



# PHARMAPOX

**Chemistry, pharmacology and bioactivity of a novel apoptotic compound - a sex regulator in decapod crustaceans with promising environmental and medical applications**

## The Challenge

It has been observed that the ingestion of selected diatoms of the genus *Cocconeis* induces the development of females shrimp *Hippolyte inermis*. This is due to the early disruption of the male gonad through apoptosis (programmed cell death) and it takes place, both in the field and in the laboratory, during the first phases of post larvae maturation. The action is specifically induced by some diatoms and selective for the male gonad and the androgenic gland, triggering the quick death of cells naturally programmed to die about 12 months after hatching. The recent discovery of the apoptotic factor and the availability of test tools offer a unique opportunity to develop pioneering biotechnological applications. Researchers in marine biology, chemistry, pharmacology and human pathology hypothesized that compounds present in benthic diatoms may play a role in triggering, in a very selective fashion and in the absence of specific toxicity, the suicide of cancer cells. The researchers also investigated if the activity of the diatoms could be extended to other species of decapods and used to produce monosex populations, for the benefit of aquaculture. The goal of the PHARMAPOX project is to study the apoptotic effects of marine algae on target cell lines, and to determine the implications for sex differentiation, aquaculture and medicine.

## Project Objective

The primary aim of the PHARMAPOX project was investigating the chemistry, the pharmacology and the bioactivity of a pro-apoptotic compound contained in some benthic diatoms and to investigate possible medical applications of the compounds.

## Key Points

- To isolate, characterise and investigate the bioactivity, the mechanism of action and the biotechnological applications of a new, peculiar apoptotic factor present in marine diatoms.
- The active compound will be extracted and isolated from cultures of diatoms. Extracts will be tested for their apoptotic potential. In vitro tests will be performed on decapod tissues to measure the specificity of effects and clarify the mechanism of action.
- The fraction containing the apoptotic factor will be analyzed and the compound will be isolated, purified, characterized, synthesized and tested in vivo and in vitro.



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## EATiP Thematic Area of Relevance

**TA1:** Product Quality, Consumer Safety and Health

**TA2:** Technology and Systems

**TA3:** Managing the Biological Lifecycle

**TA4:** Sustainable Feed Production

**TA5:** Integration with the Environment

**TA6:** Knowledge Management

**TA7:** Aquatic Animal Health and Welfare

**TA8:** Socio-Economics and Management

## Key Words

Apoptosis, sex regulation, Shrimp, Diatoms

## Project Information

**Contract number:**

4800

**Contract type:**

Specific Targeted Research Project

**Action line:**

NEST-2003-1 Adventure activities

**Duration:**

36 months (01/02/2005 – 31/01/2008)

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- Its activity will be tested on human cells, both normal and neoplastic (leukemia and solid tumours). The biochemical and molecular pathways induced by the agent will be characterized. The production of the novel apoptotic compound has biotechnological and medical significance and potential tremendous impacts through applications in aquaculture and ecology.

## Output Highlights

### Analytical methods

Guidelines and protocols for the large scale culture of diatoms have been developed. Diatoms produce several secondary metabolites having defined bioactivity so protocols developed ensured the maximum conservation of secondary metabolites. The production of secondary metabolites from benthic diatoms is still in its infancy but the guidelines produced will be beneficial for any industry aiming at massively produce these organisms.

### Analytical methods

Guidelines and protocols for the culture of small marine shrimp were developed. The experimental culture of shrimps like *Hippolyte inermis* needs procedures which differ in respect to those routinely used for large prawns in aquaculture. Accurate protocols developed through the project assure good survival with minimum stress. Small marine shrimps have no aquaculture value but they may be important models for scientific research. From an environmental perspective the protocols developed could potentially be used to recruit for re-stocking of natural populations which are a key trophic species.

### New product applications

This project yielded the first isolation of an apoptogenic factor still inactive for controlling shrimp sex. The apoptogenic factor, if applied to shrimp culture, could allow for the production of all-male generations. These all male generations of commercial prawn could provide important economical advantages since in some species males grow faster and larger, resulting in a higher economic value. Environmentally the stocking of monosex populations eliminates the risks associated with the culture of alien species.

### Biotechnology

The apoptogenic compound isolated during this project could prove to be important for medical purposes. Fundamental knowledge on the factors triggering specificity of apoptosis is of significant interest to the biotechnological field.

### The Full Report

A comprehensive description of the project is available on the website, <http://pharmapox.szn.it/index.html>

## Next Steps – Suggested Actions/Follow On



### Knowledge Transfer

- The application potential of the apoptogenic compound isolated during the project needs further investigation.

## Related Publications/Projects

Zupo V, Messina P, Carcaterra A, Aflalo E D., Sagi A (2008) Experimental evidence of a sex reversal process in the shrimp *Hippolyte inermis*. *Invertebrate Reproduction and Development*, 52:(1–2) (2008) 93–100.

Nappo M, Berkov S, Codina C, Avila C, Messina P, Zupo V, Bastida J (2009) Metabolite profiling of the benthic diatom *Cocconeis scutellum* by GC-MS. *J Appl Phycol* 21(3): 295-306 DOI 10.1007/s10811-008-9367-8