# CAMBODIA

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## I. Introduction

In Cambodia, there are some cases of food poisoning that occurred occasionally and is suspected to be associated with biotoxins that arises from the consumption of aquatic animals. Most of the cases reported were associated to the consumption of puffer fish, which is also known as blow fish. In 2007, it was reported that one child was dead and 12 members of two other families were hospitalized after having puffer fish for lunch in Takeo province. In 2010, it was also reported that one person died and 23 were hospitalized after eating poisonous freshwater puffer fish in Kampong Cham province.

Puffer fish contains toxic substances that are lethal to human. Some studies have revealed that small puffer fish inhabiting in brackish water or freshwater areas in Southeast Asia are also contaminated with toxins that are identified as tetrodotoxin (TTX) and saxitoxin (STX), toxins that belong to the paralytic shellfish poisoning (PSP) family, and are detected as the main toxic principles. However, in Cambodia, PSP contamination in aquatic animals has not been broadly investigated yet, especially toxins in marine bivalves and molluscs that feed on the toxin-producing dinoflagellate which is the potential source of PSP. On the other hand, PSP is also suggested to be a potential source of biotoxins contamination in shellfish which makes it an important indicator for biotoxins monitoring purposes.

Early studies conducted by Laymithuna Ngy *et al*[1]. about the toxicity in the horseshoe crab (*Carcinoscopius rotundicauda*) revealed the presence of TTX in this species, but nil presence of paralytic shellfish toxins. The toxicity of two species of wild Cambodia freshwater puffer fish of genus *Tetraodon T. Turgudus* and *Tetraodon* sp., was further investigated and found to have high toxicity in their skin and ovary (Ngy L. *et al*., 2008)[2]. Recent studies on toxicity and toxin profiles of Cambodia's marine puffer fish (*Takifugu oblongus*) were investigated and revealed that the toxicities ranged from 10 - 132 mouse units, with the main concentration found in ovaries. The study also found that TTX is the main component while STX is a minor component. It was confirmed that T. *Oblongus* is a hazardous species that is unsafe for human consumption (Ngy L. *et al*., 2009)[3].

[1] L Ngy *et al.* 2007. Toxicity assessment for the horseshoe crab *Carcinoscorpius rotundicauda* collected from Cambodia. Toxicon: 49(6):843-7

[2] L. Ngy *et al.* 2008. Occurrence of paralytic shellfish toxins in Cambodia Mekong pufferfish Tetraodon turgidus: selective toxin accumulation in the skin. Toxicon: 51(2):2080-8.

[3] L Ngy *et al.* 2009. Co-occurrence of tetrodotoxin and saxitoxin in Cambodian marine pufferfish *Takifugu oblongus.* African Journal of Marine Science: 31(3): 349-354.

Bivalves are considered as one of the potential hosts for biotoxins, but these species of aquatic animals have not yet been investigated in Cambodia. Bivalves have been widely distributed and preferably consumed amongst other types of seafood, especially Green Mussel. This species of bivalves is collected from the wild and widely marketed in Cambodia.

In this study, Green Mussel (*Perna viridis*) is identified and investigated for PSP contamination. It is envisaged that the results from this study would provide insights for further researches to be conducted, provide recommendations for future biotoxins monitoring programme in Cambodia, as well as contribute to the biotoxins monitoring programme in the Asian region.

## II. Objectives and Goals

The main objective of this study is to investigate PSP contamination in Green Mussel (*Perna viridis*) in Cambodia's marine water using the Mouse Bioassay Method (MBA). A monthly monitoring study would be conducted from June 2011 – May 2012. This study is part of the biotoxins watch programme in Cambodia's marine water, which contributes to the control of biotoxins contamination in Asian region.

## III. Survey Methodologies

### a. Sampling Method, Sampling Site, Target Species, Number of Samples & Sampling Size

In this study, two sampling sites were identified and selected, Koh Preap and Tumnup Rolok, as shown in Figure C1. Koh Preap and Tumnup Rolok are bivalves-inhabiting locations and they are the favourite fishing grounds for local fishermen. These sampling sites are located in Preah Sihanouk province, one amongst the four provinces in coastal areas situated in the South-western part of the Kingdom of Cambodia.
This province is a productive biodiversity area in the Cambodia's Exclusive Economic Zone (EEZ) where it produces all types of seafood including bivalves and molluscs. Most of the fishery products in this area are captured from the wild, but some are from aquaculture farming. In contrast, this province is an industrial area that potentially post risks to the marine environment in this area. With reference to the Asia's mussel watch programme conducted during 1997 – 2001, implemented by the Centre for Marine Environmental Studies (CMES), Japan discovered some level of toxins contamination in mussels collected from this area, but PSP contamination in the species has not yet been investigated. Therefore, the selected sampling sites are suitable for this study to investigate for possible PSP contamination in Cambodian bivalves in this coastal province.

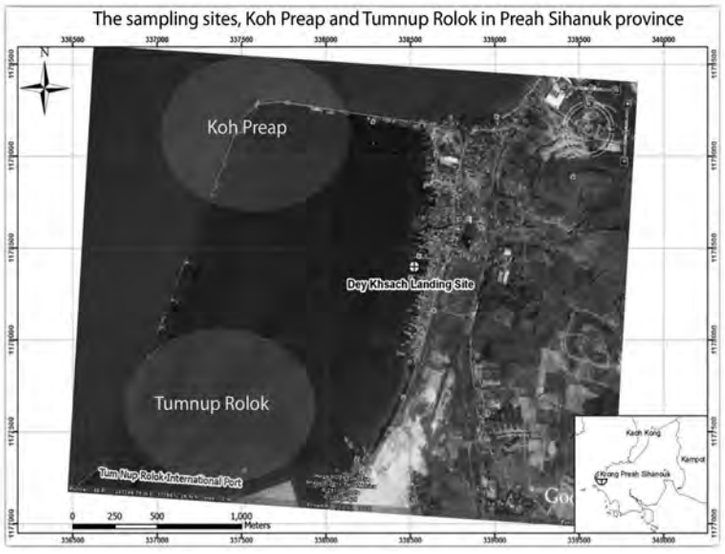


*Figure C1. Map showing the sampling sites of the study*

Green Mussels (*Perna viridis*) are widely distributed and more preferred by consumers amongst other types of bivalves. This type of bivalves is collected from the wild resource and widely marketed in Cambodia. In this study, Green Mussel (*Perna viridis*) is identified for investigation of PSP contamination.

*Figure C2. Identification and measurements of the samples of Green Mussel (Perna viridis)*

The samples were collected monthly, on the fourth week of each month starting from June 2011 - May 2012. Each time, 4 - 5 kg of Green Mussel (*Perna viridis*) samples were collected at the fishing grounds from each sampling site. Then, each piece of identified Green Mussel (*Perna viridis*) was recorded for its length and weight (Table C1). The samples were collected in live form and quickly kept in an icebox to transport to Phnom Penh city. At the laboratory in Phnom Penh, the samples were cleaned and washed several times with clean water before the shell was being cut opened. The whole tissues of weight between 350 - 500g were collected and kept in zip-locked plastic bag with proper labelling and stored in a freezer at -25°C until they are used for testing.

| Sampling Date | Sampling Location | Code | Average Weight (g) | Average Length (cm) |
|---|---|---|---|---|
| June 2011 | Tumnup Rolok | A6 | 19.60 | 7.10 |
| | Koh Preap | B6 | 36.85 | 8.73 |
| July 2011 | Tumnup Rolok | A7 | 26.39 | 8.04 |
| | Koh Preap | B7 | 24.05 | 7.20 |
| August 2011 | Tumnup Rolok | A8 | 11.58 | 5.80 |
| | Koh Preap | B8 | 15.76 | 6.72 |
| September 2011 | Tumnup Rolok | A9 | 22.41 | 7.39 |
| | Koh Preap | B9 | 22.81 | 7.43 |
| October 2011 | Tumnup Rolok | A10 | 15.40 | 6.00 |
| | Koh Preap | B10 | 22.20 | 7.50 |
| November 2011 | Tumnup Rolok | A11 | 15.79 | 6.33 |
| | Koh Preap | B11 | 24.42 | 7.46 |
| December 2011 | Tumnup Rolok | A12 | 23.01 | 7.46 |
| | Koh Preap | B12 | 24.64 | 7.32 |
| January 2012 | Tumnup Rolok | A1 | 21.87 | 7.23 |
| | Koh Preap | B1 | 25.32 | 7.62 |
| February 2012 | Tumnup Rolok | A2 | 18.35 | 6.74 |
| | Koh Preap | B2 | 31.23 | 8.24 |
| March 2012 | Tumnup Rolok | A3 | 30.84 | 8.61 |
| | Koh Preap | B3 | 20.71 | 7.55 |
| April 2012 | Tumnup Rolok | A4 | 26.21 | 8.92 |
| | Koh Preap | B4 | 30.28 | 8.35 |
| May 2012 | Tumnup Rolok | A5 | 27.50 | 8.05 |
| | Koh Preap | B5 | 32.85 | 8.52 |
| Note: A= Samples from Tumnup Rolok, B= Sample from Koh Preap | | | | |

*Table C1. Average length and weight of collected samples, Green Mussel (Perna viridis)*

### b. Method of Analysis

350 - 500g of the whole tissue of Green Mussel (*Perna viridis*) was transferred into icebox for analysis at the laboratory of the Centre of Analytical Service and Experimentation (CASE), Department of Science and Technology of Ho Chi Minh City in Vietnam. The analytical method was conducted in accordance with the CASE laboratory standard protocol for PSP detection using a Mouse Bioassay Method (MBA).

In this study, MBA is used to test the PSP level in Green Mussel (*Perna viridis*). PSP is analyzed qualitatively for the amount of positive or negative toxins present. The internationally recognized method used for analysis of PSP toxins is the standard AOAC Mouse Bioassay method (OAC 1990, AOAC International 1995). This is the only live animal bioassay method that has been fully validated in a collaborative study. The detection level of this method is about 40µg STXeq/100g wet weight of tissue. The procedures were:

1. Boil 100g of tissue with 100ml of 0.1 N HCl for 5 minutes.
2. Adjust the volume back to 200ml and pH to 3 (ideally).
3. Inject the intraperitoneal (i.p.) infection of the acidified extract 0.1N HCL solution into 3 white mice.
4. Observe white mice carefully for 1 hour.

Results are then calibrated against a STX standard and expressed in mouse units (MU) which are converted to toxicity units [STX equivalents (STXeq)] using the conversion factor which varies with the sensitivity of the mouse strain used ( 1 MU = 0.18 - 0.23µg STXeq). For standard MBA, 1ml of the solution was used and injected intraperitoneally into three male mice, where they were observed for symptoms and the number of times of death.

The median death time (i.e. period between injection and death) was used to calculate the number of MU. Toxicity level of the sample (MU $g^{-1}$) was determined from the dose–death time relationship (Japan Food Hygiene Association 2005), where 1MU is defined as the amount of toxin required to kill a 20g male mouse within 30 minutes of injection.

### c. Limit of Detection & Limit of Quantification

The detection level of this method is about 40µg STXeq/ 100g wet weight of tissue.

### d. National Regulatory Limits

No national regulatory limit has been set up yet in the Kingdom of Cambodia. Cambodia Fisheries Administration is mainly adopting limits based on the ASEAN and European Union (EU) Standards in complying with the international conformity for controlling safety of exported and imported fisheries products.

## IV. Results and Discussions

### a. Participation in Inter-Laboratory Proficiency Testing & Results

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### b. Survey Results & Discussion

PSP in Cambodian Green Mussel (*Perna viridis*) was investigated throughout a period from June 2011 to May 2012. All samples were analysed for PSP contamination by using the Standard MBA. The results of this analysis have shown that PSP was Not Detected (negative) in all analysed samples of Green Mussels (*Perva viridis*) from the above-mentioned two sampling sites, Koh Preap and Tumnup Rolok, throughout the sampling period (Table C2).

| Month and Year of Sampling | Method | No. of Replica Analysed | Average Sample Weight (g) | Confirmation | |
|---|---|---|---|---|---|
| | | | | Koh Preap | Tumnup Rolok |
| Jun 2011 | MBA | 3 | 350-500g | NE | NE |
| Jul 2011 | MBA | 3 | 350-500g | NE | NE |
| Aug 2011 | MBA | 3 | 350-500g | NE | NE |
| Sep 2011 | MBA | 3 | 350-500g | NE | NE |
| Oct 2011 | MBA | 3 | 350-500g | NE | NE |
| Nov 2011 | MBA | 3 | 350-500g | NE | NE |
| Dec 2011 | MBA | 3 | 350-500g | NE | NE |
| Jan 2012 | MBA | 3 | 350-500g | NE | NE |
| Feb 2012 | MBA | 3 | 350-500g | NE | NE |
| Mar 2012 | MBA | 3 | 350-500g | NE | NE |
| Apr 2012 | MBA | 3 | 350-500g | NE | NE |
| May 2012 | MBA | 3 | 350-500g | NE | NE |
| NOTE: MBA (Mouse Bio Assay), NE (Negative), PO (Positive) | | | | | |

*Table C2. Results of the detection of PSP contamination in Green Mussel (Perna viridis)*

PSP in humans is caused by ingestion of shellfish containing PSP toxins. These PSP toxins are accumulated by shellfish grazing on algae and eventually producing these toxins. The PSP toxins are produced mainly by dinoflagellates belonging to the genus Alexandrium, which occur in marine environment, but are also produced in freshwater cyanobaceria and associations with calcareous red macroalgae have also been reported (Deeds, J.R., *et al*., 2008)[4]. Shellfish grazing on these algae can accumulate the toxins but the shellfish itself is rather resistant to the harmful effects of these toxins. According to Food and Agricultural Organization of the United Nations (FAO)'s reports during the last 20 years, there seems to have been an increase in intoxications caused by PSP toxins. However, this is unclear yet as to whether the increase is real, or attributed to be a consequence of improved identification, detection and medical registration, or whether it is due to expanded shellfish culture and consumption.

In this study, we have investigated PSP toxins in Green Mussel (*Perna viridis*) that naturally inhabits in Cambodia's marine water. The results of this study showed that PSP toxins in all samples collected from the sampling sites, Koh Preap and Tumnup Rolok, in Preah Shihanuk province were Not Detected. In principle, PSP toxins are dominant toxins group presented especially in marine shellfish, therefore, our present results can conclude that the Cambodia's Green Mussels (*Perna viridis*) inhabiting in this coastal area are non-hazardous and safe for human consumption. On other hand, the results from this study

[4] Deeds, J.R., *et al*., 2008. Non-Traditional Vectors for Paralytic Shellfish Poisoning. Marine Drugs 6(2): 308-348.

also revealed that the environment where the Green Mussels are inhabiting is still good and not polluted or contaminated with PSP toxins yet, suggesting that probably dinoflagellates, that are the marine primary producers of the major causative agents of harmful algal blooms producing chemical biotoxins, may not be present in this area. In contrast, the absence of PSP toxins is probably due to the limitations of the detection method used in this study that is not capable of quantifying low level of PSP toxins in this species because PSP toxins level in marine shellfish is probably below the limit detection of 40µg/100g, so it may still pose possible risks to human health in future.

Therefore, it is suggested that further study should be carried out to explore the use of quantitative methods to elucidate the PSP toxins level in the samples clearly. The results from our present study would provide insight for further researches about biotoxins contamination in fisheries products as well as in contributing to biotoxins monitoring programme in the ASEAN region.

### c. Corrective Actions

PSP toxins contamination in the Cambodia's Green Mussel (*Perna viridis*) was Not Detected in all samples tested during the period from June 2011 - May 2012, so it is proven that it is safe for human consumption, and hence no corrective action needs to be taken.

## V. Problems and Challenges Encountered

The problems and challenges encountered include:

- The samples were transported under long distances for analysis at the laboratory in Vietnam due to the lack of laboratory facilities in Cambodia, thus increasing the cost of analyses. Moreover, it is difficult to make clearance at check-point at times.
- There is limited financial support for the study.
- There is limited background information (references).
- There is limited human resource capacity in this field.

## VI. Recommendations and Suggestions for Future Follow-Up Action

Cambodia's marine water may be potentially contaminated with hazardous chemical toxins due to increased industrial activities, therefore it is suggested that further studies or investigations on other potentially toxins-contaminated aquatic species should be continuously conducted and expended widely in order to provide clear background information for controlling biotoxins that are harmful to public health. In addition, long term biotoxins monitoring programme should be set up and human resource capacity building is needed for Cambodia.