Canadian Council on Animal Care
Conseil canadien de protection des animaux

Good Animal Practice in Science
Bonnes pratiques animales en science

PROGRESSING FROM THE MOUSE BIOASSAY FOR SHELLFISH TOXIN TESTING IN CANADA

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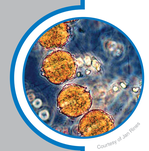


Courtesy of Jan Mead

INTRODUCTION

Paralytic shellfish poisoning (PSP) can result from the ingestion of shellfish contaminated with marine biotoxins, and can be fatal. For over 50 years, Canadian regulators have used the mouse bioassay (MBA) to protect the public from PSP. In addition to the lack of sensitivity and high variability of the test, the use of the MBA to monitor levels of shellfish toxin is ethically challenging because of the potential for extreme pain and distress. In 2006, the Canadian Shellfish Sanitation Program (CSSP) used approximately 36,000 mice to perform the MBA, representing 55% of the total number of mice used in the CCAC's Category of Invasiveness E[1] for regulatory testing (CCAC, 2007).

Canadian research has led to the development of non-animal test methods that are more sensitive and more reliable than the MBA. These methods have not yet been adopted by Canadian regulators to detect PSP toxins, despite international validation studies and the adoption of other non-animal methods to test shellfish for other types of marine biotoxins.

RESEARCH OBJECTIVE

- Characterize the opportunities and challenges involved in the adoption of alternative methods for routine monitoring of shellfish for PSP toxins in Canada.

METHODS

- **Data Collection**: Ethnographic approach using elite interviews with 10 stakeholders from the Government of Canada: 6 scientists and 4 regulators
- **Data Analysis**: Inductive coding of interview transcripts to identify themes relevant to understanding the challenges associated with adopting non-animal methods for shellfish monitoring for PSP in Canada

Footnotes

1 The CCAC's Categories of Invasiveness describe a range of procedures from Category A which encompasses experiments on most invertebrate or on live isolates to Category E which encompasses procedures that cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals.

2 LC-MS: Liquid chromatography – mass spectrometry

3 HPLC: High performance liquid chromatography

4 The Codex Alimentarius Commission was formed by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) 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.

5 AOAC: Association of Official Analytical Chemists

RESULTS

Challenge 1: The nature of the shellfish toxins

PSP is caused by a group of toxins referred to as the saxitoxin suite. The saxitoxin suite encompasses 34 toxins and toxin derivatives, making it difficult to develop non-animal methods. However, diarrhetic shellfish poisoning is also caused by a group of toxins, but, a non-animal method it is currently being used for routine testing and only a subset of samples are confirmed by the MBA to satisfy trade agreements with Europe.

Some of the toxins in the saxitoxin suite are not acutely toxic but research is currently being performed to determine the effect of chronic exposure. The non-toxic toxins may also be converted to toxic analogues in stomach acid when eaten. The reverse is also true; some toxins are quite potent when injected into a mouse's intraperitoneal cavity but when eaten are benign.

But shellfish samples, at least in North America, contain very little saxitoxin. Actually, it's mostly saxitoxin derivatives and usually a mixture of these different toxins.

- Study Participant

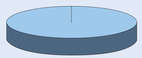
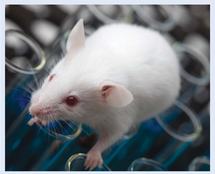


Figure 1: All of the participants cited that the nature of the PSP toxins were a challenge for adopting non-animal methods.

Challenge 2: Misconception that the MBA will detect all of the toxins in the sample



Because the MBA gives a measure of general toxicity of a sample, many stakeholders believe that this test will detect all of the toxins in a sample and, in particular, previously unseen toxins. This is a misconception because there are currently many toxins in Canada's shellfish that the MBA is not well suited to detect. In Canada, the CSSP has already moved away from the MBA and adopted an LC-MS[2] method to detect the group of toxins responsible for diarrhetic shellfish poisoning (Quilliam, 2003). Also, there is no scientific evidence to indicate that the MBA will detect previously unseen toxins. The domoic acid crisis in 1987 (Perl et al., 1990), where 3 people died and 107 people suffered toxin symptoms, is evidence that the MBA may not protect the Canadian public from emerging toxins.

I don't remember any toxins that were discovered by injecting mice. The only unknown toxin, the only case that I'm aware of, is domoic acid in PEI. And the bioassay was done in humans, not mice.

- Study Participant

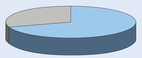


Figure 2: Most participants (70%) stated in their interview that the mouse bioassay would be able to detect unknown toxins in the sample. This opinion was held by all of the regulators surveyed but only by half of the scientists.

Challenge 3: Analytical methods lack a complete set of reference standards to fully replace the MBA for risk assessment

For analytical methods like HPLC[3], reference standards are required to calibrate the equipment. Certified reference standards are produced by the Canadian National Research Council (NRC) but the NRC has yet to develop standards for every toxin in the PSP suite. The toxins that the NRC does not currently have standards for are not particularly harmful to humans.

Also, it is thought that analytical methods, like HPLC, would not be able to detect emerging toxins because they would not have reference standards for them. However, an analyst would become familiar with the toxin profile of the geographic area from which the samples were harvested and would be able to see any unusual peaks. Currently, only one certified reference standard, saxitoxin, is required to calibrate the MBA.

We would have done chemical a long time ago if we did have the standards or if there was only one chemical.

- Study Participant

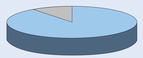


Figure 3: Most participants (90%) stated in their interview that the lack of reference standards was a challenge affecting the adoption of non-animal methods. This opinion was held by all of the regulators surveyed and all but one of the scientists.

Challenge 4: Standards for international trade

One of the reasons Canadian regulators are reluctant to adopt non-animal methods is that the Codex Alimentarius Commission[4] (Codex), whose testing methods are quoted in many international trade agreements, has not incorporated non-animal methods into its *Recommended International Code of Hygienic Practice for Molluscan Shellfish* (1978). The methods cited 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). There is resistance at the international level to accept non-animal methods because Third World countries cannot afford the technology.

If we could show that our method was equivalent to the international method…our methods could be used.

- Study Participant

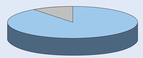


Figure 4: Most participants (90%) stated in their interview that international trade was a challenge affecting the adoption of non-animal methods. This opinion was held by all of the regulators surveyed and all but one of the scientists.

RECOMMENDATIONS

- Current opportunities to implement non-animal methods
  - The Lawrence method is an HPLC method developed at Health Canada that has been validated by the AOAC[5] as a suitable replacement for the MBA for monitoring shellfish for PSP toxins (Lawrence & Niedzwiadek, 2001). Regulators interviewed felt that this method lacks the appropriate certified reference standards to replace the MBA. Until a complete set of standards has been developed, this method could be used as a screening method.
    - use of the Lawrence method in the UK as a pre-screen has reduced animal use by 70% (Dennison & Anderson, 2007)
    - easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to test for contamination of shellfish with domoic acid
- Future opportunities to implement non-animal methods
  - research to develop more reference standards and to improve the throughput of non-animal methods
  - adoption of a two-tiered testing system for First World and Third World countries
  - application of the Lawrence method for national use, and re-test of a small sub-set of samples using the MBA to satisfy trade requirements

Once there is enough science supporting these alternative methods; we are comfortable that we have caught the toxic elements within the PSP suite; and that we are confident that we are not going to allow unsafe food on the market place, then I'm fairly confident that there will be a switch.

- Study Participant

Acknowledgement

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