PROJECTING FROM THE MOUSE BIOASSAY FOR SHELLFISH TOXIN TESTING IN CANADA

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INTRODUCTION

Traditionally, shellfish poisoning (SSP) cases result from the ingestion of shellfish contaminated with saxitoxins. In Canada, commercial and non-commercial shellfish are tested to ensure that the saxitoxin concentration is below the regulatory tolerance (Health Canada, 2007). However, the mouse bioassay (MBA) has significant problems and limitations, which has led to the CCAC’s Category of Invasiveness E1 for regulatory test-conditions. This method is used to test shellfish for PSP toxins in Canada. This method lacks the appropriate control methods and standards to fully test shellfish for other types of marine toxins. The Lawrence method is an HPLC method developed for screening shellfish for PSP toxins and is sensitive to saxitoxin and its derivatives. This method is commonly used internationally, for example, the European Union adopted this method in 2005 as a suitable alternative for the MBA. However, the Lawrence method lacks the appropriate control methods and standards to replace the MBA. Until a complete set of standards has been developed, this method could be used as a screening method. The Lawrence method has been shown to screen has reduced animal use by 70% (Dennison & Anderson, 2007) and is relatively easy to implement as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins. The Lawrence method is easy to implement because it is easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.

METHODS

- **Data Collection:** Bibliographic approach using site searches with 30 stakeholders from the Government of Canada to conduct a survey about non-animal test methods and their acceptance.
- **Data Analysis:** In-depth evaluation of non-animal methods to identify challenges and opportunities for the adoption of non-animal test methods.
- **Create a survey:** Conduct a survey of stakeholders to determine the current status and potential of non-animal test methods to replace the MBA. The survey was designed to determine the factors that are important to Canadian regulators when adopting alternative methods for shellfish testing.

RESULTS

For analytical methods like HPLC, reference standards are required to calibrate the equipment. Reference standards are produced by the Canadian National Research Council (NRC) for the MBA. The NRC has not developed standards for every toxin in the PSP suite. The toxin that the MBA does not normally have standards for are not particularly harmful to humans. It is difficult to develop standard test protocols for non-animal test methods that are sensitive and reliable for detecting all of the known saxitoxins. It is important to Canadian regulators because of the large amount of shellfish that is exported to foreign countries. If Canada were to adopt a two-tiered testing system for First World and Third World countries, there is a possibility of international trade agreements, which would make it impossible to find standards for every toxin.

- **Future opportunities to implement non-animal methods:**
- **Research to develop more reference standards:**
- **Use of the Lawrence method in the UK as a pre-screening tool:**
- **Adaptation of the Lawrence method to screen for First World and Third World countries:**
- **Applications of the Lawrence method for national use:**

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**Recommendations**

- **Commitments to implement non-animal methods:**
  - The Lawrence method is an HPLC method developed in Canada, has been validated by the NRC as a suitable replacement for the MBA for screening shellfish for PSP toxins. Government & industry commitment is required to develop standards for every toxin in the PSP suite.
  - The CCAC’s Category of Invasiveness E1 for the MBA makes it challenging to develop global agreements. However, the Lawrence method lacks the appropriate control methods and 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-screening tool could be evaluated.
  - Easily implemented alternatives to the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.
- **Future opportunities to implement non-animal methods:**
- **Research to develop more reference standards:**
- **Use of the Lawrence method in the UK as a pre-screening tool:**
- **Adaptation of the Lawrence method to screen for First World and Third World countries:**
- **Applications of the Lawrence method for national use:**

**Figure 1:** All of the participants cited that the nature of the PSP toxin was a challenge for acceptance non-animal methods.

**Figure 2:** Most participants (90% cited that the toxin that the mouse bioassay would be able to detect saxitoxins in the sample. This toxin was capturing all of the samples analyzed but only half of the scientists.

**Figure 3:** Most participants (70% stated that there were a number of issues that are important to Canadian regulators when adopting alternative methods for shellfish testing.

**Figure 4:** Most participants (80% stated that their institutions have international trade agreements, which would make it impossible to find standards for every toxin. This option was cited by all of the regulations and institutions but not all the institutions.

**Figure 5:** The Lawrence method is an HPLC method developed for screening shellfish for PSP toxins. The Lawrence method is easy to implement because it is easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins. The Lawrence method has been shown to screen has reduced animal use by 70% (Dennison & Anderson, 2007) and is relatively easy to implement as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.

**Figure 6:** The Lawrence method is an HPLC method developed for screening shellfish for PSP toxins. The Lawrence method is easy to implement because it is easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins. The Lawrence method has been shown to screen has reduced animal use by 70% (Dennison & Anderson, 2007) and is relatively easy to implement as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.

**Figure 7:** The Lawrence method is an HPLC method developed for screening shellfish for PSP toxins. The Lawrence method is easy to implement because it is easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins. The Lawrence method has been shown to screen has reduced animal use by 70% (Dennison & Anderson, 2007) and is relatively easy to implement as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.

**Figure 8:** The Lawrence method is an HPLC method developed for screening shellfish for PSP toxins. The Lawrence method is easy to implement because it is easily implemented as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins. The Lawrence method has been shown to screen has reduced animal use by 70% (Dennison & Anderson, 2007) and is relatively easy to implement as all of the Canadian testing laboratories currently have the HPLC equipment to analyze shellfish-maternal toxins.