

**1996 ANNUAL REPORT
OF
CHEMICAL AND BIOLOGICAL
MONITORING
FOR
AUSTRALIAN NEWSPRINT MILLS
Ltd ALBURY**

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1.0 AIM

To undertake biological and chemical monitoring of ANM's wastewater discharge to the River Murray in accordance with New South Wales Environment Protection Agency licence No.01272 sections W10 (Ecotoxicological and Biological Monitoring) and W11 (River Environment Monitoring Surveys).

The null hypothesis tested in all cases “that there is no difference between control water and wastewater treatments.”

2.0 METHODS

2.1 Ecotoxicological Monitoring [W10]

2.1.1 Sample Preparation

All waters were collected as grab samples in 10L buckets on the morning of the test following ASTM (1990) guidelines, the dilution/control water was obtained from the Lake Hume Resort boat ramp at the Hume Dam on the River Murray upstream of the discharge; the receiving water sample was taken from the River Murray approximately 2 km downstream of the wastewater discharge. The wastewater samples were collected on site at ANM from three locations; the final outfall, the 96 hr holding pond and the inlet to the 96 hr holding pond. All waters were sieved to 180 um to remove macro and micro fauna that could interfere with the test, whilst still retaining the samples as close as possible to actual field conditions. The temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity of all samples were measured prior to use (USEPA 1991 pp 44-46). The control and downstream river sample were tested undiluted (USEPA 1991 p47). The three on-site wastewater samples were routinely prepared at three concentrations 100%, 10% and 1%, diluted with control water and aliquots of these were distributed between replicates (ASTM 1990). The laboratory temperature was maintained at 20 ± 5 Celsius throughout the year.

Acute Toxicity Tests

Acute toxicity testing procedures are formulated from ASTM's "Standard Guide for Conducting Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates and Amphibians" (ASTM 1990) and USEPA's "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (USEPA 1991) but in some cases have been modified slightly to accommodate the local organisms in this program.

Acute toxicity tests are designed to obtain information on the immediate effects on test organisms following short term exposure to effluents under laboratory conditions. Results of these tests can be used to predict the likely effects of the effluent on aquatic organisms in receiving waters. The use of locally occurring species ensures greater accuracy of these predictions. The organisms selected for this program occupy different functional groups: *Daphnia carinata* is a pelagic microcrustacean and obligate filter feeder; *Chironomus tepperi*

is a benthic midge larva which feeds on detritus. *D.carinata* and *C.tepperi* can be reliably cultured in the laboratory in sufficient numbers for the testing program.

Acute toxicity tests were conducted monthly. Test chambers used for both organisms were 60 mL clear round glass jars (resin acids, potential toxic components of paper mill effluent may be adsorbed onto plastic surfaces), approximately 40 mm high to ensure an adequate surface area to volume ratio for gas exchange. Test solutions were prepared in a single batch and apportioned between three replicates positioned randomly.

Daphnid tests were conducted using neonates (less than 24 hr old) from laboratory cultures. The neonates were collected by combining broodstock culture solutions in a 5L aquarium after the adults were transferred to fresh culture solutions. Neonates required for the test were carefully captured using a disposable pipette and released under the surface of the test solutions to minimise trauma due to handling. The neonates were distributed randomly between treatments and replicates so that there were 10 animals per jar. (ASTM 1990 pp 758-760, USEPA 1991 pp 49-51).

Chironomid tests were conducted using final instar larvae from laboratory cultures. Chironomid larvae were sieved from their culture solution and final instars carefully transferred to test solutions using flexible forceps to minimise trauma. The chironomids were distributed randomly between treatments so that there were ten animals per jar (ASTM 1990 pp 758-760). A small strip of facial tissue was added to each jar as a substrate to help prevent clumping of animals.

The organisms were not fed for the duration of the test as faecal matter and undigested food can reduce the dissolved oxygen level and reduce the biological activity of some test materials (ASTM p761). Observations were made at 24 hours, and finalised at 48 hours. Death of invertebrates is often difficult to determine, so immobilisation, lack of response to stimuli and opaque colouration or loss of colour are the symptoms interpreted as “effect” (ASTM p761). The results of these tests were reported as EC50 values (the concentration of effluent which results in the “effect” observed for 50% of the organisms) provided sufficient number of organisms were affected. “Calculation of an EC50 is considered unacceptable if either or both of the following occurred: No treatment other than a control treatment killed or

affected less than 37% of the test organisms exposed to it; No treatment killed or affected more than 63% of the organisms exposed to it.” also if more than 10% of the controls exhibited signs of disease, stress or death (ASTM p762 , USEPA p55).

Results were reported quarterly to ANM and a summary of significant results is provided in this Annual Report.

Chronic Toxicity Tests

Chronic toxicity testing procedures for a local cladoceran, *Daphnia carinata* were adapted from ASTM’s “Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*” (1991) and USEPA’s “Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms” (1989) which contain methods based on *Daphnia magna* and *Ceriodaphnia dubia*.

Chronic toxicity tests are designed to provide information to permit the prediction of the possible long term effects of effluents on the test organism in receiving waters. These tests are primarily concerned with sublethal effects which may not be expressed in the short term. The cladoceran tests are life cycle tests, in which an animal is assessed for survival, growth and reproduction.

Chronic toxicity tests were conducted at two monthly intervals using *D.carinata*. Test chambers for these cladocerans were clear glass 60 mL jars. Test solutions were prepared in bulk and apportioned between ten replicates. *Daphnia* broodstock were transferred to a fresh solution and the neonates (less than 24 hours old) remaining were bulked in a 5L aquarium. Neonates required for the test were carefully captured using a disposable pipette and released under the surface of the test solutions to minimise handling trauma. The neonates were distributed randomly between treatments and replicates so that there was one animal per jar. (ASTM 1990 p771, USEPA 1989 p106). The cladocerans were fed a daily dose of blended food solution made up of yeast and trout feed (ASTM 1990 p775) and transferred to fresh solutions three times per week (USEPA 1989 p110). Observations of survival, stress and reproduction/number of live young produced were noted at each transfer. The tests continued for a maximum of 21 days (*D.carinata*). The results were considered acceptable if survival of the controls was at least 80% and each surviving control animal had achieved at least three

broods. Results rejected if ephippia (desiccation resistant eggs produced in response to environmental stress) were produced in any of the controls. (USEPA 1989 p122, ASTM 1990 p 774).

Results were analysed in quarterly reports to ANM providing a summary of reproductive statistics for the duration of the tests. The mean number of young produced in each treatment were compared with the control using t-tests to determine their significance @ $p < 0.05$. A summary of the results for tests conducted this year is provided in this Annual Report to ANM.

2.2 Bioaccumulation Monitoring [W10]

Bioaccumulation trials were conducted to determine the levels of bioaccumulation of metals from ANM's final outfall wastewater using a crustacean (yabby, *Cherax destructor*) and two fish species (silver perch, *Bidyanus bidyanus* and carp gudgeon *Hypseleotris spp.*).

2.2.1 Yabby (*Cherax destructor*)

Yabby trials were conducted on site at ANM using three preconditioned 8-9m³ concrete flow-through tanks each containing ~90 pieces of PVC pipe as hides. Two control tanks were fed by sand filtered river water and the test tank was fed by 50% final outfall wastewater diluted with sand filtered river water. 300 male yabbies of approximately equal size (70 to 80 mm total length) were purchased from a commercial yabby farm. These yabbies were distributed randomly between the tanks to achieve a stocking density of 19/m². A subsample (5%) was measured and nine animals (three from each tank) were retained as initial control samples for metals analysis. Temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity were measured fortnightly and 10-20 animals from each tank were measured (weight and length) monthly, and a smaller subsample (3) removed, frozen and freeze dried every three months or at the termination of the trial in preparation for metals analysis. Samples were then submitted to the MDFRC's chemistry laboratory for acid digestion and shipment to a contract lab for ICP analysis.

Mean growth data were analysed using 't'-tests to determine differences between the control and test treatments. These results were presented in quarterly reports to ANM. The results of

the metals assays were compared for each treatment using the means of the three subsamples. A summary of these results is provided in this Annual Report to ANM.

2.2.2 Silver Perch (*Bidyanus bidyanus*, Mitchell)

Perch trials were conducted following the breeding season in April 1996. The fish tanks were housed in the laboratory on site at ANM to minimise fluctuations in temperature known to adversely affect the health of fish. The fish were contained in six 90L preconditioned polypropylene flow through tanks ('Nally' Tubs) containing filter boxes with aeration and artificial weed. Three tanks were randomly assigned to each treatment. The control tanks were fed by river water filtered to 1 micron, sterilised by Ultra Violet radiation and adjusted to 1000 μ S conductivity using commercial sodium chloride, and the test tanks were fed by final outfall wastewater. Fingerlings of approximately equal size (60-80 mm total length) and similar history were purchased from a local hatchery. About 150 fish were added to each tank after treatment with methylene blue and salt to prevent infection resulting from damage during transport and transfer. The fish were fed daily on commercial fish pellets. A subsample of fish from each tank was measured weekly. Fish were anaesthetised using 1 mL/L of 'Benzocaine' stock solution (5g/100 mL alcohol) prior to being measured, then revived in fresh water and treated with methylene blue and salt before being returned to the tanks. Animals further subsampled for chemical analysis were killed by overdose with 'Benzocaine' prior to being frozen and freeze dried in preparation for metals analysis. Samples were then submitted to the MDFRC's chemistry laboratory for acid digestion and shipment to a contract lab for ICP analysis. Temperature, dissolved oxygen, conductivity, pH, hardness and alkalinity were measured in each tank fortnightly.

Mean growth data were analysed using 't' tests to determine differences between the control and test treatments. These results were presented in quarterly reports to ANM. The results of the metals assays were compared for each treatment and the feed using the means of the three subsamples. A summary of these results is provided in this Annual Report to ANM.

2.2.3 Carp Gudgeon (*Hypseleotris* spp).

Bioaccumulation studies using adult Carp Gudgeon spp. (primarily Western Carp Gudgeon (*Hypseleotris klunzingeri*, Ogilby) with some Lake's Carp Gudgeon (*Hypseleotris* sp.5, undescribed) and Midgley's Carp Gudgeon (*Hypseleotris* sp.4, undescribed)) were

conducted in November 1996, using fish captured from a local billabong during the breeding season. The trial was conducted using the 6 polypropylene flow through tanks in the laboratory at ANM. Three tanks were assigned as controls (river water filtered to 1 micron and UV sterilised) and three tanks assigned as wastewater treatments (ANM's final outfall wastewater). Low stocking densities were used to reduce the impact of territoriality on the fish. ~40 fish were added to each tank and maintained on a diet of frozen tubificid worms and trout pellets. Whole fish samples were collected at the termination of the trial for metals assays (as for silver perch above). This trial is still underway. The results will be included in the 1997 Annual Report.

2.3 River Environment Monitoring Surveys [W11]

2.3.1 Water

Sample Collection and Handling.

Grab samples were taken at three locations on the river on a monthly basis. Site 1 samples were taken from Mungabarena Reserve (approximately 4 km upstream of the outfall). Site 2 samples were taken at a point approximately 200 m downstream of ANM's outfall (adjacent to the railway bridge). Site 3 samples were taken at a point approximately 1 km downstream of ANM's outfall (adjacent to Union Bridge).

5 samples were taken at each location (for analysis of physical parameters, phosphorus, forms of nitrogen, metals and mercury respectively). All samples were collected and preserved in accordance with Australian Standards AS2031.1 and AS2051 - all preservatives were "ANALAR" grade or better and, clean polyethylene gloves were worn at all times. Sampling blanks were handled and analysed in a similar manner to the samples.

Analysis of water samples.

All metal analyses were performed by NATA registered :-

EML (Chem) Pty Ltd

425 -427 Canterbury Road

Surrey Hills Vic 3127

Concentrations of recoverable aluminium, cadmium, cobalt, chromium, copper, iron, manganese, mercury, lead and zinc were determined.

Physical and nutrient analyses were performed at the Murray-Darling Freshwater Research Centre (MDFRC). Turbidity, colour, specific conductance, total filterable solids, ammonia, oxides of nitrogen (NO_x), organic nitrogen, and total phosphorus were determined according to the methods outlined in the MDFRC Chemistry laboratory's methods manual and all except colour approved by NATA.

2.3.2 Sediment.

Sample Collection and Handling.

A series of forty sediment samples were taken on the 2nd of May 1996. Sediment samples were collected from three deposition zones on the River Murray. Deposition site A was located at Doctor's Point (about 2 km upstream of ANM's outfall). Deposition sites B and C were approximately equidistant (*ca* 500 m upstream and *ca* 500 m downstream) of ANM's outfall. Samples were collected at 10 meter intervals along the 60 cm depth contour (approximately 2 meters from, and parallel to, the river bank). A total of 20 samples were taken from each deposition zone.

Approximately the top 5 - 10 cm of sediment was directly scooped into 500 mL wide mouthed polyethylene bottles which had previously been acid washed (5% HCl) and repeatedly rinsed with Milli-Q water. Sampling was such that every effort was made to completely fill the sampling bottle with sediment. The bottle was sealed while under water to minimise the loss of fine material.

The samples were immediately returned to the laboratory and air dried. The air dried samples were sieved (2 mm) - the fraction retained by the sieve was weighed and then discarded, the fraction passing through the sieve was weighed and then thoroughly mixed. All subsequent analysis were performed only on the sieved fraction (Grimshaw 1989).

Particle Size Analysis and Loss on Ignition (LOI)

Approximate particle fractionation was carried out on all samples. Fractionation was by the method described by Grimshaw (1989). Essentially, that portion of the sample which was

retained by a 2 mm sieve was considered gravel. The portion of the sample that passed through a 2 mm sieve was considered a mixture of silt, clay and sand.

The percentage of silt + clay in this fraction was determined by the 4 minute 48 second hydrometer method described by Grimshaw (1989). The sand content was estimated by difference. No distinction between silt and clay content, or fine sand and coarse sand content was attempted.

Loss on ignition was determined gravimetrically after firing up to 20 g of dried sediment at 550 °C for 2 hours (Grimshaw 1989).

Analysis of Acid Extractable Metals.

The fraction of acid extractable metals in the samples was determined by a modification of the method of Anon (1989). 5 g of sediment was accurately weighed into 50 mL polyethylene centrifuge tubes (which had previously been washed with 5% HCl and extensively rinsed with MILLI - Q water). 25 mL of 0.1 M "ARISTAR" grade HCl was subsequently added to the sediment. The tubes were then capped and placed on a "Ratek" orbital shaking table for one hour. The samples were allowed to settle overnight and, subsequently filtered through acid washed Whatman GF/C filters. The filtrate was placed in 100 mL polyethylene bottles (which had previously been washed with 5% HNO₃ and repeatedly rinsed with MILLI-Q water) and dispatched to :-

Australian Government Analytical Laboratories (AGAL)

1 Suakin St

PYMBLE NSW 2073,

for analysis by Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES). The elements assayed for were aluminium, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lanthanum, lead, magnesium, manganese, molybdenum, nickel, silica, silver, strontium, tin, yttrium and zinc. An extraction blank and a standard reference material (Buffalo River sediment - SRM 2707) were processed in exactly the same manner as the samples.

Analysis for Total Mercury.

Approximately 10 g of air dried sample was placed in clean polyethylene bags and dispatched to AGAL for digestion and subsequent analysis by Cold Vapour Generation Atomic Absorption Spectroscopy.

Analysis for Total Nitrogen.

Total nitrogen was determined by a modification of the technique of Hosmoi and Sudo (1986). Approximately 0.25 g of sediment was accurately weighed into acid washed 50 mL centrifuge tubes. 10 mL of an alkaline persulfate digestion medium (0.9 % NaOH, 4.0 % K₂S₂O₄) and 20 mL of Milli-Q water was added to each tube. The tubes were sealed and subsequently heated in an autoclave for one hour. The solution was analysed for nitrate by an automated version of the cadmium reduction method (Clesceri *et al* 1989). All analyses were done at least in duplicate.

Analysis for Exchangeable Phosphorus.

Exchangeable phosphorus was determined by a modification of the method of Anon (1982). About 5 g of sediment was accurately weighed into 50 mL acid washed centrifuge tubes. The sediment was extracted into 25 mL of a 0.5 M sodium bicarbonate solution (pH adjusted to 8.5 with NaOH). The level of soluble reactive phosphate in the extractant was determined by an automated version of the ascorbic acid method (Clesceri *et al* 1989).

2.3.3 Macroinvertebrates

Monitoring of the macroinvertebrate fauna above and below the ANM wastewater discharge was performed using artificial substrate samplers as described in “Macroinvertebrates of the River Murray (Survey and Monitoring: 1980-1985)”, (Bennison *et al* 1989). This standard sampling technique was used to obtain results that were directly comparable with respect to both temporal and spatial characteristics. The sampler, placed in an aquatic ecosystem acts as an artificial substrate so that colonisation by benthic organisms can be assessed.

Each sampler consists of a cylinder of black plastic “gutterguard” (mesh size ~ 10 mm²) approximately 180 mm high by 180 mm diameter, the cylinder is closed on one end by a round piece of “gutterguard” and contains two knitted onion bags as complex substrate and a couple of small rocks as ballast. The top of the sampler is pinched and tied closed with a

length of nylon cord which is attached to the limb of an overhanging tree. The sampler sits on the bed of the river for ~ 4 weeks before being retrieved using a net.

Artificial substrate samplers were set at three paired sites - the controls opposite Grey's farm approximately 500m above ANM's discharge; mixing zone near the railway bridge 200m below the discharge and downstream at Union Bridge 2 km below the discharge. Ten samplers were set monthly at each of the three sites and after a minimum of four weeks, six of these were collected using a fine mesh net and all ten replaced with clean samplers. This allows for the possible loss of four samplers each month due to disturbance ensuring that sufficient samples are collected. The samples were sieved to 500 µm to remove silt and the remaining portion retained and preserved in 70% alcohol. Samples were sorted using a stereo microscope and identified with reference to MDFRC's taxonomy collection.

Site data were analysed statistically to ascertain similarity/dissimilarity in community structure between site pairs using multivariate techniques developed at Plymouth Marine Laboratories, England (Clarke 1993, Clarke and Warwick 1994). The Bray Curtis metric is used to compute similarity and construct a dendrogram linking samples based on their similarity to each other. Hypothesis testing of predefined groups is performed using ANOSIM (analysis of similarity), which is analogous to the univariate ANOVA (analysis of variance). SIMPER (similarity percentages) were calculated to determine the proportional contribution of species to the dissimilarity between the predefined groups of samples.

Comparisons of the community structure data were summarised for inclusion in this Annual Report to ANM.

2.3.4 Fish

Following consultation with NSW Fisheries, and approval by ANM, NSW Environment Protection Authority and NSW Fisheries, fish surveys were no longer required as part of this monitoring program.

2.4 Reporting

Quarterly reports containing all test results and observations including physico-chemical data were submitted to ANM. This annual report containing a summary of results from the monitoring program was submitted to ANM for incorporation as an appendix to their annual report to fulfil their requirements for Condition W16 of Licence No.01272 issued by the NSW Environment Protection Authority.

3.0 RESULTS AND DISCUSSION

3.1 Ecotoxicological and Bioaccumulation Monitoring [W10]

3.1.1 Acute and Chronic Toxicity tests

Chironomid acute toxicity tests

Seven valid chironomid EC50 tests were conducted between January and September 1996. Figures 1a-c provide a summary of acute toxicity results for the chironomid tests conducted using three sources of ANM wastewater (final outfall, 4-day pond and pond inlet respectively), in 1996. No significant mortalities (>20%) were recorded.

Daphnid acute toxicity tests

Eight valid daphnid EC50 tests were conducted between January and September 1996. Figures 2a-c provide a summary of acute toxicity results for the daphnid tests conducted using three sources of ANM wastewater (final outfall, 4-day pond and pond inlet respectively), in 1996. No significant mortalities (>20%) were recorded for the final outfall sample (Figure 2a). In Figure 2b (4 day pond wastewater), significant mortalities were recorded for the April sample at 100% concentration and for the June 1% concentration. In Figure 2c (pond inlet wastewater), significant mortalities were recorded for the April and July samples at 100% concentration.

Daphnid chronic toxicity tests

Four valid daphnia survival/maturation/reproduction tests were conducted between January and September 1996. A summary of these chronic toxicity test results using “t” values to compare the mean number of young produced in each treatment, compared with the control is provided in Figure 3. A “t” value that exceeds +2.1 ($p > 0.05$) denotes a significant reduction in the number of young produced, and conversely, a “t” value that exceeds -2.1 ($p > 0.05$) denotes a significant increase in the number of young produced. A significant reduction in the number of young produced by daphnids exposed to ANM’s treated wastewater occurred; in January, for final outfall 100% and 10%, pond 10% and pond inlet 100% and 1% concentrations; in March, for pond 100% and pond inlet 1% concentrations; in July, for pond 10%, pond inlet 100% and 1% concentrations; and in September, for final outfall 100% and pond 100% concentrations.

Given that the final concentration of ANM final outfall wastewater in the River Murray would not exceed 1% concentration, there is no evidence to suggest any acute or chronic toxicity to riverine invertebrates from ANM's discharge, based on the sensitivity of these cladoceran crustacean tests.

3.1.2 Bioaccumulation Studies

Yabby

A nine month *C.destructor* trial commenced in September 1995, terminating in May 1996. There was no significant difference in the length measurements between treatments, but the animals living in ANM's wastewater were significantly heavier than those living in river water (reported in the second quarter report to ANM, dated 8 July 1996).

The results (mean of three subsamples) for the eight metals assayed (aluminium, arsenic, cadmium, copper, iron, lead, manganese and zinc) are presented in Figures 4a-h. At each sampling date there are two control sample results and one test (wastewater) sample result. There was no consistent difference in the concentrations of aluminium (Figure 4a), cadmium (Figure 4c), copper (Figure 4d), iron (Figure 4e), lead (Figure 4f), or zinc (Figure 4h) between treatments. The lead and cadmium concentrations were below 0.5 mg/kg, and the zinc concentration was variable ranging from 77-109 mg/kg, with the control 1 sample in February 1996, an outlier at 354 mg/kg. Aluminium levels ranged from 134 mg/kg (test 3 in February 1996) to 748 mg/kg (control 1 initial sample in September 1995). Copper levels ranged from 42-63 mg/kg. Iron levels ranged from 80-262 mg/kg, the control 1 sample from February 1996, again an outlier at 1646 mg/kg.

Arsenic levels (Figure 4b), showed a higher concentration for test animals compared with control animals, although these concentrations remained below 1.6 mg/kg, significantly lower than last year's levels which peaked at 9 mg/kg. In last year's Annual Report, the possibility of contamination of the test with arsenic was discussed due to unexpected levels. The pellet feed used for both year's trials was tested along with the animal samples and found to contain arsenic at ~0.6 mg/kg.

Manganese concentrations (Figure 4g), were consistently higher in samples from the test tanks, peaking at 450 mg/kg in November, compared with the controls at ~200 mg/kg at the same time, and of similar magnitude to last years results. The concentration in test animals dropped to ~ 260 mg/kg in February. This peak and fall is probably the result of the yabbies growing and moulting the carapace they have carried through the winter in November/December as the water temperatures and daylength increase. These old carapaces are discoloured and may be covered by a type of bacterium which oxidises manganese, resulting in high concentrations of manganese attached to the surface (Ehrlich 1990, Tyler 1970, Tyler and Marshall 1967a & 1967b). A short trial to determine the validity of this theory is presently underway, and will be reported in the 1997 Annual report.

Silver perch

The 4 week Silver Perch (*Bidyanus bidyanus*) trial was conducted in April 1996. No significant difference in growth between control and wastewater treatments was recorded. (reported in the second quarter report to ANM, dated 8 July 1996).

The results (mean of three subsamples) for the seven metals detected; aluminium, arsenic, copper, iron, lead, manganese and zinc, are presented in Figures 5a-g (cadmium levels were below the detection limit). The data for one initial sample, three replicate control samples and three replicate test (wastewater) samples are plotted on the figure, and the final column depicts the commercial trout pellet feed used throughout the trial. Aluminium levels (Figure 5a) decreased in the controls and increased in two of the test replicates. Arsenic (Figure 5b) increased similarly in all control and test replicates. Iron (Figure 5d) and lead (Figure 5e) increased slightly in two of the test replicates. Zinc and Copper decreased similarly in all control and test replicates. This variation fails to indicate any consistent difference for any of the metals tested between control fish and treatment fish. The trial was confounded by the presence of metals in the feed. The commercial pellets were high in most metals (Mn, Pb, Fe, and Cu) assayed, compared with the fish samples, including ~2.6 mg/kg arsenic (figure 5b). The manufacturer was notified and the matter is currently under investigation. The pellet feed was discarded and a new feed was used for the carp gudgeon bioaccumulation trial presently underway.

These bioaccumulation studies have demonstrated little effect from exposure to 50 -100% ANM wastewater. The longer term yabby trial show increased growth and some bioaccumulation of manganese (the latter, probably due to bacterial action on the carapaces of the animals). The short term perch trial showed no consistent difference between treatments. Over a longer period the high metals levels in the feed would reduce the sensitivity of this type of trial. Fish may grow more rapidly in the warmer wastewater, consuming more feed and consequently more metals. Therefore, even if differences between control and treatment were detected it would be inaccurate to attribute this to the ANM wastewater.

3.2 River Environment Monitoring Surveys [W11]

3.2.1 Water

A summary of the water quality data is presented in Figure 6. The figure shows the variation of metals (iron, manganese, aluminium, and zinc), nutrients (total phosphorus, organic nitrogen, ammonia and oxides of nitrogen), and, physical parameters (conductivity, turbidity, total filtrable solids and colour) between the three sites over time. (Site 1 samples are represented by circles, site 2 samples are represented by squares and site 3 samples are represented by triangles; lines are included only for clarity and no interpolation between data points is intended.) All the water-quality data accumulated since the commencement of the monitoring program (January 1992) has also been included for purpose of comparison. The figure does not include those analytes not detected in any of the samples or, those whose levels remained very close to their detection limit. Cadmium (0.001 mg/L), cobalt (0.006 mg/L), chromium (0.01 mg/L), lead (0.03 mg/L) and mercury (0.0005 mg/L) were not detected in any of the samples (detection limits in brackets). Copper (detection limit of 0.004 mg/L) was detected on only three occasions - site 1 on the 20/5/96 (0.006 mg/L), sites 1, 2 and 3 on the 44/6/96 (0.006, 0.010 and 0.008 mg/L respectively) and at site 2 on the 22/7/96 (0.005 mg/L).

Generally, most of the data show little (if any) variation between sites although there may be significant variation over time (seasonal effects). The exceptions to this generality are the water samples taken in June and July of 1996. From the figure it can be seen that many of

the analytes show a peak in June/July for sites 2 and 3 but not for site 1. While it is not possible to definitively identify the cause of this increase, it is possible to speculate on its nature. From Figure 6 it can be seen that the peak corresponds to a large increase in the particulate load (turbidity) at sites 2 and 3 but not site 1. Indeed the increase at these two sites is primarily in analytes normally associated with particulates (eg. Fe, Al, P, organic N) but not in analytes normally found in the dissolved phase (conductivity, TDS, NH₃ or NO_x). This strongly suggests that the peak was caused by an increase in particulate matter rather than as a result of discharge from ANM's outfall. The source of the increased particulate load at sites 2 (above the outfall) and 3 (below the outfall) is not known but may relate to remedial earthworks at Hume Dam or inputs from a tributary stream.

3.2.2 Sediments

Mercury, tin, molybdenum, and silver (all with detection limits of 0.05 mg/kg) were not detected in any of the sediment samples. Arsenic, cadmium and barium was found in samples from above the outfall at low levels but not from samples taken below the outfall. Arsenic was detected in all of the sediments from site A (range 0.05 - 0.15 mg/kg) and 8 of the samples from site B (range 0.05 - 0.25 mg/kg). Cadmium was found in seven of the sample from site A (range 0.05 - 0.15 mg/kg) and 3 samples from site B (0.05 - 0.25 mg/kg) while barium was found in 4 samples from site A (0.05 - 0.25 mg/kg) and 8 samples from site B (0.05 - 0.10 mg/kg).

The results for the sediment analyses for total persulfate nitrogen (N), exchangeable phosphorus (P) and acid extractable aluminium, calcium, copper, iron, lanthanum, lead, magnesium, manganese, nickel, silica, strontium, yttrium and zinc are summarised in Figure 7. For each analyte a box plot showing the analytes distribution for samples taken at Doctors Point (marked A on Figure 7), directly above the outfall (B) and below the outfall (c) are presented. The solid horizontal lines of the box plot represent the 10th, 25th, 50th, 75th and, 90th percentiles of the data - the box itself represents the 25th to 75th percentile. All data outside the 10th and 90th percentiles are shown as open circles on the plots. The mean of the data is represented by a dotted line. From the figure it is clear that all of the samples from the two upstream deposition zones have a greater range and mean concentration than those of the down stream sites. Indeed it can be seen that both the highest means and ranges for all analytes were from the deposition zone at Doctors Point.

An extensive series of elements was determined including total persulfate nitrogen, exchangeable phosphorus and acid extractable aluminium, arsenic, barium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lanthanum, lead, magnesium, manganese, molybdenum, nickel, silica, silver, strontium, tin, yttrium and zinc. Unlike previous years it can be seen that the upstream sediments tend to have a greater distribution in the concentration of analytes than the down sites and further that the mean concentration for the upstream sites (A and B) tend to be significantly higher than for the downstream site (C). It is also of note that the sediments taken from Doctor's Point consistently have a significantly higher concentration of elements than the other sites.

The question remains as to whether discharge from ANM's outfall is having a measurable effect on sediment quality in the river Murray. The data presented in this and previous reports clearly show that the sediment composition found downstream of the outfall falls within the range found along this stretch of the river Murray. Data were presented in the 1995 annual report to show that for many elements there was a good relationship between the organic carbon content of the sample (determined by the surrogate measure of Loss on ignition - LOI) and the analyte of interest. With the exception of Cu, samples from upstream and downstream of the outfall tended to fall on the same continuum - indicating that the same processes were operating above and below the outfall. A similar (albeit weaker) correlation between LOI and some of the analytes of interest was also observed in this year's data (including the deposition zone from Doctors point) - Figure 8. The correlation between LOI and analyte concentration coupled with the observation that these correlations are mostly independent of site supports the proposition that variations in sediment composition is a function of the geomorphology of the particular deposition zones being studied - the more that finer material and organic material could be trapped in a deposition zone, the higher the analyte concentration found in those samples.

3.2.3 Macroinvertebrates

The complete 1995 species list and abundance data for each of three replicate baskets at the 6 sites on the River Murray, presented by month is included in Table 1 (24 pages). The totals and % totals for each species on the last two of these pages show the mayfly (ephemeroptera) larva, Caenid Genus B as most common taxa, contributing 40% and the caddisfly

(trichoptera) larva, *Ecnomus pansus* as next most common taxa, contributing 7.5% to the total macroinvertebrate abundance. The mean percentage abundance data for each site at each sampling time was analysed using a Bray-Curtis Similarity matrix (Clarke 1993, Clarke and Warwick 1994). These results are displayed in the cluster analysis dendrogram (Figure 9). The dendrogram groupings for the whole year show no biological differences between sites with limited grouping according to sampling period/season.

In an attempt to increase the sensitivity of the analyses the cluster analysis was also performed on smaller portions of the data set, separated by season and sampling date, and, by reducing the number of species used in the analyses, focussing on common groups. None of these analyses produced any clearer groupings of samples and as such, are not included in this report.

The complete mean percentage abundance data set was tested for statistically significant difference using a 2-way nested ANOSIM (Clarke 1993, Clarke and Warwick 1994), with factor one as river flow (high/low) and factor two as site classification (upstream/mixing zone/downstream). 12 to 13 high flow samples and 4 to 8 low flow samples were tested for each site classification. (High flow conditions on the River Murray are sustained during the irrigation season - September to March. During the winter the flow/depth of the river at the sites is dramatically reduced). These results (Table 2) showed that the different site classifications with respect to ANM's wastewater discharge contributed only 11.5% to the variation, whereas the difference in flow conditions contributed 77.8% to the variation between the samples. Therefore, the influence of site is insignificant compared with the influence of flow, and correspondingly season, on the variation in macroinvertebrate community composition.

A breakdown of dissimilarity between the ANOSIM groups using SIMPER (Clarke 1993, Clarke and Warwick 1994) is depicted in Table 3. The most similar (48.4% dissimilar) groups were high flow downstream and low flow downstream groups. The least similar (69.4% dissimilar) groups were high flow downstream and low flow up stream groups. This continues to highlight the spatial and temporal variability, with no correlation to position in relation to ANM's discharge.

Table 4 follows on from Table 3 and highlights the main species that contribute in total 50% of the difference between the groups tested. In all cases, 4 to 6 taxa out of a total of 100, contributed to this proportion of the variation. The dominant taxa; Caenidae Genus B (mayfly), *Ecnomus pansus* (caddisfly), Turbellaria spp. (flatworm), *Peza ops* (mite) and Oligochaete spp. (worm) generally contributed the greatest proportion of the difference. This pattern reflects the seasonal dominance of the macroinvertebrates which have aquatic larval stages and emphasises the overall dominance of seasonal effects in these data.

Overall, there was no difference in the macroinvertebrate community structure between river sites for each sampling period, and therefore, no detected effect of ANM's wastewater discharge.

The samples for 1996 are currently being identified and analysed for inclusion in the 1997 annual report.

3.2.4 Fish

Fish surveys were not required following a decision reached at the annual meeting (2 November 1995) and ratified by Allan Lugg from NSW Fisheries (29 January 1996).

4.0 COMMUNICATION

The revised monitoring program entitled "Biological Monitoring Program Proposal for Australian Newsprint Mills Ltd Albury" incorporating changes to the program since its inception in 1991, submitted to NSW Fisheries and EPA NSW in May 1995, was approved and adopted in January 1995.

The annual review meeting was held on 12 November 1996, with participants from Department of Land and Water Conservation, Environment Protection Authority, Australian Newsprint Mills and The Murray-Darling Freshwater Research Centre.

5.0 REFERENCES

Anon, 1982. "Chemical Analysis of Polluted Soils" Victorian Environment Protection Authority Publication 139.

APHA 1989. "Standard Methods for the Examination of Water and Wastewater" 17th Edition, Clesceri, L.S., Greenburg, A.E. and Trussel, R.R. (Eds), American Public Health Association Washington.

Bennison, G.L., Hillman, T.J. and Suter, P.J. 1989. "Macroinvertebrates of the River Murray, survey and monitoring 1980-1985". Murray-Darling Basin Commission, Canberra.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18, 117-143.

Clarke, K.R. and Warwick R.M., 1994. "Change in marine communities: an approach to statistical analysis and interpretation". Natural Environment Research Council, U.K. 144 pp.

Ehrlich, H.L., 1990. "Geomicrobiology of Manganese", *In Geomicrobiology*, 2nd Edition, Marcel Dekker Inc., New York and Basel .

Grimshaw, H. 1989. *In "Chemical Analysis of Ecological Materials" 2nd Edition*, Allen, S. (Editor), Blackwell Scientific Publications, London.

- Hosmoi, M. and Sudo, R. 1986. Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. *Intern. J. Environmental Studies* 27, 267-275.
- OECD 1981. "OECD Guidelines for Testing of Chemicals" (Adopted 12 May 1981).
- Tyler, P.A. 1970. Hyphomicrobia and the oxidation of manganese in aquatic ecosystems. *Antonie van Leeuwenhoek* 36, 567-578.
- Tyler, P.A. and Marshall K.C. 1967a. Hyphomicrobia - A significant factor in manganese problems. *Journal of American Water Works Association*, 59, 1043-1048.
- Tyler, P.A. and Marshall K.C. 1967b. Form and function of manganese oxidising bacteria. *Archiv fur mikrobiologie*, 56, 344-353.
- USEPA 1989. "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (2nd Edition) EPA-600/4-89/001.
- USEPA 1991. "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (4th Edition) EPA-600/4-90/027.

APPENDIX 1 (Figures)

Figure 1a-c: Acute toxicity results for seven chironomid tests exposed to three concentrations of three types of ANM wastewater during 1996. Where a percentage mortality greater than 20% is considered a significant result.

Figure 2a-c: Acute toxicity results for eight Daphnia tests exposed to three concentrations of three types of ANM wastewater during 1996. Where a percentage mortality greater than 20% is considered a significant result.

Figure 3: Chronic toxicity results for four Daphnia tests exposed to three concentrations of three types of ANM wastewater during 1996. Where the mean number of young produced by the animals in each treatment is compared, using “t-tests” with the control. A “t” value greater than ± 2.1 is considered a significant difference, the +ve exceedences indicate a reduction and the -ve exceedence indicates an increase in the abundance of young produced.

Figure 4a-h: Mean concentrations (3 subsamples) of eight metals (aluminium, arsenic, cadmium, copper, iron, lead, manganese and zinc) in pellet feed and whole yabby samples, taken at three month intervals for two controls and one test treatment exposed to ANM wastewater during the 1995/1996 bioaccumulation trial.

Figure 5a-h: Mean concentrations (3 subsamples) of seven metals detected (aluminium, arsenic, copper, iron, lead, manganese and zinc) in pellet feed, and whole perch samples at day 27, for three controls and three test treatments exposed to ANM wastewater during the 1996 bioaccumulation trial.

Figure 6: A summary of the water quality data for 1992 to 1996. The figure shows the variation of metals (iron, manganese, aluminium, and zinc), nutrients (total phosphorus, organic nitrogen, ammonia and oxides of nitrogen), and, physical parameters (conductivity, turbidity, total filtrable solids and colour) between the three sites over time. (Site 1 samples are represented by circles, site 2 samples are represented by squares and site 3 samples are represented by triangles; lines are included only for clarity and no interpolation between data points is intended.)

Figure 7: Box plots for the distribution of elements from deposition sites; A (Doctor's Point), B (directly above ANM's outfall) and C (directly beneath ANM's outfall).

Figure 8: Variation of Zn, Fe and Cu for the sediments taken from deposition sites A (Doctor's Point), B (directly above ANM's outfall) and C (directly beneath ANM's outfall) as a function of Loss on ignition.

Figure 9: Dissimilarity classification dendrogram of ANM river monitoring sites using macroinvertebrate species abundance for 1995. Where:

- Sites 1 and 2 = 2km downstream of the discharge
- Sites 3 and 4 = immediately downstream of discharge in the mixing zone
- Sites 5 and 6 = controls - upstream of the discharge.

APPENDIX 2 (Tables)

Table 1: 1995 macroinvertebrate species list and abundance data for three replicate samplers at the six paired ANM river monitoring sites:
Sites 1 and 2 = 2km downstream of the discharge
Sites 3 and 4 = immediately downstream of discharge in the mixing zone
Sites 5 and 6 = controls - upstream of the discharge.

Table 2: 1995 macroinvertebrate analyses for Australian Newsprint Mills River Monitoring.

Testing for differences between groups of samples (selected *apriori*) using 2 way nested ANOSIM (Bray-Curtis Matrix). Where: High flow (>2m at Albury) includes the summer and spring months of January, February, March, September, October, November and December; and low flow (<2m at Albury) includes the autumn and winter months of April, May, July and August.

FACTOR 1 flow/season	FACTOR 2 ANM site classification	SAMPLE SIZE (n)
High flow	Upstream (control)	12
High flow	Mixing Zone	12
High flow	Downstream	13
Low Flow	Upstream (control)	4
Low Flow	Mixing Zone	5
Low Flow	Downstream	8

ANOSIM sample statistic for differences between ANM site classification = 0.115
(averaged across all Factor 1 groups). Significance level 0.8%.

ANOSIM sample statistic for differences between flow/season groups = 0.778
(using Factor 2 groups as samples). Significance level 10.0%.

Table 3: 1995 macroinvertebrate analyses for Australian Newsprint Mills River Monitoring. Percentage dissimilarity breakdown between groups of samples using SIMPER. Groups of samples are classified into six samples: one of two flow conditions; High (>2m) and Low (<2m); and one of three site classifications with respect to ANM’s wastewater discharge; upstream (control), mixing zone and downstream.

SAMPLES	High/up	High/mixing	High/down	Low/up	Low/Mix	Low/down
High/up	0.0%					
High/mix	62.1%	0.0%				
High/down	57.6%	57.9%	0.0%			
Low/up	66.8%	66.1%	69.4%	0.0%		
Low/Mix	64.3%	63.5%	63.9%	59.0%	0.0%	
Low/down	58.1%	60.2%	48.4%	61.7%	58.2%	0.0%

Table 4: 1995 ANM Macroinvertebrate data. Species contributing 50% of the dissimilarity between samples (flow/site) listed in order of decreasing magnitude. Where; high and low refer to flow conditions, and site is describe with reference to ANM’s wastewater discharge.

SAMPLES	High/upstream	High/mixing	High/downstream	Low/upstream	Low/mixing	Low/downstream
High/upstream	0					
High/mixing	Caenid Genus B <i>Ecnomus pansus</i> Oligochaete spp. Turbellaria spp. <i>Peza ops</i> <i>Parakiefferella spp.</i>	0				
High/downstream	Caenid Genus B Oligochaete spp. <i>Ecnomus pansus</i> Turbellaria spp. <i>Ablabesmya spp.</i> <i>Trienodes spp.</i>	Caenid Genus B <i>Ecnomus pansus</i> <i>Peza ops</i> Oligochaete spp. <i>Parakiefferella spp.</i> <i>Tanytarsus spp.</i>	0			
Low/upstream	Caenid Genus B <i>Peza ops</i> Turbellaria spp. Oligochaete spp. <i>Trienodes spp.</i> <i>Ecnomus pansus</i>	Caenid Genus B <i>Peza ops</i> Turbellaria spp. Oligochaete spp. <i>Trienodes spp.</i> <i>Ecnomus pansus</i>	Caenid Genus B <i>Peza ops</i> Turbellaria sp <i>Trienodes spp.</i> Oligochaete sp	0		
Low/mixing	Caenid Genus B Turbellaria spp. Oligochaete spp. <i>Ecnomus pansus</i> Corixidae sp <i>Peza ops.</i>	Caenid Genus B. Turbellaria spp. <i>Ecnomus pansus</i> <i>Peza ops</i> Oligochaete sp <i>Rietha spp.</i>	Caenid Genus B Turbellaria spp. <i>Ecnomus pansus</i> <i>Trienodes sp</i> Oligochaete sp	Caenid Genus B <i>Peza ops</i> Turbellaria spp. Oligochaete sp <i>Ecnomus pansus</i> Corixidae spp.	0	
Low/downstream	Caenid Genus B <i>Trienodes spp.</i> Turbellaria spp. Oligochaete spp. <i>Ecnomus pansus</i> <i>Hypogasturidae spp.</i>	Caenid Genus B <i>Trienodes spp.</i> <i>Peza ops</i> <i>Ecnomus pansus</i> <i>Rietha spp.</i> <i>Tanytarsus spp.</i>	Caenid Genus B <i>Trienodes spp.</i> <i>Ecnomus pansus</i> Oligochaete sp <i>Ablabesmyia spp.</i> <i>Parakiefferella spp.</i>	Caenid Genus B <i>Peza ops</i> Turbellaria sp <i>Trienodes spp.</i>	Caenid Genus B Turbellaria spp. <i>Trienodes spp.</i> <i>Ecnomus pansus</i> Corixidae spp.	0

6 December, 1996

Our Ref: YH/6/21/1 and YH/6/21/3

Mr Ralph Coghill
Technical Services Manager
Australian Newsprint Mills Limited
Private Bag
LAVINGTON NSW 2641

Dear Ralph

1996 ANNUAL REPORT - BIOLOGICAL AND CHEMICAL MONITORING

Please find enclosed an unbound copy of the 1996 Annual Report of Chemical and Biological Monitoring for Australian Newsprint Mills Limited, undertaken by The Murray-Darling Freshwater Research Centre. This Annual Report complies with Licence Condition W16 on the ecotoxicological and bioaccumulation monitoring and the river environment monitoring surveys.

Please do not hesitate to contact me on 582355 for any additional information.

Wishing you and your staff a Merry Xmas and a Happy New Year.

Yours sincerely

Helen King

Scientific Officer

Enc. 1996 Annual Report.