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ANIMAL HEALTH LABORATORIES

The Mineral Status and Health of Sheep Grazing on Neutralised Used Acid Amended Pasture

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EXECUTIVE SUMMARY

Situation

Neutralised Used Acid (NUA) solids has been proposed as a soil amendment for poor sandy soils with a number of potential benefits cited in previous research work. The use of NUA broadly as a soil amendment requires regulatory approval for which there are no assessment criteria. There are moves to introduce a uniform national framework for assessing the use of industrial wastes in agricultural which may open the way for NUA to become a soil amendment product.

Complication

Work done at laboratory and field scale to prove the soil amendment values, had focused on the direct improvement to soil nutrient retention and pasture growth. The scale of the field trial was quite small. Questions could be raised about the impact of the amendment and the components of the amendment (particularly heavy metals) on an operating productive food chain from soil to animal. It was determined to conduct trials on a larger scale extending beyond pasture growth to animals grazing on the pasture. A preliminary trial was carried out in 2003 followed by an expanded trial in 2004.

Focussing questions

1. What are the issues to address in the design of a trial to assess the potential effects of NUA soil amendment on meat producing livestock?
2. Have the current trials been able to adequately address these issues and provide a sound conclusion?
3. Were any adverse or beneficial effects noted that could be attributed to application of the NUA as soil amendment?

Conclusions

1. Key issues in the design of a grazing trial investigating the effects of NUA soil amendment on livestock are:
    a. Investigate the potential impacts NUA may have on the mineral status and health of grazing animals; in terms of beneficial, essential and toxic (heavy metals) minerals. Any such investigation should determine if minerals accumulate to the point where concentrations in meat or offal exceed recommended or legislative levels, and so might prevent product from entering the market place. Additional factors that might influence mineral loading of grazing animals on NUA amended pasture would include seasonal effects and weathering of NUA (i.e. as some minerals are leached away).

    b. The trial design should allow sufficient sensitivity to detect changes in mineral status in grazing animals and give results that can be extrapolated to the real world. This means having a suitably long grazing period and sufficient numbers of animals in treatment and control groups.

   c. Ingestion of soil, particularly in summer/autumn, is a significant contributor of minerals to grazing animals in Western Australia. Therefore this is an important element that should be incorporated into the design of a NUA grazing trial. NUA is a fine dust, and so inhalation of NUA might also contribute minerals during that period.

2. While the trials presented here did address the key issues above, and allow reasonable conclusions to be made, there were some limitations:
   a. The ability (i.e. sensitivity) of the trials to detect changes in mineral loading in sheep due to NUA may have been compromised by insufficient numbers of animals as well as the short grazing time (i.e. time to accumulate minerals within tissues). This was due to the unexpectedly poor pasture growth and the small plot sizes.
   b. The concentrations of some minerals were close to or below the levels of detection of the analytical techniques. Initial testing of some minerals were found to be falsely elevated after re-testing at a specialist laboratory.
   c. Comparisons between trials may have been compromised by, firstly, differences in the breed of sheep (differences in mineral metabolism) and, secondly, by differences in feed-on-offer, and therefore opportunity to ingest NUA.

3. The effect of using NUA as a soil conditioner as determined in trials in 2003 and 2004:
   a. All trial sheep commended the trials healthy and remained so over the grazing periods (approximately 14 weeks for each trial).
   b. Of the essential elements measured in pasture grown on NUA amended soil, all were generally at adequate levels for sheep. Furthermore concentrations of the heavy metals measured in the pasture were within normal ranges.
   c. No deficiencies in any of the key essential minerals developed in sheep grazing on NUA treated plots in 2003 and 2004.
   d. There was evidence that copper and phosphorus statuses were reduced in sheep grazing NUA amended pasture in 2004. The mechanism by which phosphorus was reduced is unknown, but is perhaps due to retention of the phosphorus by NUA. Reduced copper is most likely due to iron antagonism, with the strong possibility of sulphur involvement. There is also evidence that NUA may supply additional cobalt to grazing sheep. This may reduce the likelihood of vitamin B12 deficiency.
   e. Grazing sheep on NUA amended pasture did not lead to heavy metals in tissues exceeding desirable or legal limits.
   f. While NUA, which is high in manganese, did not appear to elevate pasture manganese, there was some evidence that ingestion of NUA may have caused a small elevation in liver in the sheep on the 150 t/ha plot in 2004. Excessive intake of manganese can reduce performance of sheep and reduce iron and cobalt absorption. While there was no evidence of this here, under some circumstances the availability of manganese to pasture may increase and so this should be factored into future investigations.
   g. NUA contaminated wool, but was easily removed by scouring.

Recommendations

1. The results presented here provide evidence that grazing sheep on NUA amended pasture does not result in deficiencies of minerals or excesses of minerals to cause toxicity or undesirable or violative levels of heavy metals in tissues. These results could be built upon, and made more relevant, by expanding to a larger number of animals grazing for a longer period of time, and covering such factors as seasonal effects and weathering of NUA. This would mean a larger experimental site.
2. An animal house feeding trial, in which NUA is incorporated into feed pellets and fed to sheep, would be another approach. This design would use relatively small animal numbers whilst maximising the effect of NUA on tissue mineral levels, particularly heavy metals, in the animal. It would be, in effect, a simulation of soil ingestion over summer. This approach would not take into account the complex interactions that occur between soil and pasture minerals and absorption into the animal, and so would be best used to compliment a field study.
3. Factors, such as soil pH and water logging, which can affect availability of manganese to pasture, should be investigated.

1. INTRODUCTION AND OBJECTIVES

Neutralised Used Acid (NUA) is a waste by-product resulting from mineral sands processing. Treatment of iron rich ores with sulfuric acid produces an acid leachate, which is then neutralised with excess lime. The final product is alkaline (pH 8.5 to 9). The resulting NUA contains microcrystalline iron oxides as well as significant gypsum ($CaSO_4$ H_2O) and calcite ($CaCO_3$) content. Analysis of the element content of NUA used in the trial is given in Table 1.

NUA has a high phosphorus adsorption capacity, which suggests it could be used to reduce phosphorus fertiliser application and phosphorus run-off. Furthermore it has the potential to reduce water repellency of sandy agricultural soils.

Table 1. Concentrations are in mg/kg unless specified otherwise

Element		ANZECC[€] background 1992	Ecological invest. limits[£]	Average NUA used in trial	Control site soil	150 t/ha conc. in soil[∂]	Mineral category
Aluminium	Al	3–6%[a]		0.13%	0.85%	0.56%	NT, NE
Antimony	Sb	4–44	20	0.3	0.3	0.3	T, NE
Arsenic	As	0.2–30	20	5	4	4	T, NE
Barium	Ba	20–200	400	15	167	107	T, NE
Boron	B	1–75		< 50	< 50	< 50	NT, OB
Cadmium	Cd	0.04–2	3	< 0.1	< 0.1	< 0.1	T, NE
Calcium	Ca			15.9%	0.04%	4.0%	NT, E
Chromium	Cr	0.5–110	50	99	24	43	T, E
Cobalt	Co	1–190	50	181	3	42	NT, E
Copper	Cu	-	60	47	3	14	NT, E
Fluorine	F	30–100[a]		128	75	91	T, NE
Iron	Fe	2.5%[a]		12.8%	1.4–2.1%	4.2%	NT, E
Lead	Pb	< 2–200	300	3	10–13	9	T, NE
Magnesium	Mg	0.05–0.5%[b]		0.7%	0.03%	0.17%	NT, E
Manganese	Mn	4–12,600	500	27,500	430–720	6,555	NT, E
Mercury	Hg	0.001–0.1	1	< 0.01	< 0.01	< 0.01	T, NE
Molybdenum	Mo	< 1–20	40	NA (typ 2)	NA	NA	OB
Nickel	Ni	2–400	60	77	3	19	OB
Phosphorus	P	-		21	15	21	NT, E
Potassium	K	-		1,000	6,000	4,400	NT, E
Selenium	Se	0.6–1.6[a]		< 2	< 2	< 2	T, E
Sodium	Na	-		417	545	422	NT, E
Sulphur	S	-		12.3%	< 0.01%	2.9%	NT, E
Thorium	Th	6 to 15[c]		139	13–19	42	T, NE
Tin	Sn	1–25	50	1	2	2	NT, OB
Titanium	Ti	0.44%[d]		NA (typ < 10)	NA	NA	NT, NE
Uranium	U	3[e]		7	1	2	T, NE
Zinc	Zn	2–180	200	11	14–23	15	NT, E

[€] ANZECC/NHMRC (1992).

[£] Ecological Limits: Dept. of Environment (DEP), WA (2003).

[∂] This trial site. There were problems with homogeneous mixing of the NUA and soil and soil sampling which resulted in inflated values – see Lavell (2005).

a Underwood and Suttle (1999).

b Moore (1998).

c Agency for Toxic Substances and Disease Registry (ATSDR) (1990).

d International Programme on Chemical Safety (IPCS).

e Furness (2000).

T = Toxic; OB = occasionally beneficial; NE = Non Essential; NT = Non Toxic; E = Essential.

The mineral composition of NUA used in this trial, and NUA mixed with soil (at an application rate of 150 t/ha), is given in Table 1, along with standards on assessment of mineral contamination of soils as well as ranges on background or expected levels of minerals in soil. Chromium, cobalt, manganese and nickel in NUA exceeded the *Ecological Investigation Levels* as set by the Department of Environmental Protection (2003). Minerals that might be considered unusually high in NUA are iron, magnesium, manganese, thorium and uranium, with chromium and cobalt at the upper end of background ranges. Dilution with soil, at an application rate of 150 t/ha, resulted in none of the health and environmental standards being exceeded, with the exception of manganese (Table 1). Throssell (2002) made a similar observation. In terms of background soil ranges, iron, manganese and thorium would all be considered high in the 150 t/ha soil (Table 1). However Summers and Perch (1997) reported that thorium levels in NUA were similar to, or less than, those found in untreated agricultural soils of the catchment of the Peel Inlet and Harvey Estuary. Thorium is a radioactive element and so investigation of its potential impact in terms of radioactive residues (e.g. radium isotopes) in tissues is outside the scope of this study.

The use of NUA as a soil amendment requires regulatory approval for which there are currently no assessment criteria. However there are moves to introduce a uniform national framework for assessing the use of industrial wastes in agricultural which may open the way for NUA to become a soil amendment product. In the mean time, the most applicable standard is that for biosolids. The NUA used in this trial met Class 2 standards (high Ni), which is the minimum grade required for the unrestricted agricultural application of biosolids in Western Australia.

Given the data presented in Table 1 it would seem unlikely that stock grazing on pasture amended with NUA at the rate of 150 t/ha would be at risk of accumulating toxic or heavy metals to levels of any concern. However NUA could impact on the mineral load in grazing animals through several mechanisms, namely:

a. by altering the mineral composition of pasture by direct means or possibly through alterations in soil chemistry (e.g. pH);

b. through ingestion of NUA during grazing;

c. through inhalation of NUA during grazing;

d. through mineral interactions.

It appears that the availability of trace elements and heavy metals in NUA to plants may be poor. While plants grown on NUA amended soil have higher concentrations of some metals, particularly manganese (Hamon and McLaughlin 2002), Throssell considered that concentrations were "below levels considered hazardous to sheep and cattle". Summers *et al.* (2003) reported that application of NUA actually decreased the concentration of some heavy metals, namely cadmium, mercury and uranium; probably by increasing plant growth. And most likely for the same reason, zinc and manganese concentrations were also decreased. On the other hand they did observe an increase in nickel concentration, but to no where near toxic levels for ruminants. NUA may change the availability of minerals to pasture already present in the soil because of its effect on soil characteristics, such as pH and reduce water repellency (Underwood and Suttle 1999).

Ingestion of NUA amended soil during grazing, especially by sheep, is a very real possibility. Soil ingestion, especially when grazing intensity is high or when pasture availability is low, is a common occurrence in late summer/autumn in Western Australia. Soil intake by sheep can rise to 163 g per day under such conditions and can be a significant source of minerals (Underwood and Suttle 1999). Soil ingestion also occurs when pasture is contaminated by soil, for example after rainfall.

Inhalation of NUA by grazing animals, particularly during summer and when NUA has recently been applied, is also very likely. This is because NUA is a fine dust with a small crystal size (≅ 1 μm). Alveolar deposition is of considerable importance for respiratory absorption, and so very fine particles that have the potential to be inhaled deeply into the lung will have the largest potential impact on mineral load. The absorption of minerals from the lungs into other tissues will vary from mineral to mineral, depending on factors such as solubility of mineral salts and particle size.

Because some minerals in NUA are high there is a possibility of interactions that could affect mineral loading. For example, the high iron and sulphur content of NUA could reduce absorption of copper in grazing animals, while high manganese intake by ruminants has been known to reduce iron status even in the presence of high pasture iron levels (Underwood and Suttle 1999). These sorts of interactions are, however, highly complex and almost impossible to predict based on soil and or pasture mineral composition.

Tissue mineral levels should not only be assessed in terms of toxicity and adequacy to the animal, but also in terms of guideline and legislative levels, as defined under the Australia New Zealand Food Standards. Maximum Levels (MLs) are legal levels, enforced by government, to ensure food is safe for human consumption, while Generally Expected Levels (GELs) provide information about the levels of metals in food that reflect best practice for producers and are not enforceable.

As NUA is a fine dust it has the potential to contaminate wool. Prior to processing wool is scoured, or cleaned, to remove contaminating soil and grease. As part of this study we determined the extent of contamination and if it was easily removed by a conventional scouring method.

Objectives

1. To graze sheep so as to maximise the likelihood of soil NUA ingestion and inhalation by maintaining grazing pressure on senescent pasture on plots treated with NUA up to 150 t/ha.
2. To assess the health of sheep grazing on NUA amended plots (up to 150 t/ha). Assessment will be made by field observation, use of blood clinical chemistry panels and post-mortem examination.
3. To assess the status of essential minerals in sheep grazing on NUA amended plots (up to 150 t/ha) using blood and or tissue parameters.
4. To assess the load of non-essential and heavy minerals in sheep grazing on NUA amended plots (up to 150 t/ha) using tissue parameters.
5. To assess the impact of NUA on mineral contamination of wool of sheep grazing on NUA amended plots (up to 150 t/ha).

2. METHODOLOGY

Overview of the two trials

Sheep were grazed from September 2003 for about 14 weeks (on a control plot or a plot treated with 150 t NUA/ha) and then slaughtered for tissue mineral analysis. NUA had been applied in autumn of 2003. Poor pasture growth in 2003 limited the number of sheep that could be grazed. Furthermore there was a delay in placing sheep onto the plots in 2003 whilst waiting for pasture to establish. Only a small number of sheep were used with the intention of gaining preliminary information. The second trial was a more extensive repeat of the first trial, with more animals and an additional treatment of 100 t NUA/ha. The trial commenced August 2004 and again ran for about 14 weeks. Both grazing periods were on the same trial site, consisting of two NUA treated plots (100 and 150 t/ha) and an untreated control plot. In both years, sheep were held on untreated farmland (baseline plot) adjacent to the trial site before the start of the trials. There was no recent history of trace element application (e.g. copper, selenium) to the farmland.

Rehabilitation staff based at the North Capel Mine was responsible for the management and monitoring of pasture at the trial site.

Site

The trial site was located within the Demonstration Site in the buffer zone surrounding the North Capel Iluka Mine. The Demonstration Site is rehabilitated land, consisting of sand tailings from the adjacent mine and topsoil from the surrounding area; Throssell (2002) gives a detailed description of the site. The trial site consisted of two NUA treated plots (approximately 1.0 ha each), with NUA either applied at a rate of 100 or 150 t NUA/ha in May 2003. The site also included a 1.0 ha control plot. All plots were located in the same demonstration site and were of similar soil and pasture composition. Additional sheep in 2004 where held on baseline plot adjacent to the trial site which could be used to increase grazing pressure. Each plot had water supplied from a nearby bore. Mineral analysis of the water, as arranged by Iluka rehabilitation staff, indicated that it was low in minerals and so suitable for this trial.

Fertiliser (220 kg/ha super:potash 1:1) was applied to the plots in August 2003 and repeated in September 2003 and in August 2004. In June 2003 plots were sown with a mixture of annual ryegrass, clover, oats and serradella; however the species germinated at different densities across the plots. Opportunistic species, such as Brome, Silver and Veldt grasses and capeweed were also present.

Pasture growth was poor in 2003 and restricted the grazing of sheep on the plots. In 2004, growth was improved and so allowed more extensive grazing. Soil and pasture samples were collected for mineral analyses by Iluka staff.

The analysis of the soil, groundwater and pasture data from this trial is subject of another report (Lavell and Summers 2005).

Ingestion of NUA in 2003 was less likely than in 2004 because of the lower grazing pressure (Figures 1 to 8). Within 2004 ingestion of NUA was more likely on the 150 t/ha plot compared to the 100 t/ha plot (Figures 6 and 7), while on the control plot pasture growth was comparatively good and so significant ingestion of soil would have been unlikely (Figure 8).

Animals

2003

Merino cross (believed to be South African Meat Merinos) weaner wethers were purchased by Iluka in June and held on untreated farmland adjacent to the treatment plots prior to the trial. The sheep had not been given any long-term mineral supplements. In the first week of September (3/9/03) sheep were introduced onto the control (5 sheep) and 150 t/ha (5 sheep) plots and grazed until mid December (15/12/03) when feed was becoming limited, particularly on the 150 t/ha plot. The sheep grazed the plots for about 14 weeks, after which they were euthanased, examined at post-mortem and tissue and blood samples collected.

2004

Merino weaner wethers were purchased by Iluka and held on untreated farmland adjacent to the treatment plots prior to the trial. The sheep had not received any long-term mineral supplements. In the second week of August (10/8/04) sheep were randomly placed onto the control (8 sheep), 100 (8 sheep) and 150 (10 sheep) t NUA /ha plots. Sheep, which were at the extreme ends of the size range, were excluded from allocation to these plots. Fourteen sheep were also placed on baseline plot adjacent to the trial site. Henceforth, this is referred to as day 0 of the trial. As allocated to the plots sheep were drenched with anthelmintics (Levamisole and Oxfendazole; Cooper's Scanda). The sheep received a follow up anthelmintic treatment using Cydectin on 7 October (day 57 of grazing on plots), when worm egg counts indicated an ongoing worm problem.

Ten sheep, from the adjacent baseline plot were removed and transported to AHL on 9 September. There the sheep were euthanased for post-mortem examination and tissue and blood samples collected to establish baseline mineral levels. Sheep were grazed on the plots until mid November (16/11/04; day 97), but 6 sheep were removed from the 150 t/ha plot in early November (4/11/04; day 85). This was an animal welfare decision because of increased grazing pressure. All sheep removed were euthanased for post mortem examination and tissue and blood samples collected. Most sheep grazed the plots for about 14 weeks, and the 6 sheep removed early from the 150 t/ha grazed for about 2 weeks less. Sheep were grazed to a point where there was reasonable likelihood of soil ingestion, i.e. feed was becoming limited.

Specimens and measurements

Blood samples

In 2003, heparinised blood samples were collected from the sheep by venipuncture on the following dates; 27 June and 17 July (both pre-trial bleeds, about 9.5 and 6 weeks before the sheep went onto the trial plots, respectively) and 15 December (blood taken from sheep at slaughter), while in 2004 samples were collected at the start of grazing (10 August), 7 October and whenever sheep were euthanased. Parameters measured are listed in Table 2. Vitamin B12 was not measured on all occasions in 2003.

Tissue samples

At slaughter sheep were euthanased using an injection of Lethabarb. Tissues collected were; liver, lung (right cranial lobe), rib (3rd rib from floating rib, distal half), kidney, muscle (vastus lateralis and vastus intermedius) and wool. At post-mortem all sheep were examined for any gross pathological changes. Mineral concentrations in tissue samples were determined by ICP-AES and ICP-MS methodologies. Tissues were digested in acid using a microwave digester system before reading. Selected wool samples from 2004 (three randomly selected samples from each group) were split and one half scoured using a laboratory-based system that simulates the commercial scouring process (Wool Laboratory, Department of Agriculture, Western Australia). The mineral composition of scoured and un-scoured wool was determined.

Table 2. List of parameters measured in plasma or red cells, the method used and the significance

Plasma parameters	Method	Purpose
Glutamate Dehydrogenase (GLDH)	Working Group on Enzymes. 1992 Eur. J. Clin. Chem. Clin. Biochem. 30: 493-502	Assessment of liver health
Gamma-Glutamyl Transferase (GGT)	Commercial kit: Olympus Diagnostica GmbH	Assessment of liver health
Total Bilirubin	"	Assessment of liver health
Creatinine	"	Assessment of kidney health
Urea	"	Assessment of kidney health
Alanine Aminotransferase (ALT)	"	Assessment of muscle health
Creatine Kinase (CK)	"	Assessment of muscle health
Beta-Hydroxybutyrate (BHB)	"	Indicator of nutritional (energy) status
Phosphate (Pi)	"	Indicator of phosphorus status
Calcium (Ca)	"	Indicator of calcium status
Zinc (Zn)	TCA Soluble, Flame Atomic Absorption Spectrometry	Indicator of zinc status
Copper (Cu)	TCA Soluble, Flame Atomic Absorption Spectrometry	Indicator of copper status
Glutathione Peroxidase in red blood cells (GSHPx)	Paynter *et al.* (1985)	Indicator of selenium status
Vitamin B12	Enzyme Immunoassay Roche CEDIA	Indicator of cobalt status
Methylmalonic acid (MMA)	McMurrary *et al.* (1986)	Indicator of cobalt status

Feed-on-offer (FOO) was estimated on a routine basis by Iluka staff at both years to assist in determining changes, if any, in grazing pressure on the plots. Condition scores of sheep were monitored as described by Suiter (1994).

Statistical analysis

Means were compared between treatments by either Analysis of Variance or T-Test using the software package Statistix version 8.

3. RESULTS

Sheep health

In both years, all sheep appeared healthy over the 14 weeks grazing periods (Figures 1 to 5). Furthermore there was no evidence of gross disease at post-mortem examination, except for one sheep from the control plot in 2004 that had a localised infection around one kidney. However, according to a Veterinary Pathologist this condition would not be considered an unusual finding and in this case had little impact on the health of the animal (pers. comm. S. Besier).

Assessment of sheep health using clinical chemistry in both years provided additional evidence that sheep were healthy. Activities of glutamate dehydrogenase (GLDH), an indicator of liver damage, were in some cases elevated (Tables 3a, 3b), but not to an extent that would indicate any significant liver damage, and activities fell within the normal range at the end of grazing.

Elevated levels of beta-hydroxybutyrate (BHB) in plasma are a good indication of inadequate energy intake in sheep. The fact that sheep in both years had low levels of BHB (Tables 3a, 3b) suggests that energy intake was adequate, and this is supported by condition scores of 2-3 at slaughter.

Table 3a. Clinical Biochemistry values (mean ± SD) in sheep that have grazed for about 14 weeks on either a control plot or on a plot treated with 150 t/ha of NUA, 2003

Parameter	Normal ranges	Pre-trial 27 June	Pre-trial 17 July	End of grazing 15 December	
				Control plot	150 t/ha plot
GGT (U/L)	23–67	54 ± 9.8	65 ± 14.7	68 ± 8.4	73 ± 6.7
GLDH (U/L)	< 20	104 ± 87	66 ± 218	24 ± 18.3	12 ± 7
Bilirubin (umol/L)	< 15	3.7 ± 1.3	4.3 ± 1.9	3.8 ± 1.1	4.1 ± 1.5
Creatinine (umol/L)	50–150	72 ± 4.7	76 ± 6.1	97 ± 8.6	83 ± 6.4
Urea (mmol/L)	3.3–12	6.9 ± 0.8	4.9 ± 0.9	5.6 ± 0.9	4.1 ± 0.2
ALT (IU/L)	< 30	12.8 ± 2.7	14.2 ± 3.2	19 ± 2.9	14 ± 2.6
CK (IU/L)	< 500	245 ± 54	270 ± 64	116 ± 14.4	112 ± 21
BHB (mmol/L)	0.2–0.6	0.34 ± 0.09	0.32 ± 0.08	0.35 ± 0.04	0.33 ± 0.08

Table 3b. Clinical Biochemistry values (mean ± SD) in sheep that have grazed for 14 weeks on either an untreated control plot or on plots treated with 100 or 150 t/ha of NUA, 2004. Sheep were placed onto plots on Day 0

Parameter	Normal ranges	Day 0	Baseline group	7 October (Day 57)			End of grazing; 16 November (Day 97)		
		10 August	10 September	Control plot	100 t/ha plot	150 t/ha plot*	Control plot	100 t/ha plot	150 t/ha plot*
GGT (U/L)	23–67	46 ± 10.2	33 ± 11	47 ± 9	56 ± 14	43 ± 6	43 ± 8.9	47 ± 10	36 ± 6.7
GLDH (U/L)	< 20	33 ± 16	29 ± 9	68 ± 84	88 ± 95	27 ± 18	8 ± 7	4 ± 3	10 ± 14
Bilirubin (umol/L)	< 15	2.4 ± 1.05	4.1 ± 1.7	2.7 ± 0.4	2.2 ± 0.3	2.1 ± 0.4	2.9 ± 0.5	3.5 ± 0.9	3.3 ± 0.1
Creatinine (umol/L)	50–150	61 ± 6.1	75 ± 4.9	69 ± 7	69 ± 3.8	66 ± 6.5	106 ± 16	112 ± 10	83 ± 15
Urea (mmol/L)	3.3–1.2	5.0 ± 0.8	3.9 ± 1.9	9.1 ±1.1	7.1 ± 0.6	7.9 ± 0.9	3.7 ± 1.0	4.7 ± 2.0	3.3 ± 1.6
ALT (IU/L)	< 30	14 ± 3.5	17 ± 3.2	20 ± 4.0	20 ± 2.9	15 ± 2.6	11 ± 3	10 ± 2	10 ± 1.9
CK (IU/L)	< 500	169 ± 37	156 ± 54	217 ± 37	319 ± 118	177 ± 28	134 ± 74	245 ± 221	79 ± 13
BHB (mmol/L)	0.2–0.6	0.32 ± 0.08	0.36 ± 0.08	0.32 ± 0.04	0.34 ± 0.09	0.37 ± 0.06	0.2 ± 0.09	0.28 ± 0.1	0.26 ± 0.1

* Includes sheep sampled on the 4/11/04.

Minerals

Pasture minerals

Analysis of mineral composition of pasture at the experimental site, over 2003 and 2004, was supplied by Iluka Resources and attached to this report as Appendix Table 7a. Of the minerals tested it would seem that deficiency, toxicity or accumulation of heavy metals in grazing sheep would be unlikely from pasture consumption alone (Underwood and Suttle 1999, Puls 1994).

Blood measures in 2003

Sheep arrived at the trial site adequate in minerals, with the exception of several of the sheep that had marginal plasma vitamin B12 status, as determined by plasma vitamin B12 and methylmalonic acid (MMA) concentrations. Cobalt is required for vitamin B12 formation, and so cobalt intake where the sheep were previously held was probably inadequate. Grazing on the farmland adjacent to the trial plot saw an increase in vitamin B12 status to adequate levels at the next pre-trial sampling three weeks later.

There was no apparent negative impact on the mineral status of key essential minerals in sheep grazing on NUA amended land (150 t/ha), as assessed by measurement of blood based indicators (Table 4a). Plasma calcium was significantly lower compared with sheep on the control plot, but was still well within the normal range.

Table 4a. Biochemistry parameters (mean ± SD) in blood plasma that indicate mineral status in sheep, that have grazed for about 14 weeks on either an untreated plot or on a plot treated with 150 t/ha of NUA, 2003

Parameters	Normal ranges	Pre-trial 27 June	Pre-trial 17 July	End of grazing 15 December	
				Control plot	150 t/ha plot
Pi (mmol/L)	0.9–2.5	2.0 ± 0.17	1.8 ± 0.09	1.7 ± 0.09	1.7 ± 0.2
Ca (mmol/L)	2.2–3.0	2.6 ± 0.09	2.4 ± 0.12	2.7 ± 0.09^{a}	2.5 ± 0.06^{b}
Zn (mg/L)	0.6–1.0	0.78 ± 0.08	0.8 ± 0.09	0.82 ± 0.03	0.79 ± 0.09
Cu (mg/L)	0.9–1.4	1.0 ± 0.13	1.1 ± 0.10	1.0 ± 0.07	1.2 ± 0.15
GSHPx (IU/g Hb)	> 50	67 ± 24.87	74 ± 23	83 ± 19	70 ± 12
Vitamin B12 (pM/L)	> 400	382 ± 132	484 ± 150		
MMA (uM/L)	< 3	2.7 ± 1.3	2.0 ± 1.1	1.0 ± 0.23	1.09 ± 0.08

For each parameter, significant differences between means of the control group and 150 t/ha group are indicated by different superscripts.

Blood measures in 2004

Based on measurements of blood-based indicators all sheep commenced the trial adequate in key essential minerals, and remained adequate over the trial period (Table 4b). Application of NUA to plots did, however, have some effects on some minerals. Plasma phosphorus levels were significantly lower in sheep with increasing NUA application, at day 57 and at the end of the trial, while plasma copper levels were significantly lowered by NUA application at day 57, but not at the end of the trial (Table 4b). Otherwise there were no other significant effects of NUA on the blood parameters measured.

Table 4b. Biochemistry parameters (mean ± SD) in blood that indicate mineral status in sheep that have grazed for 14 weeks on either an untreated plot or on plots treated with 100 or 150 t/ha of NUA, 2004. Sheep were placed onto plots on Day 0

Parameter	Normal ranges	Day 0	Baseline group	7 October (Day 57)			End of grazing; 16 November (Day 97)		
		10 August	10 September	Control plot	100 t/ha plot	150 t/ha plot*	Control plot	100 t/ha plot	150 t/ha plot*
Pi (mmol/L)	0.9–2.5	2.4 ± 0.26	2.4 ± 0.23	2.5 ± 0.25 ^{a}	2.21 ± 0.15 ^{b}	2.01 ± 0.23 ^{c}	2.5 ± 0.2 ^{a}	2.2 ± 0.1 ^{b}	2.0 ± 0.2 ^{c}
Ca (mmol/L)	2.2–3.0	2.5 ± 0.08	2.5 ± 0.11	2.67 ± 0.15	2.68 ± 0.10	2.64 ± 0.08	2.7 ± 0.1	2.7 ± 0.23	2.6 ± 0.2
Mg (mmol/L)	0.6–0.74	0.78 ± 0.08	0.76 ± 0.07	0.86 ± 0.05	0.94 ± 0.06	0.92 ± 0.07	0.71 ± 0.1	0.81 ± 0.9	0.76 ± 0.1
Zn (mg/L)	0.6–1.0	0.8 ± 0.09	0.72 ± 0.12	0.74 ± 0.09	0.79 ± 0.08	0.71 ± 0.14	0.86 ± 0.05	0.95 ± 0.05	0.92 ± 0.07
Cu (mg/L)	0.9–1.4	1.2 ± 0.22	1.1 ± 0.34	1.1 ± 0.11 ^{a}	0.85 ± 0.12 ^{b}	0.71 ± 0.12 ^{b}	1.56 ± 0.25	1.53 ± 0.55	1.23 ± 0.29
GSHPx (IU/g Hb)	> 50	502 ± 42	482 ± 164	294 ± 103	347 ± 60	350 ± 76	294 ± 101	340 ± 61	355 ± 73
MMA (uM/L)	< 3	0.2 ± 0.3	0.32 ± 0.24	0.41 ± 0.28	0.43 ± 0.17	0.42 ± 0.22	0.46 ± 0.24	0.46 ± 0.22	0.32 ± 0.15

* Includes sheep sampled on the 4/11/04.

For each parameter, significant differences between means of plots, at either day 57 or 97, are indicated by different superscripts.

Minerals in tissues 2003

Mean concentrations of essential minerals in livers of sheep grazed on the trial plots in 2003 are given in Table 5a, while those of non-essential and toxic minerals are given in Table 5b. All sheep had adequate levels of essential minerals with no evidence of accumulation of any minerals to unusually high or toxic levels. However initial mean results for mercury exceeded the GEL in control sheep and sheep on the 150 t/ha plot (results in parentheses in Table 5b). Some samples (2 from 0 t/ha and 4 from 150 t/ha) were re-testing at the National Measurements Institute (NMI; South Melbourne, Victoria). This laboratory specialises in testing residues in foodstuffs. All results were less than the GEL and are given in Table 5b.

Grazing sheep on NUA treated land did increase liver cobalt and iron concentrations (Table 5a). Nickel (Table 5b) may also have been increased but no statistical comparison could be made as most of the results in the control group were reported as below the limit of detection (< 50 ug/kg). None of the minerals exceeded GEL or ML levels.

Mean concentrations of minerals in kidney are given Table 5c. Kidney is rarely used to monitor mineral status but it is a useful when investigating accumulation of heavy metals. Grazing sheep on NUA treated land did not significantly affect any of the elements. However, as the case with liver, initial testing for mercury gave levels greater than the GEL (results in parentheses in Table 5c). Samples re-tested at NMI (one from each group) gave levels less than the GEL (results are given in Table 5c).

Grazing sheep on the 150 t/ha NUA plot resulted in significant increases in cobalt, manganese and iron concentrations in the lung (Table 5d).

Table 5a. Concentrations of essential minerals in liver (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on a plot treated with 150 t/ha of NUA in 2003. Levels above the marginal range would indicate adequate mineral status while levels below this range would indicate increased probability of a clinical deficiency. Levels above "High" indicate levels well above normal but not necessarily toxic. Levels above "Toxic" would indicate high probability of toxicity. Generally Expected Levels (GEL) for offal, under the Food Standards Code of Australia, are given where applicable

NUA plot (t/ha)	Co ug/kg	Cu mg/kg	Fe mg/kg	Mg mg/kg	Mn mg/kg	Se mg/kg	Zn mg/kg
0	37 ± 7[a]	54 ± 5	110 ± 25[a]	168 ± 4	1.9 ± 0.2	0.09 ± 0.02	39 ± 4
150	61 ± 6.4[b]	74 ± 24	180 ± 33[b]	169 ± 9.9	2.0 ± 0.26	0.07 ± 0.04	37 ± 4
Marginal range	5–20	2–6.5	23–34	< 118	2.6-3.0	0.02–0.03	< 30
High	> 85	> 150				> 2	> 100
Toxic	> 5,000	> 250	> 9,000[a]	-		> 15	> 400
GEL	-	150	-	-	-	2	60

[a] Cattle range, but sheep would be similar.

For each element, significant differences between means of the control group and 150 t/ha group are indicated by different superscripts.

Table 5b. **Concentrations of non-essential or toxic minerals in liver (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on a plot treated with 150 t/ha of NUA, 2003. Levels above "High" indicate levels well above normal but not necessarily toxic. Levels above "Toxic" would indicate high probability of toxicity. Generally Expected Levels (GEL) and Maximum Levels (ML) for offal, under the Food Standards Code of Australia, are given where applicable. For each element, there was no significant difference between means of the control group and 150 t/ha group**

NUA plot (t/ha)	Al mg/kg	As mg/kg	Cd mg/kg	Cr mg/kg	Pb mg/kg	HgΔ mg/kg	Sb ug/kg	Mo mg/kg	Ni ug/kg	Sn ug/kg
0	1.7 ± 1.5	0.04 ± 0.02	0.26 ± 0.06	0.14 ± 0.01	0.05 ± 0.03	< 0.01 (0.024 ± 0.007)	7.8 ± 2.4	0.67 ± 0.16	< 50	18 ± 8
150	2.3 ± 1.8	0.04 ± 0.02	0.26 ± 0.08	0.14 ± 0.01	0.05 ± 0.01	< 0.01 (0.027 ± 0.017)	6.7 ± 1.2	0.75 ± 0.15	64 ± 11	13 ± 3
High			> 2			> 7	-	> 2.6	> 200	> 3,800
Toxic	6-11	> 10	> 50	> 30[a]	> 10	> 10	-	> 30		
GEL		0.1				0.010	50			
ML			1.25		0.5					

[a] This range is for the hexavalent form in bovine liver. Tissue levels here are total Cr.

Δ Selected samples were re-tested because of high initial results (parentheses). Ranges of values from re-testing are given.

Table 5c. **Concentrations of minerals in kidney (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on a plot treated with 150 t/ha of NUA, 2003. Levels above "High" indicate levels well above normal but not necessarily toxic. Levels above "Toxic" would indicate high probability of toxicity. Generally Expected Levels (GEL) and Maximum Levels (ML) for offal under the Food Standards Code of Australia are given where applicable. For each element, there was no significant difference between means of the control group and 150 t/ha group**

	NUA plot (t/ha)		High	Toxic	ML	GEL
	0	150				
Al mg/kg	1.26 ± 0.55	1.02 ± 0.22		> 4		
As mg/kg	0.024 ±0.013	0.028 ± 0.031	> 1	> 10		0.1
Cd mg/kg	0.75 ± 0.17	0.78 ± 0.37	> 4	> 50	2.5	
Co ug/kg	24 ± 9	44 ± 20	> 1,000	> 30,000		-
Cr mg/kg	0.16 ± 0.07	0.12 ± 0.02		> 15[a]		
Cu mg/kg	2.5 ± 0.4	2.7 ± 0.4	> 4	> 18		50
Hg mg/kg Δ	< 0.01 (0.04 ± 0.04)	< 0.01 (0.02 ± 0.01)	>18	> 20		0.01
Mn mg/kg	0.98 ± 0.48	0.87 ± 0.13	> 2.0	> 5.0		-
Mo mg/kg	0.33 ± 0.12	0.38 ± 0.10	> 7.4	> 200		
Ni ug/kg	103 ± 57	> 50	> 6,800			
Pb mg/kg	< 0.05	< 0.05		> 5	0.5	
Sb ug/kg	6.6 ± 7	3.7 ± 3.3				50
Se mg/kg	0.43 ± 0.12	0.39 ± 0.08	> 4	> 6		2
Sn ug/kg	14 ± 8	12 ± 7				
Zn mg/kg	13.7 ± 1.3	16.6 ± 4.3	> 50	> 240		60

[a] This range is for the hexavalent form in bovine liver. Tissue levels here are total Cr.

Δ Selected samples were re-tested because of high initial results (parentheses). Values from re-testing are given.

Table 5d. Concentrations of minerals in lung (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on a plot treated with 150 t/ha of NUA, 2003

	NUA Plot (t/ha)	
	0	**150**
Al mg/kg	2.25 ± 0.5	1.6 ± 0.2
As ug/kg	20 ± 7.5	35 ± 18
Co ug/kg	4.2^{a} ± 2.1	14^{b} ± 2
Cr mg/kg	0.12 ± 0.02	0.11 ± 0.02
Cu mg/kg	2.19 ± 0.98	1.93 ± 0.37
Fe mg/kg	116^{a} ± 4	185^{b} ± 62
Hg ug/kg	20 ± 7	21 ± 11
Mg mg/kg	104 ± 15	99 ± 13
Mn mg/kg	0.24^{a} ± 0.04	0.46^{b} ± 0.14
Mo ug/kg	104 ± 28	131 ± 24
Ni ug/kg	61 ± 55	65 ± 14
Pb ug/kg	11 ± 6	13 ± 18
Sb ug/kg	4 ± 2	2.3 ± 2.2
Se ug/kg	50 ± 20	70 ± 17
Sn ug/kg	13 ± 3	7.7 ± 4.1
Zn mg/kg	13.73 ± 2.69	13.04 ± 2.67

For each element, significant differences between means of the control group and 150 t/ha group are indicated by different superscripts.

Minerals in tissues 2004

Mean mineral concentrations in liver in sheep that grazed on NUA treated plots in 2004 for approximately 14 weeks are given in Tables 6a (essential elements) and 6b (non-essential and toxic elements). Sheep grazing on the 150 t/ha plot had increased liver iron and manganese and decreased copper compared to the control sheep (Table 6a). Initial testing gave mean concentrations for lead, arsenic and mercury (results in parentheses in Table 6b) that exceeded the relevant GEL or ML in all or some of the groups. Elevations were not associated with NUA as baseline and or control animals were also affected. Selected liver samples were re-tested at NMI and the results placed into Table 6b. Numbers of samples re-tested were:

	Baseline	NUA t/ha		
		0	**100**	**150**
Lead	-	1	2	3
Arsenic	-	2	4	1
Mercury	2	3	4	1

With re-testing, lead and arsenic levels fell within their respective limits. Mercury however fell outside its GEL for some samples, but as with the initial test results this was not associated with NUA (Table 6b). Concentrations of uranium were below the limit of detection in all liver samples tested.

The initial mean result for aluminium concentration in liver in sheep on the 150 t/ha plot (in parentheses in Table 6b) fell within the toxic range and so samples were selected for re-testing at NMI (3 samples from the 150 t/ha and 1 from the 100 t/ha treatment). The results were all within the normal range for Aluminium (Table 6b).

Concentrations of minerals in lungs are given Tables 6c. Concentrations of iron and thorium in sheep on the 150 t/ha plot were significantly higher compared to the baseline and control sheep.

Wool on sheep grazing NUA treated plots was visibly contaminated compared to control sheep (Figures 1 to 5) and this was reflected in higher concentrations of some minerals in unwashed wool (Table 6d). Minerals that were significantly elevated by NUA were cobalt, iron, manganese, nickel and thorium. Scouring the wool effectively removed these minerals (Table 6d) and resulted in wool of acceptable cleanness.

Table 6a. Concentrations of essential minerals in liver (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on plots treated with 100 or 150 t/ha of NUA, 2004. Levels above the marginal range would indicate adequate mineral status while levels below this range would indicate increased probability of a clinical deficiency. Levels above the marginal range would indicate adequate mineral status while levels below this range would indicate increased probability of a clinical deficiency. Levels above "High" indicate levels well above normal but not necessarily toxic. Levels above "Toxic" would indicate high probability of toxicity. Generally Expected Levels (GEL) for offal, under the Food Standards Code of Australia, are given where applicable

NUA plot (t/ha)	Co ug/kg	Cu mg/kg	Fe mg/kg	Mg mg/kg	Mn mg/kg	Se mg/kg	Zn mg/kg
Baseline	178 ± 75	126^{a} ± 41	246^{b} ± 55	300 ± 18	5.7^{b} ± 0.5	0.14 ± 0.04	52 ± 10
0	102 ± 44	116^{ab} ± 37	138^{b} ± 35	312 ± 17	6.27^{b} ± 0.69	0.12 ± 0.04	44 ± 11.6
100	196 ± 97	69^{bc} ± 33	343^{b} ± 149	270 ± 13	6.24^{b} ± 0.63		52 ± 24
150	186 ± 107	58^{c} ± 29	926^{a} ± 522	332 ± 11	7.5^{a} ± 0.8	0.09 ± 0.03	52 ± 13
Marginal range	5–20	2–6.5	23–34	< 118	2.6–3.0	0.02–0.03	< 30
High	> 85	> 150				> 2	> 100
Toxic	> 5,000	> 250	> 9,000[a]			> 15	> 400
GEL		150				2	60

[a] Cattle range, but sheep would be similar. For each element, significant differences between means are indicated by different superscripts.

Table 6b. Concentrations of non-essential or toxic minerals in liver (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on plots treated with 100 or 150 t/ha of NUA, 2004. Minerals listed are those that have been given a Maximum Level (ML) or Generally Expected Level (GEL) for offal under the Food Standards Code of Australia. Levels above the marginal range would indicate adequate mineral status while levels below this range would indicate increased probability of a clinical deficiency. Levels above "High" indicate levels well above normal but not necessarily toxic. Levels above "Toxic" would indicate high probability of toxicity

	Baseline	NUA plot (t/ha)			High	Toxic	GEL	ML
		0	100	150				
Al mg/kg Δ	- (2.76 ± 2)	< 0.05 (3.9 ± 3.5)	- (1.8 ± 1.3)	< 0.05 (6.9 ± 4.8)		6-11		
As mg/kg Δ	- (0.13^{ab} ± 0.03)	< 0.1 (0.07^{b} ± 0.08)	< 0.1 (0.19^{a} ± 0.02)	< 0.1 (0.05^{b} ± 0.08		> 10	0.10	
Cd mg/kg	0.59^{a} ± 0.2	0.31^{b} ± 0.14	0.37^{b} ± 0.07	0.21^{b} ± 0.09	> 2	> 50		1.25
Cr mg/kg	2.7 ± 1.7	1.04 ± 0.35	1.2 ± 0.76	3.6 ± 5.1		> 30^{a}		
Pb mg/kg Δ	- (0.026^{b} ± 0.00 3)	< 0.1 (0.22^{ab} ± 0.23)	< 0.1 (0.24^{ab} ± 0.25)	< 0.1-0.14 (0.73^{a} ± 0.71)		> 10		0.5
Hg mg/kg Δ	0.02-0.06 (0.13^{b} ± 0.04)	< 0.01-0.03 (0.11^{b} ± 0.09)	< 0.01-0.01 (0.23^{a} ± 0.05)	0.02 (0.12^{b} ± 0.09)	> 7	> 10	0.010	
Sb ug/kg	21 ± 12	-	52 ± 35	-			50	
Mo mg/kg	1.3^{a} ± 0.4	0.79^{b} ± 0.37	0.99^{ab} ± 0.23	0.72^{b} ± 0.26	> 2.6	> 30		
Ni ug/kg	400 ± 115	BLD	333 ± 115	346 ± 128	> 200			
Sn ug/kg	124^{b} ± 59	593^{ab} ± 380	301^{b} ± 179	1419^{a} ± 1530	> 3,800			
Th ug/kg	6.1 ± 8.9	3.6 ± 1.4	4.8 ± 1.9	8.5 ± 7.8				

[a] This range is for the hexavalent form in bovine liver. Tissue levels here are total Cr.

Δ Selected samples were re-tested because of high initial test results (in parentheses). Ranges of values from repeat testing, where done, are given.

Table 6c. Concentrations of minerals in lung (fresh weight, mean ± SD) in sheep that have grazed for about 14 weeks on either an untreated plot or on plots treated with 100 or 150 t/ha of NUA, 2004

	Baseline	NUA plot (t/ha)		
		0	100	150
Al mg/kg	5.9 ± 3.5	14.2 ± 13.4	7.2 ± 4.4	9.5 ± 2.8
As ug/kg	134 ± 26	102 ± 70	144 ± 42	206 ± 48
Co ug/kg	131 ± 76	85 ± 66	160 ± 100	72 ± 36
Cr mg/kg	BLD	BLD	BLD	BLD
Cd ug/kg	BLD	BLD	BLD	BLD
Cu mg/kg	3.2 ± 0.7	3.1 ± 0.5	3.4 ± 0.4	3.8 ± 0.6
Fe mg/kg	176^{bc} ± 73	98^{c} ± 29	255^{ab} ± 131	340^{a} ± 81
Hg ug/kg	34a^{b} ± 8	23^{b} ± 18	48^{a} ± 19	27a^{b} ± 18
Mg mg/kg	220 ± 11	235 ± 30	210 ± 15	243 ± 16
Mn mg/kg	5 ± 0	5 ± 0	5.2 ± 0.4	5 ± 0
Mo ug/kg	197^{a} ± 38	132^{b} ± 52	222^{a} ± 20	132^{b} ± 45
Ni ug/kg	711 ± 348	1200 ± 916	666 ± 206	500 ± 141
Pb ug/kg	184^{b} ± 97	129^{b} ± 120	126^{b} ± 113	407^{a} ± 198
Sb ug/kg	8.4 ± 4.7	33 ± 37	31 ± 35	32 ± 22
Se ug/kg	100 ± 51	64 ± 22	60 ± 28	80 ± 0
Sn ug/kg	70 ± 33	329 ± 397	192 ± 165	313 ± 366
Zn mg/kg	17.5 ± 2.8	16.7 ± 1. 8	18.2 ± 2. 6	17.6 ± 0.8
Th ug/kg	3.6^{b} ± 2.9	4.8^{b} ± 2.4	$7.9^{a b}$ ± 2.4	10.3^{a} ± 4.7
U ug/kg	BLD	BLD	BLD	BLD

Table 6d. **Concentrations of minerals in unwashed wool (as received, mean ± SD) and washed wool (i.e. scoured) from sheep that have grazed for about 14 weeks on either untreated plots or on plots treated with 100 or 150 t/ha of NUA, 2004**

	Baseline		NUA Plot Treatment (t/ha)					
			0		100		150	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
	Essential minerals							
Co mg/kg	213^{c} ± 93	126 ± 88	805^{c} ± 470	93 ± 38	6106^{b} ± 1128	165 ± 16	9866^{a} ± 794	521 ± 349
Cu mg/kg	5.2^{b} ± 1.5	23.5^{b} ± 4.4	6^{b} ± 1.4	36^{a} ± 3.4	15^{a} ± 2.3	31^{ab} ± 5	18^{a} ± 2.5	41.9^{a} ± 3.7
Fe mg/kg	979^{b} ± 472	217 ± 176	1553^{b} ± 687	64 ± 20	5945^{a} ± 1098	95 ± 40	7443^{a} ± 1141	158 ± 98
Mn mg/kg	20^{b} ± 19	11.3 ± 7.1	114^{b} ± 69	8.1 ± 1.3	1061^{a} ± 210	21.6 ± 6.6	1401^{a} ± 221	34 ± 18
Se ug/kg	240 ± 106	186 ± 83	106 ± 46	BLD	120 ± 40	60 ± 28	160 ± 40	BLD
Zn mg/kg	93 ± 28	109 ± 33	87 ± 9	104 ± 15	99 ± 8	105 ± 8	117 ± 20	133 ± 39
	Non essential and toxic minerals							
Sb ug/kg	12 ± 4	21 ± 14	18.6 ± 8.3	12 ± 4	21.3 ± 2.3	14.7 ± 4.6	69.3 ± 71.6	18 ± 12.2
Al mg/kg	1278 ± 667	254 ± 383	1051 ± 404	65 ± 19	976 ± 44	49 ± 29	847 ± 47	46 ± 14
As ug/kg	758 ± 456	60^{b} ± 39	1670 ± 901	116^{ab} ± 17	2533 ± 878	121^{ab} ± 33	1498 ± 233	212^{a} ± 70
Cd ug/kg	39 ± 19	67 ± 23	30 ± 2.8	108 ± 65	44 ± 10	73 ± 5.7	48 ± 6	112 ± 52
Cr mg/kg	4 ± 2.1	18 ± 21.5	4.6 ± 4.2	BLD	6.5 ± 1.9	BLD	8.8 ± 3.2	BLD
Pb ug/kg	1876 ± 1181	1074 ± 1479	1285 ± 365	272 ± 350	940 ± 226	250 ± 42	1337 ± 205	1088 ± 1035
Hg ug/kg	44 ± 7.2	28 ± 3.2	59.2 ± 9.1	28 ± 3.6	59.2 ± 12.6	29 ± 6	66 ± 6.9	34 ± 10
Mo ug/kg	394^{ab} ± 72	222 ± 102	234^{b} ± 56	84 ± 24	409^{ab} ± 52	92 ± 32	522^{a} ± 102	141 ± 12
Ni ug/kg	1133^{b} ± 305	466 ± 461	1333^{b} ± 642	1000 ± 282	3933^{a} ± 416	733 ± 416	5266^{a} ± 832	1133 ± 577
Th ug/kg	1282^{b} ± 252	108 ± 119	2761^{b} ± 1276	70 ± 36	6079^{a} ± 578	66 ± 33	7650^{a} ± 1366	338 ± 385
Sn ug/kg	157 ± 33	240 ± 149	150 ± 6	129 ± 30	169 ± 30	112 ± 31	204 ± 17	314 ± 319
U ug/kg	8.3^{b} ± 1.8	BLD	18.4^{b} ± 7.7	BLD	38^{a} ± 5	BLD	48.8^{a} ± 7	BLD

4. DISCUSSION

Limitations to results

There were limitations in the trial data for both years:

1. Size restrictions of the site meant that there were only small numbers of animals in the groups, reducing sensitivity to measure changes caused by NUA.
2. Concentrations of some elements were close to or below the limits of detection of the analytical methods (e.g. nickel), and as a result analytical error may have created "noise" in some data.
3. Comparisons between the two years might be compromised because of breed differences in the experimental sheep (breeds can metabolise minerals differently (Underwood and Suttle 1999)) and differences in grazing pressure between years, and therefore probability of soil ingestion. In 2003 there were fewer sheep on the 150 t/ha plot and as a result lower grazing pressure (Figures 2 and 5).
4. The length of grazing was comparatively short in both trials (around 14 weeks). For example, two grazing studies investigating accumulation of cadmium in sheep from contaminated fertiliser ran from 15 to 24 months (Lee *et al.* 1994; Lee *et al.* 1996). Obviously the probability of detecting changes in mineral load or imbalance in the studies reported here would have increased with a longer grazing period. While it was very likely that NUA was ingested by grazing sheep in the 2004 trial (Figures 6 and 7), under real grazing conditions the potential for ingestion would be far greater due to our long summer/autumn period. For comparison, in a British trial investigating bioaccumulation of heavy metals under the worst case scenario (i.e. soil ingestion whilst grazing), Hill *et al*. (1998) fed penned sheep diets with added soil and sewerage sludge (up to 300 g daily) over 16 weeks.
5. In 2004 the control plot was well covered with feed whilst the NUA plots, particularly the 150 t/ha plot, were poorly covered (Figures 6 to 8). In terms of trial design it would have been better if soil ingestion had been just as likely on the control plot.

These limitations should be kept in mind when reviewing the results of these trials.

Sheep health

In both years, sheep commenced the trials with adequate mineral statuses and in good health. Grazing them on NUA amended paddocks had no detrimental effects on their health as determined by clinical biochemistry and post mortem examination. See Figures 1 to 5.

Essential mineral nutrition

Grazing sheep on NUA amended plots did not lead to deficiencies in either year in any of the key essential minerals, namely; calcium, phosphorus, magnesium, zinc, selenium, cobalt or copper. NUA did, however, influence some of these minerals. Liver and plasma copper status was reduced by NUA in 2004, but not in 2003, while in both years liver iron was increased. The elevated iron is not unexpected as the NUA treated plots had higher soil iron concentration (Appendix Table 7b). Ingested iron, along with sulphur (which is also high in NUA, Table 1), is an antagonist of copper absorption and this could well be responsible for the reduction in liver copper (Howell and Gawthorne 1987). Copper levels in the pasture (Appendix Table 7a) were adequate, but at some times close to the upper limit of the marginal range (8 mg/kg, with no association with NUA application). This would suggest that reduced copper status in the grazing sheep may have, at times, been more likely due to interactions with other minerals.

If the control animals had been more likely to ingest soil then a downward pressure on copper status may have also been seen in those animals. Diagnostic records from the Animal Health Laboratories, Department of Agriculture, Western Australia, indicate that reduced liver copper concentrations, associated with elevated iron, is not uncommon during late summer/autumn (Masters 1982).

Plasma phosphorus was reduced with increasing NUA application at both day 57 and at the end of the trial in 2004. The reason for this decrease is not known, but NUA absorbs phosphorus (Hamon and McLaughlin 2002) and so perhaps reduced concentration in the pasture. Perhaps ingested NUA could have impacted on phosphorus absorbed within the animal.

Although manganese is considered an essential element, deficiency in grazing animals in this State is virtually unknown. Manganese concentration in NUA is high (Table 1 and Appendix Table 7b), and so this may be responsible for the small elevation of liver manganese in sheep grazing on the 150 t/ha plot in 2004 (Table 6a). This increase was most likely due to ingestion of NUA amended soil since NUA did not elevate pasture manganese (Table 7a). Summers *et al*. (2003) reported that NUA caused a small but significant decrease in pasture manganese, while in contrast Hamon and McLaughlin (2002), in a pot study, reported a large increase (average of 714 mg/kg dry weight). Since high manganese intake (e.g. 400 to 700 mg/kg) can reduce growth rate in sheep (Grace 1973), factors that could lead to increased intakes, such as its mobilisation in the soil or increased soil/NUA ingestion, should be considered in future studies. As the Mn^{2+} (available manganese) level in soil depends on oxidation-reduction reactions, all factors influencing these processes have impact on availability. These include soil pH, organic matter content, microbial activity and soil moisture (Norvell 1988).

Manganese has been reported to reduce cobalt and iron absorption (Underwood and Suttle 1999), but we found evidence of increased iron and cobalt status in sheep grazing on the 150 t/ha plot. This is not surprising given the relatively high concentration of both of these elements in NUA (Table 7b). Cobalt is required for vitamin B12 status and so grazing sheep or cattle on NUA amended paddocks might help prevent deficiency.

Minerals with GEL or ML limits

Grazing sheep on NUA amended pasture was not associated with heavy metals exceeding GEL or ML limits in liver and or kidney (Tables 5b, 5c and 6b). Initial testing of samples gave liver and kidney concentrations of mercury greater than the ML in 2003 and liver concentrations of mercury, lead and arsenic greater than the respective ML or GEL in 2004. On re-testing at a laboratory that specialises in residue testing (National Measurement Institute) it was apparent that initial results were falsely elevated. With the exception of mercury in 2004, all metals now fell within the respective GEL or ML limits. In the case of mercury, liver concentrations that exceeded the GEL occurred in the baseline and control groups, and so there was no association with NUA (Table 6b). These results are not surprising since NUA/soil concentrations for these metals would not be considered high (Table 1), and there was also no evidence from the pasture tissue analysis that these elements were concentrating in the plant tissues from the NUA treated sites compared to the control site (see Appendix Table 7a).

The reason for the higher concentrations of these elements at the first round of testing could be due to a number of analytical factors, for example the sensitivity for these elements may have been inappropriately set at calibration. Both arsenic and mercury have a reputation for being difficult to measure (pers. comm. Dr Jeff Proudfoot, CSBP).

Toxic elements

There were no indications of any element being elevated to possible toxic levels in 2003 or 2004. In 2004 some sheep in the NUA 150 t/ha group did have aluminium liver concentrations that were of concern, but at re-testing concentrations were found to be very low (Table 6c). This finding is further supported by the low aluminium concentrations in NUA (Table 1) as well as in pasture grown on the NUA treated plots (Appendix Table 7a; Underwood and Suttle 1999).

Elements in lung tissue

Absorption of minerals via the respiratory tract can be an important route for the entry of minerals into the body. Particles of NUA would be small enough to be inhaled but possibly not small enough to reach the alveolar lining of the lung (less than a few tenths of a micrometre in diameter). Measurement of minerals (particularly those high in NUA) in lung tissue could give some evidence of NUA dust being deposited into the lungs during grazing; although it is impossible to make any distinction between "endogenous" lung tissue minerals and those inhaled. In 2003, cobalt, iron and manganese were significantly increased in lungs in sheep grazing on the 150 t/ha plot, while in 2004, thorium (not measured in 2003), lead and iron increased in sheep on the 150 t/ha plot compared to controls. Sheep in 2004 would be expected to have been exposed to more dust because of the greater grazing pressure. While these results are inconsistent across years there is some evidence that particles of NUA were absorbed into the lungs. This is based mainly on thorium data collected in 2004. Thorium levels are comparatively high in NUA (Table 1), and thorium is poorly absorbed through the gastrointestinal tract but enters the body primarily through the lungs (ATSDR 1990). The extent of absorption of thorium into other body tissues from the lungs would depend largely on the solubility of the thorium salt. Liver thorium concentrations were not significantly higher in sheep on the NUA plots, suggesting that the thorium in lungs was poorly absorbed. However with larger numbers of animals and longer grazing periods a significant increase may have developed (Table 6b).

NUA particles would most likely be subjected to mucociliary clearance, with a certain percentage of particles swallowed; with the dominating factor in absorption being solubility of the mineral salt. Elucidating the impact of respiratory absorption on mineral loads in sheep grazing on NUA treated paddocks would require further experimental work.

Impact of NUA on wool

Grazing sheep on NUA amended plots resulted in significant contamination of the wool, with large increases in mineral levels compared to control sheep (Table 6d). In order to determine if the NUA could be easily removed, wool samples were cleaned by a bench method that simulates commercial scouring. The results showed (both in wool colour and mineral analysis) that NUA contamination would have no impact on wool processing. NUA might, however, impact on the effluent from scouring by increasing mineral content.

Radionuclides

While radioactive compounds in NUA are beyond the scope of this study we obtained some preliminary data. Uranium was below the level of detection in tissues but obviously present in NUA (significantly higher in unwashed wool in NUA sheep compared to controls and baseline sheep; Table 6d).

As discussed above, there were increased concentrations of thorium in lung. In humans exposed to thorium through inhalation (e.g. dust at mining sites) there is an increased risk, in the longer term, of cancer of the lung, pancreas or liver. The implications of this on the health of grazing animals is unknown, as development times for cancer differ considerable between species as well as threshold limits.

When thorium emits alpha particles it decays into other daughter radionuclides (e.g. radium-226 and radium-228). The possibility of measuring these two radium isotopes in tissues was explored. Samples of lung and liver from a sheep on the 150 t/ha plot and a baseline sheep were sent to the Australian Nuclear Science and Technology Organization (ANSTO) for measurement of radium 226 and 228 by gamma spectrometry. Unfortunately the method employed was not sufficiently sensitive. Given the levels of thorium in the lungs of sheep and the results of radium isotopes analysis ANSTO have suggested measuring alpha emission if there is to be any future testing.

Summary

Based on the results from these grazing trials, NUA poses no threat to producers in terms of toxicity to grazing sheep or accumulation of heavy metals that might impact on the saleability of meat. However these trials were limited by the number of animals and the short grazing time. While no deficiencies of essential minerals developed, there were some effects, namely phosphorus and copper status decreased and iron and manganese status increased. Although there is no evidence from these trials that manganese was of concern, its concentration in NUA is high, and high intakes can decrease production in sheep. Factors that could mobilise manganese in NUA should be considered in future trials. Thorium levels were elevated in lungs of sheep grazing on the 150 t/ha plot. Thorium can cause health problems in humans but the significance of this finding is not known. NUA contaminated wool but was easily removed.

Figure 1 0 t/ha 29 October 2003, about midway in the trial

Figure 2 150 t/ha 29 October 2003, about midway in the trial

Figure 3 0 t/ha 26 October 2004, 3 weeks before the end of the trial

Figure 4 100 t/ha 26 October 2004, 3 weeks before the end of the trial

Figure 5 150 t/ha 26 October 2004, 3 weeks before the end of the trial

Figure 6 0 t/ha, end of trial, 2004

Figure 7 100 t/ha, end of trial, 2004

Figure 8 150 t/ha, end of trial, 2004

Table 7a. Mean analysis of pasture tissue from unamended and amended

NUA rate	Date	Al	B	Ba	Ca	Cd	Co	Cu	Fe	Mg	Mn	Mo	Ni	Pb	Sn	Th	U	Zn
		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
0	11/09/2003	38	7.7	19.2	3205	< 0.1	< 0.1	13.7	82	1615	214	1.1	2.7	< 2	0.3	0.03	0.03	31
	7/10/2003	33	< 5	15.7	2568	0.2	0.2	10.3	74	1009	146	0.3	2.3	< 2	0.6	0.16	0.015	30
	12/08/2004	33	< 5	29.4	5638	0.3	< 0.1	9.3	54	1392	267	0.7	1.3	2.3	0.6	0.06	< 0.01	64
	20/09/2004	< 20	< 5	18.9	2843	0.2	0.4	8.3	43	969	136	0.3	< 1	2.7	0.8	0.09	< 0.01	39
50	11/09/2003	36	6.7	1.4	2432	< 0.1	0.2	18.3	96	1423	182	0.7	7	< 2	0.3	0.02	< 0.01	34
	7/10/2003	23	< 5	1.3	1398	< 0.1	0.1	8.0	59	1012	129	0.3	1.3	2.7	1.0	0.08	0.01	24
	12/08/2004	36	< 5	4.9	7303	< 0.1	0.2	8.3	130	1484	309	0.6	1.3	2.7	0.6	0.08	0.015	23
	20/09/2004	< 20	< 5	4.5	3746	< 0.1	0.4	9.0	85	968	228	0.2	2.3	3.3	0.9	0.15	0.01	38
100	11/09/2003	41	< 5	1.1	2210	< 0.1	0.1	19.3	81	1422	155	0.7	3.7	< 2	0.3	< 0.01	< 0.01	35
	7/10/2003	22	< 5	1.3	1420	< 0.1	< 0.1	10.0	60	1080	98	0.3	1.7	3.7	1.4	0.03	< 0.01	35
	12/08/2004	46	< 5	2.4	6946	< 0.1	0.3	11.0	277	1418	273	0.5	1.3	2.3	0.6	0.18	0.02	26
	20/09/2004	< 20	< 5	4.0	3535	< 0.1	0.4	8.0	59	912	150	0.2	< 1	< 2	0.6	0.13	< 0.01	40
150	11/09/2003	77	< 5	1.0	2176	< 0.1	0.1	8.7	81	1366	145	0.7	2.3	< 2	0.3	0.03	< 0.01	27
	7/10/2003	24	< 5	0.8	1588	< 0.1	0.1	13.7	85	1216	140	0.4	1.3	4	1.3	0.03	< 0.01	47
	12/08/2004	40	< 5	2.9	7819	< 0.1	0.6	11.3	386	1415	330	0.7	2.7	2.3	0.6	0.29	0.03	26
	20/09/2004	< 20	< 5	1.5	3787	< 0.1	0.5	6.7	76	975	157	0.2	< 1	< 2	0.6	0.11	< 0.01	36

Note:

As (< 1 ppm), Cr (< 2 ppm), Hg (< 0.01 ppm) and Se (< 2 ppm).

There appears to be an issue with all the samples taken on 12 August 2004 - there was a significant spike in the concentration of most elements for all the trial sites including the control.

Table 7b. Soil analysis of trial site including unamended site

Elements	Units	Detection	100 t/ha pre-profile		0 t/ha		50 t/ha		100 t/ha		150 t/ha	
Depth (cm)			0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20	0-10	10-20
Al	%	0.02	0.9	1.1	0.8	1.0	0.9	1.2	0.7	1.0	0.6	0.7
As	ppm	1	4.0	4.0	3.7	4.0	5.0	6.0	4.0	4.0	4.0	3.3
Ca	ppm	10	381	356	422	328	13,009	1,662	20,738	3,414	40,365	7,571
Co	ppm	0.1	3.4	2.0	2.2	2.3	14.9	4.3	22.1	5.5	41.6	9.3
Cu	ppm	1	3.3	2.3	3.0	3.0	7.3	3.3	11.3	4.3	13.7	5.0
Fe	%	0.01	2.2	1.1	1.4	1.4	3.0	2.0	2.9	1.9	4.1	2.2
Hg	ppm	0.01	0.01	0.01	< 0.01	0.01	0.01	0.02	< 0.01	0.01	0.01	0.01
Mg	%	0.01	0.03	0.02	0.03	0.04	0.07	0.04	0.11	0.04	0.17	0.05
Mn	ppm	20	717	277	429	434	2,372	721	3,577	969	6,554	1,579
P	ppm	20	97.7	67.0	90.7	71.0	149.7	99.0	170.3	121.3	185.0	128.7
P	mg/kg	1	14.7	12.0	10.7	8.0	15.0	17.0	17.7	15.3	21.3	19.7
Pb	ppm	2	12.7	10.0	10.3	10.3	11.7	12.0	13.0	11.7	9.0	10.7
Se	ppm	2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Th	ppm	0.01	18.5	9.6	12.8	11.3	25.9	15.6	28.2	15.7	42.0	19.1
U	ppm	0.01	1.2	0.7	0.9	0.8	1.7	1.1	1.7	1.0	2.3	1.2
Zn	ppm	1	22.7	11.7	14.3	12.3	17.7	15.7	19.0	17.3	15.3	20.3

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