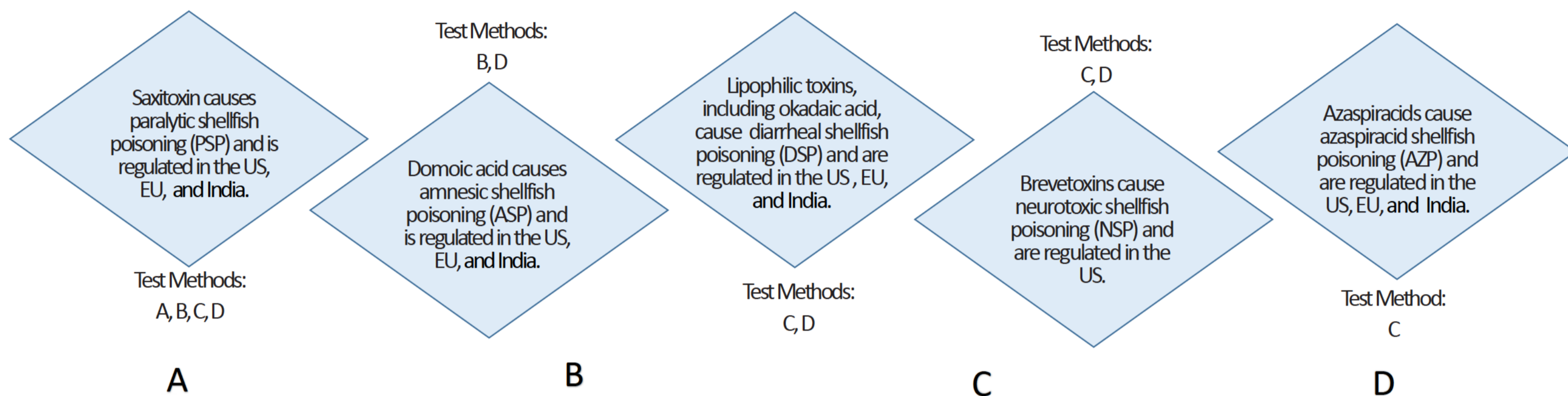


Introduction

The mouse bioassay (MBA) or biological method is commonly used to test for marine biotoxins in shellfish. Authorities such as the World Health Organization, European Food Safety Authority (EFSA), and US Food and Drug Administration have noted its lack of sensitivity, specificity, and precision, highlighting the need to transition to better test methods.^{1,2} Many fisheries have successfully implemented alternative methods, and there is a need to define the path for leveraging advances in toxicology in locations where they have not yet been adopted. Here, we describe toxins monitored in the United States, European Union, and India.

Biotoxins Monitored and Available Assays



Receptor Binding Assay (RBA): Approved in the US for full use for mussels and for screening and precautionary closure for clams and scallops. The RBA's limit of detection is 60 to 100 µg STX equiv/kg, compared to 400 µg STX equiv/kg for the MBA.³ While this method does not replace the use of all animals, it does not use live animals for testing and reduces animal use.

High-Performance Liquid Chromatography (HPLC): Approved for use in the US for PSP in clams, mussels, oysters, and scallops (HPLC PCOX) and for ASP. It is also the EU's reference method for ASP and PSP (HPLC-FLD for PSP). Because of its higher detection capacity, it has provided an earlier warning of shellfish toxicity.⁴ It provides a full toxin profile and can be conducted in a higher-throughput semi-quantitative screening procedure, followed by a full quantitative analysis as needed.^{4,5}

Liquid Chromatography with Tandem Mass Spectrometry (LC-MS): An ultrahigh-performance LC-MS/MS method was validated in a 21 laboratory study for PSP.⁶ For detection of DSP, this method is the reference method in the EU and approved for use in the US for clams. An LC-MS/MS method for NSP detection in mussels, oysters, and clams showed good sensitivity and ruggedness and acceptable recovery and precision.⁷ LC-MS/MS can be used to detect AZP in the EU.

Immunoassays: The Abraxis shipboard ELISA and the lateral flow immunosorbent assay Scotia Rapid Test are approved for limited use in the US for PSP. The Biosense ASP ELISA is approved as a screening method for ASP in the EU, and Reveal[®] 2.0 for ASP is approved as a screening method in the US. The Protein Phosphatase Inhibition Assay is an alternative or supplementary method to LC-MS/MS in the EU for DSP. MARBIONC Brevetoxin ELISA is approved for use under certain parameters for NSP in the US.

Reasons to Transition to New Methods

The MBA has numerous disadvantages, including:

- Animal strain, sex, and age as well as salt concentration, pH, and treatment of the sample affect results.⁸⁻¹¹
- While oral toxicity is the relevant human risk factor, toxicities are often based on intraperitoneal injection.^{12,13}
- Trace metals and an inability to discriminate between toxins results in false positives.^{10,14}
- Test results vary with the choice of solvents used for toxin extraction and injection.¹⁵
- A lack of precision in determining death times in the MBA can lead to inaccuracies.^{3,10}
- It involves death as a routine endpoint, often causes animals to experience severe shock and trauma shortly after dosing, and anesthesia is not used.¹⁶

Regulatory Oversight and Standards

- Codex Alimentarius, established by FAO and WHO, and AOAC International, a nongovernment association, develop and publish standards and test methods for food, including shellfish.
- In the US, the Food and Drug Administration Center for Food Safety and Applied Nutrition requires monitoring of shellfish for algal toxins. The Interstate Shellfish Sanitation Conference (ISSC), a federal cooperative body, manages the National Shellfish Sanitation Program (NSSP) under which test methods are approved.
- In the EU, the European Commission sets allowable levels of marine biotoxins and recognizes approved analytical methods for detection.
- In India, the Food Safety and Standards Authority of India (FSSAI) regulates the limits of marine biotoxins in fish and fishery products but it does not identify the test methods to be used for their detection.

Moving Forward

- As a result of the scientific, technical, and ethical limitations of the MBA, countries such as Australia, Canada, Ireland, New Zealand, and the UK no longer use the test for routine toxicity testing of shellfish.^{16,17}
- As of 2019, following implementation of Commission Regulation (EU) 2017/1980, the MBA is no longer the reference method for detecting PSP toxins in the EU, allowing for complete replacement of the MBA in the EU.
- The MBA is not listed in the US NSSP "Guide for the Control of Molluscan Shellfish" as a method to test for DSP, ASP, or AZP.
- Robust alternative methods are available that can completely replace the MBA. Political and practical (i.e., cost and implementation) hurdles must be expediently addressed.

References available at:
www.thepsci.eu/our-work/shellfish-toxicity-testing