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# Incentives and impediments to adopting alternative shellfish testing methods in Canada

Allison Guy and Gilly Griffin

Canadian Council on Animal Care

Corresponding author: Allison Guy
Canadian Council on Animal Care
1510-130 Albert Street, Ottawa, Ontario, Canada
Phone: +(1)-613-238-4031 x. 60, Fax: +(1)-613-238-2837, allisonguy@ccac.ca

**Abstract**
Bivalve molluscs are filter-feeders that readily accumulate toxic compounds which can severely affect the health of those who ingest them. These compounds include marine biotoxins, the metabolic byproducts of microscopic algae, which are highly toxic to humans and can lead to short-term memory loss, diarrhea, and in severe cases paralysis and even death. To protect consumers, shellfish harvesting countries monitor their shellfish beds regularly for the presence of these toxins. Canada is one of the world's largest harvesters of bivalve molluscs. To ensure the safety of its shellfish, the Canadian Food Inspection Agency (CFIA) manages a marine biotoxin monitoring program. Currently, CFIA uses the mouse bioassay as the gold standard for detecting biotoxins despite the existence of validated alternative methods. The purpose of this research project is to identify incentives and impediments to adopting alternative methods for regulatory shellfish toxin testing. Preliminary results from this study are presented.

**Keywords:** shellfish toxins, regulatory testing, alternatives

## Introduction

Toxic illness caused by shellfish has been recognized for hundreds of years. The earliest reported case in Canada dates back to 1793 when members of Captain Vancouver's crew died from eating shellfish harvested from what has been since named Poison Cove in British Columbia. There are three main types of shellfish poisoning that affect humans: diarrheic 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 paralytic shellfish poisoning (PSP) which, in extreme cases, can lead to death through respiratory paralysis (Table 1). Each type of poisoning is the result of an accumulation of different toxic metabolic byproducts of marine algae in filter-feeding bivalve molluscs. DSP is due to more than one toxin; namely okadaic acid and other lipophilic toxins. ASP is the only one of these three types of poisoning that is caused by a single toxin, domoic acid. PSP is attributable to a variety of toxins known as the PSP suite, which is made up of saxitoxin and saxitoxin derivatives. While not toxic to the shellfish themselves, these compounds can severely affect the health of those who ingest them. To protect consumers, countries that harvest shellfish monitor their shellfish beds regularly for the presence of these toxins.

## Canadian Shellfish Sanitation Program

Canada is among the world's largest harvesters of bivalve molluscs. To ensure the safety of its shellfish, the Canadian Food Inspection Agency (CFIA) manages a marine biotoxin monitoring program that was developed by the Interdepartmental Shellfish Committee made up of representatives from Environment Canada (EC), the CFIA and the Department of Fisheries and Oceans (DFO). The program's regulations are outlined 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 saxitoxin (and saxitoxin derivatives) known to cause PSP as required by the *CSSP Manual of Operations*:

> "Current Association of Official Analytical Chemists (AOAC) and American Public Health Association official methods shall be followed in the bioassay for PSP."
> – *CSSP Manual of Operations*, Appendix 1, p.11

 

Table 1: Shellfish Toxins

| | Type of Poisoning | Toxin | Symptoms |
|---|---|---|---|
| DSP | Diarrhetic Shellfish Poisoning | okadaic acid (OA), dinophysistoxins (DTX1-2) | gastrointestinal illness, nausea, vomiting, and diarrhea |
| ASP | Amnesic Shellfish Poisoning | domoic acid (DA) | stomach upset, facial grimace, difficulty breathing, disorientation, and in severe cases, the permanent loss of short-term memory, and in severe cases, death |
| PSP | Paralytic Shellfish Poisoning | saxitoxin (STX), gonyautoxin (GNTX1-6), neosaxitoxin (neoSTX), decarbamoyl saxitoxin (dcSTX), decarbamoyl neosaxitoxin (dcneoSTX), decarbamoyl gonyautoxin (dcGTX1-4), deoxydecarbamoyl saxitoxin (doSTX), deoxydecarbamoyl gonyautoxin (doGTX2-3), N-sulfo-carbamoyl toxins (B1/GTX5; B2/GTX6; C1-4) | tingling or numbness of the lips and extremities, and in severe cases, paralysis and death |

To perform the MBA to detect the toxins that cause PSP (PSP MBA), shellfish from the area being monitored are harvested and homogenized. A hydrochloric acid extraction method is then used to produce an extract which is then injected into the body cavities of three mice. These 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 (Yasumoto et al., 1995). Although the PSP MBA has been accepted as an AOAC approved method and is the internationally accepted standard to test for PSP, this method has its limitations. The PSP MBA cannot reveal the exact toxins in the extract, only that there was something lethal in the extract. This assay also has an inherent variability that can exceed ±20% (Quilliam, 2003), as the assay is affected by the age, sex and strain of the mice, as well as by the pH and salinity of the extract (Stephenson et al., 1955; Wiberg & Stephenson, 1960; Shantz, 1960; Prakash et al., 1971; Park et al., 1986; Nagashima et al., 1991; Stabell et al., 1992).

Another MBA is used to detect DSP toxins (DSP MBA). It requires an organic extraction method, but otherwise follows the same approach as the PSP MBA: mice are injected with the shellfish extract and observed until a lethal endpoint is reached. However, unlike the PSP MBA where a positive result is observable within minutes, mice used in the DSP MBA may not die from their symptoms for up to 48 hours. Therefore, the DSP MBA takes a considerable amount of time to perform (Oshiro et al., 2006) and, unlike many alternative methods, the DSP MBA has never been validated in an interlaboratory study for its ability to predict toxicity in humans. Also, the results of the DSP MBA are unreliable because this test can give both false positives and false negatives (Quilliam, 2003).

**Canadian Council on Animal Care**

While the Canadian public expects to be protected from shellfish poisoning, they only support animal use in science when the animals are not subjected to undue pain or distress (The Gallup Organization, 2007; Canadian Public Health Association, 2001; MORI, 2000). The Canadian Council on Animal Care (CCAC) is the national body overseeing animal use in research, teaching and testing. On behalf of the people of Canada, the CCAC ensures that the use of animals employs optimal physical and psychological care without compromising scientific integrity through programs of guidelines, assessment and education. Like other national authorities responsible for the ethical use of animals in science, the CCAC's mandate is based on the principles of the Three Rs: reducing the numbers of animals used, refining the procedures, and replacing animal use protocols with non-animal methods. Through its Assesment Program, the CCAC collects information on animal use for research, teaching and testing which is published according to the procedure's level of invasiveness. When the CCAC published the *CCAC guidelines on: choosing appropriate endpoints for research, teaching and testing* (1998), a general decrease in the number of animals used in procedures with lethal endpoints was seen. However, these guidelines did not reduce the number of mice used for the mouse bioassay for shellfish toxin testing. As all versions of the MBA use a large number of mice, cause them considerable pain and distress, and require death as an endpoint,

the CCAC is interested in having the mouse bioassay replaced with an appropriate non-animal method.

**Alternative methods**

In 1987, 107 people in Eastern Canada suffered toxin symptoms, 12 required intensive care and 3 people died as the result of a mystery toxin that was not detected by the mouse bioassay (Perl et al., 1990). Within a matter of days, Canada's National Research Council (NRC) was able to identify the toxin as domoic acid and develop an instrument-based, liquid chromatography detection method as well as the appropriate certified reference material. This was necessary because the MBA method is not sensitive enough to protect the public from ASP, as the current legal limit for the toxin responsible for this type of poisoning, domoic acid (20 μg/kg of shellfish in Canada), is far below the detection limit of the MBA (400 μg/kg of shellfish).

Since the domoic acid crisis, the NRC and Health Canada have also developed instrument-based methods to detect the toxins responsible for PSP and DSP that are more sensitive and more reliable than either MBA (for reviews see Inami et al., 2004 and Quilliam, 2003). While only some of the alternative tests have gained regulatory acceptance, the development and validation of these methods has led to a considerable debate as to whether the MBA should remain the standard reference method for the regular analysis of algal toxins in shellfish (Joint FAO/IOC/WHO ad hoc Expert Consultation, 2004).

**Case study**

Even though some alternative methods for shellfish toxin testing have been validated in international studies, they have not yet been adopted by the regulatory authorities in Canada. The CCAC has begun a case study (scheduled for completion in December 2007) surveying the perspectives of different stakeholders (government regulators, scientists and industry representatives) on incentives and impediments for greater implementation of the Three Rs (Replacement, Reduction and Refinement) in regulatory shellfish toxin testing. An understanding of these factors will enable the CCAC to work proactively with regulatory agencies to facilitate acceptance of alternative methods for shellfish toxin testing.

In 2005, Utrecht University's School of Governance and Science Shop for Biology conducted a survey of the factors influencing the implementation of the Three Rs in regulatory testing, with a focus on the authorization and release of pharmaceuticals (Schiffelers et al., 2005). The results of this study will be used as a benchmark for the results of the CCAC's study to see whether the recommendations in Schiffelers et al. (2005) could also be applicable to the implementation of the Three Rs for regulatory shellfish toxin testing in Canada.

**Participants**

Criterion-based sampling (Patton, 1990) was used to select participants for this study. Regulators from the CFIA and Health Canada were asked to participate based on their involvement in policy development for shellfish toxin testing. Toxicologists from laboratories where shellfish toxin testing is performed, scientists who have been involved in the development of alternative testing methods, and people from industry, were also asked to participate. Additional participants were found by referral from the initial participants in a process known as snowball sampling (Patton, 1990).

**Methodology**

The perspectives of the different stakeholders were collected through in-depth, semi-structured interviews conducted either face-to-face or over the telephone. Approximately 13 open-ended, prepared questions were asked, but unplanned questions were also used to clarify the participants' responses. Each interview, with one exception, was audio recorded and the transcripts were verified by the participants for clarity and accuracy. To encourage candid responses, the interviews were conducted under the condition of strict anonymity. Prior to the initiation of the study, the protocols received ethical review and approval from IRB Services.

**Preliminary results**

The preliminary results were 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.

A common factor impeding the use of alternative methods identified by both the Schiffelers et al. (2005) study and this study is the lack of validated alternative methods. Although some alternative methods have undergone international validation, other validation attempts have been unsuccessful because their results have been directly compared with the MBA. The results of an MBA can have large variance depending on the characteristics of the mice, the toxin composition in the samples and the skill of the analyst. One participant stated that this variance could be reduced by improving the training of the analysts, which would not only improve validation efforts but could also reduce animal use by 60-70%.

Even after methods have undergone international validation, their acceptance by regulators is still not

guaranteed. This is another factor common to both the Schiffelers et al. study and the CCAC study that impedes the use of alternative methods. For instance, the pre-column oxidation liquid chromatography method developed by Lawrence and Niedzwiadek (2001; Lawrence LC-FD method) at Health Canada has been validated at the international level and accepted by the AOAC as an official method to test for PSP toxins. Even though the Lawrence LC-FD method was developed at Health Canada as an alternative to the PSP MBA, Canadian regulators do not use it because they do not considered it to be as suitable for their regulatory purposes as the PSP MBA and because it is not as well established. This is in contrast to the Schiffelers et al. (2005) finding that regulators are reluctant to adopt new methods because they lack the scientific fluency to be able to accurately evaluate alternatives.

Another reason Canadian regulators may be reluctant to adopt the Lawrence LC-FD method is that the Codex Alimentarius Commission[1] (Codex), whose standards are quoted in many international trade agreements, has not incorporated this method into its *Recommended International Code of Hygienic Practice for Molluscan Shellfish* (1978). The standards set by Codex are very important to Canadian regulators because of the large amount of shellfish that is exported to foreign countries. If Canada were to adopt an alternative method before Codex, Canadian exporters could be found to be in violation of one or more international trade agreement(s). Trade also has an impact on Canadian policies with respect to shellfish toxin testing because, 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 of shellfish toxin testing protocols may help to reduce the number of tests Canada needs to perform; however, harmonization efforts are difficult because each country has different native species of shellfish that accumulate different toxins at different rates in different areas of their anatomy.

Another factor impeding the adoption of alternative methods for shellfish toxin testing is that existing alternative methods are very specific and will only detect toxins for which there are reference standards. However, if the 1987 domoic acid crisis in Canada is any indication, the mouse bioassay may be no better at protecting public health in the event a previously unseen lethal toxin emerges in Canada. According to one participant, a second factor impeding the replacement of the PSP MBA is that since it is a very well established method in Canada to test for shellfish toxins, there is very little desire within the government to change the status quo. Paradoxically, despite its conservatism on adopting validated alternatives, the Canadian government has been, and continues to be, very supportive of the development of alternative methods.

## Future directions

The results summarized above are preliminary and although they 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 the area of shellfish toxin testing for regulatory purposes in Canada should emerge once an analysis of all of the interviews has been completed. The preliminary results suggest that the Canadian government is reluctant to use alternatives for shellfish toxicity testing despite their active involvement in the development and validation of alternative methods.

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## Footnotes

[1] The Codex Alimentarius Commission was formed by Food and Agriculture Organization of the United Nations and the World Health Organization in 1963 to develop food standards and guidelines for the Joint FAO/WHO Food Standards Program. The Codex standards have become the global point of reference for international trade and World Trade Organization agreements, and are used in resolving trade disputes in international law.