Technical Memorandum 42

Laboratory procedures for assessing effects of chemicals on aquatic animals

GD Rippon & JC Chapman

Supervising Scientist for
the Alligator Rivers Region

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Abstract

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There is an increasing reliance in the protection of aquatic ecosystems on the assessment of biological impact. The type and frequency of assessment that is implemented depends on the philosophical approach of those parties involved in water management of any aquatic ecosystem. Laboratory-based toxicity testing is used to generate toxicity data for single chemicals and waste waters and is used to determine dilution rates of waste waters for regulatory purposes. These tests are also useful in post-impact studies in attempting to establish cause-effect relationships; any suspected perturbation of an ecosystem can be investigated using these tests to confirm the causative agent or process. They are also a valuable tool for establishing water quality criteria and play a large role in hazard and risk assessment. This paper discusses and gives examples of different aspects of laboratory toxicity tests using aquatic organisms. The importance of a systematic, tiered investigation with appropriate triggers, is highlighted.

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Laboratory procedures for assessing effects of chemicals on aquatic animals

Introduction

The approach taken by any organisation to environmental protection has to have a firm philosophical basis. Fry (1991) outlined the approach of the Office of the Supervising Scientist at a workshop on environmental protection of the Alligator Rivers Region (ARR), a 28,000km^2 area which includes Kakadu National Park. He considered that some definable level of *acceptable* impact as proposed by Fox et al (1977) in the *Ranger Uranium Environmental Inquiry* was not definable, and therefore has proposed that there should be no *observable* impact in a sensitive and broadly based biological monitoring program. An ecologically sustainable development philosophical approach to water management has been adopted in the new water quality guidelines for Australia (ANZECC 1992). The guidelines give a range of values for many chemicals for which enough data are available and recommend that site specific information be obtained. This reflects the approach of allowing water managers to consider local receiving water physico-chemical qualities, uses, and the identity of chemicals (if known) with which the chemical of concern will be released (Chapman 1991). Nevertheless, the guidelines also recognise that this chemical-specific approach will not allow assessment of any additive, antagonistic, or synergistic biological effects of a complex mixture of chemicals or products of their reactions. Nor will it identify the cause of any toxic effects, assess the bioavailability of a chemical, or determine effects on aquatic ecosystems. They therefore recommend biological water quality assessment be an essential tool for protecting aquatic ecosystems.

In 1984, the Office of the Supervising Scientist (OSS) published a list of water quality guidelines based on chemical criteria (SS ARR 1985) as the first approach to control of any discharge of mine waste water into an area of great cultural and conservation value, Kakadu National Park. Nevertheless, it was recognised that this approach had limitations. In particular, it could not predict toxicity because of the complexity of physical, chemical, biological and other environmental interactions (eg see Holdway 1992). The OSS therefore developed protocols for laboratory toxicity tests by which to establish dilution rates prior to release of mine waste waters. Four such tests, using species found in the ARR, are now registered with the National Association of Testing Authorities (NATA). These include survival and reproduction tests for a freshwater cladoceran (planktonic crustacean), *Moinodaphnia macleayi* (Hyne et al 1991, McBride et al 1991), a population growth test for either *Hydra viridissima* or *H. vulgaris* (Allison et al 1991), and a fish early life-stage test using an eleotrid gudgeon, *Mogurnda mogurnda* (Holdway et al 1991).

The current approach by the Office of the Supervising Scientist to environmental protection of aquatic ecosystems from the release of mine waste waters into surface waters is a three-tiered

one, involving pre-release assessment of quality using biological toxicity tests, radio-chemical standards and total solute load standards; biological monitoring during release of the adequacy of dilution; and long-term post-release monitoring for verification. Thus, the approach not only determines acceptable release rates, but provides the monitoring required to assess and validate the adequacy of these control strategies (Johnston 1991).

Laboratory-based toxicity tests have considerable value in assessment of the environmental impacts of chemicals and complex mixtures besides that of pre-release testing. They are useful also for establishing cause-effect relationships and in confirming the causative agent when perturbations are suspected in the field. They are an essential starting point for decision-making and form the basis for most recent water quality criteria, and, therefore, risk assessment. For their use in regulation, and as routine procedures, these tests need to be standard, reliable, precise, inexpensive, reasonably sensitive, and produce unequivocal responses (Mackay et al 1989, Robinson 1989).

Laboratory toxicity testing using aquatic organisms

Laboratory toxicity tests have been developed for a large number of aquatic species and certain tests have been standardised internationally. The OECD (1987) has published guidelines for the testing of new chemicals which include acute 96-hour and 14-day tests with fish and *Daphnia* (Cladocera), as well as chronic tests with an alga and fish (assessing growth) and *Daphnia* (assessing survival and reproduction). Although these tests are designed to examine three basic trophic levels, the variety of species used for freshwater testing has developed well beyond this basic set. The USEPA (1986) water quality criteria are based on acute tests of at least eight species representing different animal genera and chronic tests from at least three animal genera and at least one species of plant (Stephan et al 1985).

One of the basic tenets in toxicity testing is to use local species, found in the receiving water, under local conditions (Brown 1986). In doing this, the following factors need to be considered: feeding strategy and habit of an (aquatic) organism which is to be exposed to the chemical or waste (eg benthic, lentic, or lotic habits); ability to rear and maintain cultures of the organism; type of end point; route of exposure to a chemical (eg water, food, sediment); exposure of the organism to the chemical (eg duration, behaviours); and physiochemical conditions of the organism's environment (eg pH, redox potential, salinity). Both the OSS in the Northern Territory and the Centre for Environmental Toxicology ('the Centre') in New South Wales have developed various protocols, involving local species, to a stage where they can be used routinely. For example, Holdway (1992) reports on an extensive screening process using nineteen local aquatic species of the ARR. Eight species were finally selected as being both adaptable to laboratory conditions and also sensitive to chemicals, and were used in establishing test protocols. These included one fish, two crustaceans, two molluscs, two cnidarians (hydra) and one aquatic plant. Up to 30 lethal and sublethal effects were examined as possible end points.

Possible end points which might be assessed in laboratory tests range from the sub-cellular to that of the whole organism. The first level includes cellular and subcellular effects of chemicals, such as blood chemistry, adenylate charge determination, enzyme and protein induction (eg cytochrome P-450 and metallothionein, respectively), lysosomal fragility, steroid hormone metabolism, taurine:glycine ratio, and gross pathology and histopathology. The second (eg single species acute or chronic toxicity tests) and third (eg microcosm) levels typically include measurement of survival, reproduction, growth, and behaviour, while other morphological changes or pathological conditions can be measured on an individual basis. Those end points that have an effect on the population of an organism will give the most relevant information for determination of environmental impact (Woltering 1985). Toxicity tests are normally not used to

protect the actual test species but to assess the likely magnitude of the effect on higher levels of organisation (ie population community and ecosystem) (Brown 1986, Giesy & Graney 1989). Therefore, assessment of toxicity using sub-cellular or whole organism end points that can not be related to population effects, will only give an indication that populations might be stressed and that monitoring should be continued.

Acute toxicity tests

Acute tests used in Australia are based on a limited range of non-Australian protocols, which enhances their comparative value (eg Grothe & Kimerle 1985), although arguments are raised about their applicability to environmental protection (Cairns 1983). Nevertheless, they form the basis for identifying hazard and predicting environmental risk. A number of laboratories in Australia conduct tests with native species of cladocera, usually of 48 hours duration and a lesser number with fish. At the Centre, the eastern rainbow-fish *Melanotaenia duboulayi* has been used most commonly for 96-hour LC50 tests, but other species have also been used as their availability and environmental relevance dictate (Sunderam et al 1992).

Tests using vertebrates are becoming increasingly difficult to perform in New South Wales due to animal protection legislation with ministerial approval required for all acute LC50 tests using fish. Although such tests are firmly entrenched in the environmental regulatory processes, there will be an increasing emphasis on the use of macro- and micro-invertebrates (Cairns & Mount 1990, Sugiura 1992).

Chronic toxicity and sub-lethal tests

Chronic tests are generally more complex and time-consuming than acute tests, and hence chronic data for any chemical are often less readily available. There is a great variety of test methods and end points for chronic tests. This can make international standardisation and comparison and interpretation of results difficult. Chronic tests have been replaced by shorter term tests which use sub-lethal end points to assess toxicity. In these tests, end points assumed to be ecologically relevant, such as growth, reproduction and certain behavioural characteristics, are used at the relevant (sensitive) life stage, rather than over a complete life cycle. Methods that assess a sub-lethal response have been standardised to some extent, in particular the cladoceran reproductive impairment test (OECD 1987, Mount & Norberg 1984) and the fish early life-stage test (Norberg & Mount 1985). These tests, using the fathead minnow (*Pimephales promelas*), and a cladoceran (*Ceriodaphnia dubia*), as well as a plant growth test using a green alga (*Selenastrum capricornutum*) have been adopted by the United States Environmental Protection Agency (USEPA 1991) to evaluate the potential chronic toxicity of effluents and receiving waters.

A fish early life-stage test was developed by Holdway and Wiecek (1989) at OSS using the purple-spotted gudgeon, *Mogurnda mogurnda*. This test has hatchability (ie emergence of embryo from egg case) and survival as end points. The Centre is adapting this procedure to the eastern rainbow-fish, *Melanotaenia duboulayi*, and to a related gudgeon, *Mogurnda adspersa*. Application of this assay using different species of the genus *Mogurnda* available in different parts of Australia has been proposed (Rippon & Hyne 1992).

Test design requirements

It is important to recognise the factors which can cause wide variations in the results of laboratory toxicity tests (White & Champ 1983). These include frequency of replenishment of test solution, test container shape and size, and whether the test animals are intermittently or continuously exposed to the chemical in solution.

Laboratory test data on any one chemical can vary, depending on whether the test solution is not renewed (static), whether it is renewed only after long intervals (static-renewal) or whether renewal is continuous (flowthrough). When the eastern rainbow-fish, *Melanotaenia duboulayi*, was tested with endosulfan in static conditions the 96-hour LC50 was 5 µg/L, whereas in static renewal tests it was 2.5 µg/L, and as low as 0.5 µg/L in flowthrough tests (Sunderam et al 1992). The choice of whether the test should use static, static renewal, or flowthrough, depends on the volume of test solution to animal size, species and growth stage of the test animal, chemical and physical stability of the solution, the type and form of the chemical, and the end use of the results.

If the concentration of a chemical which degrades or is absorbed or precipitated rapidly in water is not measured, or the solution frequently renewed, then the toxicity will be underestimated. Measured 96-hour LC50 values for endosulfan, for example, were often about 40% of nominal values, even under flowthrough conditions (Sunderam et al 1992), although the difference was reduced at lower temperatures. A study using test vessels with different surface areas of solution clearly demonstrated the effect of different shaped test containers on the half-life of cyanide (Rippon et al 1991). There are numerous data demonstrating the various water quality factors which alter the toxicity of a pollutant (see White & Champ 1983, Johnston et al 1990).

For chemicals (such as non-persistent pesticides) which are deliberately applied intermittently, then test methods should mimic the problem involved. Methods have been developed for both field and laboratory studies of such events (McCahon & Pascoe 1990). Repeat dosing of methoxychlor, for example to simulate stream dosing for blackfly treatment, caused significant acute mortality in juvenile flagfish *Jordanella floridae* (Holdway & Dixon 1985) and adversely affected hatching success and juvenile tolerance of recently-fertilised flagfish eggs (Holdway & Dixon 1986). Intermittent exposure of juvenile trout to high levels of fenvalerate was more toxic than its continuous exposure at a much lower concentration, even though the mean concentration was the same for each treatment for the duration of the test (Curtis et al 1985). The peak concentration and duration of any poison can be an important factor in the effects observed.

Dealing with chemicals and dilution (control) water or sediments

Assessment of the toxicity of mixtures of chemicals based on knowledge of individual chemicals present can be difficult, and often impossible. Even if all components of the system can be identified, their complex interactions cannot, at present, be fully understood nor assessed. Although the toxicity test procedures used for single chemicals may therefore need modification to adapt them to complex mixtures, for example greater pH control (Mount & Mount 1992), they remain the most cost-effective means of assessing the potential environmental impact of complex mixtures (Cairns & Mount 1990). The source and nature (ie organic or inorganic, speciation, potential to sorb to organic or inorganic components) of the chemical should therefore be considered and an appropriate toxicity test used. Brown (1986) reiterates that 'the material to be tested must be the relevant one'. Therefore, if the chemical in question is a particular pesticide formulation, then that formulation should be used, not the pure form of the pesticide.

Sediments are a major sink for various chemicals, including pesticides (see examples in Scheunert 1985). Contaminated sediments are widespread and of considerable concern because of the potential for remobilisation of sorbed chemicals and subsequent uptake by, and toxicity to, organisms. Several approaches for assessing the toxicity of sediments have been reviewed by Giesy & Hoke (1990), Burton & Scott (1992) and OECD (1993). Associated with this is the rapid development of toxicity tests using benthic organisms for both the total and aqueous phase of sediment (see examples in Burton & Scott 1992), although conventional aquatic toxicity tests, such as the cladoceran and fish tests, have also been used on elutriates and pore water of sediments (see examples in Giesy & Hoke 1990).

The appropriate choice of dilution (control) water or sediment is often difficult and will depend largely on the objectives of the study (see USEPA 1989). For assessing the impact of a point-source industrial outfall on organisms, the diluent would logically be water from upstream of the outfall and outside the influence of the outfall. If there were other wastes entering that water and the effect of the nominated outfall only was to be studied, then a suitable control water might better be synthesised to reflect major physico-chemical qualities of the waste-free receiving water. If dealing with widespread non-point pollution of waterways, then, unless there is a system that is clearly not affected and shares similar physico-chemical characteristics, a standard water should be used for the control and diluent water. The same arguments apply for sediment but standard sediments are more difficult to characterise and manipulate. Further, in sediment toxicity testing, choices need to be made as to whether the pore (interstitial) water or bulk phase is tested, and what is then the appropriate diluent (Giesy et al 1990).

If test water or sediment is to be collected from field sites, sampling design requirements, including the sampling technique and number of samples required, should be carefully determined (Keith 1990). The design should avoid pseudoreplication (Hurlbert 1984) and have adequate statistical power (Fairweather 1991). This will have implications for whether a composite or single point sample is needed. Different samples may need different storage requirements as described in appropriate standard methods, such as those described in the Australian standards (eg SAA 1986).

Statistical analysis

The type of statistical analysis will depend on the philosophical approach taken and the type of end point used. In acute tests, the end point is frequently mortality and therefore probit analysis is used (Finney 1971) to determine the median lethal concentration (LC50) after specified times of exposure, its 95% confidence limits, and the slope of the concentration-response curve. For other types of responses there are two approaches, hypothesis testing and modelling.

Hypothesis testing is used to test the null hypothesis (H_0) that there are no significant differences between control and treatments. The alternative (H_1) is therefore that there is a significant difference between the control and at least one of the treatments. From comparative tests such as Dunnett's (1955) or Williams' (1972), the lowest-observed-effect-concentration (LOEC) is determined. This is the lowest concentration tested at which there is a significant difference from the control. The test concentration immediately below this in the series used is the no-observed-effect-concentration (NOEC). This approach is criticised because the LOEC and NOEC that are obtained are dependent on the concentration series tested; in particular, the NOEC may not actually be the no-effect level (NEL) (Hoekstra & van Ewijk 1993). Also, because it is a comparative statistical technique, some test designs have been criticised for their poor statistical power (Hayes 1987, Oris & Bailer 1993). To allow for some of the inherent errors in this approach, a safety factor is applied to the NOEC or the geometric mean of the LOEC and NOEC, commonly a factor of 10 (eg Holdway 1992).

Alternative approaches include the use of modelling followed by regression analysis (Hoekstra & van Ewijk 1993). Stephan & Rogers (1985) list twelve advantages of regression analysis compared with the well established hypothesis testing. For instance, inhibition (eg of growth or reproduction) concentrations (ICs) can be determined from a regression model (Norberg-King 1988, Oris et al 1991). It has been shown that an IC50 and an IC25 (that level at which 50% and 25%, respectively, of the population is inhibited) give similar values to that of a LOEC and NOEC, respectively. Modelling, however, also has disadvantages, one of which is that the type of model chosen will influence determination of an IC level. Also, the level at which there is an acceptable level of impact has to be decided, although the IC25 has been suggested as appropriate (Norberg-King 1988).

Using toxicity tests in regulation and research

Four major ways that laboratory toxicity tests might be used include the determination of water quality standards, testing of a waste water to determine an acceptable dilution ratio, identification of toxicants in a waste water, and determination of cause-effect relationships. An example of their usage for each of the above will be given, including results for toxicity of endosulfan to Australian aquatic species.

Determination of water quality criteria

Toxicity tests are commonly used as the basis for setting water quality standards. A recent Australian example of this is the development of Australian water quality guidelines for fresh and marine waters. A specific example is development of criteria for endosulfan based on data from the Centre.

Comparison of OSS toxicity testing results with Australian water quality guidelines

Results from OSS tests and the appropriate Australian water quality guidelines are given in table 1. For copper, a LOEC of 83µg/L and a NOEC of 26µg/L for purple spotted gudgeon was found (Rippon & Hyne 1992). The minimum copper concentration given by the Australian water quality guidelines is 2µg/L and the value applied is dependent on the hardness of the water. In very soft waters such as those of the ARR, heavy metals are much more toxic than when present at the same concentration in hard waters. The gudgeon, although moderately sensitive to copper, might not be as sensitive as algae which are very sensitive to copper. In using the guidelines therefore, considerable judgement must be made in those circumstances where relevant tests cannot be made (Greig-Smith 1992).

Table 1 Comparison of OSS toxicity test results with the Australian water quality guidelines

Chemical	Water quality guideline (µg/L)	OSS test results				Protection afforded? [a]
		Species	End point	LOEC (µg/L)	NOEC (µg/L)	
Ammonia [e]	20–30	*Mogurnda mogurnda*	Survival	120 [b]	40 [b]	yes
		Moinodaphnia macleayi	Survival	214 [c]	64 [c]	yes
		Hydra viridissima	Population growth	7 [c]	<7 [c]	no
Copper [f]	2–5	*Mogurnda mogurnda*	Survival	83 [b]	26 [b]	yes
Cyanide	5	*Moinodaphnia macleayi*	Survival	67 [d]	20 [d]	yes
Selenium	5	*Moinodaphnia macleayi*	Survival	2	ND	no
Penta-chlorophenol	0.05	*Hydra viridissima*	Population growth	56	11	yes
		Hydra vulgaris	Population growth	56	11	yes

a based on whether the NOEC is above the minimum of the range given
b actual value based on 60% of nominal value given by Rippon and Hyne (1992)
c free ammonia concentration calculated from total ammonia concentration in a mine waste water
d nominal value only with cyanide having a half-life of 14.4h in the test container
e toxicity is affected by pH
f toxicity is affected by water hardness
ND not determined

The guideline for cyanide is essentially that of the USEPA and similar to the OSS value established using cladocera, gudgeon and hydra in toxicity tests (Rippon et al 1991). Hydra

appear insensitive to pentachlorophenol (Rippon 1991). Tests with selenium at 2µg/L, to check whether this essential element was limiting, killed all test cladocera on one occasion (Hyne 1991). The range for cadmium is 0.2–2µg/L (not shown in table 1) although Baird et al (1990) present data for different genotypes indicating a 48h EC50 (starting with neonates <24h old) range of 0.6–120 ppb Cd^{2+}, while the LOEC obtained from chronic life-cycle tests had a range of 0.2–2.0 ppb Cd^{2+}. Any use of guidelines requires that they are considered as being no more than that. In areas of potential risk from chemicals, direct assessment of toxicity should always be made using appropriate species and conditions.

Endosulfan

Four native and two introduced fish species were used in laboratory tests in the local turbid waters from Moree, NSW, with the insecticide endosulfan (Sunderam et al 1992), a compound important for the control of cotton pests. Results of these tests confirmed the high toxicity of this insecticide to fish, as reported in overseas tests. The measured 96-hour LC50 in static-renewal (24-h replacement) tests varied from 0.1 to 2.4 µg/L. The introduced pest species, the European carp, *Cyprinus carpio*, was the most sensitive of the species tested with an LC50 of 0.1 µg/L, while the most sensitive native species was the bony bream, *Nematolosa erebi*, with a 96-hour LC50 of 0.2 µg/L. These species are the ones most commonly caught in the cotton growing areas.

Endosulfan is generally less toxic to invertebrates. The 96-hour LC50 to the common freshwater shrimp, *Paratya australiensis*, was 8.9 µg/L at 25°C, while a preliminary test on a Notonectid species (Insecta) gave a tentative figure of 0.1 µg/L (Sunderam 1990). Two native cladocera were less sensitive, with a 96-hour LC50 range of 215–490 µg/L, although the acute/chronic ratio, based on reproductive impairment, varied between 8 and 50 (Sunderam 1990). Tentative criteria calculated using these data were similar to that of the Australian water quality guidelines (ANZECC 1992). Toxicity to invertebrates is different for organophosphate and pyrethroid pesticides, the latter commonly have invertebrate 96-hour LC50 values less than 10 µg/L (eg Julli, in press).

Pre-release toxicity testing of waste waters

Mine waste waters are frequently screened, using toxicity test protocols developed by OSS, both by OSS itself and by the Ranger Uranium Mine Environmental Laboratory. OSS has tested mine waste water from two uranium mines in the ARR, namely Ranger Uranium Mine Pty Ltd at Jabiru and Queensland Mines Pty Ltd at Nabarlek. Retention Ponds 2 and 4 (RP2 and RP4) collect run-off from the ore-stockpile and waste rock dump, respectively, at the Ranger uranium mine and the waters are noticeably enriched, with respect to the receiving water, in uranium, manganese, magnesium, and sulphate, and other heavy metals. The mine at Nabarlek is currently being decommissioned and it was planned to release water from Evaporation Pond 1 (EP1) which is enriched in heavy metals and ammonia.

In the 1991–92 Wet season, EP1 water was tested by OSS using three of its NATA registered tests prior to a possible release, to determine the required dilution rate for release (Rippon et al, in press). The gudgeon was the least sensitive of the three species tested with a LOEC of 32% and a NOEC of 10% EP1 water. The cladoceran was intermediate in sensitivity with a LOEC of 10% and NOEC of 3.2% EP1 water, while *Hydra* was most sensitive (LOEC 1% and NOEC 0.3% EP1 water). The recommended dilution was therefore based on the *Hydra* LOEC and NOEC.

Identification of toxicants using biological toxicity tests

Toxicity tests on complex aqueous wastes are concerned with determining the biological impact of the waste. Nevertheless, it is sometimes useful to identify the major toxicant(s) in a complex waste water. For instance, identification of the major toxicant could allow remediation techniques

to be implemented, thus reducing the concentration of the major toxicant and increasing the capacity for discharge. Alternatively, it can allow regulators a method to trace any non-compliance in discharge to a particular source. A toxicity-based approach involves (1) the separating of the chemicals contributing to toxicity from other chemicals in the effluent (toxicant characterisation) prior to (2) instrumental analysis (toxicant identification) then (3) verification using correlation, relative species sensitivity and other tools (toxicant confirmation) (Burkhard & Ankley 1989). This three-phased approach is used by the US National Effluent Toxicity Assessment Center and relies on toxicity tests to indicate the presence of toxicity in each fraction or after each treatment (Burkhard & Ankley 1989).

A similar iterative process was adopted by OSS to identify the major toxicants in a uranium mine's waste waters from RP2 and RP4. RP4 water is considered to be of relatively high quality when judged by chemical criteria alone while RP2 is in a restricted release zone and of lower quality. The uranium concentration for RP2 and RP4 water was about 2500µg/L and 50µg/L, respectively, through the Wet season. RP4 water, however, had previously shown a seasonal peak in toxicity through the Wet season which could not be explained by the known chemical constituents in RP4.

Biological toxicity tests were performed using *Moinodaphnia macleayi* with survival after three to five days as the end point. RP2 and RP4 water caused significant effects at concentrations of 10% and 0.3% respectively. A metal was identified as the major toxicant in RP2 water after an ashing treatment, and further studies using Scintrex time-delay fluorimetry identified uranium as the main toxicant contributing to the year-round toxicity of RP2 water (SS ARR 1991). However, the seasonal toxicity of RP4 water was shown to be due to a toxicant which was soluble in methanol, but less soluble in dichloromethane. This, together with other experiments in which RP4 water was passed through various ion-exchange resins and then screened for toxicity, suggested that the toxicant in RP4 was an organic chemical rather than a heavy metal (SS ARR 1991). Toxicant identification using instrument analysis was not possible because RP4 water ceased to show seasonal toxicity. Nevertheless, the study identified a possible remediation technique for RP4 toxicity (if it again became prominent) because the toxicant was alkaline-labile. It also emphasises the importance of testing the toxicity of effluents and wastes rather than relating toxicity to literature concentrations of chemical constituents.

Determination of cause-effect relationships

Uranium added to creek water had a NOEC and LOEC of 160 and 190µg/L when tested with *Hydra viridissima* (SS ARR 1988), even though 100% RP2 (3900µg/L U) water was not toxic *H. viridissima* (Hyne et al 1992). However, when RP2 water was diluted with creek (control) water to 32% RP2 water, there was a clear reduction in the population growth of *Hydra*. The difference was seemingly due to a reduction in pH when RP2 water was diluted. This was confirmed in subsequent experiments involving the adjustment of pH and conductivity. It was postulated that the complex uranyl carbonate ion at a pH>8 was negatively charged and less toxic because it was less membrane permeable.

A systematic approach to the use of laboratory toxicity tests

Tiered testing approaches

Tiered testing is a systematic approach to assessing impact, such as used by the USEPA in their National Pollutant Discharge Elimination System (NPDES) and for establishing the potential hazard of a new chemical in the United States Toxic Substances Control Act (USEPA 1991). The USEPA Office of Pesticide Programs screening of pesticides for registration includes four tiers,

each of which involves an increase in the complexity of the testing regime, and therefore, increased rigour in establishing the potential hazard to an ecosystem. The first tier uses results from acute toxicity data while the second tier uses results from chronic tests. The third tier is a complete fish life-cycle test and the fourth tier is field or mesocosm testing. The European Community approach is similar but omits mesocosm testing. Testing in each tier is based on results of tests in the lower tier, with the base tier mandatory for all chemicals. The results are linked to various trigger conditions, such as half-life of the chemical, LC50 and NOEC data, its predicted environmental concentrations and its potential for bioaccumulation.

An Australian approach to pesticides already present in the environment

The philosophy of the Australian water quality guidelines is to maintain *ecological integrity*, consistent with ecologically sustainable development (ANZECC 1992). Ecological integrity is viewed as *ecological health* which, although difficult (if not impossible) to define, may be defined in terms of a system's 'capacity to perform all ecological processes'. Two categories of aquatic ecosystem are recognised: 1) pristine ecosystems (such as national parks) not subject to human interference through discharges or activities in the catchment; and 2) all modified ecosystems subject to human interference. Nevertheless, the intention of the guidelines is 'not to place a constraint on the development and long-term maintenance of a healthy biological community'. This would appear to be the first task in taking any remedial action.

Laboratory toxicity tests can help establish suitable guidelines for minimum acceptable concentrations of a chemical. If a tiered approach was taken, then results from these tests would constitute the first tier. Subsequent tiers would involve more complex interactions and levels of investigation, such as the potential for bioaccumulation and subsequent biomagnification of pesticides, and their possible sorption on suspended and bottom sediments. The subsequent investigative tiers would also involve laboratory toxicity testing to establish cause-effect relationships and help establish regulatory quality guidelines for water and sediment. More complicated field-orientated approaches would be used to verify laboratory data and help build more robust, predictive and accurate models. Maltby & Calow (1989) warn that any investigative approach should be firmly based in a hypothetico-deductive approach, rather than an inductive approach, to develop environmentally relevant, predictive models. Thus each tier of investigation should have clearly identified hypotheses and triggers that would lead to the next level of investigation.

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