

ORIGINAL PAPER

# A THEORETICAL ESTIMATION OF EXPOSURE TO SHELLFISH TOXINS OF DIFFERENT POPULATION GROUPS IN BULGARIA

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## ABSTRACT

**Introduction.** Shellfish poisoning could be caused by ingestion of shellfish contaminated by bacteria, viruses, heavy metals, pesticides, marine biotoxins etc.

**The objective of the study** was to determine the theoretical human exposure to shellfish toxins of Bulgarian consumers. The incidence of registered cases of intoxications due to marine biotoxins ingestion - “Toxic effect: Other fish and shellfish poisoning (T61.2)” in regional hospitals was investigated.

**Materials and methods.** Acute exposure (AE), chronic exposure (CE) and hazard quotient (HQ) are calculated based on experimentally-determined levels of shellfish toxins in mussels harvested from North Bulgarian coast in the period 2012 - 2018. We performed a retrospective analysis of the frequency of the diagnosis T61.2, based on medical documentation for the period 1.05.2012 - 7.12.2018 in hospitals in Varna and Dobrich, Bulgaria.

**Results.** Shellfish toxins that most often occur in mussels are domoic acid (DA), yessotoxin (YTX) and pectenotoxin-2 (PTX2). The calculated maximum

## RÉSUMÉ

### RÉSUMÉ

**Détermination théorique de l'exposition aux toxines des mollusques dans des groupes différents de la population en Bulgarie**

**Introduction.** L'intoxication par des mollusques peut être causée de l'ingestion des mollusques contaminés par des bactéries, virus, métaux lourds, pesticides, biotoxines marines, etc.

**L'objectif de l'étude** était de déterminer l'exposition théorique des hommes aux toxines des mollusques et des crustacés par les consommateurs bulgares. L'incidence des cas enregistrés d'intoxications dues à l'ingestion de biotoxines marines - «L'effet toxique: autres intoxications aux poissons et crustacés (T61.2)» a été étudiée.

**Matériaux et méthodes.** L'exposition aiguë (AE), chronique (CE) et le quotient de risque (HQ) sont calculés à la base des niveaux déterminés expérimentalement de toxines des mollusques dans les moules

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AE was 1.00 mg/kg bw for DA, 0.02 mg/kg bw for YTX and 0.0045 mg/kg bw for PTX2, respectively. Maximum CE for DA was 0.01 mg/kg bw/day. All estimated values were much lower than those legislated by European Food Safety Authority thresholds. The exposure to marine biotoxins was estimated based on contamination data of mussels harvested from the same region. The analysis for the whole period showed that its frequency is lower than 1%. Clinicians report that this diagnosis is presumptive, based on recent ingestion of shellfish and presence of symptoms consistent with shellfish poisoning.

**Conclusions.** Estimated theoretical acute and chronic exposures agree upon the incidence of clinical cases with the studied diagnosis. A parallel investigation on a prolonged period is required, to increase the accuracy of these results.

**Keywords:** acute exposure, chronic exposure, phycotoxins, mussels, Black Sea.

**List of abbreviations:**

ICD – International Classification of Diseases  
AE – acute exposure  
CE – chronic exposure  
EFSA – European Food Safety Authority  
BW – body weight  
ASP – amnesic shellfish poisoning  
PSP – paralytic shellfish poisoning  
DSP – diarrhetic shellfish poisoning  
ARfD – acute reference dose  
TDI – tolerable daily intake  
CONTAM – Contaminants in the Food Chain  
FAO/WHO/IOC – Food and Agriculture Organization of the United Nations/ World Health Organization/ Intergovernmental Oceanographic Commission

**INTRODUCTION**

Crustacean and mollusks are generally referred to as ‘shellfish’ in the context of seafood consumption<sup>1</sup>. Shellfish poisoning, linked with consuming of contaminated molluscan bivalves and other shellfish, is a worldwide seafood toxicity problem. It could be caused by ingestion of shellfish (mussels, oysters, clams, etc) contaminated by bacteria, viruses, heavy metals, pesticides, marine biotoxins, etc. Depending on the shellfish poisoning causative agent, in the International Classification of the diseases (ICD) different types of intoxications are coded with a specific letter and number.

Marine biotoxins (phycotoxins) are natural metabolites produced by micro-algae. Through accumulation in the food chain, these toxins may

récoltées sur la côte nord de la Bulgarie au cours de la période 2012 – 2018. L’analyse de la fréquence du diagnostic T61.2 est rétrospective et basée sur une enquête dans la documentation médicale pour la période du 1.05.2012 au 7.12.2018 dans les hôpitaux de Varna et de Dobrich, en Bulgarie.

**Résultats.** Au cours de la période étudiée, les toxines des mollusques et des crustacés qui se trouvent le plus souvent dans les moules sont l’acide domoïque (DA), la yessotoxine (YTX) et la pecténotoxine-2 (PTX2). L’EE maximale calculée était de 1,00 microgramme/kg pc pour l’AD, 0,02 microgramme/kg pc pour YTX et 0,0045 microgramme/kg pc pour PTX2 respectivement. La CE maximale pour l’AD était de 0,01 microgramme/kg pc /par jour. Toutes les valeurs estimées étaient bien inférieures aux seuils légiférés par L’Agence européenne de sécurité des aliments. L’incidence du diagnostic T61.2 a été étudiée dans les hôpitaux de Varna et de Dobrich, car l’exposition aux biotoxines marines a été estimée sur la base des données de contamination des moules récoltées dans la même région. L’analyse sur l’ensemble de la période a montré que sa fréquence est inférieure à 1%. Les cliniciens signalent que ce diagnostic est présumé, basé sur l’ingestion récente des mollusques et des crustacés et la présence de symptômes compatibles avec une intoxication par les mollusques.

**Conclusions.** Les expositions aiguës et chroniques théoriques estimées sont en accord avec l’incidence des cas cliniques dans le diagnostic étudié. Une enquête parallèle sur une période prolongée est nécessaire pour que l’exactitude de ces résultats soit augmentée et confirmée.

**Mots-clés:** exposition aiguë, exposition chronique, phycotoxines, moules, mer Noire.

concentrate in different marine organisms, used as food. Therefore, adverse health problems resulting from consumption of phycotoxin contaminated molluscan bivalves and other shellfish are differentiated in ICD-10 (2019)<sup>2</sup> and defined as “Toxic effect: Other fish and shellfish poisoning (T61.2)”

Shellfish poisoning is a worldwide seafood toxicity problem<sup>3,4</sup>. Growing international trade of seafood products has recently added to the popularity and frequency of consumption of mussels and other shellfish. Even more, consumers from countries with coastlines (including Bulgaria) are more likely to eat oftener fish products than consumers from land-locked countries<sup>5</sup>. This might lead to increased acute and chronic exposure to certain phycotoxins if mussels are contaminated. Symptoms of intoxication

may vary from gastrointestinal to neurological, depending on the type of the phycotoxin ingested and the level of intoxication.

**THE OBJECTIVE OF THE STUDY** was to determine the theoretical acute and chronic exposure to shellfish poisoning toxins of Bulgarian consumers and investigate the incidence of registered cases with the diagnosis "Toxic effect: Other fish and shellfish poisoning (T61.2)" in regional hospitals.

## MATERIAL AND METHODS

Human exposure was evaluated by calculating acute exposure (AE) and chronic exposure (CE) based on experimentally-determined levels of shellfish toxins in mussels harvested from North Bulgarian coast in the period 2012 - 2018 and intended for human consumption.

For the estimation of daily acute exposure (AE), the following formula<sup>6</sup> was used:

$$AE = \frac{mxw_t}{Bw}$$

where:

AE - acute exposure adjusted for bodyweight ( $\mu\text{g}/\text{kg bw}$ )  
*m* - weight of portion (kg) refers to mean portion size -  
 a) calculated in a mussel consumption survey<sup>7</sup> that corresponds to 0.234 kg; b) proposed by EFSA<sup>8</sup> - 0.400 kg

*w<sub>t</sub>* - phycotoxin level ( $\mu\text{g}/\text{kg}$ ) is the maximum concentration of each phycotoxin identified in the samples  
*BW* - bodyweight (kg) is the mean bodyweight in investigated population that corresponds to 72.5 kg for the period 2008-2013<sup>9</sup> and 74 kg for the period 2014-2018<sup>10</sup>.

The chronic exposure assessment corresponds to the level of exposure after a daily consumption of shellfish<sup>66</sup>:

$$CE = \frac{w_t \times B}{Bw}$$

CE - individual chronic exposure ( $\text{mg}/\text{kg}/\text{day}$ ) to a phycotoxin

*w<sub>t</sub>* - phycotoxin level ( $\mu\text{g}/\text{kg}$ ) is the mean positive concentration of each phycotoxin identified in the samples  
*D* is mean mussel consumption per day (consumption rate) ( $\text{kg}/\text{day}$ ). It corresponds to 0.005  $\text{kg}/\text{day}$ <sup>8</sup> for Bulgarian population.

The consumption rate and body weight for local population (Varna district) and recreational harvesters were obtained through a survey on a mussel consumption by means of interview among 78 local citizens and 31 recreational harvesters.

The exposure to shellfish poisoning toxins was calculated based on contamination of mussels with following toxins: amnesic shellfish poisoning (ASP) due to the presence of domoic acid<sup>7</sup>; paralytic shellfish poisoning (PSP) - saxitoxins and its derivatives<sup>11-13</sup> diarrhetic shellfish poisoning (DSP) - pectenotoxin-2; yessotoxin poisoning - yessotoxin<sup>14</sup>.

The estimated values were compared with the acute reference dose (ARfD) accepted by European Food Safety Authority (EFSA)<sup>15</sup> and tolerable daily intake (TDI) proposed by Kumar et al. (2009)<sup>16</sup>.

We performed a retrospective analysis of the frequency of the diagnosis "Toxic effect: Other fish and shellfish poisoning (T61.2)", based on medical documentation for the period 1.05.2012 - 7.12.2018 in the following medical units: Department of Toxicology of "Military Medical Academy - Varna", Department of Gastroenterology - Hospital "Sveta Marina" in Varna, Emergency Department - Hospital "Sveta Anna" in Varna, and in the Emergency Department and Department of Internal Diseases in the Hospital of Dobrich, Bulgaria. The investigator fulfilled in a questionnaire regarding demographic data of the patients and the medical care provided. Additionally, the results of this survey were discussed with medical consultants from the respective department.

## RESULTS

To assess the exposure, mussel's contamination data must be combined with data about physical characteristics (body weight) and consumption behaviour (portion size, consumption rate, etc) of investigated population<sup>17</sup>. Calculation of AE was complied with the body weight of Bulgarian population for different years. CE was estimated based on the data provided in Table 1.

The results on AE show that, apart from exposure to PSP toxins from consumption of a EFSA portion, dietary intake estimates derived for all detected toxins were below the ARfD (Table 2).

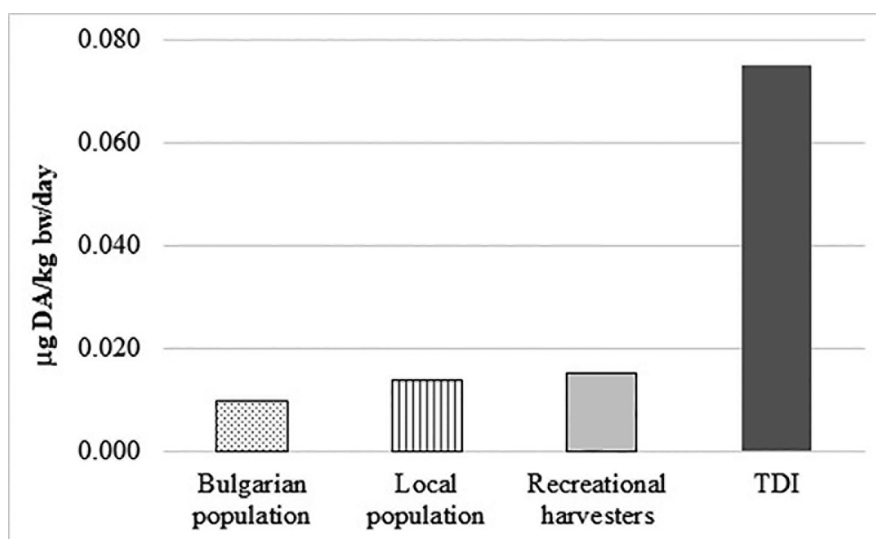
The results on CE showed that recreational harvesters are the most exposed. Even though, exposure

**Table 1.** Consumption behavior of different population groups

Population group	Number of persons in the sample	Average body weight [kg]	Consumption rate [kg/day]
Bulgarian population <sup>8</sup>	373	73	0.005
Local citizens	78	70	0.007
Recreational harvesters	31	88	0.010

**Table 2.** Calculated acute exposure of Bulgarian consumers to marine biotoxins (data is provided only for periods when marine biotoxins are detected in studied mussels)

	Exposure based on Bulgarian portion size		Exposure based on EFSA portion size	
	µg/kg bw	%ARfD	µg/kg bw	%ARfD
PSP (ARfD 0.5 µg STX equivalent/kg bw)				
2012	0.465	93	0.795	159
2016	0.162	32	0.276	55
2017	0.001	< 1	0.002	< 1
ASP (ARfD 30 µg DA+epi-DA/kg bw)				
2016	0.332	1	0.568	2
2017	0.869	3	1.486	5
2018	0.001	<< 1	0.002	<< 1
DSP (ARfD 0.8 µg OA equivalent/kg bw)				
2017	0.004	< 1	0.007	< 1
2018	0.001	< 1	0.001	< 1
Yessotoxin (ARfD 25 µg YTX equivalent/kg bw)				
2016	0.001	<< 1	0.001	<< 1
2017	0.011	< 1	0.019	< 1
2018	0.008	< 1	0.013	< 1



**Figure 1.** Calculated chronic exposure to ASP toxins of frequent consumers from different population groups for the investigated period.

is much lower than the permitted level (TDI = 0.075 mg/kg bw/day) (Figure 1).

The investigation on the incidence of the diagnosis “Toxic effect: Other fish and shellfish poisoning (T61.2)” showed that no such cases were registered in the medical units in Varna, whereas in the Hospital of Dobrich only 0.5% of the toxicological cases were diagnosed as shellfish poisoning (Table 3).

The discussion of the results of this survey with medical consultants from the medical units showed several main points that should be underlined:

- clinicians admit that shellfish poisoning diagnosis is neglected due to lack of knowledge about the presence of these toxins on the Bulgarian Black Sea coast;
- a shellfish poisoning could be misdiagnosed because symptoms of intoxication are similar to allergic reactions and viral or bacterial infections;
- no clinical diagnostic tests (e.g. blood or urine tests) are available.

**Table 3.** Incidence of diagnosis “Toxic effect: Other fish and shellfish poisoning (T61.2)” in Hospital of Dobrich, Bulgaria

Year	Cases of acute intoxications	% T61.2
2012	112	no cases
2013	153	no cases
2014	122	no cases
2015	92	no cases
2016	103	1.9
2017	56	no cases
2018	21	4.8
Total	547	0.5

## DISCUSSION

The preliminary goal of this study was to assess theoretical AE and CE to shellfish poisoning toxins of Bulgarian consumers. Thereafter, the obtained results were compared with the number of registered cases of “Toxic effect: Other fish and shellfish poisoning (T61.2)”, to investigate if there is an accordance between the theoretical results and the incidence of the diagnosis.

Human exposure to marine biotoxins can occur through a variety of mechanisms. Among them, consumption of contaminated shellfish is most commonly reported<sup>18-21</sup>. The symptoms of intoxication with marine biotoxins vary from diarrhea, nausea, abdominal pain<sup>17</sup>, confusion, disorientation, seizures, to respiratory depression and shock<sup>23</sup>, and very rarely death<sup>24</sup>. Based on the available human data on acute toxicity of marine biotoxins, the CONTAM Panel established ARfDs for them (Table 2).

Repeated dietary exposure to low levels of domoic acid could lead to problems with everyday memory<sup>25</sup> of humans and subclinical signs of toxicity in the form of increased toxin susceptibility and impaired mitochondrial function in model animals<sup>26</sup>. CE to saxitoxin of both fish and mammalian models have shown significant changes to antioxidant mechanisms<sup>27</sup>, as well as DNA damage suggestive of apoptosis<sup>28</sup>. Despite these results, the data are considered insufficient and no regulatory limits for CE are legally recognized.

In this study, calculations on exposure are based on theoretical worst-case scenario assumption. For AE estimation, the maximum detected level was used and for CE the mean positive concentration of shellfish toxins detected in mussels from North Bulgarian coast<sup>11-14</sup>. Therefore, the incidence of diagnosis T61.2 was researched in hospitals in the same region with higher capacity, namely Military Medical

Academy – Varna, Hospital “Sveta Marina” in Varna, Hospital “Sveta Anna” in Varna and Hospital of Dobrich, Bulgaria. Furthermore, for AE, the mean body weight of Bulgarian population was used as in case of emergency the regional hospitals accept all Bulgarian patients for examination. The estimates showed that AE to marine biotoxins (Table 2) based on Bulgarian consumption patterns is theoretically not exceeding the respective ARfDs. If a larger portion is consumed an acute effect of PSP could be expected only for a sample from 2012. Even though, for the same year, no cases of shellfish poisoning have been registered in the local hospitals (Table 3).

Remarkable is that for most of the detected toxins for the period of the investigation for both small and large portion size the AE is below 1% of the ARfD. This shows that no adverse effects should be observed if the investigated mussels were consumed. Still, in 2016 and 2018 few cases of T61.2 were registered in a local hospital. The inspection of the medical documentation of these patients shows that no specific tests on the presence on phycotoxins in blood or urine samples were appointed. They were diagnosed based on medical history, including consumption of mussels and symptoms such as nausea and vomiting. A consultation with medical specialists about these cases indicated that the diagnosis of shellfish poisoning based only on the recent consumption of shellfish and the development of clinical manifestations is difficult and can be inaccurate, because these findings are nonspecific and similar with other diseases. On the other hand, the occurrence of seafood poisonings might greatly differ from the estimation in Table 3. Oral communication with frequent consumers elucidates that symptoms of intoxication sometimes appear, but they are mild and fade away easily. This supports the findings of Gessner et al., who investigated the incidence of PSP in Alaska. The incidence estimated via this survey was approximately 100 times higher than the incidence calculated based on surveillance data<sup>29,30</sup>.

As the hereby reported retrospective study, an inquiry for a former period was conducted by Burri and Vale (2006)<sup>31</sup>. These authors analysed the admissions of patients with shellfish poisoning syndrome in several hospitals from around Ria de Aveiro (Portugal) and found a match between toxin levels and gastroenteritis' complaints in several periods. These results support our hypothesis that a shellfish poisoning could be misdiagnosed, because symptoms of intoxication are similar with other diseases.

For CE estimation, the mean values of consumption rates, mean proportion of a given shellfish species in a consumer diet and mean bodyweight were used. This was done following the definition of CE<sup>32</sup> and because we were unable to interview each

population representative for everyday mussel consumption. For CE assessment, estimates of dietary exposure for marine biotoxins had to be compared to the TDI. Currently, no TDI has been established by international organizations such as EFSA and Food and Agriculture Organization of the United Nations/World Health Organization/ Intergovernmental Oceanographic Commission (FAO/WHO/IOC), because of insufficient data. However, Kumar et al. (2009)<sup>15</sup> proposed the TDI value of 0.075 mg/kg bw/day for ASP toxin (domoic acid) based on data referencing non-human primate and human models. As a result, the dietary exposures to domoic acid through the shellfish consumption were more than 5-fold lower than the proposed TDI.

The comparison of the determined AE to shellfish toxins and the incidence of registered cases of shellfish poisoning (T61.2) in regional hospitals have a limited extend. The limitations are due to impossibility to investigate all the mussels intended for human consumption and to assess the real number of shellfish-poisoned consumers. Nevertheless, to our knowledge, this is the first attempt to study the relationship between expected human exposure and cases of shellfish poisoning.

## CONCLUSIONS

The theoretical determination of AE and CE to most frequent shellfish toxins in Bulgarian mussels showed that no human health risk could be expected if mussels are consumed. This conclusion is in accordance with the results obtained from the analysis of the medical records. Our study showed a negligible incidence of clinical cases with the studied diagnosis. A parallel investigation on a prolonged period is required, to increase the accuracy of these results.

## Author Contributions

*Authors Z.V.P., S.K.G., G.N.K., S.Z.Z., M.D.S. declare that all of them have contributed to the conception and design of the paper; acquisition, analysis and interpretation of the data; drafting of the paper and revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the paper in ensuring that questions related to the accuracy or integrity of any part of the paper are appropriately investigated and resolved.*

## Compliance with Ethics Requirements

*“The authors declare no conflict of interest regarding this article.”*

*“The authors declare that all the procedures and experiments of this study respect the ethical standards in the*

*Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.”*

*The study was approved by the Commission for Scientific Research Ethics at Medical University Varna, Bulgaria (Record 67/26.10.2017).*

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