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Marine Algal Toxins in Shellfish from Shanghai Markets and Original Sources Following a Large Scale Red Tide Occurrence

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The occurrences of red tide, also referred to as Harmful Algal Bloom, have been increasing along the coastal waters in the past couple of decades, causing damage to marine environment and threatening human health (Hallegraeff 1995). Of over 5000 known species of marine phytoplankton, about 300 species can proliferate in such numbers that form the red tide phenomena. Only about 40 of these species have the capacity to produce toxins which can pose risks to humans through fish, shellfish or other pathways (Felwelling et al. 2005). Generally, the toxins produced by microalgae can be divided into six groups according to the symptoms: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), ciguatera fish poisoning (CFP), and azasporacid poisoning (AZP) (Daranas et al. 2001).

Since the 1970s, red tides have become common phenomena along the coastal waters of China, where at least 512 cases were documented from 1952 to 2002 (Yan and Zhou 2004), and PSP and DSP were detected widely in China (Zhou et al. 1999; Wu et al. 2005). In 2005, a total of 82 cases of red tides were recorded in the coastal waters of the China Sea, the accumulative areas of red tides were larger than that in any previous years, especially, near the Yangtze Estuary, which was seriously polluted by inorganic nitrogen and active phosphate (Wu 2005), a large scale red tide occurred at the end of May, 2005. The area of this red tide was up to 7,000 km^2. In addition, near the Nanji Islands, Zhejiang coast, the East China Sea, a small scale red tide of about 500 km^2 broke out during May 30 to June 10. The main species forming the red tides in this period were identified as *Skeletonema costatum, Karenia mikimotoi*, *Prorocentrum dentatum.* Shanghai is the largest city in China, where it faces the East China Sea to the east and back against the Yangtze Estuary in the northeast. As the red tide had occurred off the coast of Shanghai, monitoring marine algal toxins in shellfish from the seafood markets and the original places was necessary to prevent the people in this international city from shellfish poisoning. This study might provide an important reference for the relevant management agency to take emergency measures.

MATERIALS AND METHODS

44 shellfish samples were purchased from seafood markets in Shanghai city, including the large markets, such as Fuxi market and Tongchuan market, as well

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as several small markets. At the same time, 15 shellfish samples were collected from their original places, including Lianyungang City, Chongming Island, Zhoushan Islands and Ningbo City, which are abundant in seafood. The species of samples involved scallops, gastropods, clams and mussels. The samples were delivered to the lab, analyzed immediately or stored at -20°C.

The AOAC mouse bioassay (MBA) method (AOAC, 1990) for PSP was used in the investigation and was described in detail by Wu et al (2005). The toxicity of the samples was calculated according to the average lethal time and expressed as mouse units (MU). The MBA method for DSP was conducted according to Draisci et al. (1994). In brief, the homogenated shellfish was extracted by acetone. After the acetone was removed by evaporation, the aqueous suspension was extracted with diethyl ether to remove polar substances. The combined ether phase was backwashed twice with a small amount of water, and evaporated to dryness. The residue was suspended in 5ml 1% Tween 60 solution. Then, 1ml suspension was injected into three male ICR strain mice with bodyweight about 16-20g. If two or three mice were dead, the positive response would be assumed that the shellfish was contaminated by DSP toxins.

HPLC analysis for PSP toxins was performed by pre-column oxidation with periodate (Lawrence et al.1991). HPLC methods for the determination of the DSP toxins are performed by chromatographic separation and fluorescence detection following a pre-column derivatization with 9-chloromethylanthracene (Lawrence et al. 1996). For PSP analysis, Agilent 1100 system (Agilent Comp, Palo Alto, CA ,USA) with a quaternary pump, a fluorescence detector and a Zorbax Eclipse XDB column was used. Detection wavelengths were set at λ_{EX}=330nm and λ_{EM}=390 nm. The mobile phase consisted of acetonitrile and 20mM ammonium formate(3:97, v:v) with a flow rate at 0.5 ml/min. For DSP analysis, the LC system included a Waters (Milford, MA, USA) 600 quaternary pump, a Waters 474 fluorescence detector and a Waters symmetry RP-18 column. The mobile phase was a mixture of acetonitrile:methanol:water (75:10:15, v:v:v) with a flow rate of 1ml/min. The excitation and emission wavelengths were set at 365nm and 412nm, respectively. The recovery was about 85% for PSP toxins and 75% for DSP toxins. Generally, the detection limit was 0.01μg /100g. The PSP standard materials, saxitoxin (STX), neosaxitoxin (NEO), gonyautoxins1/4 (GTX1/4), gonyautoxins2/3 (GTX2/3), as well as the standard DSP toxins, Okadaic acid (OA) and dinophysistoxin 1 (DTX1), were purchased from the National Research Council, Canada. Water used for the HPLC system was distilled and passed through the Milipore Water-purification System (Millipore Corc., Bedford, MA, USA) and chemicals for HPLC analysis were all HPLC grade.

RESULTS AND DISCUSSION

In this study, 44 samples from the Shanghai markets, included gastropods, clams, mussels, oysters and scallops, which could represent the most species of seafood sold in Shanghai city at this period. The results indicated that 3 samples were contaminated by PSP toxins by both mouse bioassay and HPLC. These

contaminated samples were *Neverita didyma* (gastropod), *Scapharca broughtonii* (clam) and *Argopecten irradians* (scallop). The toxicity ranged from 183.0 to 232.5 MU/100g shellfish tissues. Chemical analysis indicated that PSP toxins in these shellfish were GTX2/3, and the concentrations were 42.0, 49.6 and 78.5 μg /100g respectively (Table1, Figure 1, Figure 2). According to the results, only 7% of shellfish were contaminated with PSP toxins, which was less than the rate in 2003 (30%) in the same month (Wu et al. 2005). PSP toxins can block the nerve impulse conduction, which leads to neuromuscular paralysis (Bricelj et al. 2005). Therefore, many countries laid down the regulations for shellfish. Generally, the products are unsafe for consumption if the concentration of PSP toxins is above 400MU/100 g or 80 μg STX /100 g edible shellfish fresh tissues (Lefiley and Hannah 1998). According to this regulation, the PSP toxins in three samples did not overpass the security limit, but the concentration of PSP toxins in *Argopecten irradians* was very close to this value.

Table 1. PSP toxins in shellfish from Shanghai markets by MBA and HPLC

Shellfish	Toxic/total samples	Toxic species	Market name	MBA (MU/100g)	HPLC (μg /100g)
Gastropod	1/9	*Neverita didyma*	Shiliupu	183.0	42.0
Clam	1/22	*Scapharca broughtonii*	Henda	185.1	49.6
Mussel	0/4	—	—	ND	—
Oyster	0/2	—	—	ND	—
Razor clam	0/5	—	—	ND	—
Scallop	1/1	*Argopecten irradians*	Henda	232.5	78.5

ND: beyond the detection limit

The DSP toxins consist primarily of okadaic acid (OA) and its analogues dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2), which can cause gastrointestinal illness caused by consuming shellfish contaminated by toxigenic dinoflagellates (Lee et al. 1989). The guidelines for DSP in shellfish differ in various countries. In China, the temporary regulatory level of DSP is 20 μg OA/100g edible tissues. In this investigation, 21 samples in Shanghai markets monitored by mouse bioassay showed positive toxic responses. However, HPLC analysis indicated 6 samples contaminated with OA. The concentrations of OA ranged from 0.07 to 49.35 μg /100g. Only one sample (*Tegillarca granosa*) contained toxins that exceed the limit. Mouse bioassay is accepted by many countries as the standard method, since it is sensitive to a broad spectrum of activities and capable of detecting wide range of toxins. This study also illustrated that the mouse bioassay was very sensitive to monitor DSP toxins. However, the specificity was very low, which suggest that the results of mouse bioassay should be confirmed by other analytical methods, such as HPLC and LC-MS.

Table 2. DSP toxins in shellfish from Shanghai markets by MBA and HPLC

Shellfish	Sample amount	Species	Market name	MBA	HPLC (μg /100g)
Gastropod	3/9	*Babylonia areolata*	Tongchuan	+	ND
		Ampullarum crossean	Tongchuan	+	ND
		Babylonia lutosa	Tongchuan	+	ND
Clam	11/22	*Mactra veneriformis*	Fuxi	+	ND
		Mevetrix meretrix	Henda	+	ND
		Tegillarca granosa	Shiliupu	+	49.35
		Sanguinolaria olivacea	Fuxi	+	ND
		Paphia euglypta	Tongchuan	+	15.01
		Paphia undulata	Shiiupu	+	12.55
		Mevetrix meretrix	Shiliupu	+	ND
		Gomphina veneriformis	Shiliupu	+	ND
		Cyclina sinensis	Henda	+	ND
		Paphia undulata	Henda	+	ND
		Gomphina veneriformis	Henda	+	18.19
Mussel	2/4	*Mytilus edulis*	Henda	+	ND
		Pinna pectinata	Henda	+	ND
Oyster	1/2	*Crassostrea rivuldri*	Shiliupu	+	0.07
Razor clam	3/5	*Solecurlus divaricatus*	Henda	+	13.89
		Solenidae minima	Tongchuan	+	ND
		Sinonovacula constricta	Henda	+	ND
Scallop	1/1	*Argopecten irradians*	Henda	+	ND

ND: beyond the detection limit

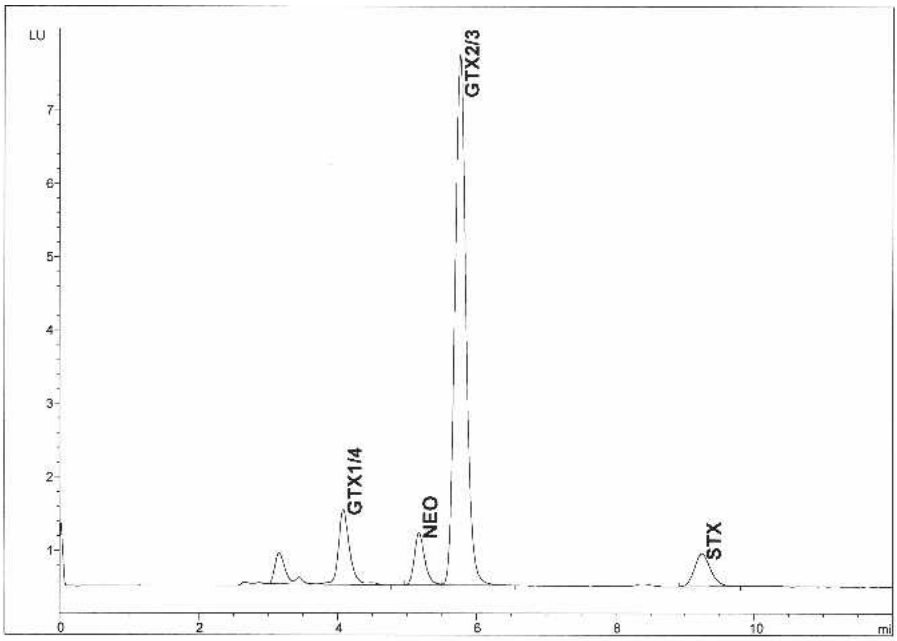

Figure 1. HPLC analysis of the standard materials of PSP toxins

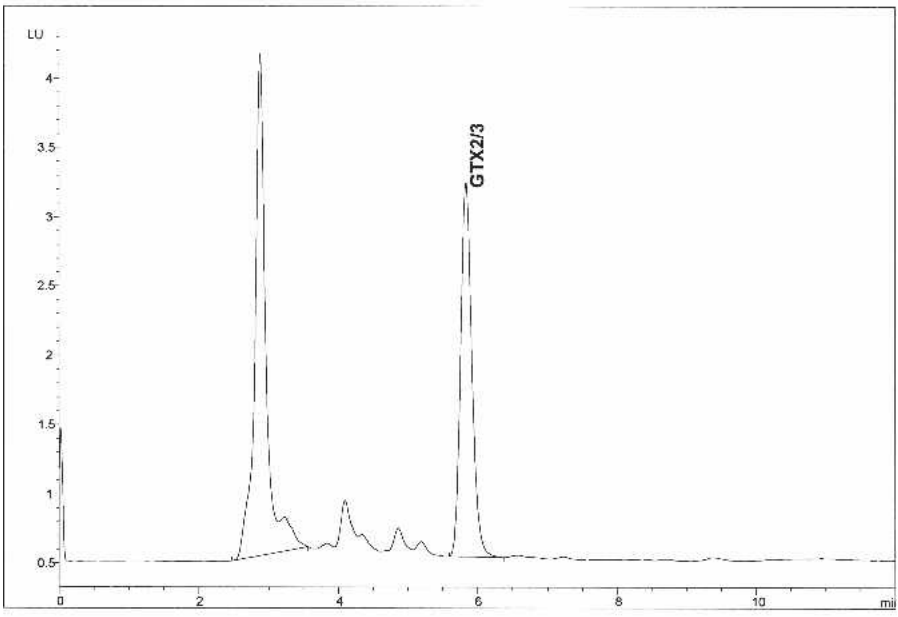

Figure 2. HPLC analysis of PSP toxins in shellfish (*Neverita didyma*)

In this investigation, PSP and DSP toxins could not be detected in 15 samples from the original place by mouse bioassay as well as HPLC analysis, though toxic algae were identified in the red tide incidents. The results suggested that the maricultures near these areas were not necessary to be closed right away. Toxic red tide occurrences do not preclude harvesting of fish and shellfish in areas if an effective surveillance program is in place. Monitoring of both shellfish and phytoplankton should be conducted further to prevent the adverse effects on public health caused by toxic red tides. In addition, early warning and prediction of algal blooms based on environmental factors over temporal and spatial scales might provide the best strategy for an emergency response.

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