NMP Mine Area Sediment Toxicity Testing

Prepared for Namibian Marine Phosphate Pty Ltd

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1 BACKGROUND

As part of their application process to gain regulatory approvals for mining Namibian Marine Phosphate (NMP) and to comply with the recommendations in the Dr Richard Newell report to the Environmental Commissioner, toxicity testing on the proposed mine area sediments was conducted. As specified by Dr Newell, this o followed accepted toxicity test procedures on sediment samples representative of the proposed mine area. NMP contracted Lwandle Technologies Pty Ltd (Lwandle) to oversee the selection of sediment samples to be analysed and the toxicity testing. Lwandle subcontracted the latter to the Durban offices of the CSIR who have extensive experience in this field.

The sediment samples available for testing were from cores previously collected in the proposed mine area. These were held in dry storage in Namibia from collection dates extending back to 2010. Lwandle advised NMP that this presented problems due to geochemical transformations that may have occurred within the cores through drying, exposure to atmospheric oxygen (e.g. Zhang and Millero 1993), especially within the deeper zones of the cores, and varying temperatures over the storage period and toxicity testing may thus not reflect actual risks that may arise during mining. As no other material was available and no sampling programme could be conducted within the required time frame of testing and reporting NMP resolved to conduct the testing on the historical core material but that data analyses be focused on surficial layers of the cores.

To address the issue of assessing toxicity in the deeper sediment profile, provision is made in the EMPR for a new set of baseline reference samples to be collected following award of the Environmental Clearance Certificate (ECC) and prior to commencement of mining, given the findings of the EIA Verification Study. This material will be subjected to toxicity testing across the planned mining depth (sediment surface to the clay footwall) to add to the sediment toxicity analyses reported on here.

2 TOXICITY TEST METHODS

The toxicity testing methods outlined below are taken from CSIR (2019).

Nine cores taken from the proposed mine area were subsampled from the 5-15 cm core depths and the extracted sediments submitted for analyses. The toxicity testing measured acute and chronic effects on sea urchin fertilisation success and larval development. The test procedures included:

- **Preparation of elutriates**

Each of the sediment samples was passed through a 2 mm mesh size sieve to remove shells and consolidated material. This was done to reduce the variable amount of shell and sediment material between samples. Elutriates were prepared by adding four parts seawater (filtered natural seawater collected at Vetches Beach in Durban) to one part sediment, as is the standard practice for assessing the toxicity of sediment identified for dredging (USEPA, 2001). The sediment-water mixtures were agitated on a rotary shaker at 800 revolutions per minute for one hour, and then allowed to settle for one hour. In all sediment-water mixtures there was still a significant amount of suspended material after the one hour settling period. To avoid physical 'toxicity' due to the presence of this material and to facilitate microscopic analysis at test termination, the supernatant was removed and centrifuged at 4000 revolutions per minute for 10 minutes. The elutriate samples were transferred to glass vials in which the tests were performed.

- **Laboratory analysis**

The salinity and pH of each elutriate was determined in the laboratory using a calibrated refractometer and pH meter. The accuracy of the refractometer and pH meter was checked after every ten measurements by reading against standards seawater and pH standards.

The toxicity of the elutriates was tested using the acute sea urchin fertilisation test and chronic sea urchin larval development test based on methods defined by the USEPA (2002). Adult sea urchins (*Echinometra mathaei*) were collected from Vetches Pier in Durban and maintained at ambient temperature in seawater in large flow-through tanks in the laboratory. Gametes (sperm and eggs) were obtained by inducing sea urchins to spawn, by injecting 1 - 2 ml of a 0.5 molar potassium chloride solution into their coelomic cavity. Gametes from males and females were collected separately. Females were inverted over glass beakers filled with seawater and eggs were allowed to settle. Sperm was collected 'dry' in pasteur pipettes. Sea urchins that provided relatively few gametes were excluded from consideration for testing.

The quality of eggs and sperm was evaluated prior to testing by adding diluted sperm from each male to eggs from each female in 20 ml of seawater in vials. Eggs were examined under a microscope for the presence of a fertilization membrane. Combinations of eggs and sperm that did not produce at least 90% fertilisation success or showed abnormal membrane development were excluded from consideration for testing.

Sperm was activated by exposure to seawater and 100 µl aliquots of the suspension were then transferred to each of four replicate vials for the control ('clean' seawater) and elutriate samples,

and a positive control (copper). The positive control provided a sensitivity reference for the sea urchins used. After ten minutes of sperm exposure, 1 ml of egg suspension was added and left for ten minutes. The test was then terminated by adding 100 µl of 37% formaldehyde to each test vial. The control and each elutriate sample comprised four replicates. Fertilisation success was determined by the microscopic examination of the egg suspension from each replicate.

For the larval development test, 1 000 µl of fertilised eggs (embryos) from a stock solution was added to each of four replicate vials for the control and elutriate samples. The vials were incubated at a temperature of 23°C in a temperature controlled environmental chamber for 72 hours (the period required for larvae to reach the 4-arm pluteus stage). The test was terminated by adding 100 µl of 37% formaldehyde to each test vial. Larval development was determined by the microscopic examination of the larvae suspension from each replicate.

- **Analysis of data**

The fertilisation and development success of gametes and embryos exposed to the elutriate samples were statistically compared to the fertilisation and development success of gametes and embryos exposed to seawater controls. The proportion of fertilised eggs and normally developed larvae were transformed (arcsine square root) prior to testing. Thereafter, a Dunnett's multiple comparison test at a level of statistical significance of $\alpha = 0.05$ was used to determine which fertilisation and development responses from the elutriate samples were significantly different to responses for the seawater controls.

In addition to statistical significance, an estimate of ecological significance was used to compare fertilisation and development success of gametes and embryos exposed to the elutriate samples to responses for the seawater controls. A reduction in fertilisation and larval development that was at least 10% lower than that for the control was considered ecologically significant (in other words, the elutriate was considered toxic) (USEPA 1998; Steevens et al., 2008). Thus, both statistical significance and ecological significance were employed to assess toxicity in the elutriates.

3 TOXICITY TEST RESULTS

The toxicity testing results for the surficial layer sediments are listed in Table 3-1 and Table 3-2 and summarised in Table 3-3.

Table 3-1: *Sea urchin fertilisation success (acute toxicity) data*

Sample ID	pH	Salinity PSU	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Replicate 4 (%)	Mean Fertilisation (%)	Standard Deviation
2253-10	8.0	36.0	98	96	98	97	97.25	0.96
2256-10	8.0	36.0	98	98	98	97	97.75	0.50
2309-10	8.0	36.0	98	99	98	97	98.00	0.82
2327-10	8.0	36.0	96	97	96	98	96.75	0.96
2355-10	8.1	36.5	96	97	97	98	97.00	0.82
2357-10	8.0	36.0	98	99	97	98	98.00	0.82
2360-10	7.9	37.0	98	98	97	97	97.50	0.58
2429-10	8.0	37.0	99	98	98	97	98.00	0.82
2556-10	8.0	36.0	98	97	99	98	98.00	0.82
SW Control	8.1	35.0	99	100	99	100	99.50	0.58

Table 3-2: *Sea urchin 72-hour larval development success (chronic toxicity) data*

Sample ID	pH	Salinity PSU	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Replicate 4(%)	Mean Normal Larvae (%)	Standard Deviation
2253-10	8.0	36.0	89	86	88	86	**87.25***	1.50
2256-10	8.0	36.0	94	96	96	97	95.75	1.26
2309-10	8.0	36.0	96	97	94	94	95.25	1.50
2327-10	8.0	36.0	98	97	98	97	97.50	0.58
2355-10	8.1	36.5	96	97	97	96	96.50	0.58
2357-10	8.0	36.0	96	97	97	96	96.50	0.58
2360-10	7.9	37.0	86	89	88	86	**87.25**	1.50
2429-10	8.0	37.0	95	97	97	96	96.25	0.96
2556-10	8.0	36.0	95	97	97	96	96.25	0.96
SW Control	8.1	35.0	98	98	97	99	98.00	0.82

* Bold text indicates >10% deviation from control results

Table 3-3: *Average (± standard deviation) fertilisation and larval development success of sea urchin gametes exposed to control and elutriate samples. Toxicity determination is based upon a mean fertilisation or development success that is statistically significantly lower than that for the control treatment and 10% lower than for the control treatment.*

Sample	pH	Salinity PSU	Average % Fertilisation Success ± Std	Toxicity Determination	Average % Development Success ± Std	Toxicity Determination
2253-10	8.0	36.0	97.25 ± 0.96 *	Non-toxic	87.25 ± 1.50	Toxic
2256-10	8.0	36.0	97.75 ± 0.50 *	Non-toxic	95.75 ± 1.26*	Non-toxic
2309-10	8.0	36.0	98.00 ± 0.82 *	Non-toxic	95.25 ± 1.50*	Non-toxic
2327-10	8.0	36.0	96.75 ± 0.96	Non-toxic	97.50 ± 0.82*	Non-toxic
2355-10	8.1	36.5	97.00 ± 0.82 *	Non-toxic	96.50 ± 0.58*	Non-toxic
2357-10	8.0	36.0	98.00 ± 0.82 *	Non-toxic	96.50 ± 0.58*	Non-toxic
2360-10	7.9	37.0	97.50 ± 0.85 *	Non-toxic	87.25 ± 1.50	Toxic
2429-10	8.0	37.0	98.00 ± 0.82 *	Non-toxic	96.25 ± 0.96*	Non-toxic
2556-10	8.0	36.0	98.00 ± 0.82 *	Non-toxic	96.25 ± 0.96*	Non-toxic
Control	8.1	35.0	99.50 ± 0.58		98.00 ± 0.82	

The toxicity testing did not show any acute level toxicity in the sea urchin fertilisation success measurements but did indicate marginal toxicity in two of the nine chronic level toxicity larval development samples analysed. The set criterion for the latter is a >10% deviation from the

development success rate measured in the control series. This equates to a success rate of 88.2% which is within 1 standard deviation of the mean success rates classed as being toxic (samples 2253-10 and 2360-10, Table 3-2). All other samples in the chronic level toxicity testing performed within a few percentage points of the control sample.

4 DISCUSSION & RECOMMENDATION

The toxicity test results obtained for the surficial sediment layers are consistent with chemical analyses and elutriation testing of gravity cores that were collected during the previous verification study, stored frozen and then analysed within weeks of collection. The chemical analyses of these showed that arsenic, cadmium and nickel concentrations exceeded the BCLME probable effect level whilst chromium and copper exceeded the guideline concentration threshold for the region. The demonstrated relationship between aluminium and trace metal concentrations reported on in the Verification survey and the location of the sampling site relative to any industrial activities indicate a natural source for these. Despite the observed particulate phase trace metal concentrations elutriation testing showed that proportions of these possible toxicants entering the dissolved phase were less than 1% of the sediment concentrations and orders of magnitude below the respective threshold, not probable, effect levels. These low releases were attributed to excess concentrations of acid volatile sulphide (AVS) compared to simultaneously extracted trace metals (SEM) present in the sediments and its propensity to combine with bivalent metals, e.g. cadmium, copper, nickel, lead, zinc, to form insoluble sulphides and iron and manganese in the sediments that complex with organic compounds and adsorb arsenic, specifically.

The low elutriated concentrations compared to sediment concentrations of primarily trace metals extended throughout the verification survey core depths that were sampled to characterise the phosphate ore body. However, despite the observed consistency between toxicity effect levels measured in surficial sediments (above) and the elutriation test results from fresh core material collected in the verification survey, it should not be assumed without further testing that the sediments deeper down in the ore body will be equally non-, or minimally toxic.

As toxicity effects linked to mining will be a critical issue it is important that as definitive as possible measurements are obtained. Accordingly, we endorse NMP's undertaking in their EMPR to conduct further testing after award of the ECC and prior to initiation of mining. For this we recommend that fresh core material is collected from a limited set of cores and appropriately curated for further toxicity testing. It is also recommended that the sediment property distributions in these cores be determined as in the verification study cores. The purpose of this would be to confirm comparability between them and allow extrapolations of toxicity responses across the proposed mine area should toxicity be observed.

5 REFERENCES

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