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Design and application of paralytic shellfish poisoning monitoring system of oysters in Owase Bay based on ELISA and simple immunochromatography in Mie Prefecture

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Abstract

Currently, paralytic shellfish poison (PSP) is officially monitored in Japan using the mouse bioassay (MBA). Ethically, the 3R principle of animal welfare recommends switching to a testing method that minimises the use of mice. In this study, we assessed the design and usefulness of an initial poisoning monitoring system for the prevention of food poisoning caused by PSP using other methods that are rapid, cost-effective, and accurate. *Crassostrea gigas* samples from consignments that were subject to voluntary shipping restrictions owing to the detection of levels of PSP above the regulatory limit (4 MU/g) through MBA were diluted to various concentrations with a non-poison containing blank sample to prepare experimental poisoned samples. The "Skit ELISA for PSP" kit and simple immunochromatography-based "MT test Immunochromato-PSP 'Nissui' " kit were used for detecting PSP in the experimental poisoned samples. The functional suitability of the ELISA and MT test kits for screening PSP was verified. We inferred that "Skit ELISA for PSP" and "MT test Immunochromatography-PSP 'Nissui' " kits are effective for monitoring poisoning by PSP. Furthermore, the use of these testing methods in combination with MBA notably reduced the number of mice and management costs of the corresponding mouse required for analysis compared with that needed for MBA alone. Specifically, a reduction of at least nine mice per sample was achieved after exceeding the regulatory limit in the first MBA. This reduction effect may become greater the longer the detection of PSP above the regulatory limit in the sample continues.

Keywords : PSP, 3R principle, *Crassostrea gigas*, enzyme-linked immunosorbent assay, immunochromatography

I Introduction

Paralytic shellfish poison (PSP) is produced by marine dinoflagellates and accumulates in shellfish primarily in the midgut gland. The poisons exhibit complex multi-component compositions that often undergo changes during the accumulation process. Complex multi-component compositions include C1 & C2 toxins, gonyautoxins-1,2,3,4,5 and -6 (dcGTX1-6), decarbamoylgonyautoxins-2 and -3 (dcGTX2 & 3) and saxitoxin (STX)[1-3]. Consuming these poison-containing shellfish causes food poisoning with an adverse impact on the nervous system. Currently, PSP is officially monitored in Japan using the mouse test or mouse bioassay (MBA)[4]. However, ethical considerations of animal welfare dictate that a testing method that minimises the use of mice needs to be adopted. Therefore, the Ministry of Agriculture, Forestry and Fisheries established the guidelines for risk management of shellfish poisoning from bivalve shellfish, etc in 2015 (Public Notification No. 6112, March 6, 2015)[5], which officially approved the introduction of screening methods. In this study, we developed an analytical method to detect PSP using a simple kit based on enzyme-linked immunosorbent assay (ELISA) and immunochromatography, which are rapid, inexpensive, and accurate techniques. The aim of this study was to establish and verify the usefulness of a screening system capable of monitoring the early stages of food poisoning to prevent paralytic shellfish poisoning.

The "Act on Protection and Management of Animals"

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