CIGUATOOLS Report Summary

Project ID: 315285
Funded under: FP7-SME
Country: United Kingdom

Final Report Summary - CIGUATOOLS (Development of a Rapid Test Kit and supporting Reference Standards Capable of Detecting the Emerging Fish toxin Ciguatoxin in European and Global Waters)

Executive Summary:
This proposal, CiguaTools has been developed to deal with a serious emerging problem, thought by many to be caused by climate change. Put in basic terms, strains of harmful marine algae which produce deadly toxins and were only associated with tropical climates are emerging in European waters. Due to the complexity of the food chain these toxins accumulate in fish. While the fish themselves show no obvious signs of contamination, eating such fish can have very serious health implications. Ciguatera fish poisoning (CFP) is a major economic and social problem worldwide, with up to 500,000 people being poisoned each year. The associated illness caused by this can last for many months.

The causative toxins are known as ciguatoxins (CTX). A consortium comprising experts in marine toxins, rapid screening test development and a group of SMEs specialising in producing vital reference standards, test kits and validating both to international standards has been formed to deliver the following objectives:

The production of a fast and simple method for high throughput analysis for CTX toxin presence in fish destined for consumption.

Project Context and Objectives:
Our oceans are a huge natural resource in terms of providing safe, healthy and nutritious foods for the European consumer. The fisheries sector employs over 140,000 fishermen with ~6.4 million tonnes of fish being caught annually. Fish farming produces a further 1 million tonnes of fish and shellfish and employs 85,000 people. Projections show an increase in the demand for seafood products due to heightened awareness of health and lifestyle trends. The consumption in the EU is estimated to increase by ~10% by 2030. However, the management of our oceans, including the important resource of fish, is an ever increasing challenge due to issues such as overfishing, acidification, climate change and pollution.

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The production of a fast and simple method for high throughput analysis for CTX toxin presence in fish destined for consumption.
The substantial benefits to be gained from this research are as follows:
• To support the implementation of EC Regulation (EC) 853/2004 of the European Parliament and of the Council which deals with issues relating to the hygiene of foodstuffs.
• To prevent human exposure to toxins which can have not only serious short term consequences but lead to chronic illness.
• To develop commercial products (standards and test kits) which will be unique in the world and will give the SMEs opportunities to develop world-wide markets for their innovative products.

Without Ciguatools we have a number of very serious challenges looming:
It is predicted that many Europeans will become ill in the short to medium term due to consuming CTX contaminated fish. There will be serious economic losses associated with this due to an increased burden on health services and loss of many working days.
• The image that fish is ‘healthy’ to eat will be questioned by many Europeans when multiple reports of poisonings start to hit the media headlines. A loss of a foodstuff rich in compounds such as Omega 3 from the diet of many Europeans may result and this in turn will have a very negative impact on the European fishing industries.

Project Results:
The results of the Ciguatools project fall into 5 categories:
1) The culture of the causative dinoflagellate Gambierdiscus in controlled conditions
2) the extraction of CTX compounds from the algal cultures, identification of active toxins, their characterisation and purification as standards for the toxin and use for production of antibodies in an ELISA type identification kit.
3) The development of techniques to identify the presence of the causative algae Gambierdiscus (6 different species) in the water column using PCR techniques.
4) Manipulation of the isolated CTX compounds from the work of (2 above) for use in producing antibodies for an ELISA based kit. The CTX extracted was too toxic for antibody production - the researchers produced a synthetic mimic which successfully produced antibodies.
5) The production of a necessary precursor (synthetic antibody tool) to the eventual production of ELISA kits for detection of the CTX toxin in fish. Further work will be needed beyond the project to develop the kits.

The results are described in more detail in the attached document.

Potential Impact:
The 5 categories of results of the project - fulfill the the original aims and objectives of the project in full and in part:-
Techniques have been developed to successfully cultivate Gambierdiscus in controlled conditions and to extract toxic compounds - which has not been before on such scale or duration.
The extracted compounds have been fully characterised into structure and toxic activity - such that standards have been identified for molecules of 2 molecular weights of CTX (ciguatoxin).
In part the extracted and identified CTX was used as the aims of the project, not directly as the compound was too toxic to produce the necessary antibodies using traditional methods, but indirectly as a synthetic mimic was used to do this using the part of the structure of the extracted.
Although the duration of the project did not allow for development of ELISA based detection kits because of the time and resources necessary to synthesise the CTX mimic - there is a baseline now for further work to develop effective kits.
During the project an opportunity was grasped to develop a successful PCR method of detection of the CTX causative organism (Gambierdiscus) in the water column in laboratory based trials. This could add a very valuable tool to the monitoring and potential management of the toxin risk in vulnerable areas.

The planned areas of economic and social impacts of the project are described below:
MARKET SITUATION

Ciguatera fish poisoning (CFP) is a foodborne illness caused by consumption of seafood containing ciguatoxins (CTXs). These toxins, originated from dinoflagellates belonging to the Genus Gambierdiscus are structurally very complex and therefore difficult to quantify. Although CFP is a global phenomenon, it is nearly restricted to tropical and subtropical regions: Pacific [1-3] and Indian Oceans [4, 5] and the Caribbean Sea [6-8]. Depending on the origin, CTXs are grouped in Pacific ciguatoxins (P-CTXs), Indian ciguatoxins (I-CTXs) and Caribbean ciguatoxins (C-CTXs), being P-CTX-1 the one suggested as the most toxic, causing CFP at 0.1 μg/kg in the flesh of carnivorous fishes [9]. In recent years, the global incidence of CFP has increased, probably due to the expansion and development of the international seafood trade [10, 11], but also because of an expansion in the habitat areas of the causative organisms, which have been recently found in more temperate waters, as those from Europe [12, 13]. Globally, it is estimated that more than 50,000 people are affected annually [14]. Despite not existing regulatory limits for CTXs in Europe, the regulation requires that no fish products containing these toxins are placed on the market [15, 16]. In addition to the monitoring of biotoxins in fishing products, the presence of toxin-producing plankton is also routinely monitored in order to fulfil European legislations [17]. Nevertheless, the European regulations do not establish a specific method for CTXs, contrarily to shellfish toxins which are clearly indicated the reference analytical methods for their detection.

The mouse bioassay (MBA) is a widespread methodology that has been widely used to detect CFP toxins, but its use causes a great deal of concern basing on statutory animal welfare provisions and ethical considerations. In addition, MBA has several detractors because of the variability in results, insufficient detection capabilities, and non-specificity. In fact, the European Commission recently announced that biological testing for the detection of marine biotoxins will be replaced by chemical methods from July 2011 [18], so it becomes necessary the development of alternative assays also for CTXs. These alternatives would be in vitro assays (cytotoxicity and receptor binding), immunological methods or analytical assays (HPLC, LC-MS/MS...).

About seventy percent of the world's catch of fish and fishery products is consumed as food and consequently checked. However, due to insufficient detection capability and ethical concerns the mouse bioassay is not considered an appropriate detection method for CTX-group toxins. Alternative assays, although more efficient detecting this kind of toxins, also present important limitations and they need further improvement conditioned, in some cases, by the use of certified standards and reference materials which are not currently available, as occur with LC-MS/MS. Moreover, none of the existing methods of analysis to determine CTX-group toxins in fish has been formally validated. Therefore, the development of the proposed kit within of this context and its subsequent validation will take place in a favourable economic environment that will yield without any doubt large profits to the institutions and companies involved in this project.

Ciguatera fish poisoning (CFP) is a major economic and social problem worldwide, with more than 50,000 and if under-reporting is taken into account may be as many as 500,000 persons poisoned annually (Dickey, 2008). The results of ciguatoxin intoxication may last for months or years, hence this intoxication is very severe, and it is often misdiagnosed given the complex symptomatology. Thus, CFP may lead to a large economic loss. For example, when 100,000 persons cannot go to their work for a week because of CFP and then estimating a loss of 500 € per person per week (with an average monthly salary of 2000 €), this will lead to an economic loss of 50 million euro annually. This is without taking into account the costs of medical care for a patient with CFP.

ECONOMIC BACKGROUND

The precise number of fish species that are susceptible to ciguatoxin accumulation is not known but it is thought to range between 300 to possibly 400 globally. These include the herbivorous Acanthuridae and corallivorous Scaridae (parrot fish), which are considered key vectors in the transfer of ciguatoxins to carnivorous fish. Many more species of carnivorous fish cause ciguatera such as Muraenidae (moray eels) and Lutjanidae (snappers such as red bass) which are notorious in the Pacific, Serranidae (groupers) including coral trout from the Great Barrier Reef, Epinephelidae, Lethrinidae (emperor), Scombridae (mackerel), Carangidae (jacks) and Sphyraenidae (barracudas). The latter two families are a particular problem in the Caribbean. More than 400 species of bony fish have been reported in the literature to have caused ciguatera poisoning. The larger carnivores such as moray eels, snappers, groupers, carangs, Spanish mackerels, emperors, certain inshore tunas and barracuda are the most toxic.

The safety of the world's fish supply is an issue of critical concern. For all fishery products, food safety authorities from fishing
areas must guarantee, with the necessary legal powers and resources, that the relevant hygiene and Public health requirements are met. Consequently, in addition to the common control requirements (e.g. good handling and processing conditions), specific official controls on this products shall be carried out at the time of landing or before first sale at an auction or wholesale market. These official controls shall include checks for the possible presence of poisonous fish species or fish containing biotoxins. Following this line, several methods are available for the determination of ciguatoxin (CTX)-group toxins, being the most important ones; biomolecular methods (including in vitro assays), chemical methods (such as liquid chromatography-tandem mass spectrometric, LC-MS/MS) and the mouse bioassay, based on the method described by Banner et al, which is presently the most widely used assay for the detection of CTX-group toxins in fish. The execution of those assays routinely involves thousands of million Euros per year.

New or expanded existing markets
Given the abandonement of the Mouse Biosassay by the European Union the market potential for New rapid testing on the one hand and for enhanced chemical testing on the other - is now clear, The SME participants are well placed to move early into this newly created market. From the data above - the global value of replacing the Mouse Bioassay is in the region of €656 Million. The incidence of CFP in European waters could reasonably be expected to lead to a European market size in the region of €50 Million per annum. This would be made up of the market for rapid testing for individual companies required to demonstrate compliance with End Product Standards and for standards for use in the precise chemical analysis at National regulatory laboratories.

The estimated market for Kits/detection systems
The size of the market for CTX-kits depends on the decision of authorities as well as of fish related industries of how much of fish and/or fish related products need to be tested for the presence of CTX. Considering the economic loss, the need to reduce the occurrence of CFP is apparent. When a target is set to reduce the occurrence of CFP with 50%, a high number of tests will have to be run.
Of course this depends on authorities and industries what budget will be released to realize this target.

Credibility in the market
When bringing an ELISA kit onto the market it is essential that the kit is robust, stable, specific, sensitive and easy-to-perform. Sample preparations need to be fast and easy-to-perform with recoveries between 70-120%. Sensitivity of the kit should meet with the current legislation.
EuroProxima is specialized in developing tests conform to these requirements. Their presence on the global market is covered by a dedicated network of specialized distribution partners. Several partners have worked with them for more than 20 years.

SMEPs will increase markets and extend/internationalise business activities.
Ciguatera is an international concern since tropical and subtropical seafood is a widely distributed product of commerce. In the modern era of world travel and rapid transportation, many warm-water fish are available commercially in markets throughout the world, and in consequence contaminated fishes may be found in any location. In addition, CTXs are appearing with increasing frequency in temperate areas such as Europe and North America leading to the encroachment of CFP into regions of the world where CFP has previously been rarely encountered. In addition to the public health and product safety problems, ciguatera causes severe economic loss to fisheries and restricts the use of seafood protein in local diets. Therefore, CTXs and CFP are not confined to specific areas but they are really a worldwide risk and in consequence, any laboratory which performs routinely marine biotoxin tests in seafood would be a candidate to use the proposed detection kit and include ciguatoxin analyses in their monitoring programmes.

Impact on Policy, Environment, Health and Employment at the European level
Environment & Health
The effects of climate change are far reaching and migration of fish species and changes in profiles and incidence of marine biotoxins create real challenges for both the environment and human and animal health. The CiguatTools project seeks to
advance knowledge of the CTX and to develop tools to mitigate the effects of the toxin by helping to avoid human consumption of contaminated fish.

Employment
In addition to creating direct employment in the SME participant organisations the potential effect, indirectly is far more significant:
• Direct days lost due to CFP illness -estimated to cost €50 million per year
• Reduction of jobs lost in the industry including the supply chain by consumer resistance following public outbreaks of CFP
• Increased employment of persons throughout the industry to use the detection devices produced as a result of CiguaTools.
EuroProxima estimates sales of 5000 kits per year by 5 years after the project. Assuming 1 person employed at each customer for regular use of the kits as required end product testing - this would mean 5000 jobs per year.

Innovation Dimension
The project offers real innovation -supplying CTX standards where these are currently inadequate, as the MBA has been the method of detection . If the ELISA kit proves accurate then the tool necessary for rapid detection in quality control rooms throughout the industry rather than reliance in expensive equipment in central laboratories will be truly innovative.

Dissemination will be through academic outlets such as:

Publications -
‘Progress in the development of immunoanalytical methods incorporating recombinant antibodies to small molecular weight biotoxins’ -
Owen Kavanagh, Christopher T. Elliott, Katrina Campbell
Analytical and Bioanalytical Chemistry - Vol. 407/Issue 10. 01/04/2015. pp 2749-2770

‘Gambierone, a Ladder-Shaped Polyether from the Dinoflagellate Gambierdiscus belizeanus’

List of Websites:
www.ciguatools.eu

Related information

| Result In Brief | A test for toxins in tropical fish |

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Last updated on 2016-06-28
Retrieved on 2018-10-17