Session I-15: Shellfish toxin testing: How are the Three Rs being progressed in this field?

Session I-15: Oral presentations

I-15-240

Evolving from the mouse to the optoelectronic mouse for phycotoxin analysis in shellfish

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Regardless of ethical and technical concerns, for phycotoxin analysis in shellfish the mouse bioassay remains the reference method of analysis for the global monitoring of diarrheic and paralytic shellfish poisoning toxins. Alternative methods of detection have been described but to date each analytical method is specific for a particular toxin and its chemical analogues, with each group of toxins requiring separate analysis. An ideal scenario for the monitoring of phycotoxins would be to evolve multiple toxin detection onto a single, easy to use *in vitro* platform.

Surface plasmon resonance biosensor technology has been demonstrated as a highly promising bioanalytical tool. This technology offers rapid real time detection requiring minimal amounts of toxin standards which is crucial because of their limited availability. A micro-fluidic immobilization device and prototype multiplex SPR biosensor designed for the detection of up to 16 molecular binding interactions in a 4 line by 4 channel array on a single chip has been utilised. This dual system was evaluated in its ability to be fit-for-purpose for the simultaneous detection of three important phycotoxin groups. Domoic acid, okadaic acid and saxitoxin calibration curves in shellfish were achieved in separate flow channels with detection limits of 4000, 36 and 144 μg/kg of mussel, respectively. The assay was designed to achieve detection below recognised regulatory action levels. This "optoelectronic mouse" detection system exhibits enormous potential for multiple phycotoxin screening as an alternative to the mouse bioassay with the additional benefit of being able to distinguish between toxin families in a single analysis.

I-15-431

Regulatory and methodical shortcomings in assessment of marine biotoxins in fish and shellfish

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As aquatic food already represents over 40% of the globally distributed animal food products, the toxicological risk assessment of these products becomes more and more a matter of special importance.

The gold standard to assess toxins in aquatic food has traditionally been and remains to date the mouse bioassay. Besides ethical issues of the *in vivo* bioassays there are specific associated problems with these *in vivo* assays, e.g. inter-species

comparability and intra-species variability. Thus there is an exigent need for more robust, sensitive, and reliable tools for toxins detection that have adequate predictivity in human risk evaluation. Responding to the growing alertness regarding this situation the European Food Safety Authority (EFSA) recommends LC-MS (Liquid Chromatography coupled Mass Spectroscopy) as a substitute for the *in vivo* bioassays for almost all classes of marine toxins.

LC-MS is a quantitative analytical method which is dependent on the availability of standardized reference toxins. However there are neither sufficient standards covering the known toxin structures nor is it foreseeable when there will be enough information on the wide range of analogues and released intermediates. Notably, for this reason the reference laboratories in this field will carry on performing *in vivo* bioassays.

This presentation will discuss the existing alternative methods as tools for risk assessment this field.

I-15-559

Removing the mouse from shellfish toxin testing – Fifteen years of the Three Rs

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Until recently, the only method acceptable in EU law for detection of the marine biotoxins paralytic (PSP) and diarrhetic shellfish (DSP) poisoning in shellfish harvested for human consumption have been rat or mouse bioassays (MBAs). MBAs cause substantial suffering to animals and have a number of potentially serious limitations including variability and false negative and positive results. It is estimated that around 400,000 mice are used annually for marine biotoxin monitoring in Europe.

Over the last 15 years, UK laboratories have used a number of strategies for refinement, reduction and finally replacement of MBAs. Using a combination of approaches, a steady reduction in the use of mice has been achieved.

From 1996 to 2005, a thirty percent reduction in numbers of animals was achieved by the use of 2 rather than 3 animals per sample. In addition, the time each animal remained on procedure was appreciably decreased. The duration of the PSP assay was reduced by one third (from 30 to 20 minutes) and that of the DSP assay by 75% (from 24 to 5 hours). A defined clinical endpoint had also been introduced for the DSP MBA. The effect of these changes significantly reduced the suffering in all animals used in the test (i.e. for both positive and negative samples).

From 2005 to 2010 further sizeable reductions in the number of MBAs have been achieved by a move to *in vitro* methods. Initially use was made of qualitative pre-screens in the PSP assay and subsequently a fully quantitative analytical method (HPLC) was introduced for the common shellfish species, with plans to extend the method into other species. In 2011, a replacement method (using LCMS) has been validated and introduced for 97% of DSP testing giving further reduction of mouse use.

Had strategies such as those above been adopted more widely in Europe, 100,000s of animals need not have been used in tests causing substantial suffering.