Import into containment any new organism that is not genetically modified

Application title: **To import into containment, for research and diagnostic purposes, marine microalgae from the Pacific region**

Applicant organisation: **Cawthron Institute, Nelson**

Please provide a brief summary of the purpose of the application (255 characters or less, including spaces)

To import into containment, for research and diagnostic purposes, marine microalgae from the Pacific region.

PLEASE CONTACT ERMA NEW ZEALAND BEFORE SUBMITTING YOUR APPLICATION

Please clearly identify any confidential information and attach as a separate appendix.

Please check and complete the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes
Confidential information identified and enclosed separately	NA
Copies of references attached	Yes
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

Signed: **Date:**

Section One – Applicant details

Name and details of the organisation making the application:	
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Note: The key contact person should have sufficient knowledge of the application to respond to queries from ERMA New Zealand staff.

Section 2: Purpose of the application

Lay summary of the application (approximately 200 words)

Note: This summary should include a description of the organism(s), the purpose of the application or what you want to do with the organisms(s).

Use simple non-technical language

We wish to import into containment, for research and diagnostic purposes, pure cultures and seawater samples containing marine microalgae from the Pacific region. Microalgae are unicellular species which exist either individually, or in chains or groups. They range in size from a few micrometers to a few hundreds of micrometers. Microalgae are a major component of the diet of many species produced in aquaculture, especially filter feeding bivalves such as mussels. The Cawthron Institute is renowned for research on marine biosecurity, harmful algal blooms, biotoxin chemistry and novel algal compounds. Many of the species of interest are present in the sub-tropical seas to the north of New Zealand, and should sea temperatures rise, could impact on the New Zealand aquaculture industry in the near future. We also aim to deliver effective tools to enable forecasting of, and a rapid and adaptable response to, any perceived micro-algal threats to the New Zealand aquaculture industry.

Describe the background and aims of the project

Note: This section is intended to put the organism(s) in the perspective of the wider project(s) that they will be used in. You may use more technical language but please make sure that any technical words are included in the Glossary.

Biosecurity is the protection of New Zealand's unique flora, fauna and economically important biological industries against invasion by unwanted foreign organisms. In pursuit of this the Biosecurity and Biotechnology Group, at the Cawthron Institute, focuses on research and advice for the biosecurity of aquatic ecosystems, seafood safety and biotechnology (Cawthron, 2006). The Cawthron Institute is renowned for research on marine biosecurity, algal blooms, biotoxin chemistry, and novel marine compounds, and it maintains a nationally significant culture collection of microalgae for New Zealand (Cawthron, 2007).

The research programme of the Biosecurity and Biotechnology Group is funded by the Foundation for Research, Science and Technology as well as other government agencies, and by seafood, shipping and port companies. This is often in collaboration with other research providers to find solutions to keep New Zealand's waters commercially productive and recreationally enjoyable.

These microalgae will be used in a number of key research areas. One of the Biosecurity and Biotechnology Group's core FRST-funded research programmes is *Seafood Safety* (CAWX0703). In collaboration with AgResearch the programme provides valuable knowledge on the risks from harmful algal toxins, and continues to improve the accuracy of marine biotoxin risk assessment, enabling the New Zealand shellfish industry to access valuable overseas markets.

We are also collaborating in a 12 year FRST-funded marine biosecurity programme lead by NIWA which has a focus on outcomes for industry. This research includes aspects of molecular biology of aquaculture species, DNA probes for harmful algae and marine pests, cryopreservation of microalgae, identifying and characterising seafood biotoxins, investigating novel marine microalgal compounds for the prevention of neurological diseases, and finding new ways of producing sustainable energy based on hydrogen production from microalgae.

With climate change, it is likely that those micro-algal species currently confined to Northland waters may extend their range south into the marine farming areas, and we aim to anticipate this possibility by understanding the nature of these sub-tropical biotoxins and by providing our biotoxin regulators with this information. In addition, there is the risk of species arriving in New Zealand from elsewhere. Note that although only a small proportion of the micro-algae that may spread south from Northland or arrive in New Zealand will be toxic – our interest in Seafood Safety means that those will be the ones of particular concern.

We will assess toxins produced by micro-algae occurring in the Pacific Region, and determine the significance of the toxins and their metabolites to human health. Members of our research team are regularly called as experts on international panels concerned with food safety and so we will ensure that new regulations are based on sound scientific evidence and are not set as non-tariff barriers. Our toxicological experts will determine if certain human population groups are particularly vulnerable to poisoning by micro-algal

toxins, e.g.: immuno-compromised, asthmatic individuals etc., and will advise and assist NZFSA of appropriate safety factors to the no-effect levels determined in toxicological studies, thus ensuring protection of all seafood consumers.

We aim to deliver significant cost savings (>$65m p.a.) to the seafood industry through cost-effective tools to enable forecasting of, and rapid and adaptable response to, any perceived micro-algal threats. This will obviate the need for costly recall of contaminated product.

Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which it is eliminated. This is of concern when marine microalgal blooms are consumed by filter feeding organisms, such as mussels, resulting in accumulation of toxins within their tissues. Even though these substances, or their metabolites, might not affect mussels, human consumers and others at the next trophic level may be more susceptible.

Microalgae have been associated with human illness such as ciguatera, a food poisoning caused by eating tropical coral reef fish containing ciguatoxins. The toxins, which come from the micro-alga *Gambierdiscus toxicus* living on coral reefs, accumulate in fish through the food chain, particularly in older and larger fish. Generally, all large reef fish are potentially poisonous. Ciguatera is a significant public health issue in the Pacific where coral reefs are found (Laurent et al. 2005).

Water samples will be collected from lagoons, and from submerged reef sites by wading through shallow water. The collecting officer will shake off biological material from seaweeds growing on the coral reef into seawater in a container at the site of collection and a sample of this water will be held in a Falcon® 50ml polypropylene conical tube container. In addition, slurry from the top 0.5mm of the sediment will be disturbed (this is where dinoflagellate cysts are found) and that seawater above the sediment will be collected. The containers, with tight screw tops, will be sealed with parafilm and bagged or carried in a polystyrene container. The samples will contain live unpreserved biological material.

The samples will be flown to New Zealand as soon as possible after seawater collection. A delegated, approved scientist will accompany samples at all times while in transit. The untreated, live biological material will be examined under the light microscope; micro-algae of interest will be isolated from the sample by standard methods (sterile glass micropipette), placed into sterile Falcon® tissue culture plate to establish growth. The growing algae of interest will be contained in PC1 facility at all times. The container with the remaining sample will be destroyed by autoclaving.

The following actions will be taken to prevent the import of other organisms in the water samples:
Seawater samples from the Pacific Islands will likely contain multiple marine micro-algal species and/or other biological material. Since there is no way to collect only selected algal species these other 'unwanted' biological materials will be separated and destroyed. It will be understood that description of the organisms of interest is not possible until sample examination and identification takes place.

The algal species we intend to obtain from the Pacific region are highly likely to be species that naturally occur in New Zealand subtropical waters, i.e. Northland where *Gambierdiscus* sp., *Ostreopsis* spp., *Coolia monotis*, *Amphidinium* spp. and *Prorocentrum* spp. have been isolated and described. The following references deal with these:

- Murray et al. (2004)
- Rhodes et al. (2000, 2001 a,b)
- Chang (1996).

Seawater samples will be examined within the Cawthron PC1 facility and species of interest will be isolated, identified and contained within PC1 facility for research purposes. The species will be tested for the presence of toxins.

For toxin extraction, micro-algae are cultured in 10L containers (all in PC1 facility). Toxin production by micro-algae is a natural phenomenon, it is not induced. Some species are toxic, some are not. After a sufficient culture density is achieved, algal cells of a single toxic species of interest are put through a filtration system, the filtrate is treated with a biocidal agent or frozen; the captured micro-algal cells are then subjected to the toxin extraction procedure which kills them.

Isolated species of interest will be cultured and maintained in the Cawthron Institute Culture Collection of Micro-algae (CICCM) for future reference and research. DNA sequence data will be obtained to confirm identification.

The CICCM is a nationally significant reference and resource for researchers, industrial users, the aquaculture industry and the public health sector and is held within our PC1 MAF approved containment facility.

The remaining seawater sample and any associated organisms will be destroyed by autoclaving or addition of biocidal agent - methods indicated in the Cawthron Quarantine Manual QSM21 (attached).

Section Three – Identification of the organism(s) to be imported

Complete this section separately for **each new organism** to be imported.

Identification of the organism to be imported

Latin binomial, including full taxonomic authority:	Unknown
Common name(s), if any:	Marine micro-algae
Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):	Marine micro-algal species amongst other biological material. Only marine micro-algal species will be propagated and stored, other organisms will be destroyed.
Taxonomic class, order and family:	Various – Micro-algae as the highest taxonomic level, in particular: Classes Dinophyceae (dinoflagellates), Diatomophyceae (Bacillariophyceae; diatoms) and Cyanophyceae (blue-green algae).
Strain(s) if relevant:	N/A
Other information, including presence of any inseparable or associated organisms and any related organisms present in New Zealand:	Biological material present in the seawater sample may include: bacteria, micro-algae, protozoa, plant material and detritus. All other material except micro-algal species of interest will be destroyed by autoclaving or by using an appropriate biocidal agent such as hypochlorite or ethanol as per the manual QSM21.

Section Four – The proposed containment system

Describe the containment facility and the proposed containment system (physical and operational)

Question	Answer
Which MAF/ERMA Standard is this containment facility approved under?	154.03.02, Facilities for Microorganisms and Cell cultures 2007
What physical containment level (AS/NZS 2243: 2002) is this containment facility registered to (where relevant)?	PC1 Reg. premises no. 386
What other physical measures do you propose to use to contain this organism?	Samples will be collected by Cawthron scientists or approved samplers in other Pacific regions (e.g. by people approved by the Ministry of Marine Resources in the Cook Islands; staff of the University of the South Pacific, etc.). All scientific work with the seawater samples will be carried out within a PC1 facility by trained and experienced staff. All open culture vessel manipulations will be conducted in a class II biosafety cabinet housed in the PC1 laboratory. Samples will be at all times contained in labelled identifiable, leak-proof screw tops containers. Tools (pipettes, culturing wells, pickers), disposable and re-usable, will be autoclaved. Any unused sample will be disposed of as per manual, see below.
What procedural or operational measures do you propose to use to contain this organism?	As described in the Transitional and Containment Manual QSM21, Issue No 5, dated: 20/03/06. (attached) The containment facility is a MAF approved PC1 facility with restricted entry and modern alarm facility that is monitored 24h/day. There are people on roster 24 hours a day who, if alerted, will attend the facility immediately. Training of all individuals re the sample handling procedures and approval controls will be carried out and documented prior to any work on samples. Documented training of individuals working with samples in quarantine has already been implemented at the Cawthron Institute PC1 MAF approved facility.

	Original, uniquely marked samples and isolated organisms will be registered in the *Biological Products in Quarantine & Containment* files in the Quarantine folder. All containers used subsequently, will be tightly sealed, labelled and registered in the Algal Cultures in Quarantine/Containment – Culturing and Disposal Record file to ensure that all containers with imported material are accounted for. All remaining seawater samples and any associated organisms will be destroyed by autoclaving or addition of a biocidal agent - methods are as indicated in the Cawthron Quarantine Manual QSM21. Non-target organisms will be kept in PC1 facility until the original seawater sample is examined, then they will be destroyed by autoclaving, the disposal recorded and filed in the relevant Quarantine folder. All scientific work with the seawater samples will be carried out within PC1 facility by trained and experienced staff. All open vessel manipulations will occur in a class II biosafety cabinet. Samples will be at all times contained in labelled identifiable, leak-proof screw top containers. Tools (pipettes, culturing wells, pickers) disposable and re-usable will be autoclaved.
Any other information relevant to the containment of the organism.	Proposed work with the biological material will be carried out by experienced staff the same way as with many previously imported micro-algae; the only difference in this instance is that the current work will start initially with unknown algal species. Micro-algae in a sample for toxin extraction are first killed by either freezing, or acid or alcohol treatment, followed by standard chemical procedure. In all cases micro-algae are dead at the toxin extraction point. The toxin extraction procedure is carried out in the PC1 facility by individuals who are experts in this field. In the event of a bloom, some toxins may cause respiratory irritation and coughing when inhaled. As a precaution, when working with large scale cultures under laboratory conditions, personal protection gear, such as a full face mask, is used. Toxic micro-algae may also be harmful if

	ingested in large quantities through contaminated seafood where toxins can accumulate. In laboratory conditions, appropriate procedures will prevent accidental ingestion. We propose the use of a class II biosafety cabinet, housed within a PCI laboratory, as a sufficient containment for samples that we intend to import from the Cook Is. and other Pacific regions. We already have organisms, which are potentially harmful to New Zealand marine aquaculture, in our PC1 MAF approved facility - some since 1994. There are strict regulations in place, such as only few designated people can enter the storage place and work with such micro-algae. MAF approval was based on close inspection of our facility and a full knowledge what we store here. Also the proposed research with micro-algae is to further protect the aquaculture industry by researching potential invaders before they enter New Zealand waters. We are audited twice yearly by MAF and we maintain close liaison with the inspector to ensure compliance.

Describe the characteristics of the organism to be imported that may influence its ability; to escape from containment, to form a self sustaining population, or to cause adverse effects. *Refer to sample applications for guidance on how to answer these questions.*

Question	Answer *attach copies of the references used in an appendix*
What are the characteristics of the organism that may prevent/enable it to escape from containment? *eg size, spore production, infectivity, seed/pollen characteristics etc.*	Microalgae exist as single cells or may form chains or a colony of cells. Their sizes vary from few microns to a few hundred microns. They are planktonic - swimmers, epiphytic - on seaweeds or benthic – attached to sediments. Some may form non-motile cysts. They reproduce by asexual and sexual cycles. They require sunlight (or alternative artificial light) as an energy source, water and carbon dioxide as raw materials - to produce oxygen and energy rich carbohydrates. Seawater pH, salinity and temperature are crucial for survival and requirements for these vary from species to species, e.g. Antarctic species and subtropical species have different requirements. Microalgae generally have simple life cycles reproducing by asexual fission particularly when environmental conditions are favourable (Anderson, 2004). When conditions begin to become unfavourable, as in the onset of winter or nutrient depletion, sexual reproduction may be triggered. The progeny arising from sexual reproduction form thick-walled, dormant cells called cysts that settle out of the water column or may be carried long distances by currents. However, evidence does not support natural long distance movement and it is more likely that global movement of microalgae is the result of inadvertent human transportation (Foissner, 2006). Generally, marine microalgae have no specialised dispersal mechanisms and are reliant on passive movement in ocean currents. The toxins do not form aerosols except if they are released from dead algal cells. Algal cells are too big to be airborne. In natural environmental conditions such as strong wind and high seas when seawater forms a spray, algae from surface water may become attached to water particles in which they may survive for a short period, but will only travel short distances (Sharma et al. 2006). In laboratory conditions, algal cells are enclosed in containers. As noted, the only mechanisms for dispersal are

	passive movement in ocean currents or in storm-formed aerosols. As these conditions are highly unlikely to occur in the laboratory the characteristics of the organism do not enable them to escape. Marine microalgae generally have specific temperature, and nutrient requirements.
How could this organism escape from containment? *i.e. what are the possible pathways for escape?* *How does the proposed containment regime address these pathways?*	Possible pathways are: -escape during transportation: This is prevented by sealed containers under personal care by approved persons at all times. The IATA Dangerous Goods Regulations contain an exemption for water samples which are not considered to pose a significant risk of infection (reference 3.6.2.2.3.4 of the Regulations). Salt water can corrode the aircraft skin if there is significant contact but these quantities are small and are not classed as corrosive by IATA. Tubes will be filled ensuring that space is provided for expansion of the liquid that will occur from the reduced pressure at altitude. All samples will be double-contained and meet the requirements of our manual or designated packaging standards. Creation of aerosols while transferring culture media: All open vessel work will be conducted in a class II biosafety cabinet to prevent any release to the environment. -accidental spillage: This is possible in the laboratory but will immediately be treated with an appropriate biocidal agent. -unintentional or deliberate removal by people: access is provided only to authorized people. All laboratory staff are trained in the requirements of the operational manual and HSNO decision controls. Entry to the laboratory is limited to staff authorised by the Containment Facility Operator. -following natural disaster like major earthquake, flood or fire. **Earthquake:** small earthquake: the trolleys are fastened to the walls to prevent movement and they have bars to prevent containers falling from shelves. With a massive earthquake destroying buildings and its contents etc., algal cultures will not survive when their

	required physical conditions such as temperature, light, media and nutrients are not maintained. **Flood:** the rooms are on the second floor - it is unlikely that flood will affect it. Flood within the building - the cultures are on mobile trolleys that can be moved to another room. Flood within the collection facilities – affected containers will be destroyed, and contaminated surfaces treated with a biocidal agent **Fire:** there are doors on both sides of the corridor adjacent to the CC room that will close automatically when fire is detected; if fire affects culture rooms the high temperature will destroy cultures, there will be no viable organisms after that to contaminate the environment. The procedural measures required by MAF for this containment facility and procedures followed up at all times prevent escape of the organisms held in the containment. Escape of these organisms to the sea is theoretically possible in the case of a major earthquake, however Cawthron is not situated by the ocean and hence this pathway is highly unlikely. Note: in our view even in the event of catastrophic earthquake there is no way a spilled sample could reach the sea.
If it were to escape, could this organism establish a population outside of containment in New Zealand? *ie what conditions are required for growth and reproduction? And are those conditions present in New Zealand? What factors might prevent this from occurring?*	Yes, some marine algae from other parts of the world are able to establish a population in New Zealand coastal waters if escape was possible. Seawater temperatures in the South Island where Cawthron Institute is situated will limit growth of some species, i.e. species from sub- or tropical waters will not grow in cooler areas and vice versa. Example: Micro-algae that occur in tropical waters require a maintenance temperature of 25^{o}C, whereas micro-algae isolated from our most northern waters grow well at 20^{o}C. However, the same species that are found in tropical locations occur in Northland waters. It is likely that most micro-algae from tropical waters are already in New Zealand – the more research we do the more species we find – at least one every year. Many other factors may limit or enhance growth: availability of nutrients (chemical composition of oceanic water), oxygen, sunlight etc (Mountfort et al. 2006). Salinity is a strong growth-limiting factor for marine micro-algae. The algae of interest are marine species and they are unable to survive in the fresh water

	environment, requiring a narrow seawater salinity range. Salinity of freshwater is close to zero (Magana, Villareal 2006); for example, tap water shows 0.01ppt salinity.
If a population did establish could it be eradicated? How? Would it be noticed immediately? How would such a population be identified?	No. No. Through IANZ accredited routine, weekly seawater sampling already in place for Tasman Bay.
Additional information	

Section Five – Identification and assessment of effects

Identify and assess the effects of the organism. Look primarily at the effects if the organism remains in containment, but also consider what might happen if the organism were to escape. If the organism were to escape think about what additional things would need to occur for these effects to be realised.

What are the beneficial effects of the organism(s) and the application? *These benefits must be relevant to the purpose and scope of the application*

The Seafood Safety programme will assess pre- and post-harvest risks from microorganisms, in particular microalgae, to New Zealand's $1.43b seafood industry. This FRST supported Seafood Safety programme is a partnership with the seafood industry and the New Zealand Food Safety Authority. By development of tools for detecting and monitoring harmful algal blooms it will enable the development of a Quality Safe Food brand, with an expected added value of $30m. The future growth of seafood exports will be facilitated through ensuring a premium status for New Zealand's unique seafood products. In addition, A strong scientific knowledge base will help prevent market exclusion caused by spurious non-tariff trade barriers.

Should sea temperatures increase, it is also highly likely that new, more tropical, microalgae species will arrive in New Zealand and we wish to be proactive in our research in this area. It is the Institute's aim to anticipate this possibility by understanding the nature of the biotoxins produced by these sub-tropical microalgae and to provide information to the regulatory agencies responsible for biotoxin events.

Furthermore, we wish to understand pre-existing algal problems besetting the South Pacific. For example some marine micro-algae occurring in subtropical waters of the Pacific region produce toxins which may be consumed by reef fish and accumulate in fish tissues. There have been reports of illnesses, even deaths associated with consumption of reef fish. We will isolate and identify the toxic micro-algae involved, characterize the toxins and assess the risk to human health.

What adverse effects could this organism have on the environment? *For all stages of the life cycle*

If released and established in oceanic waters they would become part of toxic bloom-forming species already occurring in New Zealand. Nevertheless, it is highly likely that many species isolated from the Pacific Islands are already present in New Zealand's subtropical waters.

In the unlikely event of a release from containment, sufficient propagules of each species would need to reach a suitable environment, i.e. temperature, salinity, nutrients. If all these conditions were met then a population might establish and become part of local marine flora. From time to time it possible that some of these species could cause algal blooms that may have some localised toxic effects on fauna.

Some species of micro-algae may produce toxic blooms and the toxins may enter the food chain (e.g. shellfish, fish and the humans that consume these).

Toxic blooms do occur seasonally and sporadically and are driven by many environmental and anthopogenic factors such as day length, sea surface temperatures and nitrogen inputs. Like land plants, micro-algae need water, carbon dioxide, sunlight and nutrients. Carbon dioxide is plentiful in the marine environment, but sunlight and nutrient amounts can vary. Sunlight is available near the surface of the water, so micro-algae grow readily when they

can remain near the surface. Nutrients are abundant in areas of run-off where water flows over land and picks up minerals that are then carried to the sea. Nutrients are also abundant in areas of cool, deep waters when these are brought to the surface due to tides or wind-driven mixing. When the micro-algal cells get everything they need to grow, they can divide very rapidly and potentially create a bloom. The ocean is always moving and micro-algae and nutrients move with it. In addition, many micro-algal species thrive under only certain temperature and salinity conditions (as described on pages 11, 12 and 13). And finally, for micro-algae to successfully bloom, there must be a limited number of grazers in the area - if there were enough grazers there wouldn't be a bloom.

The micro-algae are motile (either planktonic or benthic) or in cyst form. The cysts will only have an impact if environmental conditions lead to their hatching, in which case they become motile. In the motile form they would have the same impacts as the indigenous micro-flora, i.e. blooms may cause anoxia or, if toxic, may kill fish or shellfish or pass through the food web. These impacts are the same as for the many bloom-forming algae in our coastal waters already.

What adverse effects could this organism have on public health? *For all stages of the life cycle*

If released and established in oceanic waters they may enter the food chain and pass their toxins to fish or shellfish. If these fish and shellfish were to be harvested and eaten they could cause illness or, in some circumstances, death. This could also happen inadvertently *via* agencies such as ballast water or by climate change induced range extension.

It is therefore extremely important to investigate this path and fully identify toxins and their impact on human health, both to assist in the setting of biotoxin regulations and to gain knowledge for potential future "bloom" events.

What adverse effects could this organism have on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi)?

As for the previous section, in the unlikely event of an escape from containment and should establishment occur, this might affect traditional food sources such as native fish and shellfish beds. However, these are likely to be only short-term with fish populations and shellfish beds recovering after a bloom event.

Are there any other potential adverse effects (including effects on New Zealand's international obligations, society and community or the market economy)?

Yes, in the extremely unlikely event of an escape from containment and establishment occurs, this might affect society and community or the market economy. As far as we know there will not be any potential effects to international obligations.

IANZ (International Accreditation New Zealand) accredited routine, weekly seawater sampling around New Zealand is already in place to alert seafood producers and community and prevent harvesting of contaminated shellfish.

Hypothetically, if new micro-algae were released and established in oceanic waters they could become part of toxic bloom-forming suite of species species already occurring in New Zealand. They could enter the food chain and could pass their toxins to fish or shellfish resulting in contamination of seafood products.

Seawater samples routinely, weekly sampled around New Zealand from over 80 designated sites, are couriered to Cawthron's IANZ accredited Micro-algae Laboratory for processing. Results are provided to food safety regulators and commercial clients within 30 hours of receipt of samples. Toxic micro-algae concentrations above a pre-determined trigger level are highlighted to allow the appropriate regulatory or harvesting management action to take place.

Cawthron's research, funded by the government, through the Foundation for Research, Science and Technology, will provide underpinning knowledge to New Zealand's Food Safety Authority to ensure New Zealand's seafood is safe and that it is welcomed into the international market.

Are there any ethical considerations associated with the organism(s) to be imported or the proposed research?

Cawthron has a MAF permit to take marine specimens from the wild for scientific research. Organisms obtained from overseas under MAF Quarantine Service Import Permits will be maintained in Cawthron's Transitional Facility or CFR's transitional and containment facilities. Cawthron will meet all requirements of the Animal Welfare Act 1999 under the umbrella (parenting body) of Nelson Marlborough Institute of Technology's (NMIT) Animal Ethics Committee (AEC). NMIT will represent Cawthron through their Code of Ethical Conduct - Animal Welfare (2004) until 31 December 2009.

AgResearch (who will receive micro-algal extracts) has registered transitional and containment facilities under the Biosecurity Act 1993 and HSNO Act 1996. Ethical approval for carrying out toxicology research involving mice has been received from the AgResearch Ruakura Research Centre Animal Ethics committee.

Section Six – Additional information

Additional Information	Y/N	If yes, explain
Do any of the organism(s) need approvals under any other New Zealand legislation?	N	
Does New Zealand have any international obligations relating to (any of) the organism(s)?	N	We obtain permission to collect these samples each time from the appropriate Government Department.
Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere? What was the outcome?	N/A	
Is there any additional information that you consider relevant to this application that has not already been included?	Y	Dr Lesley Rhodes has been working with imported micro-algae for many years on various scientific projects. Krystyna Ponikla is an experienced curator with 10 years hands-on operation of growing and maintaining live micro-algal cultures.

Provide a glossary of scientific and technical terms used in the application:
List of appendices:
Copy of Quarantine and Containment Manual QSM 21
List of references:
• Anderson, D.M. (2004) The growing problem of harmful algae. Oceanus Magazine 43(1). http://www.whoi.edu/oceanus/viewArticle. • Cawthron (2006) Freshwater toxin and microalgae analysis. Cawthron Institute, Nelson. Cawthron Institute, Nelson. http://www.cawthron.org.nz/analytical-laboratory/downloads/freshwater_toxin_and_algae_analysis.pdf. • Cawthron (2007) Annual Report. Cawthron Institute, Nelson. http://www.cawthron.org.nz/publications/downloads/annual-report-2007.pdf. • Chang, H. (1996) Shellfish Toxin Update by NIWA. Seafood New Zealand February: 26. • Foissner, W. (2006) Biogeography and dispersal of microorganisms: a review emphasizing protists. Acta Protozoologica 45:111–136. http://www.nencki.gov.pl/pdf/ap/ap921.pdf • Laurent, D., Yeeting, B., Labrosse, P., Guadechoux, J-P. (2005) Ciguatera, field reference guide. Secretariat of the Pacific Community. 88 p.

- Magana, H.A., Villareal, T.A. (2006) The effect of environmental factors on the growth rate of *Karenia brevis* (Davis) Hansen and Moestrup. Harmful Algae 5: 192-198.
- Mountfort, D., Beuzenberg, V., MacKenzie, L., Rhodes, L. (2006) Enhancement of growth and gymnodimine production by the marine dinoflagellate, *Karenia selliformis*. Harmful Algae 5: 658-664.
- Murray, S., Jorgensen, M.F., Daubjerg, N., Rhodes, L. (2004) *Amphidinium* revisited .II. Journal of Phycology 40:366.
- Rhodes, L., Suzuki, T., Adamson, J., Mountfort, D. (2001a) Esterified okadaic acid in New Zealand strains of *Prorocentrum lima*. In: Harmful Algal Blooms. Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. (eds) IOC of UNESCO. pp. 364-366.
- Rhodes, L.L., Adamson, J., Suzuki, T., Briggs, L., Garthwaite, I. (2000) Toxic epiphytic marine dinoflagellates *Ostreopsis siamensis* and *Coolia monotis* (Dinophyceae) in New Zealand. New Zealand Journal of Marine and Freshwater Research 34:371-384.
- Rhodes, L.L., Mackenzie, A.L., Kaspar, H.F., Todd, K.E. (2001b) Harmful algae and mariculture in New Zealand. ICES Journal of Marine Science 58: 398-403.
- Sharma, N., Singh, S., Rai, A. (2006) Diversity and seasonal variation of viable algal particles in the atmosphere of a subtropical city in India Environmental Research 102: 252-259.