Bull. Environ. Contam. Toxicol. (1992) 49:471–476

Environmental Contamination and Toxicology

Purple Spotted Gudgeon: Its Use as a Standard Toxicity Test Animal in Tropical Northern Australia

G. D. Rippon and R. V. Hyne

Alligator Rivers Region Research Institute, Office of the Supervising Scientist, PMB 2 Post Office Jabiru, Northern Territory 0886, Australia

Using water quality standards based on chemical criteria alone to assess waste waters has many drawbacks because essentially they fail to integrate all physical and chemical properties of waste waters with an observed biological response. This is particularly so for the assessment of complex mixtures. Legislation is being developed in many countries, including Australia, in which the need for biological assessment is being incorporated. Biological toxicity tests (BTTs) that will be used in the enforcement of legislation will therefore ideally be standard, reliable, reproducible, inexpensive, and produce unequivocal responses (Mackay *et al.* 1989, Robinson 1989). Biological tests using single species are the most frequently used and, although the use of these tests has been questioned by several authors because of their lack of environmental complexity (Cairns 1983; Mackay *et al.* 1989), they tend to have the above characteristics required for use by government regulatory agencies (Mount 1985). To overcome their inherent lack of environmental diversity, several single species tests can be used as a "battery of tests" (Blanck 1984). Some complexity is then added when the tests use species from dissimilar taxa and trophic level, and a variety of endpoints. Also, the use of species that are found in the receiving water and which have been conditioned to the test's control water gives the test a greater degree of relevance to the local ecosystem (Brown 1986).

BTTs using local species are currently being developed by this laboratory to assess the toxicity of mine waste waters. These waste waters originate from uranium mines that lie within the Alligator Rivers Region (ARR) in tropical northern Australia. The ARR is of great environmental significance because it includes the catchment areas and associated wetlands of two major river systems, the South and East Alligator Rivers. All the South Alligator River, and most of the East Alligator River, are more thoroughly protected by their inclusion within Kakadu National Park, considered an important conservation area, entered onto the World Heritage List and the Convention of Wetlands of International Importance. The introduction of exotic species into Kakadu National Park is prohibited, and so for BTTs to be relevant to the ecosystem to which they relate, 19 local species have been screened for their potential use as BTT organisms. These include species of hydra and cladocera. On the basis of breeding and rearing success the (northern) purple spotted gudgeon, *Mogurnda mogurnda* (Teleostomi:Eleotrididae), was selected as a potential species for BTT. The fish has a long generation time, with adults commonly reaching 10 cm in length. Little is known about the biology of this species and our tests at present

Send reprint requests to Dr Greg Rippon at the above address

rely on the use of introduced wild fish to provide embryos and larvae for the tests.

MATERIALS AND METHODS

Several breeding pairs of purple spotted gudgeons were established to guarantee a supply of eggs when needed for a test. Each pair was maintained in a 70 L aquarium (70 × 35 × 30 cm). Several unpaired individuals were also kept in general stock tanks. These fish had been collected from the ARR prior to 1990. Through the 1990 Dry season, more fish were caught to supplement the general stock and increase the number of breeding pairs. The fish were maintained in tap water and fed twice daily with a diet of frozen mixed food consisting of prawns, peas, fish fillet, beef heart and protein enriched baby cereal (Leggett & Merrick 1987). Breeding pairs were established from the introduced fish at the beginning of the 1990 Wet season by placing fish in one large aquarium, providing refuges, and allowing them to pair. At that time, pairs already established were also displaying mating behaviour or spawning.

Two BTTs have been developed in this laboratory using the purple spotted gudgeon based on the Fathead Minnow Embryo-Larval Survival Test (USEPA 1985). Embryos less than 12 hours old were used in one, while the other test used larval fish 1-3 days old. The embryos or larvae were immersed in a range of concentrations of the toxicant to be assessed under 'batch-replacement' conditions. The test period for the embryo test was 4-5 days, the test finishing when all the surviving embryos in the control had hatched. Any mortality or hatching of embryos was recorded over this period. The larval/juvenile test period was normally 14 days. Through this period any mortality was recorded.

The no-observed-effect-concentration (NOEC) and the lowest-observed-effect-concentration (LOEC) for each response were statistically determined using a modified Dunnet's test on the ratio of the differences (between the control and each treatment) to their standard errors (Hyne *et al.* 1992).

The test water for the embryo test comprised either ammonium sulphate or cupric sulphate (Ajax Chemicals, Sydney) in control water while the test water for the larval test comprised waste water from Evaporation Pond No. 1 (EP1) of a uranium mine operated by Queensland Mines Pty Ltd at Nabarlek, within the ARR. Ammonium sulphate was added to the diluent (control) water to make the highest (un-ionised) ammonia test solution with a nominal concentration of 200 μg NH_3/L. This was then serially diluted to give the other test solutions of 64, 20, 6.3, and 2.0 μg NH_3/L. Cupric sulphate was added to the diluent water to make a stock copper test solution with a nominal concentration of 100 mg Cu/L. This was then serially diluted to give the test concentrations of 200, 64, 20, 6.3, and 2.0 μg/L. Similarly, EP1 water was diluted to give the highest test concentration of 10% with further serial dilution to give 3.2%, 1.0%, 0.3%, 0.1%. The diluent (and control water) used in the tests was water from Buffalo Billabong, a permanent water body seasonally connected with the Magela Creek, a tributary of the East Alligator River. Water from the Magela Creek catchment is typically a soft water with low alkalinity and conductivity through the Wet season, and poor buffering capability (Skidmore and Firth 1983).

All control and test waters were collected in clean plastic containers the morning

of the start of the test. The waters were filtered through a coarse filter (Whatman No. 1) which was capable of removing 'wild' zooplankton. Concentration of total ammonia-N and copper in samples of test waters were confirmed using HPLC ion spectrometry and graphite furnace atomic absorption respectively. Actual concentrations of unionised ammonia and copper were 60±5% and 130±20% of nominal concentrations respectively. EP1 water was analysed using Ion-Coupled Plasma Mass Spectrometry (ICPMS).

RESULTS AND DISCUSSION

Table 1 shows the NOECs and LOECs obtained for copper, un-ionised ammonia, and EP1 water. Although the experiment was designed to obtain a LOEC and NOEC for each toxicant, the dose response curves for ammonia and copper give the likely range of each toxicant's LC50. Clearly, the LC50 value for copper would be between 20 and 64 Cu μg/L and the LC50 for un-ionised ammonia would be between 64 and 200 μg NH_3/L (Fig. 1). In the 9-day exposure to copper it appeared that the 200 μg Cu/L treatment caused a precocious hatching with most hatching occurring on day 2 and 3 (data not shown). In the other treatments, most hatching occurred on day 4 and day 5. Mortality in the 64 μg Cu/L (LOEC) and 200 μg Cu/L occurred only after hatching. For ammonia, there was no precocious hatching observed with 53% mortality prior to hatching and a further 33% mortality after hatching observed for 200 μg NH_3/L (LOEC). Skidmore and Firth (1983) presented data on both the acute sensitivity of some Australian freshwater fish (*Melanotaenia splendida splendida*, *M. s. inornata* and *Ambassis* sp.) and two international reference species (rainbow trout and zebrafish) to copper and zinc with the intention of forming a sound basis for the development of Australian water quality criteria. The range of 96h copper LC50 values for *M. s. inornata* was 60-720 μg/L (for their own data as well as reviewed data). The range was considered to reflect different water hardness and total soluble organics between tests. Their results of 96h copper LC50 values for rainbow trout (*Salmo gairdneri*) and zebrafish (*Brachydanio rerio*) were 20 μg/L and 96-120 μg/L, respectively. The results for rainbow trout and zebrafish are in close agreement with other reviews (USEPA 1980; Spear and Pierce 1979). This study shows, given the constraints in making such a comparison, that the sensitivity of the purple spotted gudgeon to copper with a LC50 value between 64 and 20 μg/L is similar to *M. s. inornata* and is almost as sensitive as rainbow trout. One advantage of using the purple spotted gudgeon is the large numbers of embryos able to be obtained from one breeding pair about every 5 days, compared with the low (although daily) egg production in *M. splendida*. Enough embryos are obtained, even from very young fish, to run a toxicity test. To start a test, 180 embryos are needed.

Ammonia is acutely toxic to freshwater fish, with 96h LC50 values ranging from 0.083 to 4.60 mg/L ammonia (USEPA 1986). These concentrations were for total ammonia, not for the principal toxic species of ammonia, un-ionised ammonia (NH_3). The concentration of NH_3 and the ionised ammonia species, NH_4^+, existing in solution is dependent on pH, temperature and ionic strength (Thurston *et al.* 1979). The LOEC and NOEC for un-ionised ammonia in this study were 200 μg/L and 64 μg/L respectively (Table 1), with the LC50 value somewhere between these two values (Fig. 1). It is clear that, given the constraints in making such a comparison, the purple spotted gudgeon is quite sensitive to un-ionised ammonia.

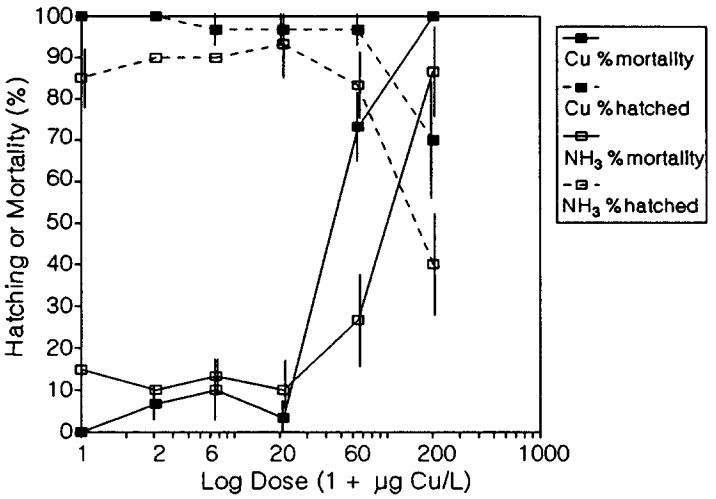

Figure. 1. The dose-response relationship of copper and un-ionised ammonia for each endpoint, hatching and mortality (means with standard error bars plotted).

Table 1. The LOECs and NOECs obtained for copper, un-ionised ammonia and EP1 water.

Toxicant (test)	Duration	End-point	LOEC	NOEC
ammonia	5 days	hatching	200 µg/L	64 µg/L
(embryo/larval)	9 days	survival	200 µg/L	64 µg/L
copper	5 days	hatching	>200 µg/L	-
(embryo/larval)	9 days	survival	64 µg/L	20 µg/L
EP1 water	4 days	survival	10 %EP1	3.2 %EP1
(larval)				

Table 2. ICPMS chemical analysis of major cations and anions in EP1 water collected in the late Dry season, 1991.

Element	Al	Mn	Cu	Zn	Pb	U
Concentration (µg/L)	<0.01	4500	78	295	6.3	820

Analyte	Ca	Mg	Na	K	Fe	Cl	SO_4	NO_3	$*NH_4^+$
Concentration (mg/L)	485	935	95	38	<0.2	235	11000	30 (as N)	1650 (as N)

* dissociated by 0.23% at pH 6.5 and 29±1°C to form 3800 µg NH_3/L.

An examination of the chemical composition of EPI water would suggest that ammonia is likely to be a major contributor to the toxicity of the water (Table 2). Under the conditions of the test of approximately 29°C and a pH of 6.5, the proportion of un-ionised ammonia (NH_3) in the EPI water would be about 0.23%. This would mean that there was about 3800 μg NH_3/L in 100% EPI water (i.e. 0.23% of 1650 mg total ammonia/L). For the LOEC of 10% and NOEC of 3.2%, the effective concentration of un-ionised ammonia would therefore be 380 and 120 μg NH_3/L, respectively (i.e. 10% and 3.2% of 3800 μg NH_3/L). This agrees fairly well (within a factor of 2) with the observed LOEC and NOEC for tests with ammonia sulphate. The differences in ionic strength and pH at the highest EPI concentration may explain the observed decrease in toxicity, since the un-ionised ammonia concentration would be lower.

This laboratory is now better placed to meet two of the basic tenets for biological toxicity testing: using local species and local water to test the actual complex waste water (Brown 1986). A closely related species, *M. adspersa*, is found in more temperate regions in southern Australia (Leggett and Merrick 1987) and may also be able to be used in toxicity testing. The two species of *Mogurnda* would allow the use of local species in BTTs throughout most of Australia.

Acknowledgments. The authors wish to thank Mrs S.M. Hunt for the establishment, maintenance and observation of gudgeon breeding stock, and Ms. J. Summerton for her excellent technical assistance.

REFERENCES

Blanck H (1984) Species dependent variation among aquatic organisms in their sensitivity to chemicals. Ecol. Bull. 36:107-119

Brown VM (1986) Development of water quality criteria from toxicological data. In: Hart BT (ed) Water quality management: Freshwater ecotoxicity in Australia. Water Studies Centre, Chisholm Institute of Technology, Melbourne

Cairns J Jr (1983) Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100:47-57

Hyne RV, Rippon GD, Hunt SM, Brown GH (1992) Procedures for the biological toxicity testing of mine waste waters using freshwater organisms. Supervising Scientist for the Alligator Rivers Region, Technical Memorandum *in press*, Australian Government Publishing Service, Canberra

Mackay DW, Holmes PJ, Redshaw CJ (1989) The application of bioassays in the resolution of environmental problems - The United Kingdom experience. Hydrobiologia 189/190:77-87

Mount DI (1985) Scientific problems in using multispecies toxicity tests for regulatory purposes. In: Cairns J Jr (ed) Multispecies Toxicity Testing. Pergamon Press, New York

Leggett R, Merrick JR (1987) Australian native fishes for aquariums. J.R. Merrick Publications, Artarmon, NSW

Robinson RM (1989) Environmental impact assessment: the growing importance of science in government decision making. Hydrobiologia 188/189:137-142

Skidmore JF, Firth IC (1983) Acute sensitivity of selected Australian animals to copper and zinc. Australian Water Resources Council Technical Paper No. 81, Australian Government Publishing Service, Canberra

Spear PA, Pierce RC (1979) Copper in the aquatic environment: chemistry, distribution, and toxicology. National Research Council of Canada, NRCC Associate Committee on Scientific Criteria for Environmental Quality, Ottawa,

Canada
Thurston RV, Russo RC, Emerson K (1979) Aqueous ammonia equilibrium - tabulation of percent un-ionised ammonia. USEPA Environmental Research Laboratory, Duluth, Minnesota. EPA-600/3-79-091
USEPA (1980) Ambient water quality criteria for copper. USEPA Office of Water Regulations and Standards Division, Washington, DC. EPA- 440/5-80-036
USEPA (1985) Fathead minnow (*Pimephales promelas*) embryo-larval survival and teratogenicity test. In: Horning WB and Weber CI (eds) "Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms". USEPA Environmental Monitoring Systems Laboratory, Cincinnati, Ohio. EPA-600/4-85/014
USEPA (1986) Quality criteria for water. Office of Water Regulations and Standards, Washington, DC. EPA-440/5-86-001

Received December 22, 1991; accepted June 24, 1992.