Modern Analytical Methods Best Ensure Food Safety

Moving Towards Effective Human-Relevant Food Safety Protocols

Samantha Dozier, Ph.D.
Regulatory Testing Division
People for the Ethical Treatment of Animals (PETA)
501 Front Street, Norfolk, VA USA 23510
SamanthaD@peta.org

Introduction

As analytical methods become increasingly capable and sophisticated, with the ability to separate and quantify each component in a sample, it is critical that we move forward and use methods such as mass spectrometry (MS) and high performance liquid chromatography (HPLC) to ensure food safety. For nearly half a century, mice have been used to test the safety of consuming many types of shellfish, which can harbor poisons accumulated from algal blooms. This testing method, termed the 'mouse bioassay', is a crude experiment in which homogenized shellfish are injected into the peritoneum of the animals. Times of death are measured in minutes and converted into an approximation of toxin content for the shellfish. Although this method has been documented to be irreproducible and inaccurate, many countries, including the United States, still use the mouse bioassay. Recently, coordinated efforts between People for the Ethical Treatment of Animals and European Union lobbyists have convinced the European Commission to replace the mouse bioassay for Paralytic Shellfish Poisoning (PSP) detection with the Lawrence method of HPLC. Work is still underway to modernize the detection methods for other toxin types in the E.U. and the U.S. This poster details the efficacy and the validation status of the Lawrence method of HPLC for shellfish toxins.

How do shellfish toxins effect public health?

There are several classes of shellfish toxins. Each class is caused by a different type of marine algae over-proliferation and poses specific threats to humans who ingest shellfish.

- Amnesiac Shellfish Poisoning (ASP)caused by domoic acid and its isomers when concentrations exceed 20mg/kg of shellfish tissue. The causative agents of ASP is detected with HPLC in the European Union
- Diarrhetic Shellfish Poisoning (DSP) is caused by Okadaic acid (OA), dinophysis toxins (DTX), yessotoxins (YTX), pectenotoxins (PTX), and azaspiracids (AZA) when concentrations of OA, DTX, PTX, or AZA exceed 160 µg/kg of in shellfish tissue. YTX causes DSP when concentrations reach 1mg/kg of shellfish tissue. Toxins responsible for DSP are detected with the mouse bioassay, although PETA has begun working towards the adoption of available non-animal detection methods for this class.
- Neurotoxic Shellfish Poisoning (NSP) is caused by brevetoxins. The EU is not affected by this class of toxins.
- Paralytic Shellfish Poisoning (PSP) is caused by saxitoxins, neosaxitoxins, gonyautoxins and their isomers when the concentrations of these toxins reach 800 µg/kg of shellfish tissue. Following PETA lobbying, the Lawrence Method of HPLC replaced the mouse bioassay for detecting this class of toxins in the European Union.

Why Should We Move Past Animal Experimentation to Protect Humans & Animals?

The mouse bioassay (intraperitoneal injection of homogenized shellfish to test shellfish toxicity) is the traditional method to test the safety of shellfish destined for human consumption.

The endpoint is the death of the mice. Thousands of mice are used for this assay each year and suffer painful convulsions prior to their death.

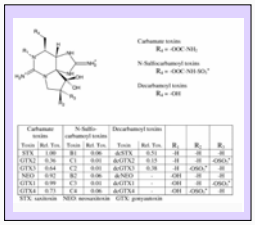
Problems associated with this method include:

- **Lack of reproducibility** (McFarren, 1959; LeDoux and Hall, 2000; Toti *et al.* 1991; BfR Expert Opinion No. 032/2005, 2005)
- **Lack of accuracy** (Stabell *et al.* 1992;
- Prakasch *et al.*, 1971;Nagashima *et al.*, 1991)
- **Lack of sensitivity** (Jorgensen *et al.*, 2004)
- **False positives** (McNabb *et al.*, 2004)
- **False negatives** (McNabb *et al.*, 2004)
- **Subject to artifacts of the extraction process** (Mouratidou *et al.*, 2006)
- **Cannot be validated** (BfR expert opinion No. 032/2005, 2005; FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs, 2004))
- **Violates animal welfare statutes since non-animal methods are validated for the same purpose** (Council Directive 86/609/EEC)

The Best Protection for Humans also Spares Animals: The Benefits of HPLC

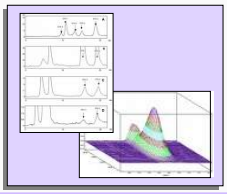
The Lawrence Method of High Performance Liquid Chromatography (HPLC) detects the causative agent of PSP

Validated by the Association of Analytical Communities (AOAC), an internationally recognized standardization body.



Benefits:

- **Reliable and reproducible ($p < 0.05$)** (Ramstad, Larsen, and Aune 2001)
- **Allows scientists to measure the actual toxin concentration (depicted below)**
- **Precise, with greater sensitivity** than 10x more sensitive than the MBA Larsen and Aune, 2004)
- **Greatly diminishes false readings** Expert Opinion 032/2005; Asp, Larsen, and Aune, 2004; Vale and Sampayo, 2001;
- **Avoids animal suffering**



What should be done to protect human health?

- **Continued use of current non-animal analytical methods for the ASP and PSP shellfish toxin groups**
- **Standardization and use of available analytical methods for the DSP class of shellfish toxins in the EU; validating testing standards for the lipophilic toxins (a subset of the DSP group) accumulated by shellfish**
- **The adoption of available, validated analytical methods in the United States**

Discussion

In vitro **methods that make the detection of shellfish toxins possible have evolved dramatically since the era when animal test results were considered by some to be the gold standard. Animal experimentation has shown itself to an unreliable and irreproducible scientific and public-health gamble. Non-animal, analytical methods such as high performance liquid chromatography (HPLC) and mass spectrometry (MS) have outperformed animal experimentation by every measure.**

The Lawrence method of high performance liquid chromatography (HPLC) is the method validated by the Association of Analytical Communities (AOAC) for PSP detection, and was implemented by the European Commission for this purpose following lobbying by PETA. It is a prime example of the kind of improvements that analytical methods can make in protecting public health and the lives of animals. Because the Lawrence method is able to detect significantly lower toxin levels than the mouse bioassay with precision and reproducibility while also providing the exact toxin concentrations in the sample, it allows public health authorities ample time to close shellfish fishing beds in order to protect human health.

The Regulatory Testing Division of People for the Ethical Treatment of Animals (PETA) works to bring together high tech scientific methods and the appropriate regulatory bodies responsible for protecting the public health. This division of scientists is committed to the modernization of toxicity testing. Our work in implementing modern, non-animal methods that are relevant to humans, helps prevent a great deal of animal suffering in laboratories and results in greater protection for the public.