INTRODUCTION

Bivalve mollusks are filter-feeders that readily accumulate toxic compounds that can severely affect the health of those who ingest them. There are three main types of shellfish poisoning that affect humans: diarhetic shellfish poisoning (DSP) which causes severe gastro-intestinal distress; amnesic shellfish poisoning (ASP) which can lead to a permanent loss of short-term memory and an inability to analyse shellfish poisoning (PSP) which, in extreme cases, can lead to death through respiratory paralysis. The different tests that cause these poisoning are the metabolic byproducts of microscopic marine algae. To protect consumers, countries that harvest shellfish monitor their shellfish beds regularly for the presence of these toxins.

Canadian Shellfish Sanitation Program

Canada is one of the world’s largest harvesters of bivalve mollusks. To ensure the safety of its shellfish, the Canadian Food Inspection Agency (CFIA) manages a marine bioassay monitoring program that was developed by an Interdepartmental Shellfish Committee made up of the Canadian Food Inspection Agency (CFIA) and the Department of Fisheries and Oceans (DFO). The program’s regulations have been published in the Canadian Shellfish Sanitation Program (CSSP) Manual of Operations which is published and maintained by the CFIA.

Mouse Bioassay

Currently, the CFIA uses the mouse bioassay (MBA) to detect the presence of saxitoxins (and saxitoxin derivatives) known to cause DSP, and as a screen to detect domoic acid, the toxin which causes ASP. All suspected cases of domoic acid are confirmed with high performance liquid chromatography (HPLC).

To perform the MBA to detect PSP shellfish from the area being monitored are harvested and homogenized to produce an extract which is then injected into the body cavities of three mice. The mice are observed for adverse affects and the latency between the intraperitoneal injection of the extract and the death of the mouse is measured. If the mice die too quickly, the extract is diluted and the process repeated until the mice die within 5-7 minutes. This latency and the dilution factor of the extract are then translated into the concentration of saxitoxin equivalents present in the extract (Tatsumi et al., 1995).

Although the MBA is the internationally accepted standard test for PSP, this method has its flaws. The MBA cannot reveal the exact toxins in the extract, only that something harmful is present. This assay is also highly variable as it is affected by the size, sex and strain of the mice as well as by the pH and salinity of the extract (Stephenson et al., 1996). Furthermore, this test causes considerable pain and uses a large number of mice.

Alternative Methods

Advances in the field of toxicology and the emergence of new technology have contributed to the development of a number of alternative methods for risk assessment that in some cases are more sensitive and more reliable than the MBA (Inam et al., 2014). While the tests have not yet gained regulatory acceptance, the development and validation of these alternative test methods has led to a considerable debate as to whether the MBA should remain the standard reference method for the regular analysis of algae toxins in shellfish (Joint FAO/IOC/WHO ad hoc Expert Consultation, 2004).

CASE STUDY

Even though some alternative methods for shellfish toxin testing that have been developed have been internationally validated, they have not yet been adopted by the regulatory environment in Canada. From the Canadian Council on Animal Care (CCAC) has approached this case study (slated for completion in December 2007) surveying the perspectives of different stakeholders on incentives and impediments for greater implementation of the Three Rs (Replacement, Reduction and Refinement) in regulatory shellfish toxin testing. By better understanding these obstacles, the CCAC will be able to work with regulatory agencies to encourage the use of refinements to the original MBA protocol and to facilitate acceptance of methods that do not use animals for shellfish toxin testing. The study focuses on the opinions of government regulators, scientists and industry stakeholders.

METHOD

We conducted semi-structured 1-to-1 interviews with Canadian government regulatory scientists in a total of 20 interviews. Interviews were conducted at either face-to-face or via telephone. Participants were asked a series of open-ended questions that focused on the implementation of the Three Rs in their respective organizations. The interviews were audiotaped and the audio files were transcribed and verified by the participants for their clarity and accuracy. Any changes were made with the participants’ consent. To encourage candid responses, the interviews were conducted under the condition of strict anonymity.

Preliminary Results

The preliminary results are based on interviews with two government regulators, one government scientist and one industry representative. Despite the small sample size, some of the same factors observed by Schiffelers et al. (2005) that affect the implementation of the Three Rs in regulatory testing in the Netherlands are also emerging in the preliminary results of this study.

Validation of Alternative Methods

Although some alternative methods have yet to undergo international validation, some validation attempts have been unsuccessful because their results have been directly compared with the MBA which can have large variance depending on the skill of the analyst. Improving the training of analysts can reduce this variance which would not only improve validation efforts but would also reduce animal use by 60-70%.

Validation vs. Acceptance

Some alternative methods have been validated, and accepted by the AOAC as official methods, but have not yet been adopted by Canadian regulators because they are not considered to be as suitable for their regulatory purposes as the MBA and are not as well established.

Regulatory Requirements

Not only is Canada required to test shellfish for toxins to protect its own population but it is subject to all of the regulations of the different countries that import its shellfish, some of which regularly inspect Canadian laboratories. For Canada to change its shellfish sanitation program, it would have to consult with its major shellfish importers.

International Harmonization

International harmonization of shellfish toxin testing protocols many help to reduce the number of tests Canada needs to perform; however, harmonization efforts are difficult because each country has native species of shellfish that accumulate different toxins at different rates in different areas of their anatomy.

Influence of Regulators

Existing alternative methods are very specific and will only detect toxins for which there are tests currently validated. Therefore, until 1997 domestic and international marine toxins are tested for, the MBA remains the gold standard for shellfish testing. For Canada to change its current method, it would need to consult with its major shellfish importers.

Future Directions

The preliminary results suggest that the Canadian government is reluctant to use alternatives for shellfish toxin testing. In contrast to the findings of Schiffelers et al. (2005), this does not appear to be a result of the lack of scientific fluency of the regulators, but rather because they believe that the existing alternatives are not yet suitable replacements for the PSP MBA.

The results summarized above are biased towards the perspectives of government regulators. A more balanced picture of the obstacles and opportunities affecting the implementation of the Three Rs in regulatory testing for purposes in Canada should emerge by increasing the sample size and including more participants from academia and industry.

Please see handout for a complete list of references.

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This study received ethical review and approval from IRB Services.

Canadian Shellfish Sanitation Program Manual of Operations

2004, Appendix 1, p.11

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