Water Quality

Testing for toxicity in our aquatic environment

Chris Hickey and David Roper

Using native species to assess the impacts of chemical discharges on ecosystems is set to become a key technique in relation to Water Quality requirements under the Resource Management Act.

THOUSANDS of different chemicals are in daily use. Their purposes range from household cleaning to contributing to large-scale industrial processes. Many will at some stage find their way back into the environment in one form or another. Those not released into the atmosphere may be discharged into streams or rivers or into the sea, perhaps with some kind of "treatment" first (e.g. in oxidation ponds). Some may gradually leach through the soil into watercourses or end up as components of marine or freshwater sediments.

The effect of these chemicals on marine and freshwater ecosystems depends on their concentration, their toxicity to organisms at this concentration, and how long the toxicity persists.

The impacts of high doses of toxic chemicals are often obvious, resulting in fish kills and extensive destruction of aquatic life. However, an increasing challenge for scientists is to assess the effects and fate of low levels of contaminants which are often associated with organic and nutrient enrichment.

Major sources and types of contaminants in New Zealand

Source	Contaminants
Agriculture *dairy, tannery, piggery*	ammonia, hydrogen sulphide, cadmium, pesticides
Mining	heavy metals, suspensoids
Forestry *pulp & paper*	resin acids, chlorinated organics, dioxins
timber treatment	copper, chromium, arsenic, PCPs, chlordane
forestry operations	
Geothermal	mercury, arsenic, boron
Stormwaters	heavy metals, PAH, suspensoids
Harbour dredging	heavy metals, organics
Municipal wastes	ammonia, hydrogen sulphide, pesticides (and numerous others)

Assessing toxicity

Techniques are available for the identification and measurement of many chemical contaminants down to extremely low concentrations, but the only way to measure toxicity is to test the effects of a substance on living organisms. There are two approaches to assessing the potential toxicity of a water or sediment sample. A detailed chemical analysis of the sample can be made, followed by reference to published water quality criteria which give "safe" concentrations for the contaminants identified. Alternatively, toxicity can be assessed directly using a biological toxicity test.

Comparison of chemical measurements versus bioassays for assessing toxicity

Chemical measurements

Advantages:
- Treatment systems are more easily designed to meet chemical requirements because the procedures are well-established.
- The fate of a pollutant can be predicted through modelling.
- Chemical analyses may be less expensive than toxicity testing.

Disadvantages:
- In complex samples, not all potential toxicants may be identified; therefore the control requirements set may be incomplete.
- It is not always clear which compounds are causing toxicity.
- Measurement of individual toxicants can be expensive, especially in complex samples, and for organic chemicals.
- The bioavailability of the toxicants at the discharge site are not assessed, and the interactions between toxicants (e.g., additivity, antagonism) are not measured or accounted for.

Toxicity bioassays

Advantages:
- The aggregate toxicity of all constituents in a complex effluent or contaminated sediment is measured.
- The bioavailability of the toxic constituents is assessed, and the effects of interactions of constituents are measured.
- It is easily understood by the public, and provides tangible evidence of environmental impact (or lack of it).

Disadvantages:
- Properties of specific chemicals (such as potential for bioaccumulation) are not assessed.
- Wastewater treatment engineers cannot identify specific toxic components and current levels of experience limit ability to design or manipulate treatment systems.
- Where there are chemical/physical conditions present (e.g., pH changes, salinity changes, photolysis) that act on toxicants in such a way as to "release" toxicity downstream (or away from the discharge point), such toxicity may not be measured.

Experiments are carried out to examine the effects of contaminated sediments on shellfish behaviour. (Photos: D. Roper)

The panel lists the advantages and disadvantages of both methods. Often, the best solution combines the two approaches.

Toxicity testing is receiving increasing attention from scientists in New Zealand, including a group based at NIWA in Hamilton.

What is a toxicity test?

A toxicity test is a relatively simple laboratory bioassay procedure. For effluents, the first step is to prepare a series of dilutions of the solution to be tested, plus a control. The control is usually the receiving water. Which is also used to dilute the test sample. The aim is to simulate as closely as possible the chemical interactions which may occur when an effluent is discharged.

Test organisms placed in each dilution and the control are incubated for a set time, under controlled temperature and lighting conditions. At the end of the exposure period a measure of toxic effect is made.

The test end point varies. It may be, for example, mortality, reduced fertilisation, lowered fecundity or reduced growth. By examining responses in the dilution series it is possible to quantify the toxicity, and to determine the lowest concentration which causes a measurable toxic effect. It is also possible to calculate the *EC50* – the effective concentration resulting in 50% of the organisms showing a response of the end point being considered. This term includes *LC50* which is the lethal concentration resulting in 50% of the organisms being killed after the chosen time.

With sediments, dilution series can also be prepared by adding clean sediment to the test sample. More commonly, however, toxicity is determined by comparing the survival or responses of test organisms placed in the test sediment with those in a reference (or control) sediment.

Most tests rely on *acute* responses (short term – relative to the life span of the organism). This is usually 48 hours or 96 hours. A *chronic* (long term – relative to the life span of the organism) test generally measures as its end point reproductive success/failure, or development and/or growth of embryonic or larval forms. Chronic tests are usually 7 days to 28 days.

Progress in toxicity testing

Toxicity testing is relatively new to New Zealand. Most work involving the technique has been carried out since 1986. NIWA scientists are undertaking toxicity-related research and consultancy studies in both freshwater and marine environments. They have established a suite of laboratory bioassays for both freshwater and marine toxicity assessment. The work has a research component involving test validation (comparing test predictions with observed field impacts), development of native species bioassays and river and estuarine process studies (biodegradation, bioaccumulation). Standard test protocols and "benchmark" test species are used for routine assessments; native species are used in site-specific comparisons. Microtest approaches are being pursued as these offer improved cost-effectiveness. Toxicity tests using native species are largely still an active research area. However, the indications are that many common native freshwater invertebrates are suitable candidates for laboratory toxicity testing. Marine invertebrates have also been used successfully.

Overall, the tests are becoming recognised as providing reliable information which can be used to meet the requirements of the Resource Management Act as well as providing useful research techniques for understanding the effects of contaminants on freshwater and marine ecosystems.

The panel gives background information on the test species which are being used routinely.

"Sensitive" species

Part of the work undertaken at NIWA has been to compare New Zealand freshwater species with internationally-used "standard" test species. A comparison of sensitivity to heavy metals and ammonia showed that native cladocerans, or "water-fleas", are similar, while the native mayfly *Deleatidium* is among the more sensitive of test species.

An ammonia sensitivity comparison of nine native invertebrate species has shown that the four most sensitive species had greater acute sensitivity to ammonia than those species, including rainbow trout, used to derive the widely-used US Environmental Protection Agency (USEPA) standards. Surprisingly, the more sensitive invertebrate species (a snail and a crustacean) were those which would normally be associated with lowland streams rather than the normally-accepted "sensitive" species (mayflies and stoneflies). The invertebrates were more sensitive than the native fish species (inanga) tested. These results suggest that EPA criteria may not provide adequate protection for New Zealand species, and that additional studies with native fish and chronic (long term) situations are required. Freshwater research is at present studying the chronic sensitivity of riverine native invertebrates and fingernail clams (*Sphaerium novaezelandiae*) to ammonia.

Freshwater sediments

Another area of research at NIWA is in developing techniques for assessing toxicity in freshwater sediments. The Hamilton team have assessed five sediment-dwelling species for suitability for 10-day sediment toxicity testing. They were compared for sensitivity to "reference" toxicants (phenol, PCP and cadmium) and to resin-acid-contaminated sediments. The results showed good survival and high sensitivity to reference toxicants for the amphipods and clams. Chronic sediment toxicity tests using freshwater amphipods (*Paracorophium lucasi*), oligochaetes (*Lumbriculus variegatus*) and clams (*S. novaezelandiae*) together with a suite of aquatic tests are being used with chemical analyses to investigate the efficacy of stormwater retention ponds for the reduction of contaminant loadings and toxicity.

Marine sediments targeted

Contaminants tend to accumulate in estuarine sediments. Therefore, sediment-dwelling species have been the subject of most of the laboratory-based toxicity studies carried out for marine environments. Effects of contaminated sediments on benthic organisms are being assessed by behavioural and growth reduction bioassays with the amphipods (*Chaetocorophium lucasi*) and bivalves (*Macomona liliana*). In one study, bivalves were exposed to copper-dosed and chlordane-dosed sediments. Behavioural responses occurred at lower levels (6 to 20-fold, respectively) than mortality, which suggests that these may provide sensitive tests for the detection of adverse contaminant effects. The Hamilton team is also involved in several marine studies evaluating the toxic effects caused by discharges into coastal waters. The tests being developed use sublethal responses of echinoid embryos (sand dollars, *Fellaster zelandiae*) and mysid shrimps (*Tenagomysis* sp.).

Background to toxicity test species

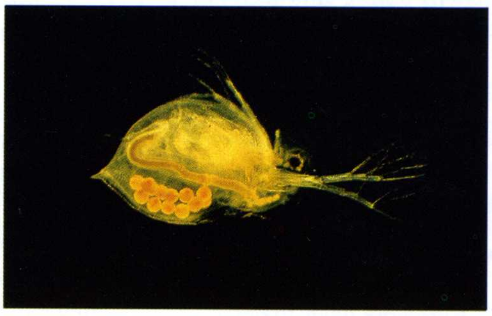
Effluent testing generally involves three test species representing different phylogenetic levels. No single species can be expected to be sensitive to all chemical contaminants. Therefore the range of test species provides a greater level of ecosystem protection. The standard test species are:

Freshwater

- *Daphnia carinata*: a native freshwater cladoceran species ("water-fleas"), found in lakes, ponds and backwaters. Cladocerans are among the most sensitive species to a wide range of chemical contaminants. They are used internationally in environmental impact assessment and monitoring of discharges. A sensitive measure of chronic (long term) toxic effects is the number of young produced.
- *Daphnia magna*: a freshwater cladoceran species. This is an international standard species used for toxicity testing.
- *Selenastrum capricornutum*: a freshwater green alga, recommended by USEPA as a standard algal test species. Reduction in growth provides a sensitive measure of the effects of toxic contaminants. The species has been recorded in New Zealand lakes.

Marine

- *Chaetocorophium* cf. *lucasi*: an estuarine amphipod species. These tube-dwellers inhabit soft sediments in estuaries but venture out at night into the water column, where they can survive for long periods. They resemble small "sand-hoppers". Juveniles (used for testing) are 2-3 mm long. They are relatively tolerant to salinity, and so are ideal for measuring the effects of freshwater effluents on a marine environment. They are quite sensitive to a wide range of contaminants.
- *Minutocellus polymorphus*: a marine alga (diatom), recommended by USEPA as a standard algal test species. It has been recorded around the Tasmanian coast so is likely to be a natural resident around the New Zealand coast.
- *Photobacterium phosphoreum*, Microtox™: a marine bioluminescent bacterium. This bioassay measures the reduction in light output in response to the presence of contaminants. The bioassay is a proprietary product and is widely used as a "standard" benchmark test in assessment and monitoring of effluent discharges.



Left: Two of the "standard" species used for toxicity testing. (Upper) Daphnia carinata *– a water flea.* (Photo: C. Hickey) *(Lower)* Paracorophium lucasi *– an amphipod whose growth rate is used to assess contamination of marine and estuarine sediments.* (Photo: D. Roper)
Above: Freshwater mussels can be used for monitoring levels of contaminants in lakes and rivers. (Photo: D. Roper)

Applying the science

The Resource Management Act (1991) contains both narrative and numeric standards which may be applied to waters and effluents through the provisions of a regional plan. In the case of the effects of contaminants, the granting of resource consents at present involves interpretation of narrative terms such as "adverse effect", "significant adverse effect" by virtue of "any discharge of contaminant into the water". For consistent and objective interpretation guidelines are required for measuring "effects" and defining "contaminants". At the same time, standards for receiving waters and effluents must be set.

Deploying mussels from a sea rider. River and lake deployment of cages of the freshwater mussel Hyridella menziesi *are used to measure bioaccumulation of chemical contaminants.* (Photo: C. Hickey)

Whole effluent toxicity testing requirements have been widely incorporated into discharge permit requirements overseas. Toxicity testing is being increasingly used in New Zealand to determine compliance with Resource Management Act requirements. The design of the testing programme (e.g., species used, acute/chronic tests, testing frequency) is tailored to the impact risk associated with a particular discharge. Associated toxicity investigations may also include "up-the-pipe" studies to identify sources of contaminants and toxicity identification evaluations (TIE) to identify the nature of the contaminants. The TIE procedures link toxicity testing with chemical treatments to modify the toxicity of specific components (e.g. air stripping, pH adjustment). These procedures assist in identifying the treatment systems required for toxicity reduction.

A comprehensive assessment of potential contaminant effects involves chemical, toxicity and biological monitoring. Together these are known as a "triad" approach. The panel outlines the relative merits of toxicity testing and environmental bioassessment for detecting contaminant effects. The relative importance of each of the triad components will differ between situations and emphasis should be dictated by the potential environmental risk involved. ■

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Biological toxicity testing versus environmental monitoring of impacts

Problems arise in attributing cause-effect relationships in complex receiving water environments with many potentially deleterious inputs. Environmental monitoring can establish that significant impacts are occurring, but may not be able to identify the major contributor(s) or establish whether other sources of contamination are important. Toxicity tests on effluent samples can help to quantify the potential impacts of contaminants on marine organisms. The biological toxicity testing approach has the following advantages and disadvantages compared with environmental impact monitoring:

Advantages:

- It deals with a more concentrated contaminant so is more likely to detect effects.
- Dilution of the effluent with the receiving water means that the measured effects are definitely attributable to that effluent discharge.
- It establishes magnitude of toxic risk.
- A rigorous testing protocol can be the basis of routine compliance monitoring.
- Testing may be used to track the source of the contaminants with "up-the-pipe" sampling.
- Having demonstrated a toxic effect on a test species, there is a high probability of impacts on local species.

Disadvantages:

- Demonstrating no measurable toxicity may not necessarily mean no environmental impact because laboratory tests use a few species only.
- Laboratory tests may not integrate other stress factors operating on local communities, particularly those operating over long periods.
- Laboratory tests cannot easily be used to predict community level ecological impacts.