

# Occurrence of marine biotoxins in bivalve molluscs available in Poland in 2014–2018

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## Abstract

**Introduction:** Marine biotoxins are toxic substances that may cause illness and death in marine organisms and humans. This article disseminates the results of a 4-year study on the occurrence of marine biotoxins in raw bivalve molluscs purchased from Polish suppliers. **Material and Methods:** A total of 256 samples of 8 different molluscs species were analysed for the presence of biotoxins using the ELISA method for paralytic shellfish poison, diarrhoetic shellfish poison, and amnaesic shellfish poison. **Results:** The permitted limits of marine biotoxin content were not exceeded in any of the analysed samples and the majority of them were free from these compounds. **Conclusion:** The results of the study indicate that the tested raw bivalve molluscs available in Poland were safe for consumers.

**Keywords:** marine biotoxins, ELISA, bivalve molluscs, food control.

## Introduction

The occurrence of toxic contaminants in food and its sources is a very important issue in food safety. Marine biotoxins are a group of chemical compounds produced by phytoplankton and other aquatic organisms. Plankton containing toxins is the main feed for marine molluscs such as clams, oysters, scallops, mussels, and shellfish (13, 20). It can produce compounds that are toxic to humans under favourable conditions, especially during algal bloom. The occurrence of harmful algal blooms (HABs) is caused by the overgrowth of marine phytoplankton (5, 19). During recent years, the amount and intensity of HABs have been increasing on a global scale due to rising water temperatures and escalating coastal eutrophication. Climate changes and hydrographic disturbance can also affect phytoplankton development (18, 29). Algae belonging to species such as *Alexandrium*, *Dinophysis*, *Gymnodinium*, and *Pseudo-nitzschia* are the main source of marine biotoxins which are produced under beneficial conditions for their growth, *i.e.* with the availability of organic matter, microelements, O<sub>2</sub>/CO<sub>2</sub>, presence of light, appropriate water temperature or change in salinity due to sea currents (5, 13, 18, 19). Consumption of

molluscs contaminated with marine biotoxins may result in human food poisoning and even death. The main biotoxins found in marine shellfish are the saxitoxins (STX), causing paralytic shellfish poisoning (PSP); domoic acid (DA), responsible for amnesic shellfish poisoning (ASP); okadaic acid (OA), inducing diarrhetic shellfish poisoning (DSP); and neurotoxins, the effect of which can be neurotoxic shellfish poisoning (NSP) (21, 24, 25). The occurrence and severity of intoxication symptoms depend on the type of marine biotoxins present in a bivalve mollusc and the consumed quantity. These include severe gastrointestinal disorders with diarrhoea, nausea, vomiting, abdominal pain, and sometimes neurological symptoms such as ataxia, disorientation, memory loss, and respiratory paralysis (20). Human exposure to marine biotoxins occurs most often after occasional consumption and is characterised by acute and short-term symptoms, therefore acute reference doses (ARfD) have been established for these toxic compounds (29). The ARfD, which correspond to the concentration of marine biotoxins per kg of shellfish meat when consuming a large portion (400 g) of shellfish is 30 µg DA eq., 0.3 µg OA eq. and 0.5 µg STX eq. per kg body weight for ASP, DSP and PSP, respectively.

The aim of the present study was to assess the marine biotoxin contamination of live bivalve molluscs available on the Polish market.

## Material and Methods

**Bivalve molluscs.** A total of 256 samples purchased in 2014–2018 were used in the study. They were fresh molluscs obtained from the market and wholesalers in Poland. The following species of bivalve molluscs were tested: blue mussel (*Mytilus edulis*), Japanese carpet shell (*Tapes philippinarum*), Pacific oyster (*Crassostrea gigas*), razor clam (*Ensis directus*), common cockle (*Cerastoderma edule*), dog cockle (*Glycymeris glycymeris*), hard clam (*Mercenaria mercenaria*) and great scallop (*Pecten maximus*). The molluscs were grown in France, Norway, the Netherlands, Italy, Spain, and Denmark. Among the eight different mollusc species used for the detection of biotoxins, the blue mussel and Japanese carpet shell were the most heavily represented with 55 and 52 samples, respectively, whereas only eight great scallops were analysed because their availability on the market was limited. The samples were delivered refrigerated to the laboratory in their original packaging. After opening and removing the shells, the meat of the molluscs was homogenised and frozen at below  $-18^{\circ}\text{C}$  for analysis.

**ELISA.** The determination of the presence of marine biotoxins was carried out using an ELISA. The RIDASCREEN FAST PSP SC test (R-Biopharm, Darmstadt, Germany) with a working range of 50–800  $\mu\text{g}/\text{kg}$  of shellfish meat

was used for analysis of PSP toxins. DSP toxins were determined by the Okatest DSP (Zeu-Immunotec, Zaragoza, Spain) and its assay range was 63–352  $\mu\text{g}/\text{kg}$  of shellfish. For the identification of ASP toxins, use was made of the ASP DIRECT ELISA (Biosense Laboratories, Bergen, Norway) with its working range of 0.01–250  $\text{mg}/\text{kg}$ . Preparation of the samples and the analysis were performed according to the procedures included in the test kits.

## Results

Out of 256 analysed samples, 126 (49.2%) contained marine biotoxins, with the most common being PSP (66 samples, 25.8%) (Table 1). Biotoxins of this group were identified in all species of bivalve molluscs tested. DSP toxin was found in all but great scallop molluscs, altogether in 50 samples (19.5%). ASP toxin was detected in 10 (3.9%) of the samples tested, and was absent from razor clams, common cockles and dog cockles.

The quantitative results of the analyses of PSP, DSP, and ASP toxins in bivalve molluscs are shown in Table 2. None of the samples tested exceeded the legal limits for biotoxins, which are 800  $\mu\text{g}/\text{kg}$  for PSP, 160  $\mu\text{g}/\text{kg}$  for DSP, and 20  $\text{mg}/\text{kg}$  for ASP (10). The highest levels of PSP and ASP were found in great scallops (783.5  $\mu\text{g}/\text{kg}$  and 2.7  $\text{mg}/\text{kg}$ , respectively), whereas DSP was identified in the highest concentration in Japanese carpet shells and Pacific oysters (112  $\mu\text{g}/\text{kg}$  in both molluscs).

**Table 1.** Bivalve molluscs samples tested and positive for biotoxins

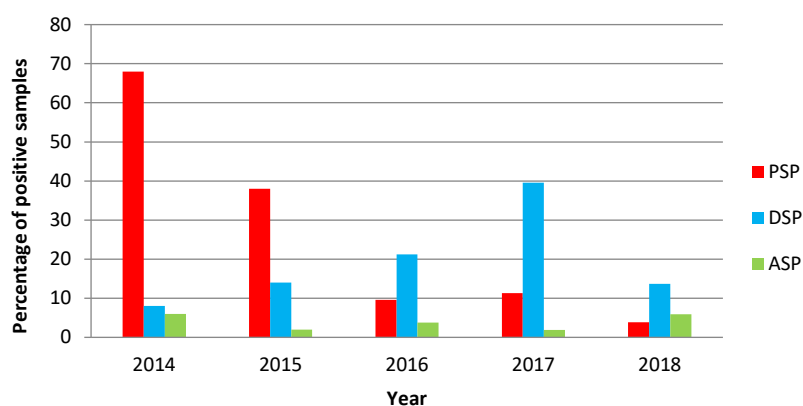
Bivalve molluscs	Number of samples tested	Number (%) of positive samples		
		PSP*	DSP**	ASP***
Blue mussel	55	16 (29.1)	14 (25.5)	3 (5.5)
Japanese carpet shell	52	12 (23.1)	6 (11.5)	1 (1.9)
Pacific oyster	42	11 (26.2)	18 (42.9)	1 (2.4)
Razor clam	29	8 (27.6)	3 (10.3)	0
Common cockle	25	4 (16.0)	4 (16.0)	0
Dog cockle	24	5 (20.8)	2 (8.3)	0
Hard clam	21	5 (23.8)	3 (14.3)	1 (4.8)
Great scallop	8	5 (62.5)	0	4 (50.0)
Total	256	66 (25.8)	50 (19.5)	10 (3.9)

\*PSP – paralytic shellfish poison; \*\*DSP – diarrhetic shellfish poison; \*\*\*ASP – amnesic shellfish poison

**Table 2.** Presence of marine biotoxins in bivalve molluscs tested

Bivalve molluscs (number of positive samples)	PSP ( $\mu\text{g}/\text{kg}$ )*		DSP ( $\mu\text{g}/\text{kg}$ )*		ASP ( $\text{mg}/\text{kg}$ )*
	Range	Mean $\pm$ SD**	Range or content	Mean $\pm$ SD	Content
Blue mussel (33)	51.7–108.5	72.6 $\pm$ 18.4	66–93	79.4 $\pm$ 8.4	0.1; 0.1; 0.5
Japanese carpet shell (19)	54.2–77.1	62.5 $\pm$ 7.9	67–112	83.8 $\pm$ 20.0	0.2
Pacific oyster (30)	50.3–128.6	72.3 $\pm$ 25.8	65–112	77.1 $\pm$ 13.4	0.2
Razor clam (11)	51.0–82.2	64.1 $\pm$ 10.1	72–85	79.0 $\pm$ 6.6	-
Cockle (8)	52.97–0.9	58.3 $\pm$ 8.5	70–81	75.0 $\pm$ 5.83	-
Dog cockle (7)	51.6–64.3	54.7 $\pm$ 5.5	77; 101	89.0 $\pm$ 17.0	-
Hard clam (9)	55.1–90.8	67.4 $\pm$ 15.8	68–81	72.3 $\pm$ 7.5	0.1
Great scallop (9)	53.9–783.5	467.3 $\pm$ 314.4	-	-	0.2; 0.9; 2.7; 2.7
Range	50.37–83.5		65–101		0.1–2.7

\*As in Table 1; \*\*SD – standard deviation



**Fig. 1.** Percentage of positive samples for marine biotoxins tested in 2014–2018  
PSP – paralytic shellfish poison; DSP – diarrhoeic shellfish poison; ASP – amnesic shellfish poison

Fig. 1 shows the percentages of positive samples identified in the 2014–2018 duration of the investigation. It was found that in the years 2014 and 2015 the molluscs were mainly positive for PSP toxin (68% and 38%, respectively). In 2016–2018 the samples were mainly contaminated with DSP toxin, especially in 2017 (39.6% molluscs). ASP toxin was identified in all years at the lowest level, ranging from 2% in 2015 to 5.9% in 2018.

## Discussion

Testing the biotoxin content in molluscs and determining the health effects of consumption of molluscs are necessary for the protection of public health, because marine biotoxins may be a cause of serious human diseases. According to EU legislation, the content of biotoxins in molluscs must not exceed established legal limits. Commission Regulation (EC) No. 853/2004 sets the maximum contents of marine biotoxins and Regulation (EC) No. 2017/625 prescribes plans for monitoring and sampling from live bivalve mollusc production (11, 12). Molluscs account for 11% of seafood consumption in the EU countries and 65% of them originate from domestic production. The most consumed species of molluscs are mussels, followed by scallops and clams (23). In 2017, 54 biotoxin-related outbreaks were reported in the EU and 170 humans were affected. Fourteen of those intoxications required hospitalisation but none were fatal (10). Data from the 2018 Rapid Alert System for Food and Feed report showed 22 notifications related to excessively high levels of natural toxins including marine biotoxins, whereas in 2019 there were 45 (26, 27). However, the real number of cases may be higher, because the current epidemiological systems for biotoxin detection are ineffective (28).

Compared to the previous investigation on marine biotoxin content performed in Poland between 2009 and 2013, the present study covering the years 2014–2018

showed lower percentages of positive samples (67.4% vs. 49.2%) (21). Similarly to the levels discovered in the earlier analyses, the highest levels of PSP and ASP were detected in great scallops and were 532.6 µg of PSP per kg mollusc meat and 1.0 mg of ASP per kg in 2009–2013 and 783.5 µg/kg and 2.7 mg/kg in the present study, respectively.

The highest number of intoxications with PSP were recorded in the Philippines in the years 1983–2002: 2,124 cases, including 120 deaths (8). In Europe and North America, 200 cases and no fatalities were reported, while in Southeast Asia and South America the number of deaths due to PSP toxin ranged from 2 to 14% (6).

Toxins of the DSP group occur most often and are detected at a high level in shellfish from southern European coastal areas (4). The largest epidemic due to this biotoxin was noted in Belgium in 2002 with 403 persons affected after consumption of blue mussels produced in Denmark (9). In France, 11 DSP outbreaks with 45 cases were reported in 2009 (14). The level of DSP toxins in mussels was eight times higher than that allowed by European law. In 2010, more than 300 consumers in northern Italy were poisoned through the consumption of mussels containing DSP biotoxins (2). High levels of DSP toxins were also identified in shellfish from China, which caused illness in more than 200 people in 2011 (17). At the same time, 57 intoxications with DSP biotoxins were detected in the country of origin of those shellfish, in Zhejiang Province (7).

In Europe, intoxication with ASP toxin has never been reported. However, in North America, there was an ASP epidemic in Canada in 1987, in which 150 people were infected, including 19 who were hospitalised, and 4 who died after eating shellfish contaminated with ASP (15). Currently, there is no poisoning with domoic acid due to effective monitoring programmes of this toxin in shellfish and coastal waters (16).

Since the beginning of the 21st century, thousands of poisonings due to the consumption of seafood contaminated with marine biotoxins have been reported.

Besides PSP, DSP and ASP, other marine biotoxins have also been detected as a cause of poisoning in humans and they are those causing CFP (ciguatera fish poisoning), TTX (tetrodotoxin) poisoning, and AZP (azaspiracid poisoning). In 2001–2015, more than 3,400, 500, and 200 cases of CFP, TTX poisoning and AZP were reported worldwide, respectively (22). Taking into account the toxicity and high occurrence of poisonings, monitoring programmes of these toxins should be developed or improved.

Consumption of seafood contaminated with marine biotoxins may result in serious diseases in humans, therefore their monitoring in bivalve molluscs is crucial for food safety. There are fewer cases of intoxications described in countries where monitoring programmes and effective relevant health protection systems were developed. Although the current and previous studies carried out in Poland showed that the shellfish available on the domestic market are safe for consumers, further investigations are required. Furthermore, the data on human risk factors and strategies for the prevention of harmful biotoxins in seafood needs to be systematised.

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