Nucella lima	USA (Washington)	*A. catenella*	180 µg eqSTX/100g	Shumway, 1995
Buccinum undatum	USA (Maine)	*A. tamarense*	3,337 µg eqSTX/100g	Shumway, 1995
Euspira heros	USA (Maine)	?	2,922 µg eqSTX/100g	Shumway, 1995
Euspira/polinices lewisii	Canada (British Columbia) USA (Massachusetts)	*A.carenella* *A tamarense ?*	176-600 µg eqSTX/100g 1,450 µg eqSTX/100g	Shumway, 1995 Shumway, 1995
Lambis lambis	Malaysia	*Pyrodinium bahamense*	31 µg eqSTX/100g	Shumway, 1995
Crepidula fornicata	?	?	46-58 µg eqSTX/100g	Shumway, 1995
Littorina littorea	USA (Massachusetts)	*A. tamarense*	72 µg eqSTX/100g	Shumway, 1995
Tectus nilotica maxima *Tectus pyramis*	Japan Japan	Macroalgae *Jania sp*	342 µg eqSTX/100g 90 µg eqSTX/100g	Shumway, 1995 Shumway, 1995
Turbo marmotata *Turbo argyrostoma*	Japan Japan	Macroalgae *Jania sp*	75 µg eqSTX/100g 360 µg eqSTX/100g	Shumway, 1995 Shumway, 1995

Haliotis tuberculata	Spain	?	Max 443 µg STXeq/100 g flesh	Bravo *et al*., 1996
Haliotis midae	South Africa	*A. catenella*	Max 1609 µg eqSTX/100g Individual.var.(55-60mm): 57-307 µg eqSTX/100g Individual.var (115-170mm): 29-314 µg eqSTX/100g	Pitcher *et al*., 2001
Haliotis tuberculata	Spain	?	252 ± 25 µg eqSTX/100g flesh with mouse assay 454 ± 86 µg eqSTX/100g flesh with HPLC Epithelium: 10 500 ± 1500 µg eqSTX/100g (9500 dcSTX and 1000 STX) Digestive tube: 28 ± 5 µg eqSTX/100g (12 dcSTX and 16 STX) Muscle: 27 ± 6 µg eqSTX/100g (2 dcSTX and 25 STX)	Bravo *at al*., 1999
Tunicates				
Phallusia nigra	Brazil	?	269 MU/100g (STX and/or TTX)	Freitas *et al*., 1996
Halocynthia roretzi	Japan	*A. tamarense*	About 700nmol PST/specimen (experimental contamination)	Sekiguchi *et al*., 2001