Determination of the toxin profile of Venus gallina, Venus verrucosa and Cardium edule mussels in Turkey

Article *in* Archiv für lebensmittelhygiene · November 2008

DOI: 10.2376/0003-925X-59-233

CITATIONS
2

READS
36

3 authors, including:

Ugur Gunsen
BANDIRMA ONYEDİ EYLÜL ÜNİVERSİTESİ
23 PUBLICATIONS **226** CITATIONS

SEE PROFILE

Ali Aydin
Istanbul University
69 PUBLICATIONS **697** CITATIONS

SEE PROFILE

Arch Lebensmittelhyg 59, 233–238 (2008)
DOI-10.2376/0003-925X-59-233

ISSN 0003-925X

Korrespondenzadresse:
ugur_gunsen@hotmail.com

University of Balikesir, Bandirma Vocational High School, Department of Food Hygiene, 10200, Bandirma, Balikesir, Turkey[1]
University of Istanbul, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, 34320, Avcilar, Istanbul, Turkey[2]
The Central Institute of Food Control and Research, 16036, Hurriyet, Bursa, Turkey[3]

Determination of the toxin profile of *Venus gallina, Venus verrucosa* and *Cardium edule* mussels in Turkey

Ermittlung des Toxinprofils von Venus gallina, Venus verrucosa und Cardium edule Muscheln in der Türkei

Ugur Gunsen[1], Ali Aydin[2], Ali Ozcan[3]

Summary

Amnesic Shellfish Poisoning (ASP), Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) toxins were determined in a total of 705 mussel (*Venus gallina, Venus verrucosa, Cardium edule*) samples, obtained from the Western Coastline of the Black Sea and commercial shellfish processing companies in Istanbul between August 2005 and October 2007. The ASP toxins were determined by High Pressure Liquid Chromatography (HPLC), PSP and DSP toxins were analysed by mouse bioassays. The presence of PSP and DSP toxins could not be detected in any of the samples during the analysis period. For ASP, an average level of 1.94 ± 1.1 µg/g mussel meat was determined in 10 (1.42%) of 705 samples during the sampling period. As a result, the levels of ASP, PSP and DSP toxins in the samples analysed were found to be in accordance with the limits set in Regulation (EC) No. 853/2004. It was concluded that the levels of toxin in the mussel samples were no problem for human consumption, because ASP was at fairly low levels in the samples obtained in the spring period when algal growth is increased, while PSP and DSP toxins could not be detected in any of the samples. These results provide a basis for effective monitoring and control of these toxins in Turkey.

Keywords: ASP, DSP, PSP, HPLC/DAD, mussels

Zusammenfasssung

Insgesamt 705 Proben von Muscheln (*Venus gallina, Venus verrucosa, Cardium edule*) wurden auf Amnesic Shellfish Poisoning (ASP), Paralytic Shellfish Poisoning (PSP) und Diarrhetic Shellfish Poisoning (DSP) Toxine untersucht. Die Probenahme erfolgte zwischen August 2005 und Oktober 2007 an der westlichen Küstenlinie des Schwarzen Meeres sowie in kommerziellen Muschel verarbeitenden Betrieben in Istanbul. Die ASP-Toxine wurden mittels Hochdruckflüssigkeits-Chromatographie (HPLC) ermittelt, PSP- und DSP-Toxine wurden durch Mäusebioassay analysiert. Während des Untersuchungszeitraums konnten PSP- und DSP-Toxinen in keiner der Proben nachgewiesen werden. Für ASP wurde ein durchschnittlicher Wert von 1,94 ± 1,1 µg/g Muschelfleisch in 10 (1,42 %) der insgesamt 705 Proben während des Untersuchungszeitraums ermittelt. Die Werte der ASP-, PSP- und DSP-Toxine lagen in den analysierten Proben unterhalb der Grenzwerte der Verordnung (EG) 853/2004. Es wurde gefolgert, dass der Toxingehalt der untersuchten Muschelproben keine Gefahr für die Gesundheit des Verbrauchers darstellt, da für ASP lediglich niedrige Werte in den Proben nachgewiesen wurden, und zwar im Frühjahr zur der Zeit des vermehrten Algenwachstums, während PSP- und DSP-Toxine in keiner der Proben ermittelt wurden. Die Ergebnisse dieser Studie stellen eine Grundlage dar für die effektive Überwachung und Kontrolle dieser Toxine in der Türkei.

Schlüsselwörter: ASP, DSP, PSP, HPLC/DAD, Muscheln

Archiv für Lebensmittelhygiene 59, Heft 6 (2008), Seiten 233–238

Introduction

The accumulation of phycotoxins is one of the most critical problems in bivalve aquaculture. To date, Amnesic Shellfish Poisoning (ASP) (domoic acid (DA)), Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) toxins are considered to be the main phycotoxin groups. Of these, DSP includes the toxin with the lowest acute effect, okadaic acid (OA) and its derivatives, which seem to produce only, albeit severe, gastrointestinal disorders (Morono et al., 2003).

Production of the biotoxin DA has been reported in ten species of diatoms within the genus *Pseudo-nitzschia* (Bates, 2000). Filter feeding bivalves such as scallops and mussels consuming these toxin-producing phytoplankton species can accumulate DA to high concentrations (Zaman et al., 1997).

Among all the known phycotoxins, PSP toxins pose one of the most serious threats due to the extreme toxicities of the compounds involved. PSP toxins, based on the saxitoxin (STX) molecule, are produced by toxic dinoflagellates such as *Alexandrium* spp. and *Gymnodinium catenatum*, and to date more than eighteen STX analogues have been reported. Dinoflagellate PSP toxins are accumulated by filter feeding bivalves and typically the *N* sulfocarbamoyl toxins are found in lower proportions in bivalves than in toxic dinoflagellates (Oshima et al., 1990; Jeon et al., 1996). PSP toxins are potent water-soluble neurotoxins (tricyclic tetrahydropurine derivatives). A mandatory limit of 80 µg of STX eq./100 g of edible mollusc tissue has been adopted in many countries (Van Egmond et al., 1992).

DSP is a toxic syndrome caused by the consumption of shellfish contaminated with algal toxins produced by marine dinoflagellates. DSP toxins can be divided into three groups depending on chemical structure: (A) okadaic acid (OA) and its derivates named dinophysistoxins (DTXs), which cause gastrointestinal symptoms and are considered more frequently responsible for mussel toxicity (Fattorusso et al., 1992), (B) polyether-lactones of the pectenotoxin group (PTXs), which cause liver necrosis (Terao et al., 1993) and (C) a sulphated polyether and its derivates, named yessotoxins (YTXs), which cause cardiac muscle damage when administered intraperitoneally in mice (Terao et al., 1990). The minimum doses of OA and DTX-1 necessary to induce diarrhea in adults have been estimated to be 40 and 36 mg, respectively (Hamano et al., 1986).

Bivalve harvesting and mariculture have considerable importance as an economic factor and a possible cause of health problems in countries, such as Turkey, where shellfish are commercially exploited. Toxic algal blooms can cause serious problems for the health of consumers and economic losses due to closure of shellfish harvesting grounds.

Although not a European Union member state, Turkey exports shellfish to the European Union and has adopted management protocols similar to those detailed in Regulation (EC) No. 853/2004. In studies performed in Turkey, Koray (1988, 1992) and Ozay (1992) reported that toxin producing dinoflagellates existed on the coastline of the Black, Marmara, Aegean and Mediterranean Seas and PSP and DSP toxins could be found in shellfish obtained from these seas.

The aim of our study is to evaluate toxicity in mussels from different locations along the Western Black Sea shore and from commercial shellfish processing companies in Istanbul, Turkey both by biological (mouse bioassay) and chemical (HPLC) analysis, which are the reference methods in the European Union for biotoxin analysis in mussels.

Material

In total, 705 mussel (*Venus gallina, Venus verrucosa, Cardium edule*) samples, obtained from shellfish producing areas at 9 locations (Igneada, Karacakoy, Catalca, Sile, Karasu, Hisaronu, Amasra, Kurucasile and Doganyurt) along the Western Coastline of the Black Sea (Fig. 1) and from commercial shellfish processing companies in Istanbul between August 2005 and October 2007 at weekly intervals were used as research materials.

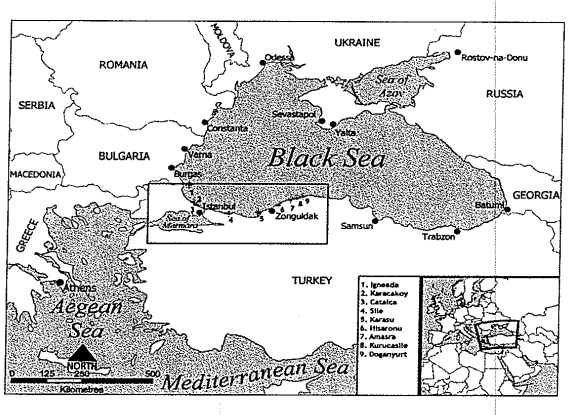

FIGURE 1: *Map of the sample collection stations in the Western Black Sea.*

Methods

ASP Analysis

Soft mussel tissues were removed from the shells and the tissue was rinsed with distilled water. After draining, the tissue was homogenized in a glass blender to produce a smooth slurry. Duplicate aliquots (50 g) of each sample were weighed into clean 250 ml beakers and the ASP toxins were extracted by gently boiling for 5 min with 50 ml 0.1 M HCl (Merck, Darmstadt, Germany). Following extraction, homogenates were centrifuged at 3000 rpm for 5 min. Each supernatant was filtered using a 0.45 µm syringe filter (Millex HV, Millipore Corp., Billerica, MA, USA) and the concentration of DA in the filtered extract was determined using Hewlett Packard Series 1100 HPLC/DAD (Diode Array Detector) equipment. All solvents used were HPLC grade. Eluant consisted of 9% acetonitrile (Merck) in deionised water plus 1% TFA (trifluoroacetic acid; Merck). HPLC flowrate was 1.0 ml/min, the injection volume was 20 µl, the column used was a Vydac 10 mm C18 25 cm x 4.6 mm column, the column temperature was +40°C and the measurement wavelength was 242 nm (AOAC, 2000a). This method was accredited according to ISO/EN 17025 and during the method validation procedures of the ASP method, the limit of detection was determined as 0.083 µg/g and linearity was found to be R^2 = 0.9999 by using eight dif-

ferent levels of calibration standards, 0.09, 0.45, 0.9, 2.25, 4.5, 9.0, 22.5 and 45 µg/ml domoic acid (Sigma-Aldrich, St. Louis, MO, USA) (Fig. 2).

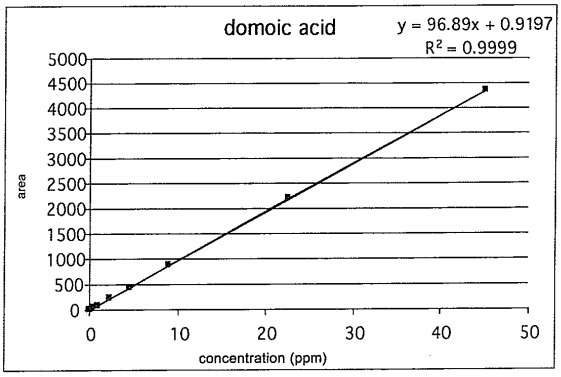

FIGURE 2: *Linearity of the ASP method using different calibration standards.*

PSP Analysis

Soft mussel tissues were removed from their shells and the tissue was rinsed with distilled water. After draining, the tissue was homogenized in a glass blender to produce a smooth slurry. Duplicate aliquots (50 g) of each sample were weighed into clean 250 ml beakers and the PSP toxins were extracted by gently boiling for 5 min with 50 ml 0.1 M HCl, according to the standard mouse bioassay procedure method. The slurry was adjusted to about pH = 3 with 1 M NaOH (Merck) and the mixture was transferred to centrifuge tubes and centrifuged for 5 min at 3000 rpm. The supernatant was carefully decanted into a 30 ml polyethylene bottle, capped and stored at –15°C until required for analysis (AOAC, 2000b).

DSP Analysis

Analysis of DSP toxicity by mouse bioassay was performed according to Yasumoto et al. (1978). The hepatopancreases were extracted with acetone at room temperature. After evaporation (Laborata 4001, Heidolph, Kelheim, Germany) of the acetone (Merck), the residue was dissolved in 1% (v/v) Tween 60 (Merck), followed by intraperitoneal injection of the diluted sample into the mouse (Swiss albino, weight limits 18–20 g). Three parallel tests were performed, and the reaction of the mice was observed over 24 h after treatment or until death. If interference occured, the extraction procedure was carried out again using dichlormethane (Merck).

Results and Discussion

In the present study, although the presence of PSP and DSP toxins could not be detected in any of the samples during the sampling period, an average level (95%, k = 2) of 1.94 ± 1.1 µg ASP per g mussel meat (minimum level 1.67 µg/g and maximum level 2.24 µg/g) was determined in 3 (7.31%) of 41 samples obtained in March 2006, in 5 (13.51%) of 37 samples taken in April 2006, in 2 (6.06%) of 33 samples obtained in September 2007, and thus in

TABLE 1: *ASP levels in mussel samples during the sampling period*

	ASP (DA µg/g)									
Year →	**2005**		**2006**				**2007**			
Month ↓	**n**	**mean**	**n**	**min**	**max**	**mean**	**n**	**min**	**max**	**mean**
January			29	none			37	none		
February			39	none			33	none		
March			**(3)[a] 41[n]** (7.31%)	1.67	1.96	1.79	36	none		
April			**(5) 37** (13.51%)	1.86	2.24	2.01	38	none		
May	PROHIBITED PERIOD									
June										
July										
August*	24	none	23	none			21	none		
September	32	none	35	none			**(2) 33** (6.06%)	1.92	2.08	2.00
October	35	none	33	none			37	none		
November	37	none	36	none						
December	30	none	39	none						
Sum	**158**	none	**(8) 312** (2.56%)	1.67	2.24	1.93	**(2) 235** (0.85%)	1.92	2.08	2.00
Total			**n**	**min**	**max**	**mean**				
			(10) 705 (1.42%)	**1.67**	**2.24**	**1.94 ± 1.1**				

a: Number of positive samples.

n: Number of samples.

*: Pre-monitoring month before opening of the fishing period.

Archiv für Lebensmittelhygiene 59, Heft 6 (2008), Seiten 233–238

10 (1.42%) of the total of 705 samples collected during the entire sampling period from August 2005 to October 2007 (Tab. 1). The HPLC/DAD chromatograms of the DA standard and a sample extract containing 2.24 µg/g are shown in Figure 3.

During DSP toxin analysis, in the mouse bioassay of 5 samples diarrheae was detected in three mice used for each sample at the end of 24 h. Next, the extraction procedure was also performed using methanol (Merck). Finally, at the end of the DSP analysis, it was decided that DSP toxins were no longer detectable in those 5 samples.

ASP was first documented in 1987, when people became ill after consuming blue mussels (*Mytilus edulis*) which were contaminated with DA, originated from Eastern Prince Edward Island, Canada (Todd, 1993). Subsequently, awareness of ASP within Europe was raised and, in 1994, DA was detected in mussels harvested from the Galician region of Spain (Arévalo et al., 1998). During 1995, the presence of DA was confirmed in Portuguese shellfish (Vale and Sampayo, 2001) and, due to the potential health risk to shellfish consumers, the EU adopted legislation (Council Directive 97/61/EC) on the maximum allowable concentration of DA permissible in shellfish marketed in Europe. These regulations state that DA should not exceed 20 mg/g in the edible part of the shellfish with evaluation of the DA concentration in shellfish determined using HPLC (Smith et al., 2006).

Programmes to monitor the DA concentrations in shellfish are in place in many countries worldwide and numerous incidents of domoic acid contamination have been reported in a wide variety of shellfish species. The highest concentration of DA recorded in hepatopancreas of an individual king scallop over the study duration was 558.6 µg/g, considerably below the highest concentrations of 1348.1 µg/g reported by Bogan et al. (2007a) for scallops from waters off the southeast of Ireland, the maximum concentrations of 2083 µg/g determined by Arévalo et al. (1998) and 2820 µg/g stated by James et al. (2005).

Researchers reported that seasonal variations could be important in the determination of DA existence (James et al., 2005; Bogan et al., 2007b). Bogan et al. (2007b) showed significant differences in DA concentrations in hepatopancreas between three sampling occasions, the two winter samples (October 2003 and October 2004) exhibiting higher concentrations than the summer sample (June 2004). The highest concentrations of DA were also determined during the winter months of 1999/2000 in king scallop from the west of Ireland despite the absence of high *Pseudo-nitzschia* cell counts (Smith et al., 2006). Temporal studies in Clew Bay, west of Ireland from February 2003 to February 2004 reported the highest concentrations of DA in hepatopancreas from February 2003 to April 2003 (Bogan et al., 2007c). Similarly, in our study ASP toxins were detected only in March and April 2006.

The bioassay also serves as the reference method in the EU and the Regulation (EC) No. 853/2004 states that the total PSP toxin content must not exceed 80 µg/100 g of mollusc flesh in accordance with the biological testing method.

In a study performed at Aveiro Lagoon in Portugal, toxicity exceeding regulatory levels (80 mg STXeq./100 g) was detected using mouse bioassay in the years 1986, 1988–1990, 1993–1995, mainly between the months of August and December. Such toxicity levels later expanded to the Southwest and South coast: shellfish resources were affected mostly in the years 1992–1996 (Sampayo et al., 1997). Taleb et al. (2003) analysed the PSP toxin level on the Atlantic Moroccan shore. The researchers detected a maximum concentration of PSP toxins of 6000 µg STXeq./100 g tissue in mussel samples near Casablanca. Mussels from other sites (Mehdia, Sale, Rabat, Mohammedia, Qualidia, Essaouira) were affected by levels of PSP toxins ranging between 1000 and 3000 µg STXeq./100 g tissue. Nagashima et al. (1990) reported that the highest level of PSP toxins was detected in *Venus gallina* as compared to crabs and *Mytilus galloprovincialis* in shellfish obtained from Japan between 1982 and 1984.

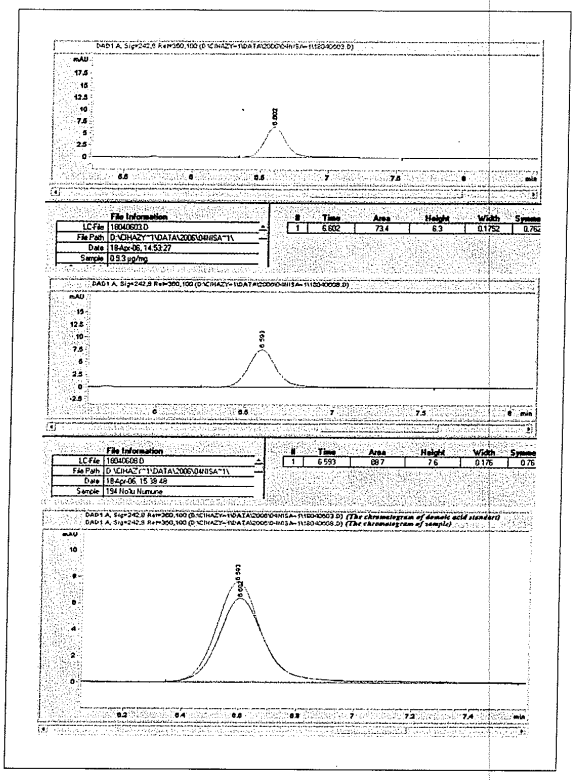

FIGURE 3: *HPLC/DAD chromatograms of a standard containing 0.9 µg/mg DA and a sample extract containing 2.24 µg/g.*

In studies performed in Turkey, Koray (1988) detected that PSP toxin producing *Alexandrium minitum, Gonyaulax polyedra* and DSP toxin producing *Dinophysis fortii, Dinophsis hastata* and *Prorocentrum micans* species were present in the Izmir Gulf region of the Aegean Sea.

Eryigit (1998) reported that although the PSP toxins could not be detected in 211 samples of *Venus gallina* and 48 samples of *Mytilus galloprovincialis*, PSP was detected in 2% of 81 *Ostrea edulis* samples. On the other hand, in a study performed by Ozay (1992) on mostly exported samples of shellfish obtained from the Marmara Sea between 1986 and 1989, PSP toxins could not be detected by mouse bioassays in a total of 47 samples

obtained from the coastline of Tekirdag, Silivri, Istanbul Bosphorus, Karasu, Gemlik, Marmara Ereglisi and Dardanel. Alpbaz et al. (1991) reported that PSP toxins were not determined by mouse bioassay in samples obtained from Izmir Gulf, Aegean Sea monthly between September 1988 and October 1989. It is seen that our results were similar to other researchers'.

In a study performed on the accumulation capacity of DSP toxins in shellfish, it was shown that *Mytilus edulis* was more toxic than *Patinopecten yessoensis* and *Chlamys nipponensis akazara* and that *Ostrea eduli* was also less toxic than the shellfish mentioned above (Yasumoto et al., 1978).

In studies performed by Yasumoto et al. (1978, 1985) in Japan, the researchers reported that the risk level of DSP toxicity was the highest in June and July. On the other hand, Lee et al. (1988) detected that the level of DSP toxin was higher in November than in the other months.

There are not many studies on the presence of DSP toxins in shellfish harvested on the coastlines of Turkey. Eryigit (1998) reported that DSP toxins were found in 17% of a total of 340 samples consisting of 211 *Venus gallina*, 81 *Ostrea edulis* and 48 *Mytilus galloprovincialis*. The same researcher detected that while the ratio of DSP and PSP toxin positive samples was 21% in 1995, the ratio decreased to 18% in 1996 and that the highest level of positive samples (25%) was determined in spring. Findings obtained from our study show differences to the results reported by Eryigit (1998).

According to the results of our study, the levels of ASP, PSP and DSP toxins in the samples analysed were found to be lower than the maximum tolerable limits given in Regulation (EC) No. 853/2004. It was concluded that the levels of toxins in mussel samples were no problem for human consumption and export, because ASP was determined at fairly low levels in the samples obtained in the spring period when algal growth is increased, while PSP and DSP toxins could not be detected in any of the samples. These results provide a basis for effective monitoring and control of these toxins in Turkey.

References

Alpbaz A, Yurekliturk O, Temelli B (1991): PSP (biotoxin) in some crustaceans obtained from Izmir Gulf. Su Urunleri Dergisi 8: 208–213.

AOAC (2000a): Domoic acid in mussel. AOAC official method 991.26. Chapter 49: 61.

AOAC (2000b): Paralytic shellfish poison, biological method. AOAC official method 959.08. Chapter 49: 59–61.

Arévalo FF, Bermudez de la Puente M, Salgado C (1998): ASP toxicity in scallops: individual variability and tissue distribution. In: Reguera BB, Blanco J, Fernandez ML, Wyatt T (eds.), Harmful algae. Xunta de Galicia and IOC of UNESCO, Spain, 499–502.

Bates SS (2000): Domoic acid producing diatoms: another genus added. J Phycol 36: 978–985.

Bogan YM, Bender K, Hervas A, Kennedy DJ, Slater JW, Hess P (2007a): Spatial variability of domoic acid concentration in king scallops *Pecten maximus* off the southeast coast of Ireland. Harmful Algae 6: 1–14.

Bogan YM, Kennedy DJ, Harkin AL, Gillespie J, Vause BJ, Beukers-Stewart BD, Hess P, Slater JW (2007b): Variation in domoic acid concentration in king scallop (*Pecten maximus*) from fishing grounds around the Isle of Man. Harmful Algae 6: 81–92.

Bogan YM, Harkin AL, Gillespie J, Kennedy DJ, Hess P, Slater JW (2007c): The influence of size on domoic acid concentration in king scallop, *Pecten maximus*. Harmful Algae 6: 15–28.

Eryigit A (1998): Investigation of Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP) toxin residues in some crustaceous hanting from the costline of our country. Istanbul, University of Istanbul, PhD thesis.

Fattorusso E, Ciminiello P, Costantino V, Magno S, Mangoni A, Milandri A, Poletti R, Pompei M, Viviani R (1992): Okadaic acid in mussels of Adriatic Sea. Mar Poll Bull 24: 234–237.

Hamano Y, Kinoshita Y, Yasumoto T (1986): Enteropathogenicity of diarrhetic shellfish toxins in intestinal models. J Food Hyg Soc Jpn 27: 375–379.

James KJ, Gillman M, Amandi MF, Lopez-Rivera A, Puente PF, Lehane M, Mitrovic S, Furey A (2005): Amnesic shellfish poisoning in Ireland. Toxicon 46: 852–858.

Jeon JK, Han MS, Lee HO (1996): Paralytic shellfish toxins in the mussel and *Alexandrium tamarense* (dinoflagellate) from Gamraepo, Korea in 1989. In: Yasumoto T, Oshima Y, Fukuyo Y (eds.), Harmful and toxic algal blooms. Sendai, UNESCO, 65–68.

Koray T (1988): Rarely known natural disaster: Red-tide and its caused toxicities. Bilim ve Teknik 21: 9–13.

Koray T (1992): Red-tide in the Gulf of Izmir and its importance on public health. Cevre Bulteni 1: 13–15.

Lee JS, Tangen K, Dahl E, Hovgaard P, Yasumoto T (1988): Diarrhetic shellfish toxins in Norwegian mussels. Bull Jpn Soc Sci Fish 54: 1953–1957.

Morono A, Arevalo F, Fernandez ML, Manerio J, Pazos Y, Salgado C, Blanco J (2003): Accumulation and transformation of DSP toxins in mussels *Mytilus galloprovincialis* during a toxic episode caused by *Dinophysis acuminata*. Aquat Toxicol 62: 269–280.

Nagashima Y, Noguchi T, Kawabata T, Hashimoto K (1990): Improved extraction method for paralytic shellfish poison to be used in mouse bioassay. Nippon Suisan Gakkaishi 56: 765–770.

Oshima Y, Sugino K, Itakura H, Hirota M, Yasumoto T (1990): Comparative studies on paralytic shellfish toxin profile of dinoflagellates and bivalves. In: Graneli E, Sundstrom B, Edler L, Anderson DM (eds.), Toxic marine phytoplankton. Elsevier, New York, USA, 391–396.

Ozay G (1992): Saxitoxin (Paralytic Shellfish Poison) contamination in some crustaceans and risks for human health. Gıda Sanayi 6: 16–24.

Sampayo MAM, Franca S, Sousa I, Alvito P, Vale P, Botelho MJ, Rodrigues S, Vieira A (1997): Dez anos de monitorização de biotoxinas marinhas em Portugal (1986–1996). Arq Inst Nacional Saúde 23: 187–194.

Smith EA, Papapanagiotou EP, Brown NA, Stobo LA, Gallacher S, Shanks AM (2006): Effect of storage on amnesic shellfish poisoning (ASP) toxins in king scallops (*Pecten maximus*). Harmful Algae 5: 9–19.

Taleb H, Vale P, Blaghen M (2003): Spatial and temporal evolution of PSP toxins along the Atlantic shore of Morocco. Toxicon 41: 199–205.

Terao K, Ito E, Oarada M, Murata M, Yasumoto T (1990): Histopathological studies on experimental marine toxin poisoning. The effects in mice of yessotoxin isolated from *Patinopecten yessoensis* and of a desulfated derivate. Toxicon 28: 1095–1104.

Terao K, Ito E, Ohkusu M, Yasumoto TA (1993): Comparative study of the effects of DSP toxins on mice and rats. In: Smayda TJ (ed.), Toxic phytoplancton blooms in the sea. Elsevier, Amsterdam, Netherlands, 581–586.

Todd ECD (1993): Domoic acid and amnesic shellfish poisoning, a review. J Food Prot 56: 69–83.

Vale P, Sampayo MAM (2001): Domoic acid in Portuguese shellfish and fish. Toxicon 39: 893–904.

Archiv für Lebensmittelhygiene 59, Heft 6 (2008), Seiten 233–238

Van Egmond H, Speijers G, van den Top H (1992): Current situation on world-wide regulations for marine phycotoxins. J Nat Toxins 1: 67–85.

Yasumoto T, Murata M, Oshima Y, Sano M, Matsumoto GK, Clardy J (1985): Diarrhetic shellfish toxins. Tetrahedron 41: 1019–1025.

Yasumoto T, Oshima Y, Yamaguchi M (1978): Occurrence of a new type shellfish poisoning in the Tohoku district. Bull Jap Soc Sci Fish 44: 1249–1255.

Zaman L, Arakawa O, Shimosu A, Onoue Y, Nishio S, Shida Y, Noguchi T (1997): Two new isomers of domoic acid from a red alga, *Chondria armata*. Toxicon 35: 205–212.

Address for correspondence:
Assoc. Prof. Dr. Ugur Gunsen
University of Balikesir
Bandirma Vocational High School
Department of Food Hygiene
10200, Bandirma, Balikesir
Turkey
ugur_gunsen@hotmail.com