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Ecotoxicological research at DIT

Introduction

The term ecotoxicology, first defined by René Truhart in 1969, essentially describes the study of the harmful effects of chemicals upon ecosystems and understanding the pathways by which these deleterious effects are elicited (Walker *et al*., 1996). Thousands of new chemicals are produced each year requiring ecotoxicological evaluation, in addition to existing chemicals for which limited or no ecotoxicity data exists. Increased public concern regarding the impact of these anthropogenic chemicals on the environment, prompted DIT to expand its research portfolio to include the niche research area of aquatic ecotoxicology. DIT's Radiation Science Centre was officially renamed the Radiation and Environmental Science Centre (RESC) in 2000 to reflect the increasing incorporation of ecotoxicological research conducted at the centre.

Research at the centre in the area of aquatic ecotoxicology was originally initiated by the centre's director Dr Carmel Mothersill. Dr Mothersill has attracted substantial funding over the past ten years, including EU Framework 4 and 5 projects, for research projects in this area. The most recent funding has come from the Higher Education Authority under the Program of Research in Third Level Institutions (Cycle 2), for University College, Cork-led collaborative projects (VITOX and BIOMASSTOX) between University College, Cork, Cork Institute of Technology and the Dublin Institute of Technology. This funding has enabled the current environmental group (postdoctoral researcher Maria Davoren and two postgraduate researchers Sharon Ní Shúilleabháin and Colm O' Dowd) to build upon and further exploit the assay systems and biomarkers previously established at the centre.

Acute ecotoxicity data for aquatic pollutants is presently derived from fish LC_{50} bioassays. The reduction or replacement of live animals in the testing of potentially hazardous environmental contaminants is, however, highly desirable for both ethical and economical reasons. Therefore, an important focus of the environmental group has been the development of novel in vitro cultures from fish and invertebrates of ecological importance for employment in ecotoxicological screening assays. The group has had considerable success in this area of research, a fact highlighted by the publication of peer reviewed papers (Mothersill *et al*., 1995; Mulford *et al*., 2001) and the contributions of several past researchers from the environmental group to a highly regarded book on aquatic invertebrate cell culture (Mothersill and Austin, 2000).

In addition to the development of novel culture systems, subsequent research focused on establishing the toxicity of chemicals of environmental concern (e.g. cadmium, copper, nonoxynol, prochloraz, and 2,4-dichloroanaline) employing established cell lines and cell culture systems developed in the centre. A significant number of publications have also arisen from this research which has been completed by former environmental researchers at the centre (Lyons-Alcantara *et al*., 1998; Dowling and Mothersill, 1999, 2001; Kilemade and Mothersill, 2000, 2001, 2003; Kilemade *et al*., 2002).

Current research at the RESC

At present, the focus of research has developed from tests with singular chemicals to assessing the potential toxicity of environmentally relevant complex mixtures of contaminants using *in vitro* test systems. Dr Maria Davoren and Sharon Ní Shúilleabháin are currently involved with the BIOMASSTOX project. The project aims to identify the potential toxicity of Irish estuarine sediments, as sediments are both a source and a sink for persistent contaminants in the aquatic environment. This research is timely, as monitoring and assessment of Irish transitional (estuarine) and coastal waters will be required in accordance with the EU Water Framework Directive (Directive 60/2000/EC) and the OSPAR convention (1992) from 2006.

The objective of the DIT. component of this project is to assess the cytotoxicity and ecotoxicity of surfical sediments (collected from the Ballymacoda and Douglas Estuaries, Co. Cork and from the Liffey Estuary at the East Wall in the Dublin Docklands), and a reference chemical (zinc chloride) using *in vitro* assays. These assays include establishing EC_{50} for continuous fish-cell lines and primary cultures, identifying and quantifying biomarkers of exposure in cell cultures and the employment of the Microtox® system (an assay using the luminescent bacteria *Vibrio fischeri* which identifies toxicity based on a reduction in the light emitted by the bacteria when exposed to a toxicant). It is also anticipated that novel cultures developed in the VITOX project may be utilised in this research.

The aim of the VITOX project is to develop new in vitro-enabling technologies for screening sediments for potential toxicity. At present Colm O'Dowd, the newest member to join the environmental team, is investigating the possibility of developing primary-culture models from a number of aquatic species including the flounder (*Platichthys flesus*), the manila clam (*Tapes semidecussatus*), the common blue mussel (*Mytilus edulis*) and juvenile larvae from the peppery furrow shell clam (*Scrobicularia plana*). Colm will subsequently concentrate his efforts on developing the most promising primary cultures to cell lines (outgrowth from the primary explant). Colm has also been focusing on the development of primers to identify and characterise consequent *in vitro* cultures using polymerase chain reaction (PCR) methods. He has had considerable success in the short time he has been working on this project.

Based on the collective results of these assays, it is envisaged that a ranking system for the risk assessment of estuarine sediments will be developed. In addition, it is hoped that comparisons between the results from *in vivo* experiments conducted in UCC and the *in vitro* data from DIT will yield significant correlations. To date a number of abstracts pertaining to this research have been presented at national and international conferences, which have been well received (see recent publications list). Maria and Sharon have also contributed to a chapter entitled '*In vitro* Ecotoxicological Assessment of Aquatic Sediments' in a second book edited by Dr Mothersill and Professor Brian Austin currently in press (Ní Shúilleabháin, 2003).

Future perspectives

While it is clear that much innovative ecotoxicological research has been conducted at the centre, the environmental section of the RESC has yet to realise its full potential. The group has established a number of highly regarded research partners and collaborators including the Environmental Research Institute, Cork, Professor Brian Austin of Heriot Watt University, Edinburgh, Athlone Institute of Technology, the Electron Microscopy Unit, University College Dublin, and the Irish Marine Institute. The group also has access to a number of exciting new technologies including a confocal laser microscope and a cell observer system, as a result of the successful SFI (Science Foundation of Ireland) award last year. Exploitation of these resources and collaborations would allow the group to enhance their ecotoxicity-testing capabilities by developing a novel battery of *in vitro* bioassays.

In addition, the research expertise of the environmental group could be advantageous in teaching courses currently offered at DIT. Involvement with teaching in the college would be mutually beneficial, as it would contribute to providing final year students with practical knowledge and expertise in novel areas of research. It would in turn increase the supervisory experience of the centres' researchers, as well as increasing the profile of the centre within the School of Science.

The current researchers in the environmental group envisage that through future collaborations and exploitation of the novel scientific technology available at the centre, the group could attract research funding and make a significant contribution to the progression of ecotoxicology in Ireland.

Glossary of Terms

Anthropogenic chemicals: man made chemicals

Biomarker: a biological response to an environmental chemical that gives a measure of exposure and sometimes a measure of toxic effect

Cell culture: Growth of cells disassociated from parent tissue by spontaneous migration or mechanical or enzymatic dispersal

Cell lines: When cells are selected from a primary culture, by cloning or some other method, the sub-line is known as a cell line, which may be propagated in culture

in vitro: In glass. This term refers to an experiment performed in an artificial environment such as a test tube or culture media

in vivo: a biological study which takes place within a living biological organism

LC50: concentration of a substance, which causes lethality in 50% of an exposed population

PCR: a technique for selectively replicating a given stretch of DNA in vitro to produce a large amount of a particular gene

Primary cultures: Tissue fragments from which cells migrate and possibly proliferate following attachment at a solid (glass/plastic) /liquid interface

VITOX: In vitro-enabling technologies

BIOMASSTOX: Biomarker Model System for Sediment Toxicity

EC50: concentration of a substance, which causes a measurable effect in 50% of an exposed population

Website Links

http://www.resc.dit.ie
http://zae.ucc.ie/biomasstox
http://zae.ucc.ie/vitox/vitox.html

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Selection of Recent Abstracts and Publications from the Environmental Group

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Ní Shúilleabháin, S., O'Halloran, J., O' Brien, N.M., Van Pelt, F.. Sheehan, D. and Mothersill, C. (2003) **Evaluating the toxicity of estuarine sediments using primary epidermal cultures of *Oncorhynnchus mykiss*.** Extended abstract delivered at The Second Symposium on Contaminated Sediments, Quebec City, May 2003.

Ní Shúilleabháin, S., Mothersill, C., O'Halloran, J., O' Brien, N.M., Van Pelt, F.. Sheehan, D. and Davoren, M. (2002) **An *in vitro* test battery approach for assessing the toxicity of Irish Estuarine sediments.** Platform Presentation presented at North American SETAC Conference, Salt Lake City, Utah.