

# Enhancing Regional Capability in Monitoring and Analysis of Contaminants in Seafood: Southeast Asian Perspective

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The “Regional Guidelines for Responsible Fisheries in Southeast Asia: Post-harvest Practices and Trade” stipulates among others, the need to ensure the trading of “safe fish and fishery products” from the Southeast Asian region. As defined in the said Regional Guidelines, “safe fish and fishery products” refers to seafood that does not cause illness, injury, or death upon consumption by consumers. Moreover, any fish products can be “unsafe” for consumption because of the presence or growth of pathogenic organisms or their toxins (e.g. bacteria, viruses, fungi), the presence of biotoxins (e.g. biogenics, amines, and ciguatoxins) and parasites, or contaminated with chemicals, drug residues, or unsafe materials (e.g. heavy metals). In view of the prevailing presence of chemical contaminants and drug residues in seafood products and their serious implications to trade and human health, SEAFDEC launched in 2004 the five-year Program “Chemical and Drug Residues in Fish and Fish Products in Southeast Asia” which was implemented by the Marine Fisheries Research Department (MFRD) of SEAFDEC and funded by the Japanese Trust Fund (JTF)-II of the Government of Japan. Intended to obtain an understanding of the levels of chemical contaminants in fish and fish products in Southeast Asia, and to transfer the developed testing technologies and methodologies to the region through capacity building, the Program was implemented through regional consultations and training, and conduct of surveys to monitor and analyze the occurrence of chemical contaminants and drug residues in seafood products.

Furthermore, the consumption of shellfishes and fishes that had been contaminated by biotoxins could lead to incidence of human intoxications and even deaths. Comprising poisonous substances that are naturally present in fish and fishery products or accumulated by aquatic animals that feed on algae that produce toxins or through the water that contained such toxins, biotoxins when consumed represent a significant and expanding threat not only to public health risk worldwide but also to the risks of mass kills in fishes and shellfishes as well as deaths in marine mammals and birds. In an effort to address such concerns, MFRD continued to implement the aforementioned JTF Program from 2009

to 2012 with funding support from JTF-II, by focusing on biotoxins monitoring in the Southeast Asian region. The main objective of the program was to increase the attention of concerned stakeholders in expanding and improving the initiatives to monitor, detect, and share information on biotoxins to reduce the public health risks associated with the consumption of contaminated seafood. The Program was implemented through capacity building in the analytical methods for monitoring and analyzing the occurrence of the diarrhetic shellfish poisoning (DSP) toxins, lipophilic toxins, paralytic shellfish poisoning (PSP) toxins, and tetrodotoxin (TTX), as exemplified through the conduct of monitoring surveys on PSP toxins in the ASEAN Member States (AMSs). Upon its completion in 2012, the Program was extended from 2013 to 2019 under the JTF-VI as a continuing effort to support and strengthen the regional capabilities in biotoxin monitoring. This extension had enabled the AMSs to enhance their capabilities in monitoring and analyzing other biotoxins such as the amnesic shellfish poisoning (ASP) toxin (domoic acid), azaspiracids (AZA) toxin, and brevetoxins (BTX) which causes neurotoxic shellfish poisoning (NSP), as well as in identifying and analyzing the occurrence of harmful algal blooms (HABs) in the AMSs.

As a result, this Program which was implemented for 15 years and received sustained funding from the JTF, had enabled the AMSs to gain greater understanding of and enhanced their knowledge of the occurrences and incidences of chemical and drug contaminants in seafood as well as those of biotoxins and on HABs. Through the Program’s capacity building activities, the regional capability of the national testing laboratories of the respective AMSs had been improved allowing them to monitor and analyze such contaminants in seafood. Eventually, the Program has empowered the AMSs to achieve high integrity of their respective laboratories and facilities as stipulated in the 2001 and 2011 Resolution and Plan of Action for Food Security for the ASEAN Region, especially in terms of analyzing the occurrence of chemical and drug contaminants, biotoxins, and HABs in seafood, and ensuring the quality and safety of the region’s fish and fishery products for local consumption as well as for export.

## Monitoring Chemical and Drug Residues in Fish and Fish Products of Southeast Asia

This part of the long-running JTF Program focused on monitoring chemical and drug residues in seafood products of Southeast Asia, and included four main activities, namely: Survey of Heavy Metals in Fish and Fish Products in Southeast Asia; Survey of Pesticide Residues in Fish and Fish Products in Southeast Asia; Survey of Histamine Levels in Fish and Fish Products in Southeast Asia; and Survey on Drug Residues in Southeast Asian Fish and Fish Products (Tan &

Saw, 2008). The development of these activities was guided by the Regional Guidelines for Responsible Fisheries in Southeast Asia: Post-harvest Practices and Trade (SEAFDEC, 2005) which stipulated among others, the need to ensure the trading of “safe fish and fishery products” from the Southeast Asian region, as well as the relevant provisions in the series of Resolution and Plan of Action for Food Security for the ASEAN Region (SEAFDEC, 2001; SEAFDEC, 2011). Results of the surveys were used as inputs for the Technical Compilation that was produced as output of the Project, and were deposited in the Database of the Fish and Fish Products Safety Information Network.

After the five-year implementation of this part of the Program, an understanding was gained by the stakeholders on the levels of chemical contaminants in seafood, skills in monitoring the presence of such contaminants had been developed, and the methodologies in testing chemical contaminants were established and promoted in the Southeast Asian region through human resource capacity development activities. As its final output, the “Technical Compilation of Heavy Metals, Pesticide Residues, Histamine and Drug Residues in Fish and Fish Products in Southeast Asia” (Tan & Saw, 2008) was published, which includes the results of the surveys and the established technologies for testing the presence of chemical contaminants in seafood products of the Southeast Asian region. Having been disseminated through massive regional training sessions, the Technical Compilation had enabled the ASEAN Member States (AMSs) to enhance their capability in testing for contaminants in seafood, and in monitoring and analyzing their presence, especially in seafood bound for export to the European Union and other countries. Moreover, experiences had been shared through the meetings and seminars that were organized to confirm the reliability of the respective countries’ analyses, and help the exporting countries in identifying and addressing technical problems, and eventually, fulfill the requirements of importing countries that benefited the seafood trade of the Southeast Asian countries.

### **Survey of Heavy Metals in Fish and Fish Products of Southeast Asia**

In determining the levels of contamination of heavy metals, *i.e.* total mercury, lead, and cadmium, in fish and fish products of the AMSs, a regional survey was carried out in seven (7) participating countries, namely: Cambodia, Indonesia, Malaysia, Myanmar, Singapore, Thailand, and Viet Nam. Economically and socially important fish and fish products of the participating countries were surveyed, and to ensure the accuracy and comparability of the different methods used by the AMSs in testing and analyses of the levels of the contaminants in fish and fish products, the countries were encouraged to participate in the Inter-laboratory Proficiency Testing which was aimed at harmonizing the test methods and standards. Furthermore, through the on-site training sessions provided by MFRD, the skills of personnel from the national laboratories had been enhanced, especially in the analysis of heavy metals contamination using the Atomic Absorption Spectrometry (AAS).

Results of the survey showed that in many cases, heavy metals were not detected in the samples otherwise the levels of heavy metals were lower than the national and international standards used by the AMSs, suggesting that the fish and fish products of the region were safe for consumption and export (Tan & Saw, 2008). However, the participating countries encountered some issues and concerns during the regional

surveys, but these had been addressed in the future relevant activities of SEAFDEC.

### **Survey of Pesticide Residues in Fish and Fish Products of Southeast Asia**

Prior to the Regional Survey of Pesticide Residues in Fish and Fish Products, on-site training sessions on analyzing pesticide residues (organochlorines) were conducted to upgrade the skills of the staff of national laboratories in conducting the analysis using the gas chromatography-mass spectrometry (GC-MS). Fish and fish products that are of economical and social importance to the participating countries were targeted, where the samples were sent to their respective local/collaborating laboratories for analysis. Dried products from both marine and freshwater species, as well as dried fish and fish products from inland and estuarine waters, and rivers were also targeted for the survey. A total of 35 fish and fish products were surveyed in seven participating countries, namely: Cambodia, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Viet Nam. Results of the survey had indicated that the level of contamination did not exceed the minimum residue levels (MRLs) set by the importing countries suggesting that the target species are safe for consumption and export (Tan & Saw, 2008).

### **Survey of Histamine Levels in Fish and Fish Products of Southeast Asia**

Regional surveys were conducted on the naturally occurring toxins and histamine in fish and fish products of the AMSs involving the national laboratories in seven participating countries, namely: Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Thailand, and Viet Nam. Fish and fish products from scombroid species were targeted for the survey that also included the analysis of fermented fish and fish products such as prawn paste and fish sauce, although each country was free to decide on the type of fermented fish and fish products to be analyzed within the allocated budget. A total of 28 fish and fish products were surveyed.

Moreover, on-site training courses on histamine analysis using the fluorometric and high-performance liquid chromatography (HPLC) methods were conducted to upgrade the technical capability of the AMSs in histamine testing. The results of the survey showed that the histamine levels in fish and fish products from the AMSs were at the acceptable range (Tan & Saw, 2008).

### **Survey on Drug Residues in Fish and Fish Products of Southeast Asia**

Regional surveys of chloramphenicol and nitrofurans in fish and fish products for seafood safety were conducted by the respective national laboratories of six participating countries,

namely: Indonesia, Malaysia, Myanmar, Philippines, Thailand, and Viet Nam. In addition to the aforementioned drugs, malachite green and leuco-malachite green were included in the survey. In view of the different analytical methods used in the survey, the countries participated in the Inter-laboratory Proficiency Testing for chloramphenicol in prawn samples, while the countries that surveyed malachite green and leuco-malachite green participated in the proficiency testing of fish muscle samples. This had ensured that the countries would come up with comparable and accurate results in spite of the different methods used. A total of 15 fish and fish products were surveyed, and the results of the survey showed that the drug residues in fish and fish products from almost all the AMSs were within the standard limits set by the EU and other importing countries (Tan & Saw, 2008). Training for laboratory personnel on the analysis of antibiotics especially in the detection of prohibited drugs such as chloramphenicol and nitrofurantoin used in aquaculture farms, was considered essential as the analysis requires the use of sophisticated instruments such as the liquid chromatography-tandem mass spectrometry (LC-MS/MS) for detection and determination of the residues.

From the results of the surveys on heavy metals, pesticide residues, histamine, and drug residues conducted in the participating AMSs, few samples were found to have exceeded the national or international regulatory limits. Nonetheless, the participating countries had been exerting efforts to improve their capabilities in surveillance and monitoring of heavy metal contents, pesticide residues, histamine contents, and drug residues in fish and fish products to ensure that the fish and fish products from the Southeast Asian region are safe not only for domestic consumption but also for export. It should be noted that only a few laboratories of the participating countries regularly took part in recognized inter-laboratory proficiency testing, notwithstanding the need for national laboratories to participate in proficiency testing as means of assessing the laboratories' proficiency and staff competency in conducting tests based on the prescribed analysis methods. The inability of laboratories to participate in inter-laboratory proficiency testing was mainly brought about by insufficient funds and lack of competent laboratory staff. Furthermore, other constraints encountered during the surveys were also identified, such as the small sampling size and limited sampling locations which could generate results that might not reflect the actual situation of the chemical contamination level in the countries. Thus, through this JTF-II Project, a number of on-site and regional training sessions had been organized generating more than 100 laboratory personnel who have been empowered to transfer the techniques in analyzing heavy metals, pesticide residues, histamine, and drug residues. However, there is a need to continuously train and upgrade the skills of laboratory personnel to ensure their competency and efficiency. In conclusion, the participating AMSs had benefited from this JTF-II Project through the baseline information compiled on the level of contamination

for heavy metals, pesticides, histamine, and drugs. Such information would be useful for the planning and monitoring of programs on chemical residues and contamination of fish and fish products in the region. The results of the surveys had also offered the assurance and confidence about the safety of the fish and fish products from the AMSs.

## Biotoxins Monitoring in the ASEAN - Phase I

Upon the completion of the research and analysis of chemical residues and contamination in fish and fish products, the AMSs recommended during the 2008 JTF-II End-of-Program Meeting that SEAFDEC should consider extending the Program to also cover biotoxins that had caused increasing number of human intoxications upon consuming contaminated seafood. This led to the development of activities under the Program on biotoxins monitoring in the AMSs that continued to receive funding from JTF-II.

With the main objective of developing the methodologies for biotoxin analyses, biotoxin surveys, and training, the corresponding activities that focused on biotoxins monitoring and analyses, were implemented from 2009 to 2012 through consultations and meetings, capacity building, and surveys. As defined in the Codex Alimentarius Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003), biotoxins are poisonous substances naturally present in fish and fishery products or accumulated by the animals feeding on toxin producing algae, or in water containing toxins produced by such organisms. Therefore, biotoxins monitoring is essential to manage food risks and ensure food safety (Joint FAO/WHO Codex Alimentarius Commission, 2009). MFRD therefore worked together with the AMSs to carry out the activities on biotoxins monitoring in order to increase the awareness of the stakeholders on the need to monitor, detect, and share information on marine biotoxins so as to reduce the public health risks associated with the consumption of contaminated fishes and shellfishes.

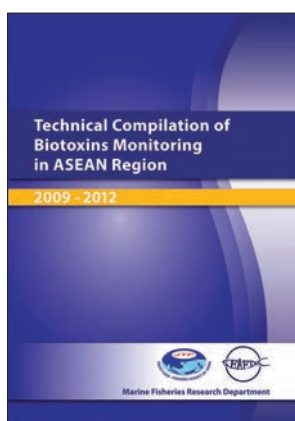
Under this phase of the JTF Program, five methods of analyzing various biotoxins were promoted, *i.e.* multi-component testing of diarrhetic shellfish poisoning (DSP) and lipophilic toxins (yessotoxin (YTX), pectenotoxins (PTX)) using LC-MS/MS method; rapid testing for DSP; paralytic shellfish poisoning (PSP) testing using HPLC; PSP ELISA rapid testing; and tetrodotoxin (TTX) testing using LC-MS/MS. Introduced through capacity building, these methods were selected to address the need for the AMSs in building up their respective capabilities (Neo *et al.*, 2012).

Moreover, the increased harvest of exotic species of shellfishes (*e.g.* moon snails, whelks, and barnacles) could also bring about new food safety issues and raise the responsibilities in managing them. Therefore, it has become important to manage

the risks that the biotoxins generate by monitoring the toxicity of seafood, although such process poses some challenges that could include: variations in toxin contents among individual fishes and shellfishes; different detection and extraction methods for various types of toxins; and awareness of the instances that would require prioritized decisions on the types of toxins to test for, the frequency of sampling, and the sampling locations.

In an effort to address such concerns, Biotoxins Survey was conducted from 2011 to 2012 in nine AMSs, namely: Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Viet Nam, and targeted at monitoring the PSP in green mussels (*Perna viridis*), except for Indonesia and Viet Nam which monitored the PSP in baby clams (*Meritrix* spp.). Meanwhile, Myanmar and Singapore had also expanded their survey to include the monitoring of amnesic shellfish poisoning (ASP) and DSP in *P. viridis*. During the survey, the countries used the mouse bioassay (MBA) as the screening method followed by HPLC as the confirmation method (Neo *et al.*, 2012). Based on the results of the surveys, the participating AMSs had also been able to upgrade their respective national laboratory capabilities and enhance their credibility for testing and analyzing DSP, PSP, and TTX biotoxins, and subsequently established their respective countries' monitoring activities for routine surveillance and testing of fish and fishery products, which were of particular benefit to countries that still have not established their respective methodologies.

Meanwhile, the AMSs had also deepened their knowledge and understanding on the levels of biotoxin occurrences and incidences in fish and fish products in the Southeast Asian region, which in turn, facilitated the exchange of information among the AMSs through the establishment of a directory of biotoxin experts, and the responsible persons and national authorities in each AMS. Finally, the Program came up with the Technical Compilation of Biotoxins Monitoring in ASEAN Region, 2009-2012 (Neo *et al.*, 2012), which had been promoted in the Southeast Asian region. As the final output of the Project, this Technical Compilation would be beneficial to policy makers, technologists and scientists as well as regulatory personnel in the fisheries sector.



From the results of the survey on PSP in green mussels (*Perna viridis*) and baby clams (*Meritrix meritrix*) conducted by the participating AMSs, a few samples were found to have exceeded the permitted regulatory limits. Nonetheless, it could be concluded that the shellfish products from the

AMSs were generally free of PSP toxin. Moreover, active participation in inter-laboratory proficiency testing has been strongly encouraged as it would ensure the credibility of the test results produced by the concerned laboratories and enhance the confidence level of the laboratory personnel.

During the survey, however, various constraints were encountered by the participating countries, *e.g.* insufficient funds for sampling and analysis, inadequate laboratory resources such as personnel with the scientific know-how, and in some countries, poor logistic arrangements resulting in undesirable preservation of samples collected from distant sampling areas. In order to address these issues, it had been recommended that the scope of investigation should be extended to cover other potential toxin hazards in fish and fish products; monitoring scheme should be established under the comprehensive quality management system; continuous upgrading of human resource capacity and laboratories' capability in biotoxins testing should be promoted; and advanced reliable rapid test method should be introduced to shorten the test cycle time.

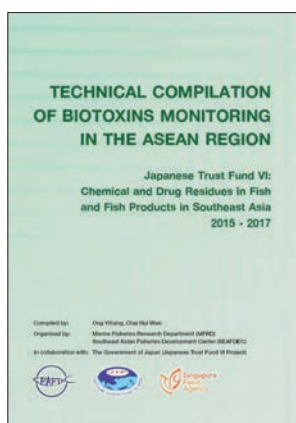
Through this Project, the participating AMSs enhanced their respective capabilities in developing methodologies for biotoxin analyses and establishing monitoring schemes for biotoxins detection. The Project has also provided a platform for knowledge-sharing and networking not only among the participating countries but also among the AMSs, bringing about better understanding of the frequency of biotoxin occurrences and incidences in shellfishes of the Southeast Asian region.

## Biotoxins Monitoring in the ASEAN - Phase II

In response to the concerns of the AMSs, the JTF-II Project on biotoxins monitoring was extended from 2013 to 2019 under the JTF-VI as a continuing effort to support and strengthen the regional capabilities in biotoxin monitoring and analyses of other biotoxins such as the amnesic shellfish poisoning (ASP) toxin (domoic acid), azaspiracids (AZA) toxin, and brevetoxins (BTX) which causes neurotoxic shellfish poisoning (NSP), and also on the identification of toxic harmful algal bloom (HAB) species. Implemented mainly through regional technical consultations and meetings, regional training courses in biotoxin analyses, and biotoxins monitoring surveys, the Project had been able to enhance the capabilities of and confirm the credibility of the laboratories of the AMSs in testing for ASP, AZA, and BTX biotoxins, facilitate the establishment of biotoxins monitoring programs for routine surveillance testing of fish and fishery products in the AMSs, improve the knowledge and understanding of technical personnel from the AMSs on the levels of biotoxins occurrences and incidences as well as of toxic HABs in fish and fishery products, and enhance the capabilities of the AMSs

in the identification of toxic HABs to ensure that fish and fishery products of the region are safe for human consumption. Moreover, a number of technical persons from the AMSs have been trained on AZA, ASP, and BTX biotoxin analyses and in the identification of toxic HABs. Thus, technical personnel from the AMSs had acquired the skills in preparing samples and conducting instrumental analysis using HPLC for ASP and the LC-MS/MS method for AZA and BTX.

Thereafter, monitoring survey was conducted by seven AMSs, namely: Indonesia, Myanmar, Malaysia, Philippines, Singapore, Thailand, and Viet Nam, allowing the participating AMSs to put into practice the knowledge gained from the training in identifying and determining the biotoxin occurrences, and facilitated the establishment biotoxins monitoring programs in their respective countries. The “Technical Compilation of Biotoxins Monitoring in the ASEAN Region” (Ong & Chai, 2019) was published at the end of the Project in 2019 as a collaborative effort of the participating AMSs to enhance regional capabilities for testing and analyzing ASP, AZA and BTX biotoxins as well as the identification of toxic HAB species.



This Technical Compilation would be beneficial to policy makers, technologists and scientists as well as regulatory personnel in the fisheries sector, and could serve as valuable learning tool for all AMSs by including the biotoxins analytical methods and outcomes of the biotoxins monitoring surveys, and the reports on toxic HABs occurrences and incidences that bridge the existing gaps in the management of HABs. The methodologies and recommendations of the biotoxin monitoring surveys contributed by respective AMSs with insightful sharing of problems and challenges encountered during the surveys are also included in the Technical Compilation. Moreover, the directory list of responsible national authorities and HABs experts in the SEAFDEC Member Countries also forms part of the said Compilation.

The successful completion of the Project under the JTF II and VI had resulted in the regional upgrading of the capabilities of national laboratories and heightened the credibility in testing and surveillance of eight types of biotoxins, as well as identification of the toxic HAB species (Yeap & Sulit, 2017). The Project also strengthened the fish quality and safety management systems that support the competitive position of the fish and fish products from the ASEAN in the global market, as provided for in the Resolution and Plan of Action for Food Security for the ASEAN Region Towards 2020 (SEAFDEC, 2011).

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