Vanadium Mining and Cattle Health

Sentinel studies, epidemiological and veterinary public health issues

(met een samenvatting in het Nederlands)

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Chapter 1

GENERAL INTRODUCTION

B. Gummow

The White Rabbit put on his spectacles. *"Where shall I begin, please your majesty?"* he asked.
"Begin at the beginning," the King said gravely, *"and go on till you come to the end; then stop."* - Carroll (1865)

Vanadium: What is it?

The chief credit for the discovery of vanadium must be given to Nils Gabriel Sefström, who when working on a sample of iron ore from Taberg in Sweden, recognised an unusual constituent, whose salts could be prepared from slags resulting from the treatment of cast iron. In 1831 he succeeded in making an oxide of an entirely new element. Because this formed beautiful multicoloured compounds, his assistant Berzelius named it vanadium, after Vanadis the legendary goddess of beauty from the far north. The separation of the element from its salts proved a hard task and it was only in 1869 that Roscoe was able to tell the Royal Society of the isolation of a little powdery vanadium, of 95.8% purity (Faulker Hudson, 1964). However it was not until 1927, nearly 100 years after its discovery, that Marden and Rich (1927) made the first ductile metal of 99.7% purity.

Vanadium is classified as a transition element on the periodic table with the atomic number 23. It has a molecular weight of 50.94, a melting point of 1890 °C and boils at 3380 °C. It has six oxidation states (l-, 0, 2+, 3+, 4+, and 5+) of which 3+, 4+, and 5+ are the most common (CRC handbook of chemistry and physics, 1977).

Where is vanadium found?

Vanadium is widely distributed in nature and the average level of vanadium in the earth's crust is normally 100-150 ppm (Faulker Hudson, 1964; Richie, 1985; Waters 1977). The prevalence of vanadium exceeds that of such well-known metals as copper and lead (Nriagu, 1998), and equals that of zinc and tin (Byerrium *et al*., 1974; Windholz, 1983; Greyson, 1983). Vanadium compounds exist in over 50 different mineral ores at concentrations of between 10-4100 ppm and in association with fossil fuels, particularly coal (at concentrations of between 19-126 ppm in ash) and crude oil (at concentrations of between 3-257 ppm) (Nriagu, 1998). There are few ores from which vanadium can be recovered economically as a single product.

Most of the vanadium production comes as by-products and co-products in the extraction of other elements such as iron, phosphorus, and uranium. Ores from which vanadium can potentially be recovered are found in many parts of the world. About one third of the vanadium resources are located in Africa and North America, and about 24% are found in Europe and <4% in both Asia and South America (Nriagu, 1998). About 83% of vanadium recently

produced from mines comes from vanadiferous magnetite (Fe_3O_4) in South Africa, China and Russia (Hilliard, 1992). The remaining 17% of worldwide vanadium production from primary sources is recovered from the oil industry.

South Africa is the worlds leading producer of vanadium and accounts for 50% of the current global output. Other producing countries include Russia, China and the USA. Western Australia has large deposits of magnetite ores containing vanadium, and the tar sands of Alberta, Canada, represent a huge reservoir of vanadium (Nriagu, 1998). These sources of vanadium remain largely untapped.

Industrial use and economic importance of vanadium

The vanadium oxides are most often used by industry, primarily in the manufacturing of steel, where it is used as ferrovanadium or as a steel additive (Reilly, 1991; Toxicological profile for Vanadium, 1992). It has good corrosion resistance to alkalis, sulphuric and hydrochloric acid, and salt waters, but the metal oxidizes readily above 660 °C. Vanadium is thus used in producing rust-resistant, spring, and high-speed tool steels. It is also used in the production of components for aircraft engines and weapon systems, making it a strategic mineral for armament manufacturers. In addition, because the metal has good structural strength and a low-fission neutron cross-section, it is useful in nuclear applications.

Vanadium foil is used as a bonding agent in cladding titanium steel. Vanadium pentoxide is used in ceramics and as a catalyst. It is also used as a mordant in dying and printing fabrics and in the manufacture of aniline black (CRC handbook of chemistry and physics, 1977). Small amounts of vanadium are used in making rubber, plastics, and certain other chemicals (Reilly, 1991; Toxicological profile for Vanadium, 1992).

The presence of vanadium in living organisms

When examining various fruits, vegetables and plants, Bertrand (1941) detected vanadium in all the 62 specimens studied. He found its average concentration in the higher plants to be 1 ppm, roots usually containing more than seeds and leaves; root nodules of most leguminous plants had about 4 ppm, occasionally up to 12 ppm. Fungi contained less, usually 0.5 ppm, but exceptionally as much as 112 ppm.

Vanadium is found in high concentrations in certain marine organisms, particularly ascadians and sea-squirts belonging to the family tunicates. It seems that many of these creatures accumulate the element in their blood, gut and other tissues. It is thought that vanadium may function as a form of oxygen carrier in these animals in the same way that erythrocruorin of invertebrates, chlorocruorin, the green blood pigment of certain annelid worms; haemerythrin in spinuculds worms; and haemocyanin in various molluscs and arthropods act as oxygen carriers. Unlike haemoglobin and haemovanadin, these pigments are not confined to blood cells but are present in the plasma as conjugated proteins, having a metal incorporated in the molecule (Faulkner Hudson, 1964; Nriagu, 1998).

Vanadium is also found in a wide variety of tissues of higher vertebrate animals. Puls (1989), gives a summary of vanadium levels found in cattle, sheep, dogs, pigs, chickens and ducks. Normal liver concentrations for cattle are reported as 6-7 μg/kg (wet weight). These appear to be much lower than the liver concentrations reported for sheep (100—220 μg/kg), dogs (30-50 μg/kg) and chickens (18-38 μg/kg) but of the same order as ducks (0.7-2 μg/kg).

Various people have looked for vanadium in human tissues (Koch *et al.*, 1956; Perry & Perry, 1959). One of the more rigorous studies using emission spectrography was done by Tipton (1960). Her specimens were taken at autopsy from the victims of sudden death, usually from trauma and sometimes from cardiovascular and other diseases. The tissues examined were aorta, brain, heart, kidney, liver, lung, ovary, pancreas, prostate, spleen, and testis, all of which appeared macroscopically normal. In a very small percentage of specimens, vanadium could be found in the liver, prostate and spleen. In about half the specimens it was found in the lungs. The average concentration in these organs was usually less than 1 mg of vanadium/kg of ashed material, except for the lung, which had levels of up to 5 mg/kg.

Vanadium as an essential trace element and therapeutic agent

Vanadium has been shown to be an essential trace element for a variety of animal species (Puls, 1989). Vanadium deficiency is associated with stunted growth, impaired reproduction, altered red blood cell formation and iron metabolism and changes in blood lipid levels. There is a growing belief among health experts that the metal can play a similar role in humans.

During the 1980's, vanadium was reported to mimic the metabolic effects of insulin in rat adipocytes (Lu *et. al.*, 2001). In the 1990's, vanadium was found to act in an insulin-like manner in muscle and liver as well. Subsequent studies revealed that the action of vanadium salts is mediated through insulin-receptor independent alternative pathways. The investigation of the antidiabetic potency of vanadium soon ensued. Vanadium therapy was shown to normalise blood glucose levels in STZ-rats and to cure many hyperglycemia-related disorders. Therapeutic effects of vanadium were then demonstrated in type II diabetic rodents, which do not respond to exogenously administered insulin (Goldwaser *et. al.*, 2000). Overcoming vanadium's toxicity, has however, remained the major obstacle in using vanadium as a therapeutic agent.

Several reviews describe the interactions of vanadium with tissues, cells and enzymes in more detail (Rehder, 1992; Dafnis & Sabatini, 1994; Leonard & Gerber, 1994; Zelikoff & Cohen, 1995; Nriagu, 1998).

Vanadium as a pollutant and its toxic effects

Biochemical effects

It has long been known that vanadium is toxic to both man and animals and many of the symptoms of acute poisoning were already described at the turn of the 20th century. Yet the pathogenesis of vanadium poisoning is still poorly understood. Scientists are only now beginning to understand the biochemistry of vanadium and with this understanding, the pathogenesis of vanadium poisoning is gradually being unravelled. It has been established that certain forms of vanadium are more toxic than others (Table 1) and we now know that vanadium is capable of modifying the activity of a number of enzymes, including Na and K-ATPase, which is important in muscle contraction, tyrosine kinase, which is located in growth factors, oncogenes, phosphatases and receptors for insulin (Berner *et. al.* 1989, Nriagu, 1998). Vanadium also inhibits the enzyme cholinesterase, which results in deficiency of choline and affects the metabolism of the sulphur-containing amino acid cysteine. The vanadate and vanadyl ions (Table 1) further inhibit Ca and Mg-ATPase, which are important in synaptosomal membranes in nervous tissue as well in facilitating muscle contraction (Nriagu, 1998).

Table 1 Toxicologically significant vanadium compounds

Name of compound	**Chemical Formula**
Vanadium pentoxide	V_20_5
Sodium metavanadate	$NaV0_3$
Sodium orthovanadate	Na_3V0_4
Vanadyl sulphate	$V0S0_4$
Ammonium metavanadate	NH_4V0_3

Toxicity in man

Dietary vanadium does not appear toxic to man, at least at low levels normally encountered in foods. Industrial exposure of workers to vanadium dusts is a well-recognised occupational hazard. Vanadium pentoxide (V_2O_5) dust is usually encountered in occupational settings, and the primary route of exposure for humans is via inhalation. Responses to industrial exposure are thought to be acute rather than chronic, involving irritation of the eyes and respiratory system in the form of conjunctivitis, bronchospasm, bronchitis and asthma-like symptoms. An estimated 25 % of soluble compounds may be absorbed from the lungs (WHO, 1987; Lauwerys & Hoet, 1993). V_2O_5 is specifically reported to be nearly 100 % absorbed by inhalation (Lewis, 1995). Information on the effects of the other vanadium compounds comes mainly from oral studies in animals. Absorption via the oral route appears to be low (Friberg, 1979).

Other symptoms of vanadium poisoning described in humans include weakness, nausea, vomiting, anorexia, tinnitus, headache, dizziness, green discolourisation of the tongue, palpitations, transient coronary insufficiency, bradycardia with extra systoles, dermatitis, anaemia, leucopoenia, leukocyte granulation and lowering of cholesterol levels (Faulkner Hudson, 1964; Hunter, 1975; Friberg, 1979; Reilly, 1991). Recent studies in humans have shown that correlations existed between the vanadium levels in urine and serum and cognitive deficits. When vanadium concentrations were around 14.2 µg/l in urine a reduction in

neurobehavioral abilities were observed, particularly visuospatial abilities and attention (Barth *et. al.*, 2002).

The long-term effects of exposure in humans do not appear to have been extensively studied (Toxicological Profile for Vanadium, 1992; SIMRAC, 2000).

Biomarkers specific for exposure to vanadium include the presence of vanadium in the urine (Gylseth *et. al.* 1979; Kiviluoto *et. al.* 1981; Lewis, 1959; Orris *et. al.* 1983; Zenz *et. al.* 1962) and a green discoloration of the tongue (Lewis, 1959), the latter resulting from the direct accumulation of vanadium pentoxide.

Toxicity in monogastric animals

A considerable amount of work has been done on vanadium over the years using mongastric animals as models. These began as long ago as 1876 when Priestley (1876) showed that sodium vanadate solutions, when given by various routes, was intensely poisonous to the pigeon, guinea-pig, rabbit, cat and dog (Faulkner Hudson, 1964). He found that 9.18-14.66 mg V_2O_5/kg could kill a rabbit when injected subcutaneously. It was apparent in these experiments that vanadium had two chief modes of action; a central effect on the nervous system causing drowsiness with convulsions, followed by a gradual paralysis of respiration and motion, and an effect on the alimentary tract causing abdominal pain, with diarrhoea and bloody stools. These original observations have been confirmed by others (Puls, 1989). Proescher, Seil & Stillians (1917), went on to use both large and small experimental animals, including birds and fish, and some of these results are reported by Faulkner Hudson (1964). The LD_{50} in rats, injected subcutaneously with ammonium metavanadate, has since been estimated more precisely at 22.7 mg V_2O_5/kg (Massmann, 1956). The horse and rabbit were shown to be especially sensitive to vanadium, while the rat and mouse were relatively resistant. Jackson (1911 and 1912) showed that vanadium, when administered intravenously to dogs, produces intense vasoconstriction in the spleen, kidney and intestine, with associated rise in temperature. The dominant findings of this early work were therefore the depression of the respiratory centre, marked vasoconstriction of the visceral arteries, and inflammatory lesions in the lung, kidney and intestine. A summary, which includes more recent work, is given by Puls (1989) and differs little from the original work carried out. All these observations however, were based on acute or subacute exposure,

usually by iatrogenic means. Until recently little work had been done to examine the effects of low dose, long term exposures. One of the first long term studies to be carried out in rats and mice was published as recently as 2001 (National Toxicology Program technical report, 2001) and because of the nature of the results, there has been some debate over the methodology used. The study reported neoplastic effects for the first time in the form of bronchiolar and alveolar adenoma or carcinoma in rats that had been exposed to inhalation of 2 mg/m^3 V_2O_5 for 2 years. No genetic toxicity however, could be shown when using *Salmonella typhimurium* gene mutations. The other lesions described in this study were non-neoplastic lesions of the nose, larynx, and lung in the form of epithelial hyperplasia, inflammation, fibrosis and alveolar histiocytosis. There don't appear to have been any studies of this duration carried out in other animal species or man.

Toxicity in ruminants

The work in this thesis looks in more detail at the effects of exposure to vanadium in ruminants. Prior to this study little work had been done in ruminants and those studies that had been carried out appear to have been primarily acute or sub-acute experimental studies or field outbreaks, usually involving exposure to high doses of vanadium. Hansard *et al*. (1978, 1982a, 1982b, 1982c) did a number of studies using sheep. They found that in sheep doses of 9.6-12 mg vanadium / kg body weight given in a gelatine capsule an hour after feeding caused a decline in feed intake and diarrhoea. Necropsy of these animals showed extensive mucosal haemorrhage of the small intestine and diffuse subcapsular haemorrhages of the kidneys. No difference in toxicity could be shown between ammonium metavanadate, calcium orthovanadate and calcium pyrovanadate. A dose of 40 mg NH_4VO_3 / kg body weight in gelatine capsules caused death in two out of three sheep within 80 hours.

One of the first experimental studies on vanadium poisoning in cattle was that of Platonow and Abbey (1968). These were acute experimental studies carried out in calves and are the ones usually referred to by others when vanadium toxicity in cattle is described (see Chapter 2 for a more detailed description of signs in cattle). Frank *et al*. (1996) reports an earlier paper published in 1964 of toxicity in cows that ingested fuel oil soot (ter Heege *et. al*., 1964). Frank *et al.* (1990) reported acute vanadium poisoning of cattle in northern Sweden after grazing on pastures fertilized with basic slag and followed this work up in 1996 with a second paper on the

event that included tissue levels in cattle slaughtered in other parts of Sweden as well (Frank, *et al.* 1996). McCrindle, *et al*. (2001) reported on acute vanadium poisoning in cattle illegally grazing in an area where a vanadium spill had occurred on a South African mine.

Comparative kinetics of vanadium

Animal data (Conklin *et. al.*, 1982; Oberg *et. al.* 1978; Rhoads & Sanders, 1985; Roshchin *et. al.* 1980) and limited human data (Diamond *et. al.* 1963; Gylseth *et. al.* 1979; Schroeder *et. al.* 1963) are available on the kinetics of vanadium. From studies done in humans, rats, mice, and dogs it would appear that vanadium kinetics between monogastric animals and humans are similar. Despite this however, as with any particulate substance, extrapolations on inhalation absorption rates from animals to humans is still difficult. Only one set of kinetic studies appear to have been done in ruminants (sheep). From these studies it appears that the kinetics in ruminants may be slightly different to those of monogastic animals due to the difference in the physiology and anatomy of the rumen versus stomach (Patterson *et al.*, 1986). Significant absorption of vanadium occurs from the upper gastro-intestinal-tract (GIT) of sheep and concentrations of vanadium in the upper and lower GIT of sheep were respectively ten and hundred fold greater than in the blood (Patterson *et al.*, 1986).

The relationship between vanadium mining and cattle farming in South Africa

Although vanadium poisoning is uncommon, vanadium forms a large component of the mining industry in South Africa and increasing concern is being expressed about the impact of this industry on livestock in South Africa (Wates, 1996). South Africa is one of the world's greatest sources of vanadium and has a substantial amount of industry associated with vanadium mining and processing. The areas where these mining and processing industries are located are in many cases the same areas that farmers utilise for farming. However, there appears to have been little work done in ruminants with respect to vanadium exposure. Yet, all the documented field outbreaks of vanadium toxicity in livestock in South Africa have involved cattle and the mining industry (Unpublished archival records of the Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110, 1961/1962, 1975/1976, McCrindle, 2001).

Very little is known about the effects of chronic low dose exposure of cattle to vanadium or what constitutes safe grazing for cattle in areas of the country where vanadium is known to occur.

The use of cattle as environmental sentinels for the mining industry

The primary goal of an animal sentinel system is to identify harmful chemicals or chemical mixtures in the environment before they might otherwise be detected through human epidemiological studies or toxicological studies in laboratory animals. Once identified, exposures to them could be minimised until methods can be devised to determine specific aetiological agents or until suitable prophylactic measures can be established. Animal sentinel systems might provide additional valuable time in which to search for the answer.

Animal sentinel systems therefore have potential value as early warning systems for new hazards, as indicators of potential human exposure to complex mixtures or in complex environments, and as monitors of the effectiveness of remedial measures or other environmental management actions. In these applications, data from animal sentinels are usually used qualitatively, but there is at least potential for semi-quantitative assessments.

Animal sentinel data should include data obtained from epidemiological (descriptive and analytic) studies and from animal and food chain monitoring programmes. Data from animal sentinel studies can often be obtained more quickly than data from human epidemiological studies, because the ideal sentinel responds to toxic insults more rapidly than humans do (long before clinical manifestations of disease) and at environmentally relevant doses. In addition, animal sentinels, like humans, are simultaneously exposed to complex and variable mixtures of chemicals and other environmental agents. Some environmental mixtures have been shown to be more toxic than would be predicted based on their principal chemical constituents (Hornshaw et al., 1983). These characteristics of animal sentinel studies offer important advantages over laboratory animal studies, in which animals are usually exposed to high, constant doses of a single chemical substance that is under investigation. Thus, the use of animal sentinels constitutes an approach to identifying hazards and estimating risks in circumstances similar to those in which actual human or surrounding animal exposures occur.

For example, animal sentinels were used as a community and public health tool in a study of sheep living around a zinc smelter in Peru. This study demonstrated the feasibility of establishing animal sentinels around point sources of pollution (Reif et al., 1989). Heavy-metal exposures were documented in sheep pastures up to 27 Km downwind from the smelter. A mortality database for the population of 177 000 sheep was used in an attempt to relate heavy-metal burdens to health effects, including cancer. No relationship between hepatic arsenic concentrations and other heavy metals was found for pulmonary adenocarcinoma, a neoplasm hypothesised as a *priori* to be related to arsenic exposure.

Other industries where cattle have been successfully used as sentinels are those that produce fluoride. These industries include aluminium, steel and copper smelting, chemical manufacture, ceramic production, and coal-based electricity generation (Shupe *et al.*, 1979). Dairy and beef cattle near such facilities have been severely affected by fluorosis because of airborne contamination of forages. These animals have acted as suitable sentinels of fluoride emissions and have been used by industry and regulatory agencies to assess the effectiveness of emission control measures.

More recently, reindeer in the Arctic and other foraging animals were used as sentinels of radioactivity resulting from the April 1986 nuclear-reactor accident at Chernobyl, USSR, by virtue of the radioactivity in their flesh and milk (National Research Council, USA, 1991)

In South Africa, cattle proved to be a suitable sentinel for detecting copper air pollution originating from a copper smelting plant, long before pollution monitoring authorities using conventional methods were aware that pollution was indeed taking place (Gummow *et al.*, 1991).

Despite these examples, most uses of domestic animals to monitor environmental pollutants have been unplanned by-products of veterinary services directed at alleviating health problems in the animals involved, rather than organised monitoring programmes. Few programmes for using healthy domestic animals as biologic monitors have until recently been proposed (Swabe *et al.*, 1971; Buck, 1979). The work in this manuscript serves as an example of an organised monitoring programme.

Scope of the thesis

In 1990, a farmer began experiencing losses amongst his calves, which were literally wasting away before dying. The private veterinarians consulted were at a loss as to what the cause of the problem could be. Initially they thought it might be copper deficiency due to high background molybdenum levels. The farmer was also blamed for poor husbandry and eventually an epidemiologist (the author) was called in to try and solve the problem. A disease outbreak investigation was carried out to find out what the cause of this "new disease" could be and the results of this investigation are published in Chapter 2. This was the first published article on vanadium poisoning in South Africa and the work went some way to try and resolve the pathogenesis of vanadium poisoning in cattle, which is still poorly understood.

Faced with a vanadium-contaminated environment, an attempt was made to find a prophylactic treatment for the farmer. No treatments for vanadium poisoning had ever been tried in ruminants before and so an experimental study was designed to test one of the treatments reported to have been successful in mice, calcium disodium ethylenediamine tetraacetate. The results of this attempt at finding a suitable prophylactic treatment for cattle are published in Chapter 3. In doing this work it became apparent that a lot more knowledge of the disease in cattle was needed before a suitable prophylactic treatment was likely to be found.

In 1998, a vanadium spill at another mining site resulted in the death of some communal cattle. It was uncertain if the deaths were due to vanadium but the mine compensated the farmers for their losses, despite the fact that the cattle were trespassing on mine property. This resulted in a flurry of claims against the mine by surrounding farmers. Having heard of the previous work done during the 1990 outbreak, the mine approached the author (B. Gummow) for a solution that would indicate whether the mine was putting the surrounding farmers' cattle at risk or not. From this was born the idea of running a sentinel herd of cattle on the mine's property, with the objectives of studying the chronic effects of vanadium, of seeing whether cattle were in fact being affected by mining operations, and whether cattle could be used as an early warning system for detecting problems in pollution control. This had never been done before and the concept of farming was as foreign to the mining industry as the concept of mining was to the veterinary profession. To sell this idea to the mine was unique on its own; to set up a trial that

would meet these objectives required many hurdles to be overcome, beginning with the infra-structure and management of the cattle on a mine.

It soon became apparent that methodology did not exist that would enable a reasonably accurate measure of how much vanadium the cattle were taking in. Existing plume dispersion models took no account of oral intake, which was a major component of intake for cattle. Failure to get an accurate assessment of intake would mean that no assessments of risk could be made. The methodology for this took two years to develop as it involved inputs from experts in the mining field, physicists, chemists, soil scientists and veterinarians. Finally a working model that encompassed environmental and physiological inputs was created and a novel way of quantifying intake of vanadium was developed. This model is applicable not only to vanadium studies but can also be used for other environmental pollution studies involving similar hazardous substances. Details of the model are presented and discussed in Chapter 4. The model allowed for the first time an estimation of the no-adverse-effect-level for vanadium in cattle.

Acceptance of the model by independent referees allowed the exploration of other questions that were arising as a result of the research. The mining industry had been using urine as a biomarker of exposure for many years, yet it was always suspected that this was an unreliable biomarker. Was this really the case? If cattle were to be used as sentinels, what should be measured that would indicate problems? If cattle died from vanadium poisoning, could tissue concentrations of vanadium be used to make a diagnosis? The question of biomarkers therefore became of increasing importance for both veterinarians and the mining industry. For this reason, a wide spectrum of tissues and blood parameters were monitored at great expense in the hope of finding a better way of monitoring vanadium exposure in both animals and man. The results of this work are discussed in Chapter 5.

If cattle were being exposed to higher vanadium levels around mining or industrial areas in the world or even in areas just rich in vanadium, did this pose any threat to the consumer? Particularly as adult cattle from these areas showed little evidence of vanadium poisoning and the milk and meat of these cattle was entering the food chain even though calves were becoming ill. Chapter 6 discusses public health aspects of farming cattle in areas rich in vanadium.

Finally the question of cattle health and the pathogenesis and treatment of chronic vanadium poisoning in cattle needed to be looked at in greater detail. These were the consequences of being exposed to chronic intake of vanadium. Some aspects of this are mentioned in the general discussion (Chapter 7), but this work is ongoing and still to be written up and properly evaluated.

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Chapter 2

VANADIUM AIR POLLUTION: A CAUSE OF MALABSORPTION AND IMMUNOSUPPRESSION IN CATTLE

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ABSTRACT

An epidemiological investigation into an "illthrift" problem occurring on a dairy farm adjacent to an alloy processing unit established that the probable cause of the problem was chronic vanadium poisoning. The disease manifested initially in animals 4-18 months old which showed emaciation, chronic diarrhoea and in some cases, rhinitis, conjunctivitis and recumbency followed by death. Post-mortem (n = 17) and clinical pathology findings (n = 60) indicated that malabsorption and immunosuppression were the basis of the pathogenesis in affected animals. Eight months after the commencement of the investigation adult cows began showing evidence of emaciation, reduced milk production and an apparent increase in the number of abortions, stillbirths and dystocias.

Over a 2-year period 134 surface-soil samples, 134 subsoil samples and 134 grass samples from the farm were analyzed for various fractions of vanadium. Thirty-four of each of these samples were collected at different time intervals (autumn 1990, summer 1991 and winter 1991) and at varying distances and directions from the processing unit in order to gauge the magnitude of the problem, the distribution pattern of vanadium and to identify any seasonal trends. The remaining 100 of each of these samples were taken at 100 m intervals over an area of approximately 1 140 000 m^2 directly adjacent to the processing unit so that concentration isolines for vanadium could be drawn and the source more conclusively identified. The levels of vanadium were found to be highest closest to the mine and surface-soil levels were consistently higher than subsoil levels suggesting aerial pollution, which was confirmed by air sampling. In addition washed grass samples were considerably lower in vanadium than the unwashed samples, indicating most of the vanadium was in the dust on the plants. The highest levels of vanadium were found in the soil during the summer and on the grass during the winter. These analyses confirmed the presence of high vanadium levels ($\leq$ 1122 ppm) in the surface-soils and grass ($\leq$ 558 ppm) on the farm and showed that the major source of vanadium was the adjacent alloy-processing unit.

INTRODUCTION

In April 1990 the Onderstepoort Veterinary Institute (OVI) was approached by a private veterinarian to do an epidemiological study into the cause of "illthrift" occurring in a dairy herd in the eastern Transvaal. This paper describes the investigation and its findings.

MATERIALS AND METHODS

An initial investigation comprising 3 farm visits, was conducted. These farm visits were undertaken on the 27 April 1990 (late autumn), 15 January 1991 (midsummer) and 17 June 1991 (midwinter). On the first visit environmental factors were examined, a complete history was obtained and clinical signs were observed. The second visit confirmed the environmental findings and clinical pathological trends within the population were examined. The final visit confirmed the environmental and population trends previously found. A second investigation was initiated in April 1992 to provide more concrete information on the source of vanadium.

Environment

Soil and Plant Material

To test an initial hypothesis of copper deficiency due to high background levels of molybdenum (Mo), soil and grass samples were taken at 10 and later 12 points on the farm (Fig. 1). At each sample point approximately 500 g of subsoil at a depth of 300 mm and 500 g of surface-soil was collected. Grass in the immediate vicinity of the soil sampling point was then collected. Sampling was done in late autumn, mid summer and winter (3 times). After the first visit, each specimen was analyzed for copper (Cu), Mo and zinc (Zn) as these minerals are generally associated with Cu deficiency.

An alloy-processing unit (APU) was situated immediately adjacent to the farm and it was therefore decided to include within the analysis profile iron (Fe), chromium (Cr) and vanadium (V), which are associated with steel production, in order to rule out environmental pollution as a cause. The samples collected in April 1990 were thus analyzed for Fe, Cu, Zn, Cr, Mo and V, using standard analytical methods for atomic absorption spectrophotometry (Perkin-Elmer

Corporation, Norwich, Connecticut, USA). The samples collected in January and June 1991 were analyzed for Cu, Fe and V only.

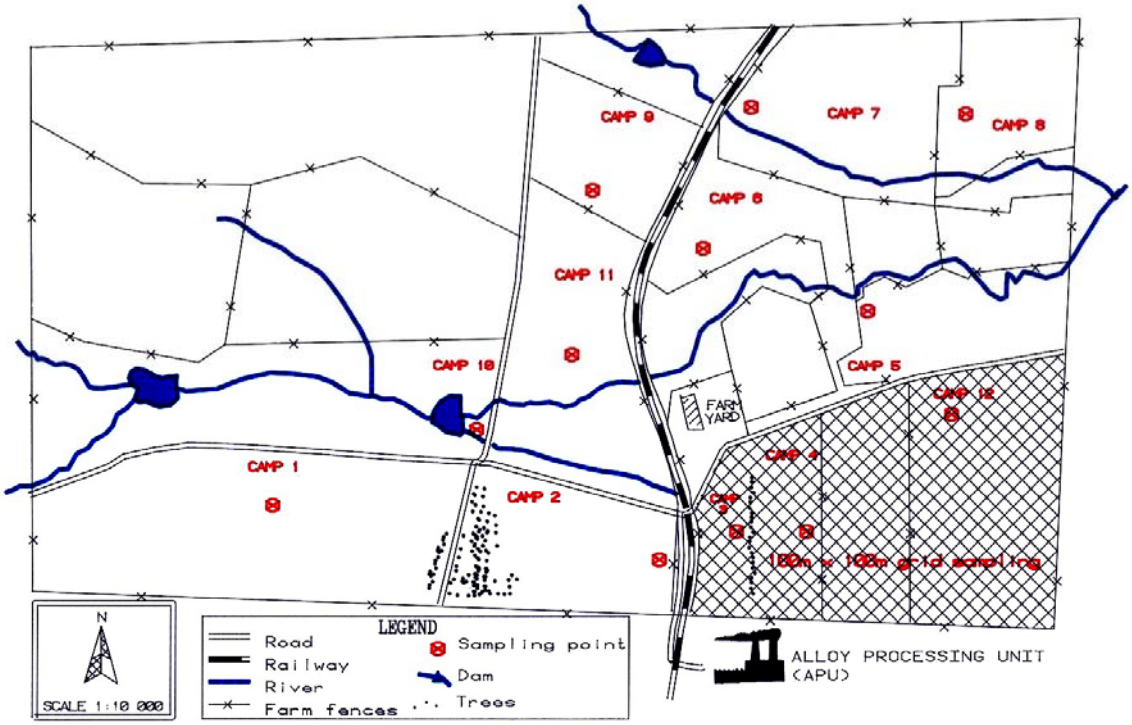


FIG. 1 Map of affected farm showing sampling points and camp numbers

A second study undertaken in April 1992 concentrated on camps 3, 4 and 12 (Fig. 1), immediately adjacent to the APU and measured V levels only. A 100 m x 100 m grid sampling was carried out over this area resulting in 100 sampling points. At each sampling point surface-soil (0-20 mm), subsoil (20-300 mm) and plant material was collected for analysis (Fig. 2 & 3). Analysis of the soil and plant material was done according to the Handbook of Standard Soil Testing Methods for Advisory Purposes (Soil Science Society of South Africa 1990). Two soil extracts were prepared, an ammonium-EDTA (NH_4EDTA) extract and a water soluble extract. Vanadium analysis was carried out on the extracted solutions using an inductive coupled plasma mass spectrometer (VG Plasmaquad PQ2 turbo plus ICPMS) and a high temperature graphite oven (Perkin Elmer) coupled to an atomic absorption spectrometer (Varian techtron AA6). Standards and blanks were tested at regular intervals during the analysis.

The plant material from the second investigation was divided into 2 equal portions. The first portion was washed with deionised water to remove any dust from the leaves and the second was left unwashed. After drying, the plant material of both portions was ground and digested with HNO_3 and $HClO_4$ to obtain a solution. The vanadium concentrations of these solutions were then determined using the methods described above.

Air Sampling Methods

An air-sampling site in the farm yard (Fig. 1) was selected since this offered an available power supply and security for the equipment. Air was drawn through a 47 mm, 8 μm pore size Nucleopore filter at about 5 *l*/min. Sampling was carried out over varying time intervals (Table 3) during the winter of 1990 and 1991 and summer of 1991. Deposit samples were collected in open plastic containers 13 cm in diameter at distances of 150 and 250 m from the processing unit over a 14 d period in the summer and winter of 1991.

Animals

Chemical pathology

Ten to 25 animals were examined clinically during each of the three farm visits. On the second and third visits blood was collected from a total of 60 animals. On the second visit animals to be bled were randomly selected from various groups as follows:

0-3 m	(n = 3)
4-7 m	(n = 5)
8-11 m	(n = 5)
Heifers	(n = 6)
Dry cows	(n = 6)
Lactating cows	(n = 6)

The animals selected on the third farm visit included those bled on the second visit and additional animals as detailed below:

0-3 m	(n = 9)
4-7 m	(n = 5)
12-15 m	(n = 4)
16-19 m	(n = 5)
Adult cows	(n = 11)

On both visits venous blood was collected for the determination of the following parameters:

Total serum protein (TSP)
Albumin (Alb)
Globulin (Glob)
Blood-urea nitrogen (BUN)
Gamma-glutamyltransferase (GGT)
Aspartate aminotransferase (AST)
Creatine phosphokinase (CK)
White cell count (WCC)
Red cell count (RCC)
Haematocrit (Ht)
Blood glucose

Blood obtained on the third farm visit was also tested for blood creatinine (Creat) levels and lymphoblast transformation responses to plant lectins. Standard methods of analysis were used in all cases (Kristensen, Kristensen & Lazary 1982; Jain 1986; Kaneko 1989).

Bacteriology

During the course of the investigation, serum was collected from nine cows which had aborted on the farm and tested for leptospira antibodies using the Microscopic Agglutination Test.

Pathology

A total of seventeen animals (cases 1-17) were necropsied between the period May 1990 to January 1992. Tissues for vanadium analysis were collected from twelve of these cases (Table 4). Complete necropsies were performed on thirteen of the seventeen cases and the remaining four cases (cases 12-15) constituted formalin-fixed tissues submitted for histopathological examination. Formalin-fixed tissues were sectioned and stained with haematoxylin and eosin (HE) according to standard methods. Where complete necropsies were performed, blood and brain smears were examined for the detection of protozoal blood parasites and heartwater colonies and, when necessary, faeces was collected for the detection of nematode eggs, coccidial oocysts and viral particles and various tissues were also collected for aerobic and anaerobic bacterial isolation.

The seventeen cases were divided into four age groups - groups A, B, C and D-as follows:

- Group A comprised five foetuses/perinatal calves (cases 1-5) which were examined according to standard methods employed at the OVI for the detection of causes of abortion/perinatal mortality in cattle.
- Group B consisted of three suckling calves (cases 6, 7 & 8).
- Group C included seven calves between 3 and 12 months of age (cases 9 - 15) and was compared to a euthanased healthy calf of about 6 months obtained from the OVI.
- Group D was made up of two adult cows, 4 and 7 years old (cases 16 and 17).

Vanadium analyses

During the second farm visit faecal samples were collected for V analyses from nineteen adult cattle. Tissues and ruminal or abomasal fluid were collected from twelve of the seventeen animals necropsied (Table 4). Samples were analysed for V content using standard methods (Boyazoglu, Berrett, Young & Ebedes, 1972).

RESULTS AND DISCUSSION

Soil analyses

The soil V results of the first investigation are shown in Table 1 and those of the second investigation in Fig. 2 & 3. The results of the first farm visit showed that Cu, Cr, Mo and Zn were not present in excessive levels in the vegetation or soil on the farm (Table 2). The pattern of distribution of these elements showed no correlation to distance or direction from the APU (Table 2) implying that these minerals occurred naturally on the farm and reflecting what should be seen if no air pollution was taking place.

The average level of V in the earth's crust is normally 100-150 ppm (Faulkner Hudson 1964; Richie 1985; Waters 1977). The average levels of V in the surface-soil of seven of the 12 camps sampled (camps 3, 2, 4, 5, 12, 7 & 8) were higher than normal (Table 1). Six of the 7 camps were situated either adjacent to the APU or north to north-east of the APU, and 1 camp (camp 2) was more north-west, but bordered on the APU. None of the other camps to the west or north-west of the APU had abnormally high levels of V.

TABLE 1 Vanadium levels (ppm) of surface-soil, subsoil and grass in relation to approximate distance and direction from APU

Camp No.	Approx. Distance (Km)	Approx. Direction	Autumn Surf. Sub. Grass	Summer Surf. Sub. Grass	Winter Surf. Sub. Grass	Mean V Surf. Sub. Grass
3	0.32	W	1055 206 290	745 461 27	991 115 180	930 261 166
2	0.49	NNW	514 508 31	313 127 4.9	204 152 85	344 262 40
4	0.52	N	274 198 8	1122 338 9.7	522 155 75	639 230 31
5	1.43	N	149 93 10	333 256 ND	86 67 70	189 139 27
2	1.49	ENE	- - -	328 264 4.6	182 127 ND	255 196 2
11	1.56	WNW	- - -	67 59 10.8	36 31 ND	52 45 5
6	1.88	NNW	58 41 12	120 80 0.8	67 98 30	82 73 14
10	1.92	WNW	44 18 ND	70 36 10.3	50 36 30	55 30 13
9	2.14	WNW	42 41 ND	51 51 0.6	48 54 ND	47 49 ND
1	2.40	W	35 22 3	92 57 0.8	61 39 20	63 39 8
7	2.73	NNE	354 215 ND	558 590 4.3	263 247 20	392 351 8
8	2.86	N	385 291 15	494 361 1.0	165 283 30	348 312 15
LEGEND: ND = not detectable (< 0.5 ppm); "-" = not determined						

TABLE 2 Analytical results of samples collected on the farm during the first farm visit (27/4/1990)

Sample No. & description		ppm Fe	ppm Cu	ppm Zn	ppm Cr	ppm Mo
Water no. 1.		1	0	0	0	
Water no. 2.		1	0	0	0	
Grass						
1	Hyperrhemia hirta.	35	0	5	0	< bl
2	Eragrostis curvula.	225	5	5	0	< bl
3	Eragrostis curvula.	30	0	0	0	< bl
3b	dead grass.	225	5	5	0	< bl
3c	Jan 1989.	95	0	5	0	< bl
3c	Jan 1990.	75	5	5	0	< bl
4	Eragrostis curvula.	45	0	0	0	< bl
4	hay bale.	50	5	385	0	< bl
5	Hyperrhemia hirta.	35	0	5	0	< bl
6	Cymbopogon excavatus.	25	0	0	0	< bl
7	Grass unidentified	45	0	25	0	< bl
8	Hyperrhemia hirta.	30	0	5	0	< bl
9	Sporobolas fimbriatus	20	0	10	0	< bl
10	grass unidentified	10	0	15	0	< bl
Soil						
1	surface.	523	2	11	0	0
1	deep.	378	2	28	0	0
2	surface.	458	3	12	1	1
2	deep.	550	3	2	0	1
3	surface.	324	3	20	1	< bl
3	deep.	228	3	2	0	3
4	surface.	358	3	12	1	< bl
4	deep.	227	4	2	0	4
5	surface.	169	3	14	12	1
5	deep.	135	3	3	0	1
6	surface.	449	4	6	0	0
6	deep.	460	5	3	0	< bl
7	surface	320	6	11	1	5
7	deep.	368	6	1	0	5
8	surface.	433	10	10	1	5
8	deep.	436	7	2	0	5
9	surface.	125	1	10	1	1
9	deep.	228	2	2	0	4
10	surface.	416	3	6	0	1
10	deep.	495	3	2	0	1
Soil and plant = dry mass basis; bl = blank sample						

These findings suggest a link between direction from the APU and V levels. The farmer claimed that the wind blew predominantly towards the north for at least 6 months of the year, which supported the postulation that the APU was the probable source of V pollution. To try and verify this, weather data from one of the nearest weather stations (Lydenberg) was

examined (unpublished data, Department of Environmental Affairs, R.S.A., 1988). This showed that during the period 1960-1988, the predominant average wind direction was from the south-east for the months December, January, February, March, April and June, which supported the farmer's observations that the wind blew predominantly in a northerly direction over the farm for at least 6 months of the year.

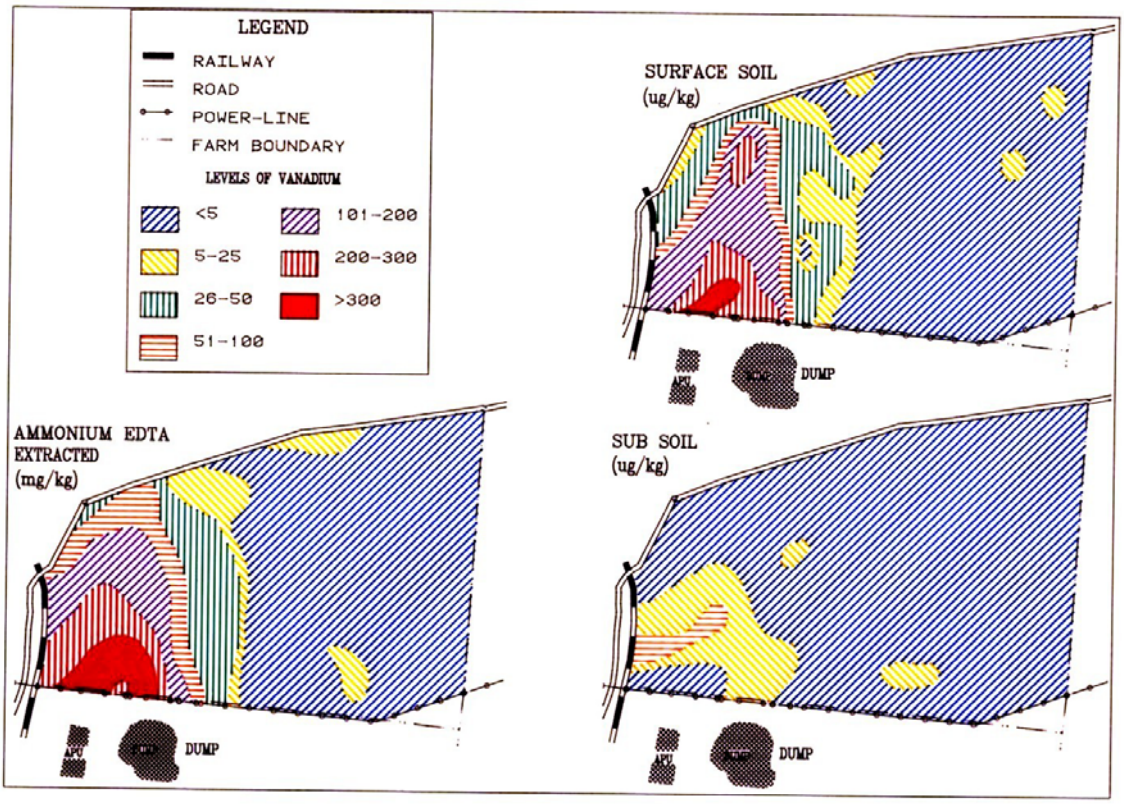


FIG. 2 Diagram of the concentration isolines for vanadium found in the surface-soil (water soluble and ammonium EDTA extracts) and subsoil (water soluble extract), in the area adjacent to the alloy processing unit (Fig. 1)

When the surface-soil V levels were examined in terms of distance from the APU a definite trend could be shown which indicated that as the distance from the APU increased so the V levels fell. This was shown most clearly in the detailed study of the area immediately north of the APU where plotted isolines showed distinct concentration gradients stemming from the APU (Fig. 2). This spatial distribution pattern of V was seen for both water and NH_4EDTA extractable V, indicating that the APU was the centre of a V "hot spot". The presence of high levels of water soluble V is significant since naturally occurring outcrops of V are unlikely to

contain large amounts of water extractable V. A large amount of the detectable V was therefore most probably due to pollution and not of natural origin.
Camps 7 and 8 appear to have inconsistent results (Table 1) as they are relatively distant from the APU, yet have high levels of V in the soil. These high levels could probably be ascribed to the topography of the farm as these camps, though further from the APU than camps 5 and 6, were at the same altitude as camps 3 and 4. It is possible that pollutants from the APU could overshoot camps 5 and 6, situated in a valley, and settle on camps 7 and 8.

In the camps to the north and north-east of the APU, the V levels in the subsoil were almost always lower than the surface-soil levels. This was particularly evident in the grid samples taken during the second investigation (Fig. 2). Such a finding is inconsistent with soils formed *in situ* on parent material naturally rich in V and lead to the assumption of V air pollution, the most likely source of which was the adjacent APU. In those camps north-west of the APU the difference between the sub and surface V levels was very small, which is what can be expected if there were no aerial pollution.

Camps 4, 5, 7, 8 and 12 had much higher levels in the soil during summer than winter. This is the converse of what occurred in the grass samples and suggested that during the high rainfall summer months much of the V which may have been trapped on the grass was washed off into the soil resulting in higher soil levels and lower grass levels during the summer. This seasonal variation supported a postulation of air pollution and could explain why there was no cumulative effect over the sampling period.

Grass analyses

Grass V levels showed a decrease with distance from the APU (Table 1, Fig. 3), the highest levels being those closest to the APU. This finding supported the postulation that the source of the V was the APU. The grass iron levels (Table 2) showed no correlation with distance, but a good correlation to soil levels and illustrate what can be expected if air pollution was not playing a role.

The low V grass levels in camps 7 and 8 (Table 1) suggest that there was little correlation between soil levels of V and aerial grass levels. Normal levels of V in higher plants are given as an average of 1 ppm (Faulkner Hudson 1964; Platonow & Abbey 1968, Waters 1977). The average levels on the farm all exceeded 1 ppm with the exception of camp 9. Most of the farm therefore had abnormally high levels of V in or on the grass. The highest levels occurred in camp 3, closest to the APU, where levels ranged from 32-533 ppm (Fig. 3). Seven of the 12 camps sampled had average levels greater than 10 ppm (Table 1).

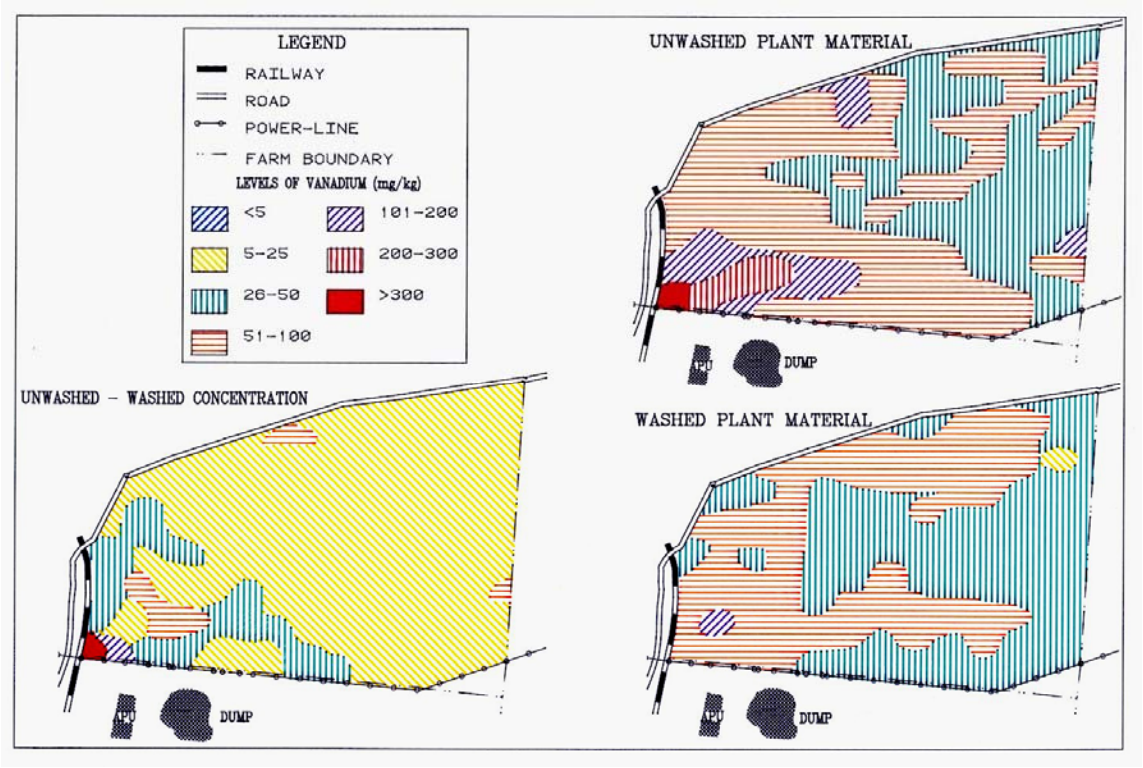


FIG. 3 Diagram of the concentration isolines for vanadium found in and on plant material, before and after washing, in the area adjacent to the alloy processing unit (Fig. 1)

Higher levels of V occurred on the grass during winter than summer (Table 1). This trend supported a postulation of aerial pollution as it can be assumed that during the rainy season (summer) dust particles will be washed off the grass resulting in lower levels on the grass. The difference in V content between washed and unwashed grass samples further supported this postulation. The unwashed grass V concentrations were up to 430 times higher than washed grass samples from the same sampling points (Fig. 3).

Little work has been done to determine the toxic level of V for cattle in grass. Fox (1987) specifies that the usual feedlot diet for cattle contains ±0.57 ppm V. Most of the camps on this farm exceeded 0.57 ppm and levels in the grass in the camps closest to the APU at times reached 533 ppm. Most of the experimental feed trials that describe V toxicity have been carried out over fairly short periods of time (Faulkner Hudson 1964; Kubena & Phillips 1986; Platonow & Abbey, 1968) and there is thus no available data on the chronic effects of V intake in cattle. It can be concluded that because the V levels in the grass were abnormally high (in terms of accepted cattle diet V levels) the potential for chronic vanadium toxicity definitely existed on the farm.

TABLE 3 Results of aerial sampling for vanadium

Season	Sampling period		Distance from APU	Concentration of V
Airborne vanadium at farmhouse				
Winter	29/6/1990-29/7/1990 29/7/90 - 13/8/90	30 d 14 d	750 m (N)	0.12 µg V/m^3 0.19 µg V/m^3
Summer	27/1/1991–14/2/1991 14/2/1991-28/2/1991 28/2/1991-13/3/1991	18 d 14 d 14 d	750 m (N)	ND 0.16 µg V/m^3 0.15 µg V/m^3
Winter	3/6/1991-7/6/1991 7/6/1991-11/6/1991 17/6/1991-13/3/1991	4 d 4 d 12 d	750 m (N)	0.25 µg V/m^3 0.13 µg V/m^3 0.64 µg V/m^3
Vanadium deposition rate				
Summer	1/2/91 - 14/3/91 1/2/91 - 14/3/91	150 m (N) 250 m (N)		3.90 mg V/m^2.day 3.10 mg V/m^2.day
Winter	29/7/91 - 12/8/91 29/7/91 - 12/8/91 29/7/91 - 12/8/91	150 m (N) 250 m (NE) 250 m (NNW)		4.70 mg V/m^2.day 0.54 mg V/m^2.day 0.54 mg V/m^2.day
ND = not detectable				

The grass analyses showed, that the northern side of the APU had high V grass levels and that the V on the grass probably arose from the air. The detection of V in the aerial samples (Table 3) supported the postulation of aerial contamination of the grass. The fact that the levels of V in the grass samples were highest closest to the APU indicated that the APU was the most likely source of aerial V. Since animals are outdoors most of their lifespan, they can potentially breathe in large quantities of V from the air and furthermore when grazing may breathe in V present in the dust near the ground. Inhalation is recognised as one of the major routes of V

toxicity in humans (Waters, 1977) and cannot be ignored as a possible route of V intake in cattle. High levels of V on the grass indicate that V was ingested with the feed, but it could not be conclusively determined in this report whether or in what quantities V was being inhaled.

Air sampling

The results of the air sampling are given in Table 3 and show that V was present in the air over the farm at levels which exceeded those measured at general sites in South Africa or elsewhere in the world (Environmental Options report 1993). They could serve as a basis for introducing legislation pertaining to maximum permissible emission levels for V since such legislation currently appears to be lacking in South Africa (Environmental Options report 1993).

Clinical signs

Signs of ill-thrift occurred primarily in 0-12 m calves and comprised: poor growth, emaciation, intermittent diarrhoea, sub-mandibular oedema, pot-belly, lacrimation/conjunctivitis, rhinitis, congested mucous membranes, intermittent fever, dull staring hair coats and stiff gait. Animals became progressively weaker over a varying periods of time before dying of cachexia. Force-feeding failed to reverse the course of the disease. Some animals have survived for more than two years despite being stunted. They tend to walk stiffly and are not as active as other animals of similar age.

The above signs are consistent with those described for V toxicity (Fox 1987; Faulkner Hudson 1964; Hansard, Ammerman, Fick & Miller 1978; Hansard, Ammerman, Henry & Simpson 1982; Hansard, Ammerman & Henry 1982; Kubena *et al.* 1986; Platonow *et al.* 1968; Van Vleet, Boon & Ferrans 1981; Waters 1977). Similar clinical signs can be associated with a variety of conditions and considerable effort was taken to exclude the following differential diagnoses: poor management practices, poor genetic material, poor ration quality and quantity, verminosis, paratuberculosis, arsenic poisoning, gossypol poisoning, Vit E/Se deficiency, bovine viral diarrhoea, *Brucella, Campylobacter, Leptospira* infections and Cu, Zn, Mo toxicities and deficiencies. It is postulated that the underlying cause of the signs was a malabsorption problem coupled to an immunosuppression phenomenon. Frank, Kristiansson & Petersson (1990), describing field cases of acute toxicity in dairy cattle, reported seeing facial

paralysis and blindness and put forward an hypothesis that there may be some effect on the CNS system as well. No clinical evidence of facial paralysis or blindness was however seen in the current investigation.

At the onset of the investigation no clinical signs were noted in the adult cows. However during the last 12 months there was a dramatic rise in abortions, stillbirths and neonatal mortalities. Pregnant cows have never experimentally been exposed to V so there is no evidence to show whether V can cause stillbirths in cattle. Wide (1984) reports an increased frequency of spontaneous abortions in Finnish women that was correlated to exposure to Al, Co, Mo and V in metal industries. As no evidence could be found that any of the common causes of abortion were playing a role on the farm it was assumed, by a process of elimination, that V was possibly the cause of the stillbirths. The farmer also reported that at about the time abortions occurred there was a drop in milk production in his herd. These findings correlated with a previous report from the Bon Accord area of South Africa (1962) of V toxicity (unpublished OVI archival data). It is postulated that V may result in a decreased contractibility of muscle, resulting in the cow being unable to adequately expel the foetus causing it to suffocate in the birth canal.

Pathology results

The gastrointestinal tract (GIT), lymphoid and haemopoeitic tissues, and respiratory system in calves and adults were primarily affected.

Group A (foetuses/perinates)

The standard tests/examinations on all five cases yielded negative results as did the MAT for antibodies to leptospirosis.

Group B (suckling calves)

Case 6 was weak from birth and death was ascribed to an *Escherichia coli* endotoxaemia due to *E. coli O26:K60*. Case 7 developed acute bloat and died as a result of *Clostridium perfringens* type A enterotoxaemia. Case 8 developed watery diarrhoea and was dehydrated and emaciated at the time of death. No pathogenic bacteria could be isolated from this calf and no viral particles could be detected in the faeces. Gross and microscopic lesions in the gastro-intestinal

tract (GIT), lymphoid tissues, and lungs were similar to those described for cases in group C and D (*vide infra*)

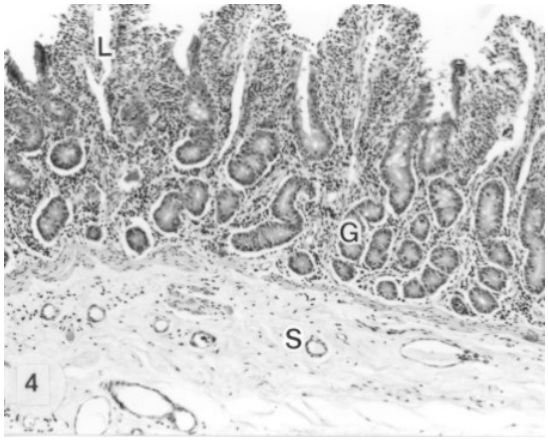
Group C (calves 3-12 m)

Case 9 was a 10-weeks-old calf and cases 10 - 16 were 6 - 12 month-old calves. The calves were all emaciated and pot-bellied with harsh staring haircoats. Signs of diarrhoea were evident in cases 9 and 11. The presence or absence of diarrhoea could not be determined in the other five cases.

No pathogenic aerobic bacteria were isolated from cases 9-11 and blood and brain smears were negative. Signs of cachexia were present in all three calves. Gross lesions were evident in the gastro-intestinal tract (GIT), lymphoid tissues and respiratory system of these three cases. The GIT lesions included: watery ruminal contents or ruminal stasis, mucoid small intestinal contents, ileal dilatation and rectal dilatation with hard mucoid-covered faecal balls. Lesions of the lymphoid tissues comprised moderate to severe atrophy of the spleen, lymph nodes and gut-associated lymphoid tissue. The respiratory lesions were mild and characterised by multifocal lobular areas of pulmonary atelectasis and/or emphysema.

Histopathological examination revealed significant lesions in the GIT, lymphoid tissues and lungs. The GIT was examined in six cases and lesions included a mild to moderate granulomatous and eosinophilic enteritis and typhlocolitis (six cases), moderate villous atrophy characterised by fusion and stunting of villi and dilatation of the lacteals (four cases) (Fig. 4), and mild parakeratosis of the ruminal and omasal mucosa (one case).

The spleen, prescapular and prefemoral lymph nodes were examined in five cases. There was moderate to severe splenic and lymph nodal atrophy characterised by absence or paucity of lymphoid follicles and hypocellularity of the medullary and red pulp cords (Fig. 5). In the cases where lymphoid follicles were evident, they appeared inactive with depleted centres or occasionally showed evidence of active necrosis. The thymus revealed moderate to advanced signs of regression.



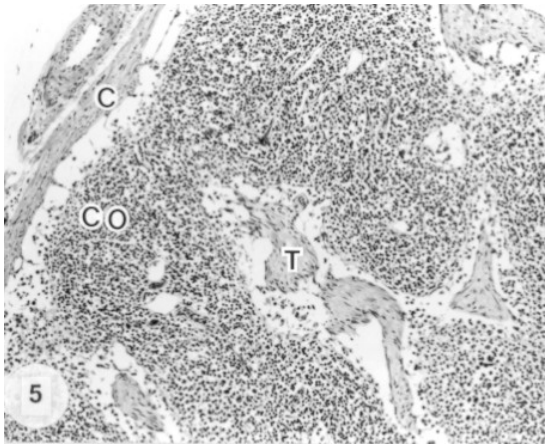
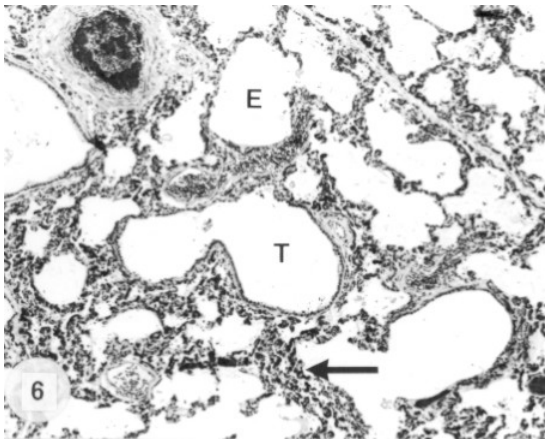


FIG. 4 Case 17. Small intestine. Villous atrophy characterised by short, blunted villi and lacteal dilatation (L). Note also round cell infiltration in mucosa, irregular proliferating basal glands (G) and oedema of the submucosa (S). HE x 90

FIG. 5 Case 8. Lymph node. Atrophy of lymph node evidenced by prominence of the capsule (C) and trabeculae cortical (CO) hypocellularity and absence of lymphoid follicles. HE x 90



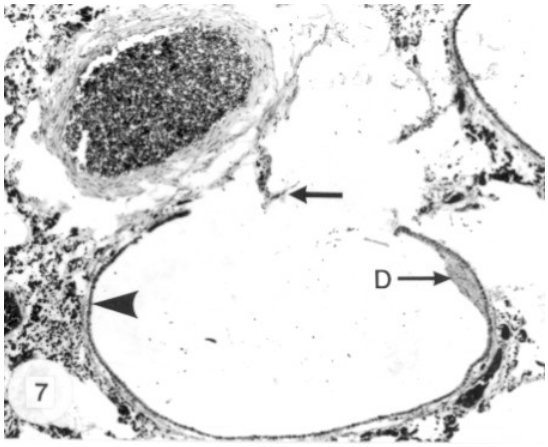


FIG. 6 Case 8. Lung. Ectasia of the terminal bronchioles (T) with multifocal alveolar atelectasis (arrow) and emphysema (E). HE x 90

FIG. 7 Case 8. Lung. Ectasia of a terminal bronchiole. Note flattened epithelium (arrowhead), desquamated cell debris (D) and rupture of alveolar walls (arrow). HE x 200

Sections of the lungs were examined in seven cases. Moderate bronchiolar ectasia with flattening and occasionally necrosis or desquamation of the bronchiolar epithelium and associated alveolar and septal emphysema was present in three cases (Fig. 6 and 7). Mild, multifocal areas of atelectasis, interstitial pneumonia and alveolar emphysema were evident in two cases and mild alveolar oedema in two cases.

Group D (adult cows)

The clinical symptoms in the two cows necropsied included emaciation, soft to watery faeces, tachycardia or cardiac arrythmia and anasarca of the neck and brisket in case 16. Case 16 (4 years old) had experienced a dystocia a few weeks prior to presentation for necropsy. Case 17 calved normally in May 1989 and then returned to oestrus several times. She produced a stillborn calf in July 1990 and again returned to oestrus several times before being necropsied in September 1991.

Blood and brain smears and faecal flotation tests were negative in both cows. No pathogenic aerobic bacteria were isolated from Case 17. *Actinomyces pyogenes* was isolated from the pericardial pus from case 16 which showed lesions of a traumatic reticulopericarditis. Evidence of a previous dystocia was present in case 16 and moderate lesions of a chronic hepatic fascioliasis in both cows. Gross lesions not associated with the above conditions involved the gastro-intestinal tract, lymphoid tissues and lungs. In both cows the ruminal ingesta contained moderate amounts of whole maize kernels whilst copious amounts of whole maize kernels were present in the abomasum. In case 16 the spleen and superficial lymph nodes were moderately atrophic and in case 17 the lungs revealed mild multifocal areas of septal and alveolar emphysema. In case 17 pneumonic lesions attributable to the traumatic reticulopericarditis obscured any other pathological lesions which may have been present. Histopathological examination revealed GIT, lymphoid tissue and respiratory lesions similar to those described for cases in Group C.

Pathology discussion

The pathological lesions indicated that exposure to toxic levels of V over a prolonged period had chronic, wide ranging effects in older calves and adult cattle, evidenced by cachexia, GIT, respiratory and reproductive disorders and atrophy of the lymphoid and haemopoeitic tissues.

The gross GIT lesions in the adult and sub-adult cattle and 2-week calf are suggestive of impaired ruminal and intestinal motility. Stasis and impairment of GIT motility would ultimately lead to inadequate nutrient uptake and absorption from the GIT with subsequent malabsorption and cachexia. Platonow & Abbey (1968) noted adherence of food material to the

mucosa of the entire GIT which could also imply impairment of GIT motility. The detection of high levels of V in the ruminal fluid compared to non-detectable or low levels in other organs (Table 4) suggests that a high proportion of the ingested V is retained within the GIT lumen where it could adversely affect the absorption of nutrients. These findings are supported by those of Frank *et al*. 1990 who recorded similar high levels of V in the rumen, reticulum and abomasum. Vanadium is known to interfere with the active transport of Na^+ and K^+ across membranes and high levels within the GIT would conceivably interfere with the motility of the GIT and uptake and absorption of nutrients with consequent malabsorption and eventual cachexia (Bracken & Sharma 1985; Kubena *et al*. 1986; Macara, Kustin & Cantly 1980; Ramarsama, Mackellar & Crane 1981). Recent *in vitro* work has shown that glucose absorption rates from the jejunum of rats rapidly decrease to a point of no absorption of glucose within 60 minutes after being exposed to a Krebs' solution containing V (J. Keegan, unpublished data, 1993). These findings add further support to the postulation that V can lead to malabsorption. The histopathological findings of villous atrophy and granulomatous enteritis are consistent with the theory of a malabsorption syndrome. Similar intestinal lesions have been reported in pigs fed high levels of V (van Vleet *et al*., 1981).

Bone marrow atrophy was present in two calves and splenic and lymph nodal atrophy in six of the nine sub-adult and adult cattle necropsied and the 2-week-old suckling calf. These lesions may be a reflection of the cachexic state of the animals but on histopathological examination there were indications of immune hypoactivity as evidenced by lack or paucity of lymphoid follicles, depletion of germinal centres and hypocellularity of the medullary and splenic cords. The lesions of hypoactivity correlate with the finding of suppressed lymphocyte transformation responses in the circulating lymphocytes (see later). The finding of an *E. coli* endotoxaemia and *C. perfringens* type A enterotoxaemia in two of the suckling calves may be indicative of a bacterial infection secondary to immunosuppression as a result of the ingestion and uptake of V. The presence of V in the ruminal fluid of the 2-week-old calf (Table 4) indicates that V was indeed ingested shortly after birth and the inability to detect V in the younger calves may be a reflection of the limitations of the detection methods employed.

The respiratory lesions were mild in nature and characterised by multifocal areas of oedema, interstitial pneumonia and atelectasis, and septal and alveolar emphysema. Of interest at the microscopic level was the presence of multifocal bronchiolar ectasia with flattening and metaplasia of the epithelium in three cases. According to Symanski (1939 cited by Waters 1977), bronchiectasis can occur in humans following prolonged exposure to V. Various respiratory symptoms and lesions such as conjunctivitis, rhinitis, pharyngitis, bronchiectasis, bronchospasm, bronchitis, emphysema and a chronic, productive cough have been recorded in humans exposed to V dust in industrial concerns. These effects are ascribed to the extremely irritant effect of V dust (usually the pentoxides) on the mucous membranes of the eyes, nose, throat and respiratory tract (Waters, 1977). The presence of respiratory lesions in particular bronchiolar ectasia and emphysema in the animals in this series implies V uptake via the respiratory passages.

No significant pathology was evident in the foetuses and perinatal calf necropsied. Aerobic bacteria, leptospires, *Brucella*, *Chlamydia* and *Campylobacter spp.* were eliminated as possible causes. No V could be found in the abomasal fluid, liver or kidney of the three cases analysed. This suggested that there was no transplacental absorption of V but may on the other hand again be a reflection of the limitations of the detection methods employed. Uterine fluids or specimens of the uterine wall were not analyzed for their V content. If one considers that high levels of V were present in the tissues of the two adult cows necropsied (Table 4) one can surmise that V may indeed have been present in the uterine lumen or tissue.

Vanadium analyses

The V levels in the faeces of the heifers, dry and lactating cows during the second farm visit ranged from 2.1-8.8 ppm with a mean of 4.5 ppm (n=19). There were no significant differences between the groups. The V organ levels obtained during the period of investigation are shown in Table 4.

Normal tissue V levels for cattle are reported to be in ppb (ng/g) and the level for liver is given as 0.006-0.007 ppm (WM) (Puls 1988). The levels of V in bone and liver were consistent with that described for V toxicity while the levels in the kidney were not (Faulkner Hudson 1964;

Hansard *et al.* 1978; Hansard *et al.* 1982; Hansard, Ammerman & Henry 1982; Nechay, Nanninga, Nechay, Post, Grantham, Macara, Kubena, Phillips & Neilson 1986, Puls 1988). No reported reference values for rumen content could be found, but the fact that V was consistently present in the rumen of the dead animals and faeces of living animals supported a postulation of V exposure and uptake via the GIT.

TABLE 4 Results of organ analyses for V (ppm WM)

Animal group	Animal necropsy case no.	Age	Tissue levels				Date
			Liver	Kidney	Bone	Ruminal fluid	
A	1	Foetus (6 m)	0	0	ND	ND	June '91
	4	Stillborn foetus	0	0	0	ND	Nov. '91
	5	Neonate (1-2 h)	0	0	ND	ND	Dec. '91
B	8	2 w	0	0	ND	4	June '91
C	9	10 w	0.3	0	1.3	4	Oct. '90
	10	11 m	0.4	2.1	2.8	1.3	May '90
	11	11 m	0.1	0	2.4	2.0	May '90
	13	6-12 m	0.7	0.2	ND	1.0	Jan. '91
	14	6-12 m	2.0	0	ND	0	Jan. '91
	15	6-12 m	ND	ND	ND	2.6	Jan. '91
D	16	4 y	0	0	ND	23.0	Sept. '91
	17	7 y	0	0	ND	27.0	Sept. '91
Negative (limit of detection = 0.05 ppm); ND = Not determined							

Clinical pathology

The main thrust of the clinical pathology was to try and establish underlying causes for the symptomatology and pathological findings. The areas concentrated on were:

- evidence that may indicate organ damage
- evidence to support a malabsorption syndrome; and
- evidence to support an immunosuppressive effect.

Evidence to support organ damage

The enzymes used to assess liver damage were GGT and AST, and the activities of neither were abnormally high for any of the groups studied. There was therefore little evidence of acute liver damage in the herd.

The enzyme used to test for muscle damage was CK. Raised CK activity was seen in the 0-3 (CK:$\bar{x}$ = 74 ± 15 U/*l* excluding one value = 2895; range: 47-2895 U/*l*) as well as the 12-15 month-old-group (CK:$\bar{x}$ = 68 ± 14 U/*l*; range: 52-83 U/*l*). This would support evidence of muscle breakdown as would occur with a cachexic state.

Indicators used to assess kidney function were BUN and Creat. A decrease in BUN (< 3.6 mmol/*l*) was seen in individuals in all the age groups. The prevalence of low BUN levels increased with increasing age. All animals 12-19 months old or which had aborted showed low BUN levels. This finding supports the postulation made by others that V causes an increased glomerular filtration rate (Faulkner Hudson 1964; Heinz, Robinson & Grantham 1982; Patterson, Hansard, Ammerson, Henry, Zech & Fisher 1986). The Creat levels increased with increasing age and all the animals in the 12-19 months-old group (Creat:$\bar{x}$ = 150 ± 14 µmol/*l*; range: 135-194 µmol/*l*) had abnormally high Creat levels. High Creat levels together with high BUN levels are usually an indication of glomerular damage. However non-Creat chromogens may cause false high values and the most significant of these are ketones (Duncan & Prasse 1986). Animals that are energy deficient such as would occur with malabsorption could therefore have false high Creat levels. Since the BUN levels were not raised the latter explanation for high Creat is more feasible than glomerular damage and would give added evidence for malabsorption.

Evidence for malabsorption

The parameters used to assess malabsorption were TSP and its various fractions. The TSP levels were low in all the 0-3 month-old calves (TSP:$\bar{x}$ = 55 ± 9.6 g/*l*; range: 43-68 g/*l*) and in the majority of animals in the 4-7 (TSP:$\bar{x}$ = 63.8 ± 8 g/*l*; range: 50.5-70.5 g/*l*), 8-11 (TSP:$\bar{x}$ = 67 ± 5 g/*l*; range: 59-71.4 g/*l*), 12-15 (TSP:$\bar{x}$ = 68 ± 4 g/*l*; range: 65-73 g/*l*) and 16-19 months-old groups (TSP:$\bar{x}$ = 69 ± 3 g/*l*; range: 65-73 g/*l*). The adult cattle had normal TSP (70-78 g/*l*) levels. The albumin:globulin (A/G) ratio in the 0-3 months-old (A/G:$\bar{x}$ = 1 ± 0.4 ; range: 0.62-1.86) and 8-11 months-old groups (A/G:$\bar{x}$ = 1.1 ± 0.2; range: 0.84-1.29) was close to normal (0.9) indicating that the low TSP levels were probably as a result of low levels of both alb and glob. This would occur with protein losing enteropathies, malabsorption, malnutrition or chronic liver disease together with immunodeficiency and/or failure of passive colostral transfer

(Duncan & Prasse 1986). Hence the low TSP values in animals < 12 months old appeared to confirm a diagnosis of malabsorption as supported by the pathology. It is interesting to note that it was only the calves that showed an overall decrease in TSP and it was only these animals that showed severe clinical signs.

Animals 4-7 months-old and > 11 months-old had low A/G ratios. In the 4-7 months-old (A/G:$\bar{x}$ = 0.87 ± 0.2; range: 0.62-1.86), 12-15 months-old (A/G:$\bar{x}$ = 0.72±0.09; range: 0.64-0.84) and 16-19 months-old groups (A/G:$\bar{x}$ = 0.86 ± 0.12; range: 0.73-1.09) this decrease was primarily due to a decrease in alb suggesting a protein-losing enteropathy in this group but not immunosuppression as the glob fraction was normal. The adult animals had low A/G ratios (A/G:$\bar{x}$ = 0.75 ± 0.15; range: 0.42-0.99) but these appeared to be due to an increase in glob levels rather than a decrease in alb levels suggesting an over-stimulation of the immune system in adult cattle.

Glob fractions were also examined for each age group. The 0-3 months-old group showed a deficiency in gamma globulin (γ-glob) levels (γ-glob:$\bar{x}$ = 9.2 ± 6 g/*l*; range: 2.7-21.6 g/*l*), which supported the findings of malabsorption and immunodeficiency. The 4-7 months-old and adult cattle groups showed a low γ-glob fraction (< 16 g/*l*) and a high ß-glob fraction (> 9 g/*l*). The ß-glob fraction represents transferrin, ß-lipoprotein, complement-3 and some immunoglobulins (Duncan *et al.* 1986). It is reported that V competes with iron for transferrin (Nechay 1984; Nechay *et al.* 1986; Patterson *et al.* 1986; Ramasarma *et al.* 1981) and it can therefore be assumed that with V toxicity there would be an extra demand for transferrin causing more of it to be produced and thus higher serum levels. The high ß-globs could therefore be as a result of increased transferrin which would be consistent with a diagnosis of V toxicity. Similar findings were seen in the 12-15 month-old and 16-19 month-old groups with the additional finding of a low α-glob fraction (< 9 g/*l*) which together with the low γ-glob fraction strengthens the argument for immunosuppression.

Evidence for immunosuppression

Cellular immunity was examined by means of differential WCC. The WCC were above normal (> 10 x 10^3 cells/*l*) in all the age groups studied, with the exception of the 0-3 months-old group

(WCC:$\bar{x}$ = 9.5 ± 3 x 10^3 cells/*l*), implying that some form of chronic immune stimulation was taking place.

From 4-12 months-old there is evidence of a lymphocytosis which becomes less common in the adult cattle. High lymphocyte counts are usually a reflection of white cell production and function and are associated with chronic infections (Duncan & Prasse 1986). Hence there appears to be evidence of a chronic stimulation of the immune system in the majority of the animals in the herd. Persistent exposure to V dust could act as a chronic irritant with subsequent chronic inflammation. An increase in immature neutrophils was seen in the 0-3 (N_i:$\bar{x}$ = 4.2 %) and 4-7 months-old groups (N_i:$\bar{x}$ = 4 %). These findings indicate that there is an increase in demand for neutrophils which is usually associated with an inflammatory condition (Duncan & Prasse 1986) such as, rhinitis, conjunctivitis and enteritis.

A monocytosis (> 7 %) was seen in all the age groups <15 month-old and >19 months-old, but was most obvious in the 0-3 months-old group ($\bar{x}$= 11.7 %). Causes of monocytosis include, protozoal infections, suppuration, haemolysis and immune injury (Duncan *et al.* 1986). A monocytosis therefore fits in with evidence to support some form of immune injury.

The absence of eosinophils in all groups gave added evidence that helminths were not playing a significant role on the farm.

A puzzling question was why were there so many white cells if the immunity of the animals were compromised? To try and solve this problem *in vitro* tests were carried out to establish how active the lymphocytes were (Kristensen *et al.* 1982). Lymphocytes from twnety cattle were examined, ten calves < 7 months-old and ten cows > 19 months-old. None of the adult cows had normal lymphoblast transformation responses to plant lectins and only four of the calves showed a response but this was nevertheless suppressed. Hence although the lymphocytes were present they were not active implying that V was possibly preventing them from responding normally.

A further finding supporting V toxicity was the presence of Heinz bodies in the erythrocytes of cattle less than 7 months-old but not in the adults. Heinz bodies usually indicate an impairment of the glucose-6-phosphate dehydrogenase pathway or a depletion of glutathione (Duncan & Prasse 1986). Glutathione is thought to reduce vanadate to vanadyl and thus render it less toxic (Faulkner Hudson 1964; Hansen, Aaseth & Alexander 1982; Heinz *et al.* 1982). Continual exposure to V could thus lead to a deficiency in glutathione and result in denaturation of haemoglobin leading to Heinz bodies.

Although the clinical pathology results alone cannot be used to make a diagnosis, it becomes clear from them that they support evidence for malabsorption and immunosuppression as well as V toxicity.

CONCLUSION

Due to the vague nature of the clinical signs of V toxicity and the lack of definitive tools for diagnosing this disease it is very difficult to conclusively prove that an animal has died or is suffering from V toxicity. What this investigation did was to accumulate a library of circumstantial evidence which lead us to believe that the animals on the affected farm were suffering from the effects of excessive levels of V. Where it was thought that another known disease may result in a similar picture steps were taken to satisfy ourselves that such a disease was not playing a role. In this way we feel confident that chronic V toxicity was the underlying cause of the ill-thrift problem experienced on the farm.

Because little has been published on the effects of V air pollution on animals there are few precedents upon which to base our results. We have accumulated circumstantial evidence suggesting that air pollution was taking place on the farm and that the source of the air pollution was the adjacent APU.

On the basis of all our findings we conclude that there was sufficient evidence to make a diagnosis of chronic V toxicity, which was caused by air pollution from the nearby APU.

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Chapter 3

CHRONIC VANADIUM POISONING IN CALVES AND ITS TREATMENT WITH CALCIUM DISODIUM ETHYLENEDIAMINE TETRAACETATE

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ABSTRACT

Sixteen Friesland heifer calves aged between 96 and 157 days were removed from a dairy farm that had been polluted with vanadium and randomly allocated into two equal groups (n=8). The objective of the trial was to determine if calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$) could be used as a treatment for cattle running in environments high in background vanadium. The treatment group received 80 mg $CaNa_2EDTA$ per kg body weight intraperitonealy (IP) twice a week over a 10-week period. The control group received normal saline IP over the same period. During the trial calves were exposed to a daily intake of vanadium in the form of contaminated tef hay derived from the farm of origin. In addition the total mixed ration was spiked with a further 20 mg V_2O_5 / kg feed to compensate for possible on farm inhalation exposure. A stochastic model was used to estimate daily intake of vanadium as a distribution function. The model estimated that the daily intake of vanadium varied between an absolute minimum of 33 mg vanadium per day to an absolute maximum of 124 mg vanadium per day. The average intake was 71.8 mg vanadium per day per calf. Various chemical pathology parameters were measured throughout the trial as well as urine excretion rates of vanadium and lymphocyte stimulation counts. All calves were slaughtered and necropsied in cohorts of 4-6 animals at monthly intervals after completion of the trial and withdrawal of vanadium from the ration. Tissue concentrations of vanadium were determined and necropsy findings were noted. The study found that $CaNa_2EDTA$ appears to enhance the excretion of vanadium in calves, but could not prove that the treatment had a protective effect against vanadium exposure. Calves were able to tolerate the prolonged treatment with $CaNa_2EDTA$ without side effects.

Key words: cattle, $CaNa_2EDTA$, chronic toxicity, excretion, symptoms, treatment, vanadium

INTRODUCTION

Although vanadium poisoning is uncommon, vanadium forms an important part of the mining industry in South Africa (Nriagu, 1998) and increasing concern is being expressed about the impact of vanadium pollution on livestock production in South Africa (Wates, 1996). Several therapeutic agents have been used experimentally to treat acute vanadium toxicity in laboratory animals (Domingo *et al.*, 1990). Since the 1940's there have been three main therapeutic

approaches for the treatment of vanadium poisoning; the use of di-thiols (Barron & Kalvitsky, 1947; Lusky *et al.*, 1949; Dalhman *et al.*, 1953), the use of chelating agents (Mitchell, 1953a; Braun & Lusky, 1959; Domingo, 1995) and the use of reducing agents (Mitchell & Floyd, 1954; Domingo *et al.,* 1990). It had already been established by the late 1940's that di-thiols such as dimercaprol (BAL) were inefficient in protecting animals against vanadium poisoning. By the end of the 1950's it was ascertained that subcutaneous Vitamin C injections protected mice, rats and dogs against LD_{95} levels of vanadium (Mitchell & Floyd, 1954), thus warranting further investigation of reducing agents. It had also been shown by then that chelators, such as calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$) and disodium catechol disulphonate (DCD) had been successful in treating or protecting laboratory animals exposed to lethal doses of sodium vanadate or ammonium metavanadate (Mitchell, 1953b; Braun & Lusky, 1959). In the early 1980's, Hansen *et al.* (1982) experimented with another similar chelator, calcium trisodium diethylene triamine pentaacetate (DTPA) and concluded that DTPA increased both urinary and faecal excretion of vanadium in rats. These concepts have been expanded on in more recent years (Gomez *et al.*, 1988, 1991a and 1991b) and a wide range of chelators and reducing agents have since been tested as antagonists for vanadium poisoning in laboratory animals (Domingo *et al.*, 1990). From this work it appears as if 4,5-dihydroxybenzene-1,3-disulfonate (Tiron) is currently, the most effective *in vivo* chelator of vanadium in laboratory animals (Gomez *et al.,* 1988, 1991a, 1991b; Domingo *et al.*, 1992). There appears, however, to have been little work done in ruminants with respect to treatment of vanadium poisoning. Yet, all the documented field outbreaks of vanadium poisoning in livestock in South Africa have involved cattle (Unpublished archival records of the Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110, 1961/1962, 1975/1976; Gummow *et al.,* 1994; Gummow, B. unpublished data, 1998; McCrindle *et al.*, 2001). Because of these field outbreaks it was decided that a practical treatment for cattle needed to be found. The primary purpose of this trial was therefore to assess the value of $CaNa_2EDTA$ as a possible on farm treatment (prevention and reversal of clinical signs and deaths) for cattle exposed to high background concentrations of vanadium. $CaNa_2EDTA$ was chosen because of its availability as a treatment for lead poisoning in ruminants (Osweiler *et al.*, 1985). A secondary objective was to examine vanadium tissue concentrations and to determine

if any pathological damage caused by exposure to vanadium, could be reversed if the source of exposure was removed.

MATERIALS AND METHODS

Model system

Sixteen Friesland heifer calves, aged between 96 and 157 days, were randomly allocated into two equal groups of eight. The calves originated from a farm that had been polluted with vanadium and were purposively selected on clinical signs consistent with chronic vanadium toxicity. One group was designated the EDTA Treatment Group and the other Group the Saline Control Group.

Vanadium exposure

All calves were fed a total mixed ration that included tef hay, which had been cultivated and baled on the polluted farm from which the calves originated. In addition, the total mixed ration was spiked with 20 mg vanadium pentoxide (V_2O_5) per kg feed to compensate for vanadium that was being inhaled or otherwise ingested on the polluted farm. The total mixed ration was then analysed each time a new batch of feed was made up during the trial to ascertain the amount of vanadium in the complete ration. The total mixed ration containing vanadium was fed to surviving calves up until Day 93 of the trial.

The total mixed ration comprised Eragrostis tef hay (50 kg), maize concentrate (38 kg), cotton seed oil cake (12 kg), fish meal (5 kg), urea (0.5 kg), limestone (0.5 kg), lucerne (11.5 kg) and salt (0.5 kg).

Formulation of vanadium intake model

A stochastic model was formulated to give a distribution function for vanadium ingested by the calves over the period of the trial. The steps in the model were as follows.

1. The amount of vanadium in the complete ration was estimated using the measured vanadium concentration in the complete ration for each mix. This was weighted by the number of days that that particular ration mix was used for as follows; concentration of vanadium in ration mix x days fed / total number of days that batch was fed. In this way a more accurate estimate of the average concentration of vanadium and its standard

deviation could be calculated for that input into the model.

2. Body weight was modelled using a truncated normal distribution function (Vose, 2000), based on the mean weight and standard deviation of both groups for the period of treatment, with the truncation parameters being the absolute minimum and maximum weights of calves in the trial.
3. The amount of feed ingested by calves was modelled using a uniform distribution function of between 2.3 and 3 % of body weight (Pers. Comm. Professor D. Lourens, Department of Production Animal Studies, University of Pretoria, Pretoria).
4. The mean vanadium intake per calf per day was calculated using the above inputs and the model was run using the programme @Risk (Palisade Corporation, 31 Decker Road, Newfield, New York) and a Latin-hypercube sampling technique for 10000 iterations to give a distribution function of possible values for vanadium intake and the probability of them occurring.

Treatment

$CaNa_2EDTA$ was prepared as a 2% m/v solution for the Treatment Group. Sterile normal saline (0.9 % NaCl), taken from commercial intravenous fluid drips (SABAX), was used as a placebo in the Control Group. Each calf was treated intraperitoneally with a volume (ml) equal to 4 ml/kg body weight, of drug or placebo on Monday and Thursday of each week for 10 weeks. The Treatment Group received a dose equivalent to 80 mg/kg $CaNa_2EDTA$ twice a week. Each calf was weighed, using an electronic scale, just prior to the trial and weekly thereafter. The volume of drug/placebo to be administered was adjusted weekly according to these weights. Calves were treated until day 71 of the trial.

Growth rate

Because of the malabsorption component of the pathogenesis of vanadium toxicity in calves, the body weights obtained for adjusting the treatment dose were also used to monitor growth rates. Growth rates were measured by calculating the average daily gain (ADG) and the area under the growth curve (AUC) for each calf up until Day 71, when treatment stopped.

Calves were tested at slaughter for nematodes, by means of egg counts, and for coccidiosis to rule them out as confounders for weight loss.

Collection and analysis of urine and faeces for vanadium excretion

Metabolic cages were used to facilitate the collection of an initial 18 h urine and faecal sample from three control and three treated animals on Day 7 of the trial. This was followed by a 6 h metabolic cage urine and faecal sample, collected from the same animals on Day 17, 38, 52 and 70 of the trial. The volume of urine was recorded and the volume of urine voided per hour was calculated for each of these six calves. The excretion rate of vanadium in the urine was calculated using the volume of urine voided over a measured time period multiplied by the concentration of vanadium measured in the urine. The concentration of vanadium in each faecal and urine sample was determined using a standard Atomic Absorption Spectrophotometer method (Boyazoglu *et al.*, 1972).

Collection and analysis of blood for haematological and chemical pathology changes

A serum and plasma sample was collected from each animal on Day –11, 22, 43, 64 and 101 of the trial. The serum and plasma samples were used to evaluate the following parameters: Total Serum Protein (TSP), albumin/globulin fractions, Aspartate aminotransferase (AST), Gamma-glutamyltransferase (GGT), Blood Urea Nitrogen (BUN), serum creatinine, White Cell Count (WCC), differential counts, Red Cell Count (RCC) and Haematocrit (Ht). Enzyme activities, urea and creatinine concentrations were analyzed using an automated chemical analyser (Technicon RA-XT system, Miles Inc., Diagnostics Division, Tarrytown, New York) and the manufacturer's methods and reagents. Complete blood counts were determined using a Cell-dyne 3700 (Abbott Laboratories, South Africa). Electrophoresis was performed with a Beckman Model R-100 Microzone Electrophoresis System (Econoscan, Helena Laboratories). The Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria performed the analyses and interpretation of the results was done according to Jain (1986) and Kaneko (1989) since there was no unexposed control.

Lymphocyte stimulation tests

Lymphocyte stimulation tests using the plant lectin phytohemagglutin (PHA) were carried out according to Kristensen, *et al.,* (1982), on serum collected initially from three EDTA Group calves and three Control Group calves on Day 7, 22 and 63 of the trial. Numbers were limited because of the costs involved; however it was felt after examining the first bleed data that for

better interpretation of the results a single calf, independent of the trial, should be bled on Day 22 and 63 of the trial to obtain a "normal" value for calves. Because of variability in the data after the second bleed data was examined, the sample size was increased to seven Control Group calves and eight EDTA Group calves for the final bleed on Day 63.

Lymphocyte activity was estimated using the difference in cell culture activity (△CPM), expressed in counts per minute (CPM), between PHA stimulated cells and unstimulated cells. It was also expressed as a stimulation ratio or stimulation index (SI).

Assessment of organ concentrations of vanadium

Samples of the brain, muscle, lung, kidney, bone and liver were collected from each animal at necropsy for the determination of vanadium concentrations using standard methods (Boyazoglu *et al.*, 1972).

In line with the secondary objectives of the trial, animals were slaughtered in cohorts at monthly intervals (Day 73, 105 and 136). Five calves (three Treatment Group + two Control Group calves = Cohort 1) were euthanased by intravenous administration of sodium pentobarbitone (Euthanase, Bayer Animal Health) in the 13th week (Day 73) of the trial, two days after receiving their last dose of $CaNa_2EDTA$. The remaining calves continued to receive a vanadium-enriched ration for a further two weeks, but received no further treatment. On the 16th week (Day 93) of the trial the contaminated tef hay in the ration was replaced with uncontaminated hay and the spiking of the ration with vanadium was discontinued. Four calves (two Treatment + two Control Group calves = Cohort 2) were euthanased and necropsied during week 18 (Day 105) and the last six calves (n= three Treatment and three Control Group animals = Cohort 3) were euthanased and necropsied during week 22 (Day 136).

A single control animal (D17) died on Day 6 of the trial and liver, kidney and rumen content of this animal were also analysed for vanadium and included in the results.

Statistical Analysis

Statistical analysis was done using Microsoft Excel (version 2000). A student t-test assuming equal variance was used to compare the means where applicable.

RESULTS

Vanadium Exposure

The feed included in the total mixed ration and obtained from the polluted farm of origin comprised unmilled harvested tef grass, which contained 18 ppm vanadium (unwashed, dry mass basis) and hammer milled grass that contained 8 ppm vanadium. The vanadium concentrations in the total mixed ration varied from 15.5 to 32.7 ppm, with an average of 26.46 ppm (sd=2.6). Table 1 shows the trial day when the total ration was sampled, the concentration of vanadium in that batch of ration, the number of days each batch was fed and the weighted input used in the vanadium intake model (see materials and methods).

TABLE 1 Concentrations of vanadium in the complete ration over the duration of the trial

Day of trial	Vanadium (ppm)	Days fed	Weighted input
8	30	21	8.87
21	32.7	4	1.84
25	31	12	5.24
37	24.6	15	5.2
52	25.8	8	2.91
60	15.5	11	2.4

Vanadium intake model

Table 2 shows the inputs for the vanadium intake model and the formulae and input distribution functions used to estimate the amount of vanadium ingested per day.

TABLE 2 Vanadium exposure assessment model

MODEL INPUTS	
Mean body weight (bwt) and (sd)	102 (11.6) kg
Body weight range (kg)	102 RiskNormal(bwt, sd, RiskTruncate(56,159))
% ration ingested	0.027RiskUniform(0.023,0.03)
Feed eaten	2.71 kg/d
Vanadium in feed (mg/kg)	26.46RiskNormal(mean, sd, RiskTruncate(10, 50))
MODEL OUTPUTS	
Mean vanadium intake per day (mg/d)	71.8 RiskOutput
Range (mg/d)	91
Min (mg/d)	33
Max (mg/d)	124
5 Percentile (mg/d)	53
95 Percentile (mg/d)	93

Figure 1 shows the output for the vanadium intake model as a distribution function for vanadium ingested by an individual animal on a daily basis during the trial. The distribution function shows that calves would have consumed on average 71.8 mg vanadium per day and that vanadium intake would not have been less that 33 mg vanadium per day or greater than 124 mg vanadium per day. There is a 90% probability that vanadium intake would have been between 53 and 93 mg vanadium per day.

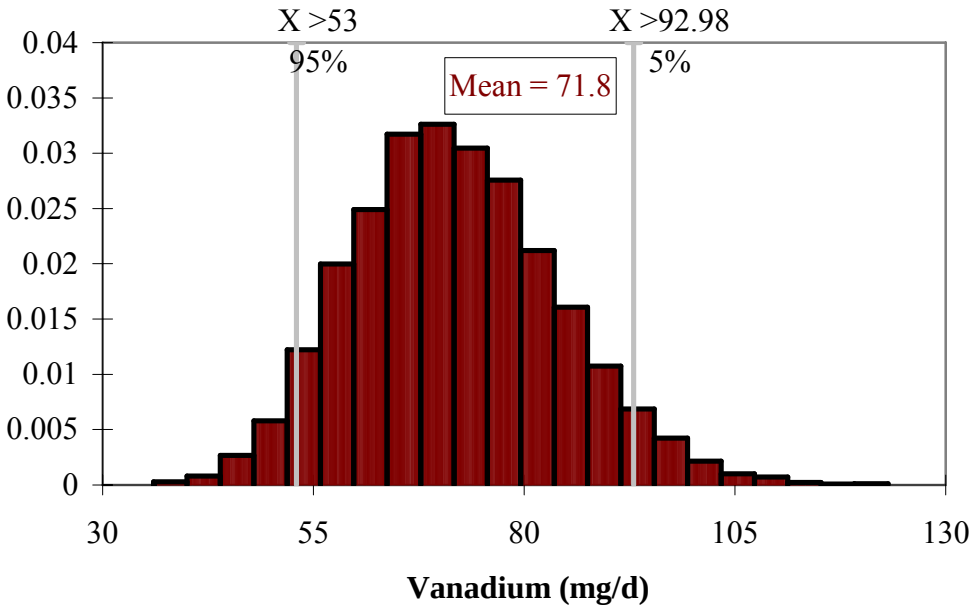

Figure 1. Distribution curve for the amount of vanadium ingested per day by a calf during the trial

Symptoms

All calves upon arrival showed signs of chronic vanadium poisoning of varying degrees, i.e. stunted growth, potbelly, sub-madibular oedema, diarrhoea and staring hair coats (Figure 2). One calf in the Control Group (D17) exhibited clinical signs reminiscent of acute vanadium poisoning on Day 5 of the trial and died on Day 6, despite intravenous fluid therapy (Maintolyte, Adcock Ingram Pharmaceuticals), treatment with $CaNa_2EDTA$ (80 mg/kg) and 10 mg Betamethasone (Betsolan, Schering-Plough Animal Health) and 5 ml of a multivitamin (Phosamine stimulans, Bayer Animal Health) containing 12 mg/ml pyridoxine (Vit B_6).

Figure 2. Calf showing typical symptoms of chronic vanadium exposure upon arrival

As the trial progressed the condition of animals in both groups appeared to improve and no further deaths were recorded.

Excretion of vanadium

Figure 3 shows the excretion rates of the Control and EDTA treated calves in mg vanadium excreted in the urine per hour. Calves in the Control Group had marginally lower excretion rates than the EDTA Group. The average excretion rate for the period of the trial (5 measurements) for the EDTA Group was 9.74 µg/h compared to 5.28 µg/h in the Control Group. This difference over the trial period could not be shown to be statistically significant ($p<0.05$). The failure to show a difference may be due to the small sample size of each group (n=3), as Figure 3 shows a trend towards increased excretion rates in the EDTA Group near the end of the trial.

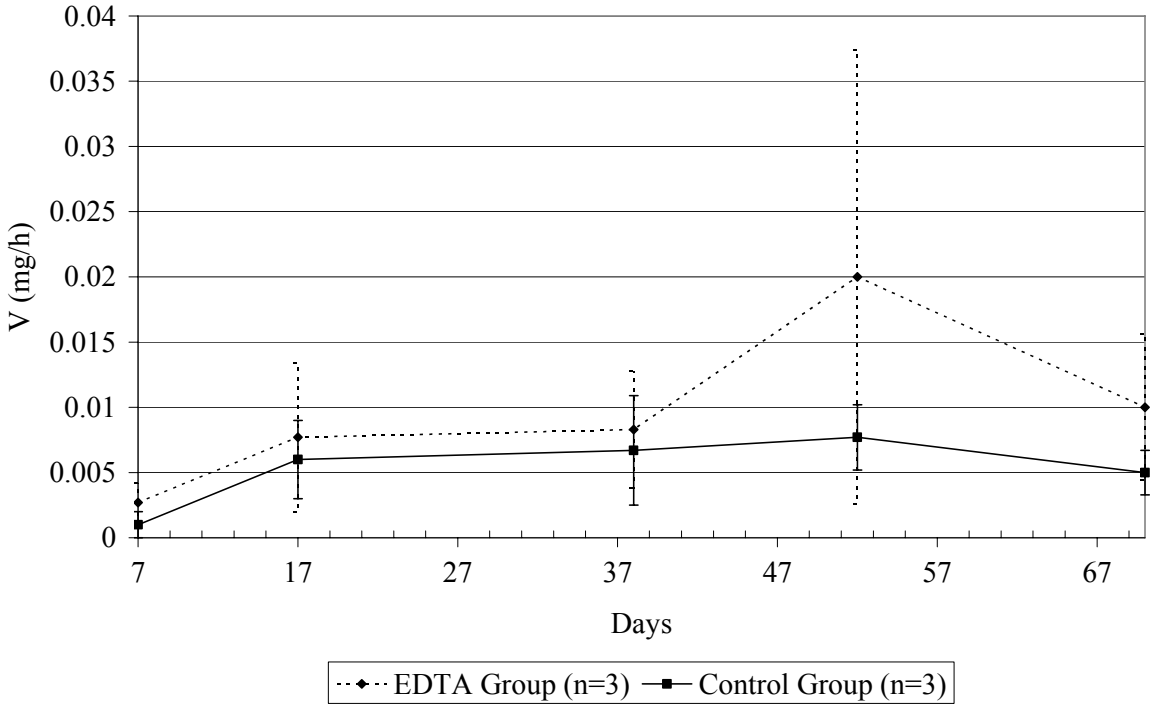

Figure 3. Mean excretion rate and standard deviation (bars) of vanadium in the urine of each group over the duration of the trial

Using the average oral intake of vanadium of 71.8 mg vanadium per day together with the excretion rate for the Control Group, it is possible to estimate the percentage vanadium excreted in the urine of calves after oral intake: = 0.00528 mg/h x 24 h / 71.8 mg x 100 = 0.18%.

Figure 4 shows the average concentration of vanadium present in the urine and faeces of treated and untreated animals over the period until the last animals were slaughtered. The average concentration of vanadium in urine for the period of treatment (i.e. until Day 71) was significantly higher ($p=0.01$) in the EDTA Group (0.134 ppm) than the Control Group (0.064 ppm). The urine vanadium concentrations in the EDTA Group decreased once treatment was stopped (Day 71). When the vanadium was removed from the ration (Day 93 of trial), vanadium concentrations in urine dropped in both groups to undetectable concentrations (<0.05 ppm) by the time the last group was slaughtered (Day 136).

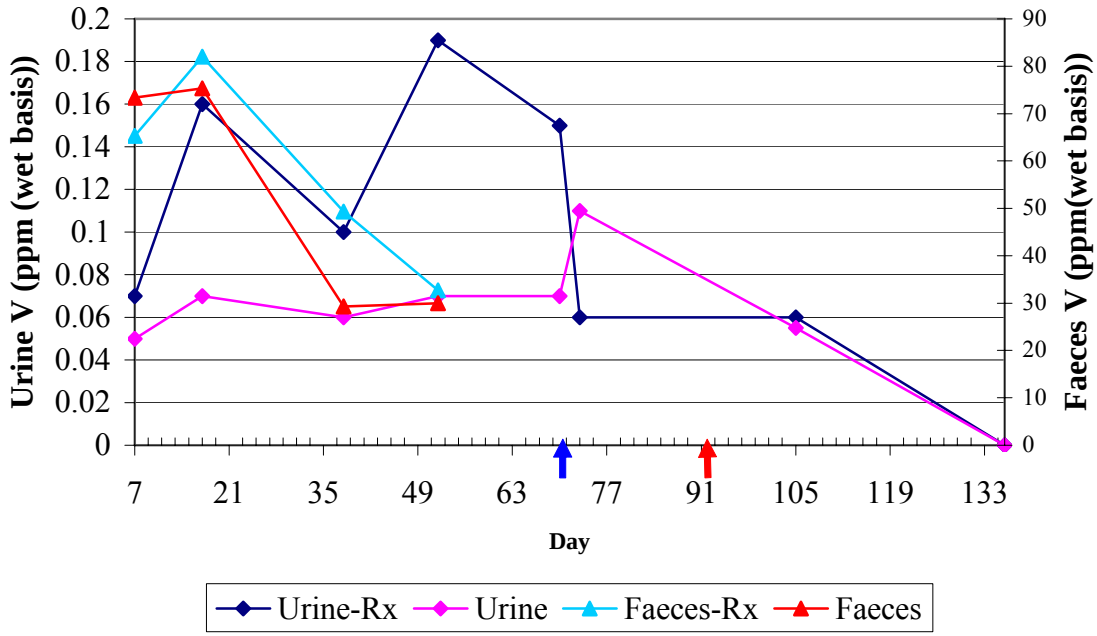

Figure 4 Concentration of vanadium in urine and faeces over the period of trial for each group (blue arrow = treatment stopped; red arrow = vanadium free ration introduced; Rx = EDTA treated group)

Faecal concentrations of vanadium followed a similar trend to urine concentrations. Faecal concentrations of vanadium over the period of treatment (4 observations) were 57 ppm (std dev = 21) in the EDTA Treatment Group and 52 ppm (std dev = 25) in the Control Group. This difference could not be shown to be significant ($p<0.05$). Unfortunately, no faeces was taken at slaughter, only rumen content, so the extent to which faecal concentrations dropped after removal of vanadium from the diet could not be determined. Rumen concentrations of vanadium at the times of slaughter are shown in Table 3.

Growth rate

Calves continued to gain weight during the trial. The mean ADG for the EDTA Treatment Group was 0.40 kg per day and for the Control Group 0.48 kg per day. No statistical difference ($p < 0.05$) could be shown between the Treatment Group and Control Group with respect to mean ADG or mean AUC. Due to the variability in mass within each group the geometric mean ADG was also compared. The geometric ADG for the EDTA Group was 0.36 kg per day and 0.45 kg per day for the Control Group.

Calves were found to be negative for both nematode eggs and coccdiosis.

TABLE 3 Vanadium concentrations (ppm wet-mass) in the rumen content of slaughtered animals

Day	EDTA Group		Control Group	
	n	Mean (sd)	n	Mean (sd)
Day 6 of trial*			1	14.7*
12 days after removal of vanadium	2	0.35 (0.07)	3	0.25 (0.07)
43 days after removal of vanadium	3	0.43 (0.4)	3	0.70 (0.4)

* Calf (D17) that died on Day 6.

Haematological and chemical pathology changes

No difference ($p < 0.05$) could be shown between the Treatment Group and Control Group with respect to mean AST and GGT activities, serum creatinine, albumin concentrations, globulin concentrations, haemoglobin concentrations, RCC, WCC, Ht, MCHC, TSP, BUN and thrombocyte count.

WCC's were slightly elevated when the calves arrived and remained just above the upper normal for the duration of the trial (mean WCC = 11.9 million for Control Group and 10.4 million for EDTA Group). It was suspected this was due to increased globulins, as they were slightly elevated when calves arrived.

The albumin:globulin (A:G) ratios were lower than normal in both groups for the first 43 days of the trial and became normal as the globulins decreased and the albumins increased with time.

BUN was lower than normal in both groups at the beginning and end of the trial. It is postulated that this may be related to low protein catabolism.

The corpuscular volume (MCV) was found to be significantly higher in the Control Group than the EDTA Group but remained within normal ranges for the duration of the trial.

The chemical pathology parameters indicated that there was no apparent liver or kidney damage or concurrent infections in these animals.

Lymphocyte stimulation test

Table 4 shows the $\triangle$CPM and SI values over the trial period.

TABLE 4 Lymphocyte stimulation test results

Animal No.	Day 7		Day 22		Day 63	
Control Group	**△CPM**	**SI**	**△CPM**	**SI**	**△CPM**	**SI**
C9	-6124	0.24	51709	26.6	143512	263.84
C10	10240	22.29	-129	0.72	3414	7.32
B03	45694	1.84	45126	79.48	47796	87.59
D16					4824	2.85
C13					53797	7.76
B06					76	1.34
B08					22907	46.72
Mean activity	16603	8	32235	36	39475	60
Sd	26489	12	28221	40	50705	95
Geomean	6905	2	8530	12	10081	16
EDTA Group						
C12	82223	5.98	26942	39.05	47	1.03
D18	3704	1.24	231530	297.45	2692	3.31
B05	-26207	0.67	13409	29.71	-356	0.76
B07					-339	0.7
B04					-666	0.67
D15					-231	0.86
C11					531	1.3
D14					-89	0.96
Mean activity	19907	3	90627	122	199	1
Sd	56001	3	122213	152	1066	1
Geomean	19299	2	43733	70	56	1
Non-exposed calf*			10210	13	-13	0.98

△CPM = difference in cell culture activity and SI = stimulation index
*Non-exposed calf was a single healthy calf at the Veterinary Faculty used only to obtain base line data for the lymphocyte stimulation test

Organ concentrations of vanadium

Table 5 shows the concentration of vanadium in various organs at time of slaughter. The main difference between the two groups 2 days after treatment stopped (Day 73) was in kidney and urine concentrations, which were higher in the Control group. A month after treatment was stopped (Day 105) and 12 days after animals were placed onto normal feed, organ concentrations in both groups appear to be similar. Organ concentrations appear to have continued to decrease slightly by the time the last animals were slaughtered 30 days later.

TABLE 5 Mean organ concentrations of vanadium (ppm wet mass) in organs of calves at slaughter

Day	7	73	105	136
		EDTA GROUP		
Brain	ND	0.12 (0.11*)	ND	0.027 (0.05)
Muscle	ND	0.09 (0.08)	0.05 (0.07)	0
Lung	ND	0.03 (0.05)	0.05 (0.07)	0
Kidney	ND	0.18 (0.10)0	0.1 (0.00)	0.027 (0.05)
Urine	ND	0.06 (0.08	0.06 (0.00)	ND
Bone	ND	0	0	0.04 (0.03)
Liver	ND	0.2 (0.09)	0.05 (0.07)	0
Rumen	ND	ND	0.35 (0.07)	0.433 (0.40)
		CONTROL GROUP		
Brain	ND	0.09 (0.01)	ND	0.063 (0.06)
Muscle	ND	0.03 (0.04)	0	0.02 (0.03)
Lung	ND	0.04 (0.05)	0.05 (0.07)	0
Kidney	0.8	0.52 (0.22)	0.1 (0.00)	0.033 (0.06)
Urine	ND	0.11 (0.00)	0.055 (0.02)	ND
Bone	ND	0	0	0.05 (0.05)
Liver	0.4	0.2 (0.05)	0.13 (0.04)	0.017 (0.03)
Rumen	14.7	ND	0.25 (0.07)	0.7 (0.40)

ND = Not Determined; 0 = < limit of detection, *standard deviations are given in brackets

Necropsy findings

The gastrointestinal tract (GIT), lymphoid and haemopoeitic tissues, and respiratory system were primarily affected. There was no obvious difference between calves that received treatment and those that did not. The control calf D17, which died on Day 6 of the trial, showed severe emaciation and cachexia, and the calves euthanased on Day 71 of the trial (Cohort 1), when treatment stopped, remained in poor condition. The second group of calves to be euthanased 35 d post treatment (Cohort 2), but still being exposed to vanadium in the diet had better body condition than the first group, but were still emaciated. The last cohort of calves (Cohort 3) slaughtered 65 d post treatment and 43 d after vanadium was removed from the ration showed no signs of emaciation and were in good condition (Table 6).

The lungs of calf D17 showed extensive areas of acute fibrinopurulent lobular pneumonia. Histologically the pneumonia was typical for that seen in acute pneumonic pasteurellosis. *Pasteurella multocida* was isolated from the lungs and liver of this calf, which probably contributed to its death. The calves in all the cohorts showed histological evidence of chronic

interstitial pneumonia, which was characterized by fibrosis and mononuclear cell infiltration in the alveolar walls and partial to complete atelectasis of alveoli. The degree varied from mild to severe (Table 6).

Animals in Cohort 1 showed rumen stasis but this was not evident in the other cohorts. A mild to severe enteritis was found in all calves except D17 (Table 6). The severity of enteritis showed a definite decrease from Cohort 1 calves to Cohort 3 calves. The enteritis was characterized by villous atrophy and fusion, oedema of the lamina propria, infiltration of eosinophils and mononuclear cells in the propria and the presence of immature epithelium.

TABLE 6 Summary of main pathological findings graded for each cohort of calves slaughtered

Euthanasia Cohort	Body Condition	GALT hyper-plasia	Enteritis	Interstitial pneumonia	Lymph-node hyperplasia	Spleen hyper-plasia
Cohort 1 No Rx (n=2)	Poor	3+	3+	1+	-	-
Cohort 1 Rx (n=3)	Poor	3+	3+	1+	-	-
Cohort 2 No Rx (n=2)	Fair	-	3+	2+	1+	1+
Cohort 2 Rx (n=2)	Fair	-	3+	2+	2+	-
Cohort 3 No Rx (n=3)	Good	-	2+	2+	2+	1+
Cohort 3 Rx (n=3)	Good	3+	1+	2+	1+	-
- = none seen; 1+ = mild; 2+ = moderate; 3+ = severe – values are median values for each cohort; GALT = gut associated lymphoid tissue						

The spleen in calf D17 was severely atrophied and the spleens of some of the other calves (n=4) showed mild red and white pulp hyperplasia (Table 6). Calf D17 showed severe atrophy of the lymph nodes, while 10 of the remaining 12 calves showed mild to severe lymph node hyperplasia, especially of the mesenteric and hepatic lymph nodes. Calf D17 and Cohort 1 calves had lymph nodes with a washed-out appearance due to lymphocyte loss. In eight of the Cohort 2 and 3 animals the most obvious abnormality in their lymph nodes was the presence of small to moderate numbers of foamy macrophages, especially in the paracortical sinuses. This was more marked in the Cohort 2 calves than the Cohort 3 calves. The thymus was seen to be macroscopically enlarged but histologically normal in four of the Cohort 3 calves.

DISCUSSION

Treatment

Faulkner Hudson (1964) reported that $CaNa_2EDTA$ gave 80 to 90 % protection from vanadium poisoning in mice when administered intraperitoneally 15 minutes prior to injection of a lethal dose of sodium vanadate, and it was for this reason and its availability for treatment of lead poisoning that this chelator was chosen for this trial. It was assumed that calves originating from a vanadium polluted area would have high baseline organ concentrations of vanadium. However, because the calves were removed from the polluted environment and vanadium is reportedly rapidly excreted it was necessary to try and maintain a level of exposure to vanadium that was consistent with those on the polluted farm if the $CaNa_2EDTA$ treatment was to be realistically evaluated. Since exposure on the farm also included aerial exposure, a combination of contaminated hay from the farm of origin and the addition of vanadium pentoxide to the complete ration was used in the trial.

The primary objective of the trial was to try and find a practical treatment for animals required to live in vanadium polluted areas. From the results, it was difficult to come to any firm conclusion that $CaNa_2EDTA$ had a protective or therapeutic effect on calves exposed to vanadium. The significant difference ($p=0.01$) in average concentrations of urine vanadium between the EDTA Treated Group and the Control Group, together with the trends shown in Figure 3, does however, provide some evidence that $CaNa_2EDTA$ enhanced the excretion of vanadium, but whether that was sufficient to protect animals could not be established. Based on conclusions by Albert (1961), it is anticipated that the chelation process would affect the oxidation-reduction potential of vanadium resulting in a change of valency to a less toxic form.

The recommended therapeutic regimen for treating lead poisoning in cattle is to administer a 6.6 % m/v solution intravenously at a dose rate of 73 mg/kg daily in 2-3 divided doses (Osweiler *et al.*, 1985). The cattle in this trial were only treated twice a week using a 2% m/v solution. Hence, it could be argued that more frequent administration might have had a better result, but this would have made the treatment impractical at a farm level. This trial did establish that the administration of $CaNa_2EDTA$ at 4ml/kg bwt produced no major side effects in calves when administered for a prolonged period. It however, remains a challenge for a suitable prophylactic treatment to be found for cattle farmed in areas with high background levels of vanadium.

Growth rates

Normal ADG for dairy calves in the 3-6 m age group is considered to be between 0.59-1.05 kg per day (Brand *et al.*, 1996). Hence in both groups calves failed to grow at the optimal rate, resulting in end weights that were below what is considered normal for calves of this age. The study was therefore able to quantify farmer claims that growth rates were being suppressed by vanadium. This is an important finding because it impacts on claims made by farmers to industry for damages due to vanadium pollution. The precise mechanism for poor growth remains unclear but is probably related to the lesions that occur in the gastrointestinal tracts of calves exposed to high background levels of vanadium.

Lymphocyte stimulation tests

The results of the lymphocyte responses are difficult to interpret since very little appears to have been published with respect to cattle. The SI can only be used to evaluate whether a stimulation test is positive or not since the CPM in unstimulated cultures is partially influenced by irrelevant factors (Kristensen *et al.*, 1982). According to Kristensen *et al.* (1982), if the SI is above 2.5-3, a test can be considered positive. On the other hand, the △CPM can, when all variables are considered, be used to estimate the magnitude of a response. In general, the reported results following stimulation with potent mitogens such as PHA, under optimal conditions, vary between 10000 and 200000 CPM/culture (Kristensen *et al.*, 1982). Using these parameters, individual animals showed reduced lymphocyte responses in both groups on Day 7 and in the Control Group on Day 22. On Day 63, all the animals in the EDTA Group showed reduced lymphocyte activity compared to one animal in the Control Group. Based on the previous findings of Gummow *et al.* (1994) one would have expected the majority of the Control animals to have a reduced lymphocyte activity. The interpretation of these results is further complicated by the fact that the single quality-control animal also showed reduced lymphocyte activity on Day 63, hence making it possible that some other confounder was playing a role. While the results in this trial remain inconclusive, lymphocyte responses in cattle remain an interesting aspect to consider for future studies on the effects of vanadium in cattle.

Organ and Necropsy findings

The organ concentrations of vanadium in liver and kidney were consistent with previous findings (Gummow *et al.*, 1994) for chronic vanadium poisoning. A new finding is that some vanadium may be retained in the tissues for some time after exposure and that vanadium is not as rapidly excreted in cattle as was initially suspected.

The lesions found in calf D17 and Cohort 1 were consistent with lesions described by others for vanadium poisoning (Gummow *et al.*, 1994; McCrindle *et al.*, 2001). Concurrent infections such as pasteurellosis are also often found in cases of chronic vanadium poisoning, probably as a result of a compromised immune system. This is however the first time that pathology was monitored for some time post exposure in calves and one of the most important findings was that animals may be able to recover once they are no longer exposed to vanadium, since previously it was thought that this damage may be irreversible.

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Chapter 4

A STOCHASTIC MODEL FOR ESTIMATING INTAKE OF VANADIUM BY BEEF CATTLE USED AS SENTINELS WITHIN THE VANADIUM MINING INDUSTRY

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Abstract

A sentinel herd of 30 Brahman Cross cattle was run as an extensive beef commercial herd and experimental cohort on a vanadium mining property over a five-year period. The cattle were farmed as two groups. A high exposure group that grazed downwind in an area immediately adjacent to the processing plant and a low exposure group whose grazing began approximately 2 km upwind of the processing plant. A primary objective of the study was to investigate whether a long-term animal sentinel system for the vanadium mining industry could act as a monitoring system for pollution problems and thus address the concerns of surrounding farmers. Various potential biomarkers and production and health parameters were monitored in the cattle over the five-year period. However, before the consequences of exposure and the value of the biomarkers could be assessed it was necessary to produce a method for quantifying how much vanadium the cattle had been taking in over the duration of the trial and what forms of vanadium were playing a role. The method had to account for chronic field exposures where there is a fluctuation of exposure over time and account for individual animal variability.

A systems approach was used to assess the risks related to the cattle in the vicinity of the mine. This approach offered a structured framework for dealing with the five years of accumulated data. This paper describes, within this framework, a qualitative method for hazard identification and release assessment within the vanadium mining industry and a quantitative stochastic model for assessing persistent long-term environmental exposure to vanadium. The stochastic nature of the model deals with uncertainty and variability within the available data. The primary inputs were direct measurements at the point of exposure and account for oral (grass, soil and water) and inhalation exposure (aerial fallout). This distinguishes it from Gaussian plume dispersion models. The primary inputs are combined with the physiological parameters of the species concerned to provide an accurate estimate of the dose of vanadium intake. The final output of the model is a distribution curve of probable vanadium intake values based on the variability within the inputs. The exposure model estimated an external dose over the five-year period of between 0.05 and 23.96 mg vanadium/kg body weight/day ($\bar{x}$=2.6) for the High Exposure group and between 0.01 and 12.72 mg/kg/d ($\bar{x}$=1.2) for the Low Exposure group. There was only a 5% probability of values being <0.56 or >6.58 mg/kg/d for the High Exposure group and <0.33 or >2.73 mg/kg/d for the Low Exposure group.

Keywords: Environmental epidemiology, vanadium pollution, beef cattle, release assessment and exposure assessment

1. Introduction

1.1. Why is Vanadium important?

Vanadium (V) is classified as a transition element on the periodic table with the atomic number 23. It is widely distributed in nature and the average level of vanadium in the earth's crust is normally 100-150 ppm (Faulkner Hudson, 1964; Richie, 1985; Waters, 1977). The prevalence of vanadium exceeds that of well-known metals such as copper and lead (Nriagu, 1998), and equals that of zinc and tin (Byerrium et al., 1974; Windholz, 1983; Grayson, 1983). Vanadium compounds exist in over 50 different mineral ores at concentrations of between 10-4100 ppm and in association with fossil fuels, particularly coal (at concentrations of between 19-126 ppm in ash) and crude oil (at concentrations of between 3-257 ppm) (Nriagu, 1998). About one third of the vanadium resources are located in Africa and North America, and about 24% are found in Europe and <4% in both Asia and South America (Nriagu, 1998). About 83% of vanadium recently produced from mines comes from vanadiferous magnetite (Fe_3O_4) in South Africa, China and Russia (Hilliard, 1992). The remaining 17 % of worldwide vanadium production from primary sources is recovered from the oil industry.

South Africa is the worlds leading producer of vanadium and accounts for 50% of the current global output. Other producing countries include Russia, China and the USA. Western Australia has large deposits of magnetite ores containing vanadium and the tar sands of Alberta, Canada, represent a huge reservoir of vanadium (Nriagu, 1998). These remain largely untapped sources of vanadium.

The vanadium oxides are most often used by industry, primarily in the manufacturing of steel, where it is used as ferrovanadium or as a steel additive (Reilly, 1991; Toxicological profile for Vanadium, 1992). Vanadium is thus used in producing rust-resistant, spring, and high-speed tool steels. It is also used in the production of components for aircraft engines and weapon systems, making it a strategic mineral for armament manufacturers. In addition, because the metal has good structural strength and a low-fission neutron cross-section, it is useful in nuclear applications. Vanadium foil is used as a bonding agent in cladding titanium steel. Vanadium pentoxide is used in ceramics and is a catalyst. It is also used as a mordant in dying and printing

fabrics and in the manufacture of aniline black (CRC handbook of chemistry and physics, 1977). Small amounts of vanadium are used in making rubber, plastics, and certain other chemicals (Reilly, 1991; Toxicological profile for Vanadium, 1992).

Vanadium poisoning has long been an occupational health risk within industry (Faulkner Hudson, 1964). In South Africa, mining companies and farmers are often found in close proximity to one another and it is no surprise that sporadic incidents of vanadium poisoning in cattle have been reported in South Africa over many years (Unpublished archival records of the Onderstepoort Veterinary Institute (OVI), Private Bag X05, Onderstepoort, 0110, 1961/1962, 1975/1976; Gummow et al., 1994; McCrindle et al., 2001). Outbreaks of vanadium poisoning in cattle are however, not confined to South Africa and a number of reports from other parts of the world (mainly Europe) have been published since the 1960's (ter Heege et. al., 1964, Frank et al., 1990; Frank, et al., 1996). Little work has however been done on the impact of chronic exposure to vanadium pollutants in ruminants or on the public health implications of that exposure (Toxicological profile for Vanadium, 1992). It was therefore decided in 1999 to set up a sentinel cattle herd within the bounds of a vanadium mine to study the effects of chronic vanadium exposure in more detail.

1.2. The use of animals as environmental sentinels

The primary goal of an animal sentinel system is to identify harmful chemicals or chemical mixtures in the environment before they might otherwise be detected through human epidemiology studies or toxicology studies in laboratory animals. Once identified, exposures to them could be minimised until methods can be devised to determine specific aetiological agents or until suitable prophylactic measures can be established. Animal sentinel systems therefore have potential value as early warning systems for new hazards, as indicators of potential human exposure to complex mixtures or in complex environments, and as monitors of the effectiveness of remedial measures or other environmental management actions (Hornshaw et al., 1983). A primary objective of this study was to investigate whether a long-term animal sentinel system for the vanadium mining industry could act as a monitoring system for pollution problems and thus address the concerns of surrounding farmers. Various potential biomarkers and production and health parameters were monitored in the cattle over the five-year period. However, before the consequences of exposure and the value of the biomarkers could be assessed it was

necessary to produce a method for quantifying how much vanadium the cattle had been taking in over the duration of the trial and to establish what forms of vanadium were playing a role. This paper describes a method that accounts for individual animal variability and the complexity of chronic field exposures, where there is a fluctuation in exposure over time.

2. Materials and Methods

2.1. Animal model system and justification of model

The animal model system comprised 30 *Bos indicus* beef breeding females with a 20% heifer replacement rate farmed extensively in a 200 ha area adjacent to a vanadium mine. *Bos indicus* beef cattle were used because they require less intensive management than dairy cattle, are more resistant to tick borne diseases, can be farmed extensively and thus have the most exposure to contaminated grazing. *Bos indicus* cattle were also more representative of the surrounding farmers cattle, which were farmed extensively. Cattle have been the species most involved in vanadium pollution problems in South Africa.

The area adjacent to the vanadium mine was used because it was not required for mining at this stage and it made economic sense to find an alternative use for the land. In addition, the area was known to have high background concentrations of vanadium in the surface soil and was adjacent to land farmed by farmers that had complained in the past that vanadium mining was having an influence on their animals welfare.

2.2 Experimental design

A cohort study was carried out using 30 *Bos indicus* beef cattle of the same approximate mass. They were purchased in 1999 as heifers and randomly divided into two groups; A High Exposure (HE) Group of 10 and a Low Exposure (LE) Group of 20 cattle. The HE Group was farmed in an area immediately adjacent to the mine where high background concentrations of vanadium were thought to occur. The LE Group was farmed approximately 2-3 km from the first group in an area thought to have much lower background vanadium concentrations (D. Steyn, Environmental officer, and L. Ford, Technical director and engineer, personal communication, 1999). Both groups were farmed as an extensive beef cattle enterprise. The herd size was limited by the amount of available grazing and a stocking density of approximately 1 animal per 5 ha was used as a guideline.

2.3 Observations and analytical procedures

The farm was visited by at least one of the veterinary co-workers once every 3 months, to monitor the health status of the herd, collect samples and bring records up to date. A record system was kept by the Department of Production Animal Studies at the Faculty of Veterinary Science, University of Pretoria for analyses purposes.

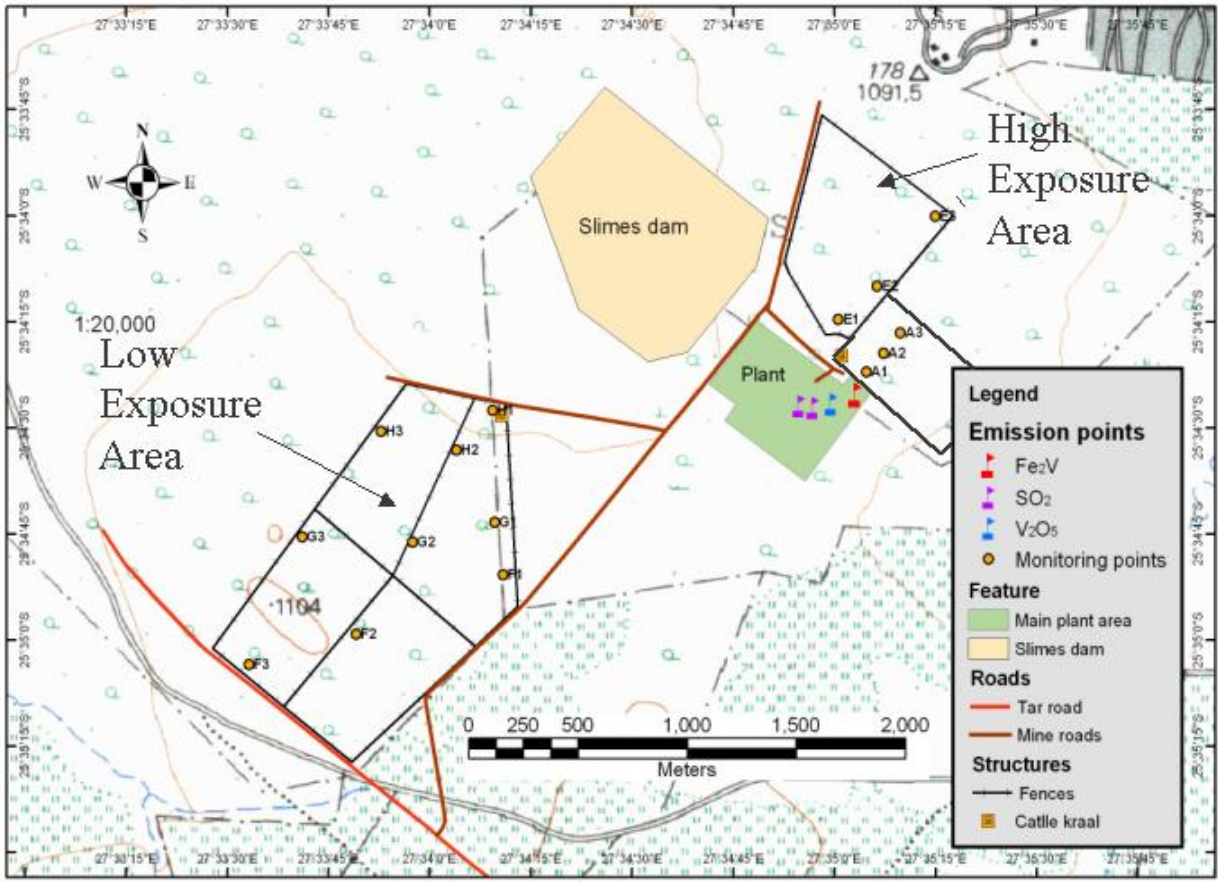

Fig. 1 Map of the area where the trial took place showing the primary sources of vanadium release in relation to the LE and HE camps and the points where samples were routinely collected in those camps.

A weather monitoring station was placed on the mine's property to measure wind direction, wind speed, ambient temperature, humidity and rainfall.

Nine deposit samplers per camp were placed at strategic points along three transections within each camp to capture airborne particulate matter. Each transect began near the processing plant and extended out to the boundary of the camps (Fig. 1). Soil and grass samples were collected

once every 3-4 months at the same points as the nine deposit samples per camp. A variety of grass species were sampled at each sampling point in each transect. At the same time, water samples were taken from the drinking troughs. The soil, grass and water samples were analysed by the Institute of Soil, Climate and Water, Pretoria, South Africa, for concentrations of heavy metals and in particular vanadium, using standard internationally accepted methods and quality control procedures (USEPA Standard Methods, 1986; Handbook of standard soil testing methods for advisory purposes, 1990).

At the same time the soil and grass samples were taken during the year, various potential biomarkers and production and health parameters were monitored in the cattle. These included bone, hair, milk, urine and faeces for vanadium determination and blood for haematology and chemical pathology.

2.4 Risk Analysis Model

Because of the complexity of the exposure and consequences it was decided to use a systems approach to deal with the information. The system was based on that first described by Covello and Merkhofer (1993) and is also used by the Office International des Épizooties (OIE) for quantitative import risk assessment. It focuses the investigation and structures the information into four categories: Hazard Identification, Risk Assessment, Risk Management and Risk Communication. The Risk Assessment portion of the system is further sub-divided into Release Assessment, Exposure Assessment, Consequence Assessment and Risk Estimation and the Risk Management portion is viewed in terms of Risk Evaluation, Option Evaluation, Implementation and Monitoring and Review. The work in this paper deals only with the Hazard Identification, Release Assessment and Exposure Assessment components of the systems analysis.

2.4.1. Hazard Identification

Hazard Identification was done using a qualitative process based on examination of the literature with respect to vanadium mining, interviews with mine management, previous case histories and preliminary findings of tissue concentrations of metals in cattle in the trial. Some years into the study, unexplained symptomatology in affected calves led to the inclusion of SO_2 and NH_4 gasses as hazards and confounding factors. Hazards identified were those that were

applicable to the cattle running on the mine property, which were not necessarily the same as those to which workers were exposed.

2.4.2. Release Assessment

Release Assessment was based on expert opinion obtained from interviews and validated using three independent environmental studies carried out by the mine between 1998 and 2003 (Strass Environmental Science & Engineering, 1998; Grundling, 1999; Burger & Watson, 2003). The experts included the mine's technical director who was also an engineer with many years experience in the vanadium industry, the site environmental and production manager who was also responsible for occupational health aspects and the group environmental manager who was responsible for environmental issues concerning several of the company's mines. Once the experts had identified the potential sources for the vanadium compounds released into the environment and the types of vanadium compounds that were produced during the production process, the same experts were asked to weight the importance of the sources of exposure with respect to the cattle. This was done for the following vanadium compounds: V_2O_5, $NaVO_3$, NH_4VO_3, V_2O_3 and also for SO_2.

2.4.3. Exposure Assessment

Exposure Assessment was carried out using stochastic simulation models that incorporated distribution functions defined by the input data and Latin-Hypercube sampling. The objective of the models was to simulate the amount of vanadium that cattle would have taken in during the project. The following is a description of a generic model that pools all the data for the duration of the trial. To reduce variance and to allow the exposure data to be correlated to the biomarker data, the data was also stratified into seasonal wet (October to February) and dry (March to September) periods for 1999/2000, 2001/2002 and 2003/2004 and a separate model created for each of these strata. It was decided not to stratify the periods any further because of the relatively small number of sampling points used for fall out and because, during certain periods of the trial, data from certain points could not be collected because of changes in mining operations, veld fires or staff problems.

The generic model was divided into three segments. The first segment comprised collation of the exposure data that had been recorded during the duration of the trial. This data comprised the aerial fall out data, the grass concentrations of vanadium, soil concentrations of vanadium,

background feed concentrations of vanadium and background water concentrations of vanadium over the duration of the trial (referred to below as exposure inputs). As explained above, the data was pooled for each year of the study into a wet season (October to February) and a dry season (March to September) based on meteorological data that was collected on site. This provided the first input into the model and each exposure input for that period was defined using a truncated lognormal distribution function generated by Bestfit version 4.5 (Palisade Corporation). The lognormal distribution function was consistently within the top ranking distribution functions that Bestfit fitted to the data and is commonly used for environmental data. The function was truncated with a lower bound of zero to prevent the generation of negative values, which would be implausible, and an upper bound dependent on what was regarded as an unrealistic maximum for that exposure input. So the maximum aerial fall out was truncated at 100000 $\mu g/m^2/d$, unwashed grass vanadium at 1000 mg/kg/d and soluble (EDTA) soil vanadium concentrations at 50 mg/kg/d. This generated a distribution function for possible concentrations of vanadium representative of each exposure input. For the purpose of the generic model, a uniform distribution function was then used to combine the seasonal data over the years into a single distribution function representing the possible vanadium concentrations over the entire period of the trial for grass, soil, water and air respectively. The resultant outputs of this segment of the model were therefore distribution functions that represented aerial fall out ($\mu g/m^2/d$), concentrations of vanadium in unwashed grass (mg/kg), concentrations of soluble vanadium in soil (mg/kg) and concentrations of vanadium in water sources used by the cattle ($\mu g/l$) for each camp over the entire period of the trial. The structure of the model was designed to allow examination of vanadium concentrations for a particular year or season in order to examine trends related to the consequence assessment, but these results are not discussed in this paper.

The second segment of the model involved modelling the physiological intake parameters for cattle. These were divided into vanadium that would be ingested orally through the intake of feed, water and soil, and vanadium that would be inhaled. Percutaneous absorption was not considered to be a route of intake for vanadium and thus was not included in the model. This assumption was made because of the relatively small amount of vanadium that is known to be absorbed across the gastro-intestinal barrier of monogastric animals (Friberg, 1979). Similarly, while background concentrations of vanadium were monitored in the commercial winter

supplementary licks and lucerne that cattle received, the small concentrations and short period of ingestion (2-3 m per year) of the lick and lucerne resulted in a negligible amount of extra vanadium per day; so this source of intake was also not included in the generic model.

The intake of veld grass for adult cattle (>24 m) is reported to be 1-2.5% of their body mass (Smith, 1990). This range of possible intakes was simulated using a Uniform(0.01,0.025) distribution function. This function was multiplied by a Normal distribution function based on the mean mass and standard deviation for each group of cattle. The result was a distribution function for the mass of grass ingested per day per adult cow (dry matter intake (DMI)). This was multiplied by the concentrations of vanadium in unwashed grass for the respective HE and LE camps, that was simulated as described above, to give a distribution for the amount of vanadium ingested by eating grass per cow per day.

The volume of water ingested per day by adult cattle was calculated using the equation 0.075 + 4.234 x DMI (La Manna et al., 1999). The DMI used in the equation was calculated as described above for each group. The volume of water ingested per day was then multiplied by the concentration of vanadium present in the water of each group as described above, to give a distribution for the amount of vanadium imbibed per cow per day.

The amount of soil ingested per day during the course of grazing is estimated to be between 1 and 18% of DMI (Healy, 1968; Healy, 1970; Thornton & Abrahams, 1981). This distribution of potential values was again modelled using a Uniform(0.01, 0.18) distribution function and multiplied by the DMI value calculated above to give a distribution for the amount of soil ingested per cow per day. This was multiplied by the concentration of soluble vanadium (designated as EDTA from its method of determination) in the soil as described above to give a distribution for the amount of vanadium ingested by soil intake per cow per day. Total oral intake of vanadium was then the sum of the grass, water and soil intakes per cow per day.

The mean ground air speed over each relevant time period was calculated using data captured by the weather station set up on the mine for this purpose. The wind speeds were stratified according to wind direction, and the mean wind speed calculated for winds blowing in the direction of the HE and LE camps. To take into account the difference in predominant wind direction, the mean wind speeds were weighted by the frequency that the wind blew in the direction of the HE and LE camps respectively.

To estimate the amount of vanadium inhaled, the vertical terminal velocity of a V_2O_5 particle was calculated according to the formula;

$$v_t = \frac{2r_v^2(\rho - \sigma)g}{9\eta}$$

Where r_v = radius of vanadium particle; ρ = density of V_2O_5; σ = density of air; g = gravitational acceleration and η = viscosity of air (Whelan & Hodgson, 1979). Inputs for the formula were obtained from the CRC Handbook of Chemistry and Physics (1977), with the exception of the radius of vanadium particles, which was derived from expert opinion obtained from the mines engineers. The radius was therefore modelled as a Uniform distribution function representing the range 30 to 60 μm.

The time for particles to move horizontally over the fall out bucket (Δt) was calculated by dividing the diameter of the fall out bucket (ϕ) by the weighted wind speed (ws). To determine the height of the column of air that contributed to the vanadium deposited in the bucket, this time was then multiplied by the vertical terminal velocity (v_t) to give the change in vertical distance of particles in this time (Δs_{vert}). The volume of air sampled in time delta t was then calculated using the formula

$$V_{air} = \Pi r^2 \times \Delta s_{vert} = \frac{\Pi r^2 \times v_t \times \phi}{ws}$$

where r is the radius of the fall out buckets. The volume of air sampled in a 24 h period could then be calculated as: $\frac{V_{air}}{\Delta t} \times 24\,h \times 3600\,s = \Pi r^2 \times v_t \times 24\,h \times 3600\,s$. Hence the volume of air sampled in 24 hours is directly proportional to the fall out surface area and vertical terminal velocity of the vanadium particles.

The fall out concentration of vanadium was then divided by the volume of air (V_{air}) to give the concentration of vanadium per m^3 of air. This was converted to vanadium concentration per litre of air by dividing by 1000. The respiratory rate per minute of an adult bovine was modelled using a Normal distribution function with a mean of 30 and standard deviation of 1.8, truncated at 15 and 60 (Svendsen & Carter, 1984; Reece, 1991). This was multiplied by 60 x 24 to give the 24 h respiratory rate. The tidal volume was modelled using a Normal distribution function with a mean of 3.5 and a standard deviation of 0.4 (Svendsen & Carter, 1984). The daily respiratory volume could then be calculated by multiplying the tidal volume by the 24 h

respiratory rate. This was then multiplied by the vanadium concentration per litre of air to give an estimation of the amount of vanadium inhaled by an adult bovine per day.

The output of the model was then the sum of the oral daily dose of vanadium and the daily inhalation dose of vanadium to give a distribution for the daily exposure dose for cattle in the HE camp and LE camp.

2.5. Data Analysis

All the RA models were constructed in MS Excel version 2000 (Microsoft Corporation) and simulated using the Excel add in @Risk version 4.5 (Palisade Corporation). Each simulation was run using Latin Hypercube sampling with 100000 iterations. The selection of distribution functions was done according to guidelines set out by Vose (2000) in conjunction with the software programme BestFit (Palisade Corporation).

2.6. Reliability of Model Inputs

Duplicate soil and grass samples were sent on an ad hoc random basis to independent accredited laboratories to ensure that the methods of analysis remained reliable. Results were consistently examined for outliers and, when present, analysis of these samples was repeated. The grass results were also compared with the results of a once off independent survey carried out in 1998 by Strass Environmental Science & Engineering (SESE) where grass samples were taken for the mine on a 50x50 m grid basis in the area of the HE camp. The grass samples taken by SESE (1998) were washed prior to being analysed for vanadium and hence were compared to washed grass samples taken in this study.

An independent company was contracted by the mine to model atmospheric dispersion of air pollution (Burger & Watson, 2003). The model used for the dispersion simulation was a traditional steady state Gaussian plume dispersion type model referred to as the Industrial Source Complex model (EPA, 1995[a] & 1995[b]). This model was used to predict the fall out of vanadium at the grid reference points used to measure aerial fall out of vanadium for the trial. The predicted concentrations were compared to the fall out values in this study in an attempt to test the reliability of the aerial fall out portion of the exposure assessment model.

3. Results

3.1. Hazard Identification

The following substances were identified as potential hazardous substances. Together with each substance is given a brief reason why it was identified as a hazardous substance (see also Fig. 2).

1. Vanadium
    a. Presence of vanadium processing plant
    b. Elevated organ concentrations – live and slaughter animals
    c. Elevated soil and vegetation concentrations
2. $S0_2$ – $S0_3$ – $S0_4$ (Plant gasses) - Sulphates – Fugitive gasses
3. NH_3 – NH_4 (Ammonium sulphate processing)

Vanadium was further subdivided into the compounds released during its purification and processing. These were V_2O_5, $NaVO_3$, NH_4VO_3 and V_2O_3. Fig. 2 shows the processing stages for the extraction and purification of vanadium to get the final product, V_2O_5, and the points where vanadium compounds can be released into the environment.

3.2. Release Assessment

The release assessment concentrated on the release of the vanadium compounds identified as hazards into the areas where cattle were grazing. The possible sources of vanadium are shown in Table 1. Table 1 also shows the weight of each source as a contributing factor to the release of vanadium into the environment. The contribution of each source to total vanadium release is summarised as a percentage. Hence stack emissions were regarded as the primary source of vanadium release into the environment of the cattle, followed by the calcine dumps.

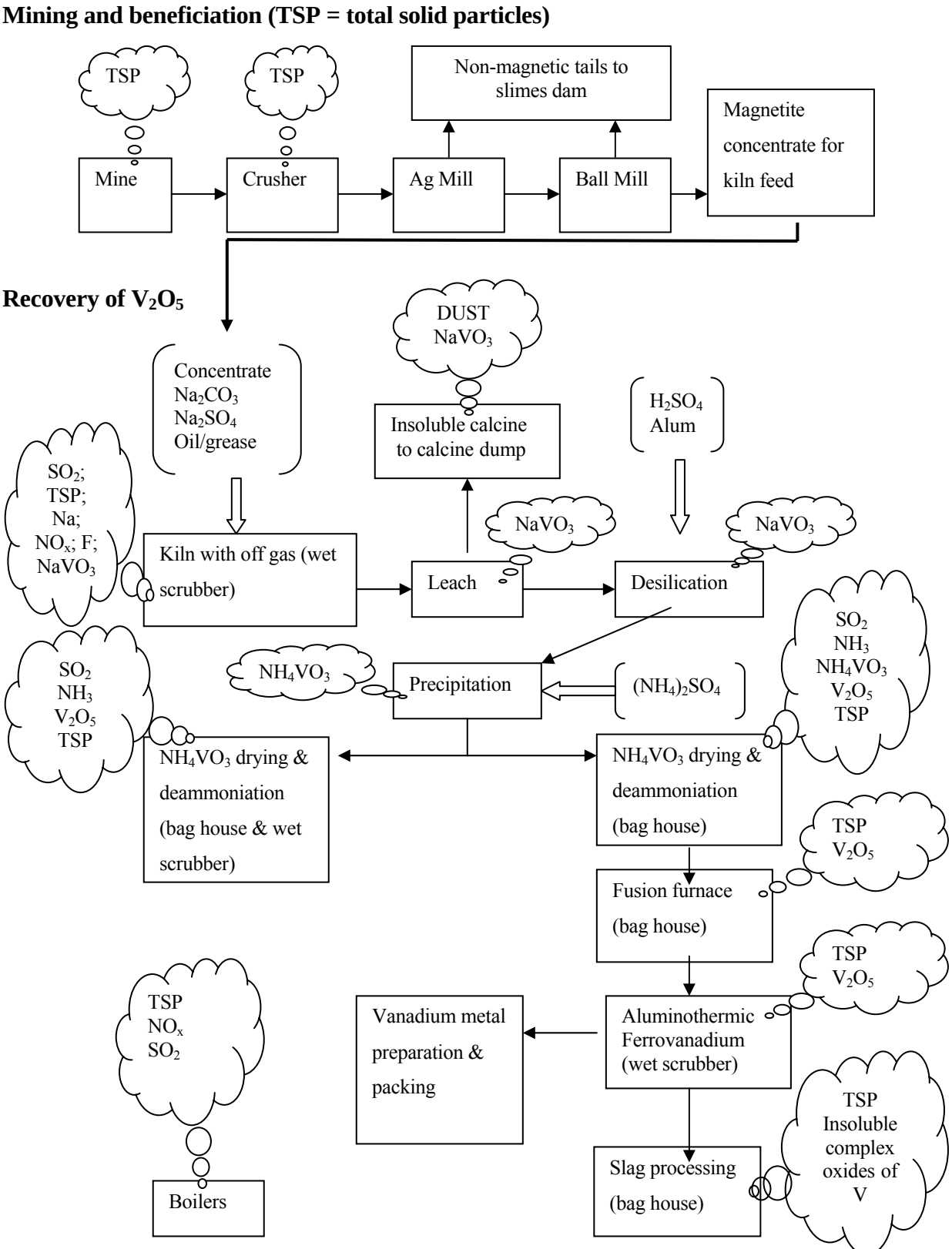

Fig. 2 Flow diagram showing the process for recovery and extraction of vanadium-pentoxide, the potential pollutants and their points of release

Table 1 Sources of vanadium from a South African vanadium processing unit and the weight of their contribution to the release of vanadium into the environment in 2003

Source	Total V (%)	V_2O_5	$NaVO_3$	NH_4VO_3	V_2O_3	SO_2 (%)
Stack Emissions	66	High	Low	Medium	No	50
Calcine Dumps	23	V Low	High	No	No	0
Natural and Historical Background	0.5	No	No	No	No	0
Roads	9.5	No	No	No	No	0
Underground Seepage	3	No	Low	No	No	0
Scrubber Pond	0	No	No	No	No	50

The total vanadium shown in Table 1 can however, be further classified as soluble vanadium and insoluble vanadium, where insoluble vanadium refers primarily to ore based vanadium that is tightly bound and thus not readily available for absorption. The mine experts (see above) estimated that 2/3 of the vanadium that is derived from the calcine dumps could be classified as insoluble vanadium compared to approximately 1/3 of the vanadium derived from stack emissions. Hence, although the calcine dumps were a major contributor of vanadium, more than half of this vanadium was probably not in a form that would be readily absorbed. The primary contributor of the insoluble vanadium from stack emissions is the Kiln main stack, which accounts for about 2/3 of the insoluble vanadium derived from stacks. This is reflected by the high proportion of total suspended particles (TSP) released from the Kiln main stack (Table 2).

Table 2 stratifies the stack emissions into the various stacks and quantifies the extent to which each stack contributed to the release of potentially hazardous substances. It can therefore be clearly seen that release of vanadium was from multiple sources and comprised various vanadium compounds (Fig. 1 and Fig. 2).

Many environmental release assessment models include in their calculations factors that play a role in transmission of the hazard to the point of exposure. These include wind direction, wind speed, stack height, ambient temperature and production output. Because this model is based on data collected at the point of exposure most of these factors do not form an integral part of this exposure assessment model. However, they become important in understanding the consequences. The primary mode of transmission of vanadium from the sources mentioned was wind. The strength of the wind and its direction influenced the volume of vanadium transmitted. In relation to the stacks and plant, the HE camps were situated between N and ESE and the LE

camps were situated between WSW and WNW of the processing plant. The wind blew in the direction of the HE camp approximately 52 % of the year and in the direction of the LE camp approximately 23 % of the year. Hence the wind blew a lot more frequently in the direction of the HE camp (Fig. 3). The wind direction data was also stratified according to year and wet and dry season for the five-year study period and there was no significant difference between years or season with respect to wind directions. Average wind speeds were calculated in relation to the predominant wind directions with respect to the HE camps and LE camps. The average wind speed in the direction of the HE was 6.8 km/h (sd=1.9) and in the direction of the LE camp was 7.5 km/h (sd=2.4) (Fig. 3). Hence, although the wind blew less frequently in the direction of the LE camps, when it did blow in that direction it blew on average more strongly.

Table 2 Sources of stack emissions form a South African vanadium processing unit and the degree to which each compound is released from that source (2003)

Stack Sources	V_2O_5	$NaVO_3$	NH_4VO_3	V_2O_3	SO_2	H_2SO_4	NH_3	PM10	TSP
1. Old deammoniator stack	High	Low	Medium	No	Low	No	High	Low	Low
2.New deammoniator stack	High	Low	Medium	No	Low	No	High	Low	Low
3. Kiln main stack	No	V Low	No	No	High	Low	Low	High	High
4. FeV stack	Low	No	No	No	No	No	No	Low	Low
5. Slag Bag house stack	Low	No	No	No	No	No	No	Medium	Medium
6.Dispatch bag house stack	High	No	No	No	No	No	No	Low	Low
7. Furnace stack	High	No	No	No	V Low	No	No	Low	Low
8. Desilication stack	Low	V Low	No	No	No	Low	No	No	No
9. Raw materials bag house stack	Medium	No	No	No	No	No	No	Medium	Medium
10. Boiler stack 1	Low	No	No	No	High	No	No	High	High
11. Boiler stack 2	Low	No	No	No	High	No	No	High	High

PM10 = particulate matter with an aerodynamic diameter of <10 μm; TSP = total suspended particles

Another important factor was distance from the source of hazards (Fig. 1), with the closest camp being the HE camp which began approximately 50 m from the processing area where

most of the sources of vanadium were found, while the LE camp began approximately 2 km west of the processing area.

The correlation between V_2O_5, FeV production figures and measured aerial fall out of vanadium (total) in each group was analysed to see if mine production output influenced the fall out of vanadium. There was no correlation between V_2O_5, FeV production and aerial fall out, and no correlation between V_2O_5 production and FeV production. A significant correlation was shown between aerial fall out in the LE and HE camps (r=0.52, p=0.006).

As part of the release assessment, note was also taken of rare events that comprised changes in the processing methods or temporary failure of equipment.

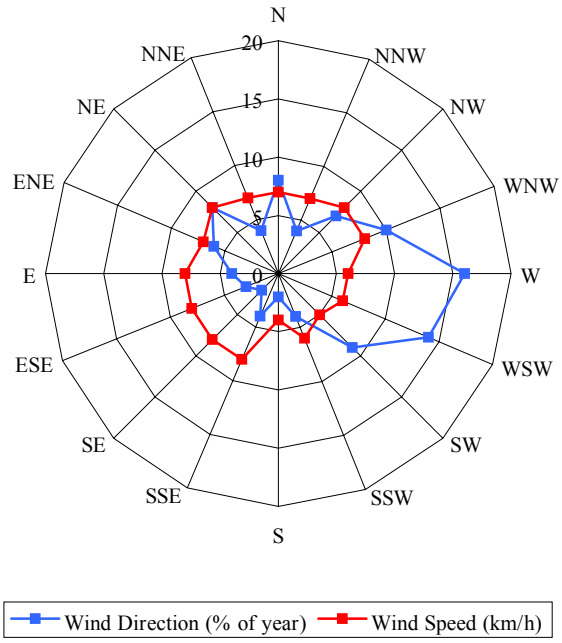

Fig. 3 Prevailing annual wind direction and the frequency (%) with which it blew in a specified direction, and the average annual wind speed (km/h) for each compass point relative to the processing plant.

3.3. Exposure Assessment

3.3.1. Unwashed grass

The results of the outputs for concentrations of vanadium in unwashed grass over the entire five year period are shown in Table 3 and Fig. 4. Fig. 4 shows a clear difference in unwashed

grass concentrations between the LE and HE camps, with the magnitude of difference decreasing in probability with a decrease in vanadium concentration.

Table 3 Summary results of the model output for the simulated concentration of vanadium in unwashed grass (mg/kg) over five years

	Low Exposure	High Exposure
Mean	68	148
Standard Deviation	34	92
Min	1	4
Max	527	880
5 Percentile	23	39
50 Percentile	64	127
95 Percentile	129	330

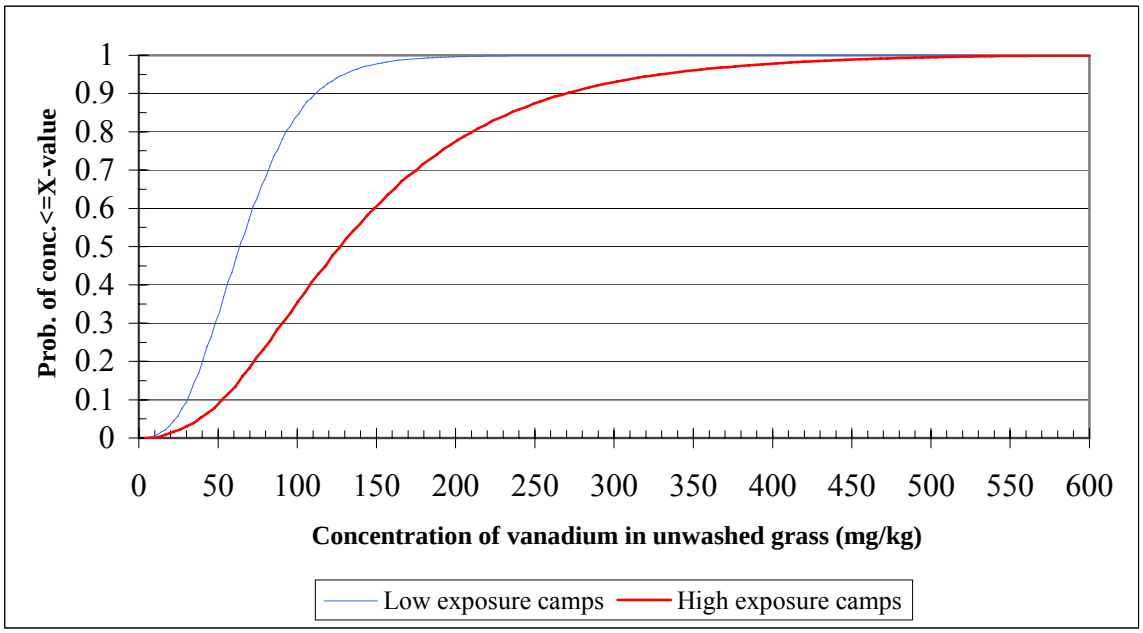

Fig. 4 Distribution curves for the concentration of vanadium in unwashed grass over five years

3.3.2. Soil vanadium concentration (EDTA)

The outputs for soil concentrations of soluble vanadium are shown in Table 4 and Fig. 5. Fig. 5 shows a fairly stable soluble vanadium soil profile with little difference between the camps.

As a point of interest it was noted that the total vanadium concentrations in the soil of the LE camp ($\bar{x}$ = 673 mg/kg) were consistently higher than those of the HE camp ($\bar{x}$ = 434 mg/kg), yet the soluble fractions reflected the inverse (0.8% LE camps and 1.5% HE camps).

Table 4 Summary results of model output for the simulated concentration of soluble vanadium in soil (mg/kg) over five years

	Low Exposure	High Exposure
Mean	5.2	6.1
Standard Deviation	4	5.9
Min	0	0
Max	284	230
5 Percentile	1.5	2
50 Percentile	4.5	4.5
95 Percentile	11.5	14

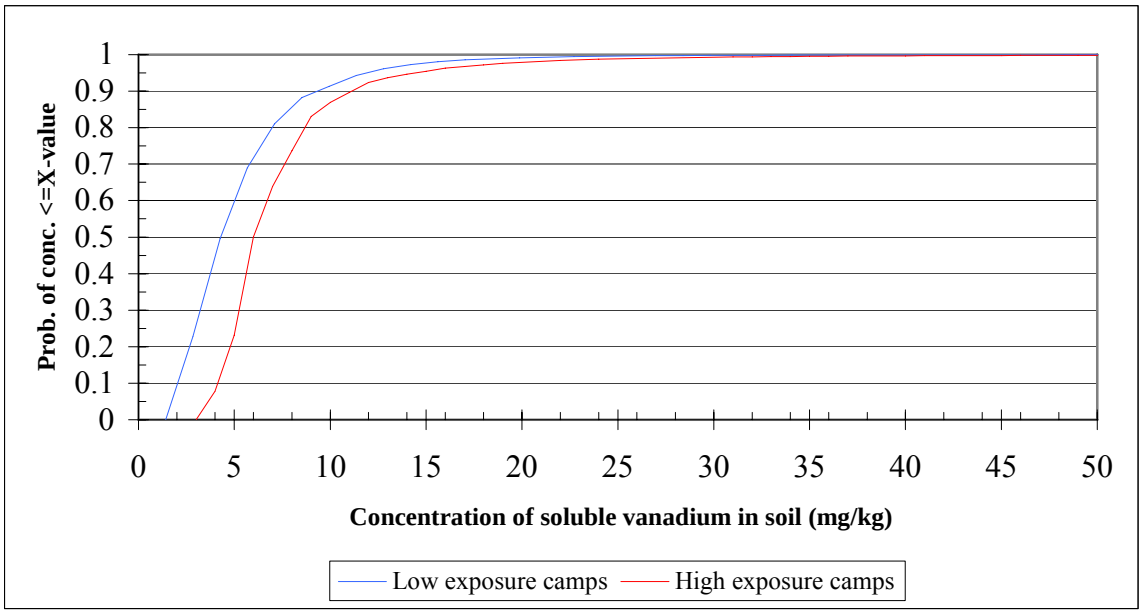

Fig. 5 Distribution curves of the concentration of soluble vanadium in soil over the five year period

3.3.3. Aerial fall out of vanadium

Table 5 and Fig. 6 show the outputs for the aerial fall out concentrations of vanadium over the five years of the trial. Fig. 6 illustrates the difference in fall out between the LE and HE camps

and also shows that there was more variation in the values for the HE camp than the LE camp. As with grass concentrations, the magnitude of difference between the LE and HE camps increases with increasing concentrations. While high extreme values could occur in the HE camp, the probability of these occurring becomes extremely remote.

Table 5 Summary results of simulated aerial fall out vanadium concentrations (µg/m^2/d)

	Low Exposure	High Exposure
Mean	1664	6979
Standard Deviation	1299	5177
Min	16	77
Max	59154	72981
5 Percentile	342	1169
50 Percentile	1320	6210
95 Percentile	4323	15067

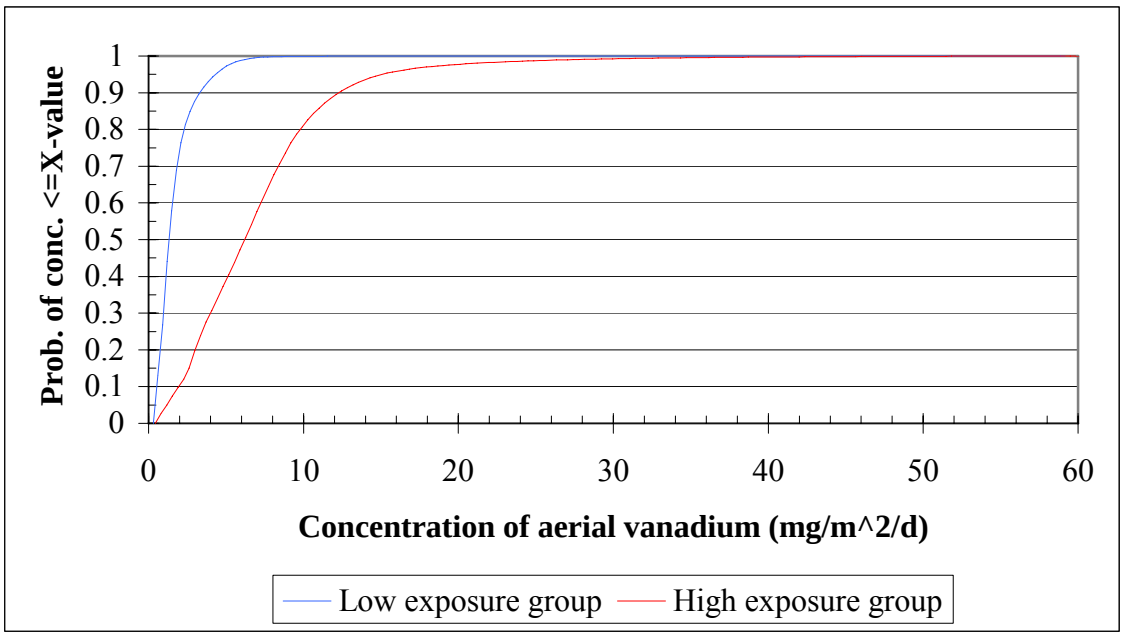

Fig. 6 Distribution curves for the concentration of aerial fall out vanadium over five years (mg/m^2/d)

3.3.4. Intake portion of model – Exposure dose of vanadium

Table 6 Model layout for simulation of intake of vanadium by high exposure (HE) and low exposure (LE) cattle, showing important inputs, outputs and distribution functions

	HE	LE	Comment / function
Oral Intake Per Day			
Grass (veld) diet			
Adult Cows (>24 m)			kg
Body Weight (stdev)	74	67	From trial data
Body Weight (mean)	472	442	From trial data
Body Weight function	471	442	RiskNormal,Risk truncate(250,650)
% of Body Weight	0.0175	1-2.5%	(RiskUniform(0.01,0.025))
Mass of Grass Ingested per day - DMI	8.2	7.74	kg
Vanadium in grass (mg/kg)	148	68	From unwashed grass model
Vanadium ingested in grass	**1219**	**526**	mg/d
Water			
Volume of water ingested (Cows (>24 m)) (l)	34.95	32.84	WI=0.075+4.234*DMI+-1.15
Vanadium in water	23.5	21	µg/l
Vanadium ingested in water	821.37	689.66	µg/d
Vanadium ingested in water	**0.82**	**0.69**	mg/d
Soil Ingested			
% of DMI	0.095	1-18% DMI	RiskUniform(0.01,0.18)
Amount of soil ingested	0.78	0.74	kg
Soluble Vanadium in soil (mg/kg)	6	5	From EDTA soil model
Vanadium ingested with soil intake	**4.7**	**3.68**	mg/d
Total vanadium ingested per cow per day	**1225**	**531**	mg/d
Inhalation			
Vanadium concentration in air (µg/m^2/d)	**6971**	**1663**	From aerial fall-out model
Diameter of collector	0.185	0.185	m
Radius of collector	0.0925	0.0925	m
Gravitational acceleration constant [g]	9.8	9.8	m/s^2
Pi constant	3.14	3.14	
Radius of V particles [r] (m)	0.00005	0.00005	RiskUniform($4x10^{-6}$,$6x10^{-6}$)
Density of V_2O_5 [p]	3357	3357	g/ml
Density of the air [σ]	1.2	1.2	g/l @ 25C = kg/m^3
Viscosity of the air [η]	0.000018	0.000018	Pascal sec
Vertical terminal velocity (v_t)	1.01	1.01	m/s
Volume of air sampled in 24 hrs	2357	2357	m^3
V conc. in m^3 air	2.96	0.71	µg/m3/d
V conc. in litre air	0.003	0.0007	µg/l
Respiratory rate	30	30	RiskNormal(30,1.8,RiskTruncate(15, 60))
Tidal volume	3.5	3.5	RiskNormal(3.5, 0.4)
Respiratory rate per day (24 h)	43200	43200	
Respiratory volume= Resp freq x tidal volume	1296000	1296000	l/d

	HE	LE	Comment / function
V inhaled per day	3832	914	µg/d
Amount of V inhaled per day	**3.83**	**0.914**	mg/d
Exposure dose of vanadium per day	1229	532	mg/d
Mean dose of vanadium per kg per day	**2.6**	**1.2**	**OUTPUT (mg/kg/d)**
Standard deviation	1.99	0.78	
Min	0.05	0.01	
Max	23.96	12.72	
5th Percentile	0.56	0.33	
50th Percentile	2.14	1.07	
95th Percentile	6.58	2.73	

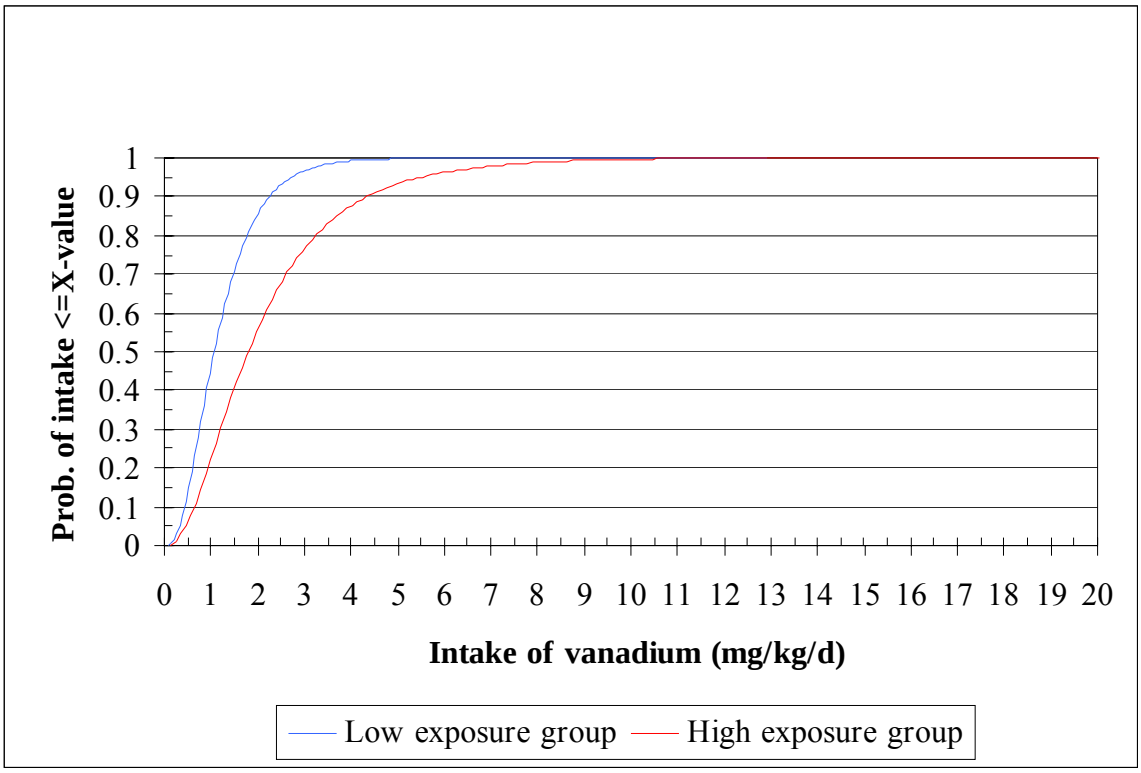

Fig. 7 Distribution function for exposure dose (mg/kg bwt/d) of vanadium for adult cattle over five-year period

3.4. Reliability of Model Inputs

The washed grass results (n=37) of SESE (1998) were modelled using a normal truncated distribution function, as used in the model, and compared to the output for the washed grass results recorded in this trial. A summary of the outputs is shown in Table 7. The SESE (1998) results were lower but similar in magnitude to the results recorded in this trial. Quality control

done by SESE (1998) on their own results (n=6) also showed their results to be lower on average than those of the laboratory that tested the duplicate samples ($\bar{x}$=159 mg/kg versus 174 mg/kg respectively).

Table 7 Simulation results for vanadium concentrations in washed grass samples taken during the trial compared to an independent companies (SESE) findings in 1998 (mg/kg)

	1999-2004 results	**1998 SESE results**
Mean	137	83
Standard Deviation	72	51
Range	450	334
Min	0	0
Max	450	334
5 Percentile	26	10
95 Percentile	263	177

Use of a Gaussian plume dispersion simulation model created for the mine by Burger & Watson (2003) was made to simulate fall out of V_2O_5 at the geographic coordinates used for collection of fall out samples. Their model predicted mean ambient concentrations of V_2O_5 for the period September 1999 to July 2001 as 1.24 µg/m^3 (sd=1.69) in the LE camp and 2.83 µg/m^3 (sd=1.98) in the HE camps. Inputs into the intake model estimated ambient concentrations of V_2O_5 as 0.71 µg/m^3 and 2.96 µg/m^3 over the five-year period of the trial for the LE and HE camps respectively. These values are thus similar to the plume dispersion model predictions.

4. Discussion

The final output of the exposure assessment model was an average intake dose of 2.6 mg V/kg body weight/d for the HE animals over the five years of the study and 1.2 mg V/kg body weight/d for the LE animals. The difference in exposure between the two camps reflects the difference in distance from the primary release sources of vanadium and the differences in wind direction and wind prevalence. The intake dose varied over the five years but, as shown in the output distribution function, is unlikely to have exceeded 23.96 mg/kg/d in the HE camp and 12.72 mg/kg/d in the LE camp, with an absolute minimum dose of 0.05 mg/kg/d and 0.01 mg/kg/d in the respective camps. In all previous field outbreaks of vanadium poisoning in cattle, the actual dose of vanadium never appears to have been quantified. What is normally reported, are the high background concentrations of vanadium, which by inference were the cause of

toxicity (Frank et al., 1990; Gummow et al., 1994; Frank et al., 1996; McCrindle et al., 2001). Even pollution control guidelines normally break up exposure limits into compartments of air quality, water or "other", which usually refers to oral intake (WHO, 1987; Toxicological Profile for Vanadium, 1992). There are therefore no known previous studies reflecting a composite intake of vanadium by several intake routes over a long period of time. Hence, the result of this study is important in providing methodology for establishing guidelines for a no-observed-adverse-effect-level (NOAEL) for cattle farmed in areas of high background levels of vanadium, which include the vicinity of vanadium producing industries. It is also important in providing the means for calculating time series exposure doses that are necessary before one can evaluate biomarkers for assessing vanadium exposure. It thus provides a useful platform for future studies.

The results of the model show that by far the largest component of vanadium intake is from the grass ingested by the cattle (99% for the HE and LE camps). The fact that most of the vanadium intake came from the grass ingested by the cattle means that grazing animals will take in more vanadium than other animals and humans in close proximity to a source of vanadium pollution and should act as good sentinels of vanadium pollution. Most human-health environmental epidemiology and occupational health studies focus on inhalation and do not account for other routes of intake (Scott et al, 2003a). These methods are therefore not applicable to grazing animals (Scott et al, 2003b) as they exclude the major route of intake found in this study. Scott et al. (2003a) in their paper debate the merits and weaknesses of various approaches to exposure assessment for point sources. They categorise these approaches into "simple" methods based on the assumption that exposure decreases as a function of increasing distance from a pollution source, which include application of mathematical decay functions, and "complex" atmospheric-plume-dispersion models, the most common being the Gaussian plume dispersion models. The exposure model put forward in this paper differs from most other approaches in several ways:

1. It does not rely heavily on extrapolating information from the point source (e.g. stack height, exit velocity, exit diameter, etc.) to the point of exposure.
2. It incorporates the physiological constraints of the species exposed.

3. It takes into account oral as well as inhalation exposure. This is important as it accounts for intake from contaminated grazing, vegetables and crops grown in the area of pollution.
4. It addresses to some extent one of the most common weaknesses of most other models, that of insensitivity to terrain specifications, by using measurements at the point of exposure.
5. It accounts for existing background concentrations of pollutants and pollutants from other origins, while most other models assume exposure is derived solely from a few known sources.
6. It accounts for variability in the data over time, which is usually a problem with long term studies.

One of the arguments put forward against "static deposition" or "area monitors" is the cost and availability. This aspect still needs to be looked at within the South African context before any firm conclusions can be drawn in this respect.

Published results show a NOAEL of 0.7 mg V_2O_5/kg/d in the drinking water of rats (Schroeder et al., 1970) and 0.54 mg V_2O_5/kg/d in the drinking water of mice (Schroeder & Mitchner, 1975) exposed for 2.5 years. Schroeder & Balassa (1967) reported a NOAEL of 4.1 mg V_2O_5/kg/d in the feed of mice exposed for 2 years. The values found in this study for oral intake of vanadium from the grazing were 2.58 (sd=1.99) mg/kg/d for the HE camp and 1.23 (sd=0.78) mg/kg/d for the LE camp. This puts cattle in both camps on the threshold of developing adverse effects. In fact it was found that the calves in the HE Group developed symptoms of vanadium poisoning while calves in the LE Group did not (Gummow, 2004, unpublished results), thus putting the NOAEL for cattle somewhere between the LE and HE doses and in the same region as that of mice and rats.

The vanadium determined in the unwashed grass samples consists of both vanadium dust deposited on the surface of the grass from airborne pollution and vanadium contained within the grass itself. Comparison of the vanadium levels in washed and unwashed grass from the HE camp (Tables 3 and 7) show that a high percentage (±92 %) of the vanadium is actually contained within the grass itself. The mean total vanadium grass levels found in this study in both the HE and LE camps, 148 mg/kg and 68 mg/kg respectively, were significantly higher than the recommended feedlot supplement of 0.57 mg/kg in a total ration (Fox, 1987) and the

level of 1 mg/kg given as an average normal value for higher plants. (Faulkner Hudson, 1964; Platonow & Abbey, 1968; Waters, 1977). Gummow et al. (1994) when examining grass vanadium concentrations around another vanadium smelter also found grass to have concentrations of vanadium higher than 10 ppm. The possibility that certain species of grass may accumulate or reflect soil concentrations of vanadium therefore needs to be investigated further.

Although the inhalation component of this exposure model accounts for less than 1% of the intake, the average levels measured in the HE camp (2.96 $\mu g/m^3/d$) exceeded the WHO air-quality guidelines for the time-weighted-average (TWA) for vanadium of less than 1 $\mu g/m^3/d$. While the average levels measured in the LE camp (0.71 $\mu g/m^3/d$) were within the guidelines. This finding alerted mine management to the risk to the human population in the area and action was taken to reduce these levels. It also supports the predicted NOAEL.

The inhalation component of the model allows comparison of the outputs of this model with plume dispersion models to ensure its validity and can be used as an alternative to plume dispersion modelling to assess the risk of exposure to the human population.

Little has been published on the EDTA soluble fraction of vanadium in soils. Publications usually refer to total vanadium in soil, as this is the easiest to determine. However, only a small fraction of the total vanadium is soluble (0.8% LE camps and 1.5% HE camps), the rest is tightly bound and therefore probably not readily available for absorption by ruminants ingesting soil. The question of different fractions of vanadium and other minerals in exposure studies needs to be investigated more thoroughly. In this study the total vanadium concentrations in soil were consistently higher in the LE camps ($\bar{x}$ = 673 mg/kg) than the HE camps ($\bar{x}$ = 434 mg/kg), yet the soluble fractions reflected the inverse. This was expected since most of the fall out occurred in the HE camps, but it highlights the danger of simplifying metal exposure assessments to just looking at total concentrations of a particular metal. Coupled to this is the potential confounder of the different vanadium compounds that are released and which occur in the environment. These factors emphasise the importance of hazard identification and release assessment when doing a sentinel study and highlighted one of the weaknesses of this study. From the release assessment it can be seen that, unlike in experimental studies, cattle in the field are exposed to a mixture of vanadium compounds of varying solubility and toxicity. As Mendz (1998) points out, “the hundreds of studies on the effects of vanadate and vanadyl ions on living

systems that have appeared in the last few years reflect the complexity of these interactions". Yet modellers using stack emissions as a measure of exposure rarely differentiate between the different vanadium compounds. For example, the modellers doing Gaussian plume dispersion models for this mining company based their model on the assumption that all emissions were V_2O_5, which was clearly not the case. This aspect need to be looked at more closely in future studies.

5. Conclusions

The exposure model was shown to provide a reasonable estimate of external exposure dose of vanadium for cattle grazing in the vicinity of a vanadium processing plant. It provided the basis for estimating a composite NOAEL for cattle exposed to vanadium under field conditions. It also provides a platform for assessing more accurately the value of biological biomarkers in cattle. The model is adaptable enough for application to other species, including man and could be employed as an alternative to plume dispersion modelling in certain situations.

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Chapter 5

THE ASSESSMENT OF BIOMARKERS IN SENTINEL CATTLE FOR MONITORING VANADIUM EXPOSURE

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Abstract

Various potential biomarkers were sampled for vanadium every 3-4 months from *Bos inducus* beef cattle farmed extensively immediately adjacent (High Exposure (HE) Group) and two km away (Low Exposure (LE) Group) from a vanadium processing plant, respectively. Vanadium intake (mg vanadium/kg bwt/d) was modelled using environmental and physiological data as inputs. The vanadium intake ranged from 0.57 to 5.44 mg vanadium/kg bwt/d in the HE group and 0.41 to 2.61 mg vanadium/kg bwt/d in the LE group over a five-year period of monitoring. Samples collected from live sentinel animals over the five-year period included caudal coccygeal vertebrae, tail-switch hair, milk, urine, faeces, rib-bone biopsies and a wide range of blood clinical pathology and haematological parameters. The data was analysed for differences in response between the HE and LE Groups. Where differences were found, a linear mixed-effects regression model was fitted to model the relationship between the exposure dose and the response variable. The model included the effects of age, duration of exposure and response, and allowed the prediction of the exposure dose given these inputs. Moreover, forty-two adult cattle were slaughtered over the five years. A wide range of tissue samples, rumen content and whole blood were taken from the cattle at slaughter for vanadium determination.

In live animals, a difference in response was found between the HE group and LE group with respect to serum albumin (n=36), monocyte (n=36) and thrombocyte (n=36) counts, and hair (n=12) and faeces (n=34) vanadium concentrations. No difference in vanadium concentrations could be shown for urine (n=36), the traditional occupational health biomarker. Regression models are described for serum albumin, monocyte counts, faeces and hair, which showed the most promise as biomarkers.

Average concentrations of vanadium in the tissues of slaughtered cattle ranged from 0.08 to 2.94 mg/kg (wet-weight basis) and rumen content contained 16.67 mg/kg. Significant correlations were found between the exposure dose (end-dose) just prior to slaughter and the concentrations of vanadium in the coccygeal vertebrae, liver, diaphragm and rib-bone in descending order of magnitude. Other tissues showed poor correlation to the end-dose. Tissue levels of vanadium in healthy cattle include a much wider range than is currently reflected in the literature. The best tissue from slaughter animals for assessing chronic vanadium exposure is probably the liver.

Keywords: Environmental epidemiology, vanadium, cattle, biomarkers, clinical pathology, haematology, tissue concentrations, chronic studies

1. Introduction

Biomarkers, biological markers and biologic markers are for all practical purposes equivalent terms (Aldrich *et al*., 1993). However, a distinction is currently arising through common usage between these terms and the term biomonitoring. Biomonitoring is tending to be equated with residue analysis because residue analysis (detection of parent compound or metabolite in tissue, fluid or breath) is considered by some to be chemical dosimetry and a measure of body burden (Aldrich *et al*., 1993). For the purposes of this paper, a biomarker is defined as any measurable chemical, biochemical, physiological, cytological, morphological, or other biological response obtainable from tissues, fluids or expired gasses that is associated directly or indirectly with exposure to an environmental pollutant (NRC, 1989a, 1989b), and therefore includes biomonitoring. Biomarkers can be used to either determine the exposure to the causative agent, to predict adverse health effects or as a measure of susceptibility. Furthermore, biomarkers can be used as surrogates for or in combination with other measures of exposure.

In the vanadium industry the most common biomarker used in occupational health is urine concentrations of vanadium (Gylseth *et al*., 1979; Kiviluoto *et al,*. 1981; Lewis, 1959; Orris et al., 1983; Zenz et al., 1962) and a green discolouration of the tongue (Lewis, 1959), the latter resulting from the direct accumulation of high doses of vanadium pentoxide. Urine concentrations of vanadium have never been an ideal biomarker due to the wide variability in results and a better, preferably non-invasive, biomarker is needed by the industry. Concentrations of minerals in tissues are also frequently used by veterinarians as biomarkers to make a diagnosis of exposure in cases of toxicity, and environmentalists are known to use tissue mineral levels as indicators of environmental pollution. This paper discusses the usefulness of various potential biomarkers in cattle sentinels that were monitored over a five-year period. It also looks at tissue concentrations of vanadium in slaughtered animals and their usefulness as a monitoring and diagnostic tool for public health authorities, environmentalists and veterinarians.

2. Materials and methods

2.1. Experimental design

A cohort study was carried out using an initial cohort of thirty *Bos indicus* beef cattle of the same approximate mass. They were purchased in 1999 as heifers and randomly divided into two groups; a High Exposure (HE) Group of ten and a Low Exposure (LE) Group of twenty cattle. The HE Group was farmed in an area immediately adjacent to a vanadium mine where high background concentrations of vanadium occurred ($\bar{x}$=1229 mg V/d). The LE Group was farmed approximately 2-3 km from the first group in an area where exposure was roughly half that of the HE Group ($\bar{x}$=532 mg V/d). Both groups were farmed as an extensive beef cattle enterprise with the resultant heifer offspring used as replacements or additions to the herd over the five-year period. Bull calves were slaughtered between 18 m and 24 m of age. Bulls used for breeding were fertility tested Bonsmara bulls, hired for the breeding season from various independent farmers. Separate bulls were used for the HE and LE camps. The herd size was limited by the amount of available grazing and a stocking density of approximately 1 animal per 5 ha was used as a guideline. The area where cattle grazed was bushveld and comprised a number of grass species; the predominant ones being *Aristida congesta, Panicum maximum, Themeda triandra, Heteropogon contortus, Eragrostis rigdior* and a *Urochloa* species. The only supplemental feeding provided was a commercial winter lick and baled lucern (fed at 1% body weight/d), which was fed for approximately 2-3 months during mid-winter (July-September), when the grazing was insufficient to sustain the animals.

2.2. Observations and analytical procedures

The farm was visited by at least one of the veterinary co-workers once every 3-4 months, to monitor the health status of the herd, collect samples and update records. A record system was kept by the Department of Production Animal Studies at the Faculty of Veterinary Science, University of Pretoria for analysis purposes.

Various potential biomarkers were collected every 3-4 months from the cattle and evaluated as indicators of vanadium exposure. These included the most caudal ossified coccygeal vertebra (tail-bone), hair from the tail-switch, milk from lactating cows, naturally voided urine and faeces from the rectum. Two years into the trial, once 2-3 caudal coccygeal vertebrae had been removed from the original cows, it was decided for ethical reasons to take rib-biopsies to enable

bone sampling from these animals to continue. Rib-biopsies were taken from the last rib approximately 1/3 of the way down and the piece of bone removed was approximately 1 cm in diameter by 0.5 cm deep. Vanadium analysis of tissues and milk was done by the Institute of Soil, Climate and Water (ISCW) using atomic emission spectrometry (ICP-AES) according to internationally accepted methods and quality control procedures (USEPA Standard Methods, 1986; Handbook of standard soil testing methods for advisory purposes, 1990, USEPA Standard Methods, 1996).

In addition to the tissue samples and body fluids, a serum and whole blood (EDTA) sample was collected from the jugular vein of sentinel cattle to examine haematological and clinical chemistry parameters. The haematology parameters monitored were: haemoglobin concentration (Hb), red cell count (RCC), heamatocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), white cell count (WCC), mature neutrophils (N mat.), immature neutrophils (N imm), lymphocytes, monocytes, eosinophils, basophils and thrombocyte count (Thr C). The clinical chemistry parameters monitored were: total serum protein (TSP), albumin (Alb), globulin (Glob), the albumin:globulin ratio (A:G), the alpha-globulin fraction (α-glob), beta-globulin fraction (β-glob), the gamma-globulin fraction (γ-glob), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), creatine phosphokinase (CK) activities and blood urea nitrogen (BUN) and blood creatinine (Creat) concentrations. Enzyme activities and serum protein, urea and creatinine concentrations were analyzed using an automated chemical analyser (Technicon RA-XT system, Miles Inc., Diagnostics Division, Tarrytown, New York) and the manufacturer's methods and reagents. Complete blood counts were determined using a Cell-dyne 3700 (Abbott Laboratories, South Africa). Electrophoresis was performed with a Beckman Model R-100 Microzone Electrophoresis System (Econoscan, Helena Laboratories). All haematological and clinical chemistry analysis was performed by the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria.

At the same time as samples were collected from the cattle, grass, soil, and aerial fall-out samples were collected at 18 sampling points along nine transects within the camps for determination of vanadium concentrations. These inputs together with drinking water vanadium concentrations were then used in an exposure dose model described by Gummow *et al*. (2005a)

to model the dose of vanadium taken in by the cattle (in mg/kg/d). The exposure dose includes intake of vanadium by both the oral and inhalation routes.

During the five-year period of monitoring, non-productive cows or bull calves were slaughtered annually at a nearby abattoir. Six cohorts of animals were slaughtered over the five-year period on the following dates; 25-Aug-99, 09-Feb-01, 06-Mar-01, 31-May-02, 20-Jun-03, 07-May-04. A total of 42 cattle were slaughtered over this period (Table 6). All animals underwent the same slaughter process routinely carried out at the abattoir, with the exception that tissue samples were taken from the carcasses as they moved along the slaughter line. Each carcass was inspected by two veterinarians from the Faculty of Veterinary Science, University of Pretoria as well the abattoir's meat inspector for lesions or abnormalities. Specific tissues were taken for analysis of vanadium concentrations and for histopathology. Tissues taken for vanadium analysis were approximately 5 cm^3 in size where possible and were stored in a deep freezer at –18 °C prior to analysis. Analysis was carried out within 3 months of the collection of samples.

The following tissues were taken for vanadium analysis from animals slaughtered: the most caudal ossified coccygeal vertebra (tail-bone), rib-bone, rib-bone biopsy, *Musculus iliopsoas* (fillet muscle), *Musculus triceps brachii* (triceps muscle), *Pars costalis* (diaphragmatic muscle), cerebellum, cerebrum, lung (cranial lobe), liver (right lobe), kidney, mesenteric lymphnode, spleen, rumen content, testes and whole blood. The rib biopsy was a 1 cm diameter x 0.5 cm deep punch of bone taken from the dorsal lateral aspect of the last rib-bone.

2.3. Data analysis

The results were analysed using Microsoft Excel (version 2000) and the statistical packages NCSS 2004 (Hintze, 2001) and R 2.1.1 (R Development Core Team, 2004). The data collected covered a five-year period of monitoring.

2.3.1. Live Animals

Data collected from live animals for each sample period comprised selected animals from all generations on the farm at that time. Live animals were sampled twenty-four times over the five-year period. On each occasion between six and twenty-nine cattle were sampled per experimental group with an average sample size of twelve per group. The variation in sample

size was because animals from successive generations were added to the numbers as the trial progressed.

The data was tested for normality and equal variance using NCSS 2004 (Hintze, 2001). The assumption of normality and equal variance could not be met for all biomarker response variables so it was decided to treat the data as non-parametric data for the purposes of comparison. Differences between the HE and LE groups with respect to biomarker response were assessed by means of confidence intervals (Tables 1, 2 and 3); where a difference in response was apparent, the data from the HE and LE groups was pooled and a linear mixed-effect model was used to model the relationship between the exposure dose and the biomarker response (Table 5). The model was defined as: mean-exposure-dose (exp-dose): age group (age) + duration-of-exposure (time-to-bleed) + age × biomarker-response-at-bleed (response). The model included random animal effects because the observations on the same animal are correlated and random time to bleed because these correlations depend on time. This was the starting model. Then the same model was fitted without the interaction: exp-dose = age + time-to-bleed + response and Akaike's information criterion (AIC) was compared with that of the starting model. If the AIC was smaller, then this model was used in the same procedure for the fixed effects. The final model was the one with the lowest AIC. The residuals of this final model were checked with a plot of the residuals vs. the fitted values and a quantile-quantile (qq-plot) graph. The age groups used in the model were: (1) 0-6 m old, (2) 7-12 m old, (3) 13-18 m old and (4) >18 m old. The age groups were based on previous findings that suggested responses may be related to these age groups (Gummow et al., 1994).

2.3.2. Slaughter animals

Data from slaughter animals was first analysed descriptively to establish the concentrations of vanadium in sampled bovine tissues. A range of possible values that could be expected for each tissue was then modelled using @Risk version 4.5.2 (Palisade Corporation, USA) and a lognormal distribution function. The lognormal distribution function for each set of tissue concentrations was defined using the data fitting software Bestfit version 4.5.2 (Palisade corporation, USA). A lognormal distribution function was used because it is a distribution function commonly used to describe environmental data (Vose Consulting, 2004) and because it consistently fitted the raw data well.

To check for differences in vanadium concentrations between the various tissues, the Kruskal-Wallis Z multiple comparison test was used (Dunn, 1964). This test uses a distribution-free multiple comparison, meaning that the assumption of normality is not necessary. It is used for testing pairs of medians following the Kruskal-Wallis test. The Bonferroni z-value ($z > 2.39$) was used for assessing significance, which is a z-value that has been adjusted for multiple tests.

Slaughter data was analysed for correlations between the independent predictor variables given below and the tissue vanadium concentrations, and between the various tissues with respect to vanadium concentrations. The Spearman-rank test was used to evaluate correlations (Hintze, 2001).

The predictor variables were:

1. The length of time the animal had been exposed to vanadium, based on the number of days from when the animal entered the trial or was born until slaughter.
2. The median dose of vanadium that the animal had been exposed to during the trial (median dose). This was modelled using the method described by Gummow et al. (2005a). It was possible from the available environmental data to model the exposure dose at 18 time points, which corresponded to when samples were collected over the five-year project. The median exposure dose for an individual animal was then estimated using a Discrete Uniform distribution function (Vose Consulting, 2004) that incorporated only the exposure doses for the months relevant to the period the animal was in the trial. The output was a distribution function of the dose (mg vanadium per kilogram body weight per day) that individual animals would have been exposed to while they were in the trial. The median of this distribution function was then used as the predictor variable for the correlation analysis. Slaughter animals fell into one of nine groups of exposure, which were designated A to I (Table 7). It must be noted that the exposure dose calculated by the model includes intake from both oral and inhalation routes. A median of the output distribution function was used because the input environmental data was not normally distributed.
3. The dose of vanadium that animals were being exposed to around the time of slaughter (end-dose). This was taken as the median dose in mg vanadium per kg body weight per day that was simulated by the model using the environmental inputs at the time closest to slaughter (Table 4).

3. Results

Table 1 shows the average responses and normal ranges of the clinical chemistry parameters over the duration of the trial. It also gives the results of the confidence intervals for the differences between the HE and LE groups with respect to the clinical chemistry parameters. The normal ranges are those used at the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria, except for the protein fractions, which are those reported by Tumblson et al. (1973). There were 24 observations over the five year period. From the Table it can be seen that the HE group had significantly (α<0.05) lower Alb levels than the LE group. This is further reflected in the A:G ratios, which were also lower in the HE group. Despite the Alb levels being lower in the HE group they were still within the lower normal range for cattle. None of the other clinical chemistry parameters showed evidence of a difference between exposure groups. The mean Glob, and in particular the β-glob fraction, and CK levels of both groups were above what is considered normal for cattle.

Table 1 Clinical chemistry parameter means and 95% confidence limits for the difference in means of high exposure (HE) and low exposure (LE) South African sentinel cattle (1999-2004).

Biomarker	Normal Range	Mean		95% LCL	95% UCL
		HE Group	LE Group		
TSP (g/l)	65-78	74.33	74.88	-2.47	1.37
Alb (g/l)	28-37	30.68	32.07	**-2.59**	**-0.19**
Glob (g/l)	28-42	44.01	43.14	-1.01	2.75
AG	0.61-1.18	0.71	0.76	**-0.10**	**-0.004**
α-glob (g/l)	10-13.5	11.83	11.54	-1.13	1.72
β-glob (g/l)	8.3-10.3	15.33	14.90	-2.27	3.12
γ-glob (g/l)	13-42	15.08	14.80	-3.01	3.58
AST (U/l)	10-80	30.66	31.61	-2.96	1.06
CK (U/l)	5-60	98.04	92.19	-13.69	25.40
GGT (U/l)	0-25	15.91	11.06	-1.23	10.92
BUN (mmol/l)	3.6-10.7	4.52	4.30	-0.53	0.97
Creat (μmol/l)	10-133	122.69	124.75	-14.06	9.94

LCL = lower confidence limit; UCL = upper confidence limit

Table 2 shows the average cell counts or responses of the haematology parameters over the duration of the trial. Normal ranges are also given. There were 24 observations over the five-year period. Monocyte numbers and thrombocyte counts were significantly higher in the HE cattle than the LE cattle. The mean WCC were above the normal ranges for both groups.

Table 2 Haematology parameter means and 95% confidence limits for the difference in means of high exposure (HE) and low exposure (LE) South African sentinel cattle (1999-2004)

Biomarker	Normal ranges	Mean		95% LCL	95% UCL
		HE Group	LE Group		
Hb (g/l)	80-140	124.98	123.69	-3.06	5.64
RCC ($x10^{12}$/l)	5-9	8.52	8.57	-0.34	0.24
Ht (l/l)	0.24-0.4	0.38	0.38	-0.01	0.01
MCV (fl)	40-60	44.53	44.15	-1.84	2.60
MCHC (g/dl)	30-36	33.08	32.89	-0.51	0.89
RDW (%)	19.5-27	24.63	24.05	-0.56	1.63
WCC ($x10^9$/l)	4-10	12.04	11.41	-0.63	1.88
N mat. ($x10^9$/l)	0.6-4	3.60	2.90	-0.15	1.56
N imm. ($x10^9$/l)	0-0.12	0.06	0.03	-0.04	0.10
Lymph ($x10^9$/l)	2.5-7.5	6.99	7.18	-1.00	0.63
Mono ($x10^9$/l)	0.03-0.84	0.70	0.56	**0.04**	**0.24**
Eos ($x10^9$/l)	0-2.4	0.70	0.67	-0.13	0.18
Baso ($x10^9$/l)	0-0.2	0.05	0.05	-0.03	0.02
Thr C ($x10^9$/l)	200-600	443.22	295.14	**8.56**	**287.59**

LCL = lower confidence limit; UCL = upper confidence limit

Table 3 shows the average concentrations of vanadium in tissues and body fluids taken from live animals. The number of observations for each parameter is shown in the table. The variability in the number of observations is due to the difficulty in obtaining samples on occasion, the decision that rib-biopsies would have to be taken as a replacement for coccygeal vertebrae and the late realisation that hair may be a good biomarker and should be included. Concentrations of vanadium in the hair and faeces were found to be higher in cattle from the HE group than the LE group. Normal concentrations of vanadium in milk are given as 0-6 µg/kg (mean= 1 µg/kg) (Puls, 1988), hence milk concentrations of vanadium are much higher than normal in both groups. Normal concentrations of vanadium in hair, urine, faeces and bone of cattle could not be found. Normal tissue levels in general foodstuffs do not usually exceed 1 mg/kg (Toxicological Profile for Vanadium, 1992), so the levels in these tissues are probably higher than what is considered normal.

Table 3 Tissue and body fluid (mg/kg wet-weight) means and 95% confidence limits for the difference in means of the HE and LE South African sentinel cattle for (1999-2004)

Biomarker	No. per group	Mean		95% LCL	95% UCL
		HE Group	LE Group		
Hair	6	57.67	38.33	**-4.26E-05**	**38.67**
Urine	18	247.89	335.22	-308.61	133.94
Faeces	19	29.72	18.45	**0.82**	**21.71**
Milk	15	0.42	0.37	-0.26	0.36
Tail-bone	15	1.76	1.00	-0.55	2.08
Rib-biopsy	6	2.00	1.16	-0.23	1.91

LCL = lower confidence limit; UCL = upper confidence limit

Table 4 shows the exposure doses calculated for adult cattle at the 18 time periods when samples were taken. It also shows the exposure dose at the time when animals were slaughtered (end-dose). The exposure dose for the HE cattle was approximately twice that of the LE cattle.

Table 4 Model outputs for dose of vanadium (mg V/kg bwt/d) taken in by South African sentinel cattle in the HE and the LE groups at the time of each sample taking over the five-year period of the trial. Bold numbers indicate exposure doses corresponding to the slaughter cattle and time when they were slaughtered (end-dose).

Date	Low Exposure Camps					High Exposure Camps				
	Min	5 %tile	50 %tile	95 %tile	Max	Min	5 %tile	50 %tile	95 %tile	Max
15-Jul-99	0.22	0.53	**1.18**	2.45	6.36	0.43	0.73	1.47	3.04	7.92
10-Nov-99	0.33	0.8	1.68	3.32	7.61	0.23	0.91	2.74	7.81	27.94
23-Feb-00	0.04	0.15	0.37	0.89	2.26	0.06	0.19	0.49	1.27	3.66
30-May-00	0.17	0.39	0.89	1.75	4.45	0.25	0.72	1.71	3.69	9.78
23-Aug-00	0.27	0.65	1.52	3.15	7.07	0.58	1.4	3.34	7.28	20.7
2-Nov-00	0.12	0.25	0.6	1.28	2.33	0.27	0.94	2.55	5.8	10
5-Mar-01	0.17	0.55	**1.2**	2.34	4.72	0.17	0.84	**2.73**	8.55	35.87
20-Jun-01	0.44	1.09	2.45	5.04	9.87	1.55	2.64	5.2	9.44	18.45
31-Jan-02	0.1	0.28	0.64	1.39	3.31	0.2	0.53	1.2	2.53	5.62
17-Apr-02	0.16	0.41	**0.88**	1.77	4.35	0.14	0.51	**1.44**	4.08	12.49
24-Aug-02	0.25	0.72	1.65	3.4	7.78	0.25	1.04	3.37	10.64	70.44
01-Oct-02	0.21	0.62	1.71	4.39	14.27	0.32	0.91	2.1	4.65	11.1
23-Jan-03	0.07	0.25	0.66	1.64	4.74	0.15	0.44	1.21	3.03	10.85
23-Apr-03	0.12	0.31	**0.75**	1.76	6.92	0.08	0.34	0.96	2.65	10.43
23-Jul-03	0.29	0.85	2.02	4.66	15.44	0.21	1.1	4.07	14.89	71.39
29-Oct-03	0.38	0.84	1.72	3.26	6.17	1.09	1.71	3.23	5.12	6.23
24-Jan-04	0.07	0.23	0.68	1.97	9.9	0.08	0.25	0.69	1.92	9.47
21-Apr-04	0.1	0.25	**0.55**	1.12	2.18	0.19	0.52	**1.19**	2.64	6.3

Table 5 Linear mixed effects mean-exposure dose model for serum albumin, hair, faeces and monocyte counts of South African sentinel cattle, 1999-2004

	95% LCL	Value	95% UCL	t-value	p-value
Albumin					
Exp-dose ~ age2+age3+age4+time-to-bleed+respose					
(Intercept)	2.10	2.88	3.67	7.20	0.00
factor(age)					
age 2	-0.89	-0.47	-0.055	-2.21	0.03
age 3	-0.17	0.29	0.74	1.24	0.21
age 4	-0.74	-0.33	0.075	-1.59	0.11
Time-to-bleed	-0.00	-0.00	0.00	-1.09	0.28
Response	-0.05	-0.03	-0.01	-2.55	0.01
Monocyte count					
Exp-dose ~ age2+age3+age4+time-to-bleed+respose					
(Intercept)	1.71	2.08	2.4	11.05	0.00
factor(age)					
age 2	-0.67	-0.23	0.21	-1.01	0.31
age 3	-0.10	0.37	0.84	1.53	0.13
age 4	-0.58	-0.13	0.31	-0.59	0.55
Time-to-bleed	-0.00	-0.00	0.00	-1.22	0.22
Response	-0.50	-0.23	0.04	-1.65	0.10
Hair					
Exp-dose ~ age2+age3+age4+time-to-bleed+respose					
(Intercept)	1.13	1.65	2.17	6.2	0.00
factor(age)					
age 2	-0.80	-0.27	2.57	-0.10	0.32
age 3	-0.96	-0.34	2.73	-1.08	0.28
age 4	-1.22	-0.64	-7.10	-2.19	0.03
Time-to-bleed	-0.00	-0.00	9.09	-1.48	0.14
Response	-0.001	0.003	6.41	1.37	0.17
Faeces					
Exp-dose ~ age2+age3+age4+time-to-bleed+age1:respose+age2:respose+age3:respose +age4:respose					
(Intercept)	1.44	1.81	2.18	9.43	0.00
factor(age)					
age 2	-0.82	-0.26	0.30	-0.91	0.36
age 3	0.50	1.12	1.74	3.53	0.00
age 4	-1.61	-0.78	0.06	-1.81	0.07
Time-to-bleed	-0.00	-0.00	-0.00	-3.39	0.008
Response					
Age1:respose	0.003	0.008	0.01	3.28	0.001
Age2:respose	-0.00	0.01	0.01	1.49	0.14
Age 3:respose	-0.01	-0.00	0.00	-1.16	0.25
Age 4:respose	-0.01	0.01	0.03	1.24	0.21

Table 5 shows the results of the linear mixed effects mean-exposure-dose model for parameters that differed significantly between the HE and LE groups (Table 1, 2 and 3). It includes the effects of age group, duration of exposure and biomarker response at the time of bleeding. Hence, if serum albumin is monitored, the biomarker model for determining exposure dose in 0 to 6 m old (Group 1) calves is mean-exposure-dose = 2.88-(0.03 × albumin response), while for 7 to 12 m old (Group 2) calves mean-exposure-dose) would be 2.88-0.47-(0.03 × albumin response).

Table 6 gives a summary of when the cattle were slaughtered, the number of cattle slaughtered, their sex and how many of these came from the HE area or LE area of the trial.

Table 6 Slaughter dates, exposure group, number and sex of sentinel cattle slaughtered in South Africa on the date shown

Date of slaughter	No. from HE camp	No. from LE camp	Male	Female
25-Aug-99	0	3	0	3
09-Feb-01	5	2	3	4
06-Mar-01	0	10	3	7
31-May-02	2	6	7	1
20-Jun-03	0	8	4	4
07-May-04	3	3	6	0
Total	10	32	23	19

HE = High exposure; LE = Low exposure

Table 7 gives a summary of the model outputs for the calculated median (50^{th} percentile) exposure dose for the groups (A-I) that slaughter cattle fell into, depending on how long an individual animal had been in the trial. These values were also used as independent predictor variables for the correlations shown in Tables 10a and 10b.

Table 7 Model outputs for the nine median dose groups of vanadium that slaughter cattle from a South African sentinel herd were exposed to depending on how long they were in the trial

Model output parameter	A LE	B HE	C LE	D HE	E LE	F LE	G LE	H HE	I LE
Total V per day (mg/d)	552	1089	610	1506	606	510	571	1370	667
Min (mg/kg/d)	0.22	0.08	0.13	0.12	0.13	0.11	0.05	0.11	0.09
5 Percentile (mg/kg/d)	0.53	0.38	0.36	0.69	0.35	0.31	0.31	0.43	0.31
50 Percentile (mg/kg/d)	1.18	1.97	1.03	2.47	1.03	0.83	1.03	1.73	1.1
95 Percentile (mg/kg/d)	2.45	6.22	3.36	7.46	3.31	2.64	3.12	7.16	3.31
Max (mg/kg/d)	6.36	27.24	9.21	32.56	9.7	9.15	10.67	80.47	15.35

LE = Low Exposure Group; HE = High Exposure Group; A to I refers to respective chronic dose groups

Table 8 summarises the mean concentration of vanadium found in each tissue and the results of the lognormal distribution function simulation used to describe the range and probability of values occurring, given the variability in each set of data.

Table 8 Concentrations of vanadium in tissue (mg/kg wet-weight basis) and milk of South African sentinel cattle (1999-2004) and results of the related lognormal simulation model

			Model outputs						
Tissue	Mean	Stdev	Mean	Stdev	Min	5th %tile	50th %tile	95th %tile	Max
Rumen	16.67	14.61	16.11	10.19	1.42	5.25	13.62	35.33	142.5
Mesenteric Lymphnode	2.94	2.76	3.21	4.69	0.03	0.31	1.81	10.56	122.81
Liver	2.79	2.96	2.72	3.29	0.04	0.36	1.72	8.29	68.64
Kidney	1.94	1.57	1.94	1.79	0.07	0.39	1.42	5.19	28.31
Rib	1.82	1.71	1.76	1.53	0.07	0.38	1.32	4.57	23.86
Spleen	1.32	0.89	1.35	1.15	0.07	0.31	1.03	3.47	16.84
Tail-bone	1.65	1.91	1.66	2.5	0.01	0.15	0.92	5.5	75.36
Rib-biopsy	1.01	0.46	1.01	0.48	0.17	0.44	0.91	1.91	7.15
Lung	1.4	1.35	1.5	2.26	0.01	0.14	0.83	4.94	76.12
Cerebellum	0.8	0.76	0.83	1.22	0	0.08	0.47	2.75	27.58
Diaphragm	0.75	0.76	0.8	1.32	0.01	0.06	0.42	2.74	44.32
Cerebrum	0.78	0.83	0.88	1.78	0	0.05	0.38	3.21	49.75
Fillet	0.86	0.99	0.96	2.19	0	0.04	0.37	3.59	68.38
Triceps	0.61	0.63	0.61	0.87	0.01	0.06	0.35	2	18.23
Testes	0.28	0.27	0.27	0.22	0.01	0.06	0.21	0.67	4.16
Milk	0.39	0.46	0.47	1.09	0	0.02	0.18	1.75	45.79
Whole Blood	0.08	0.02	0.08	0.02	0.03	0.06	0.08	0.12	0.21

Table 9 shows the results of the Kruskal-Wallis multiple comparison tests for differences between the mean concentrations of vanadium in the various tissues. It also ranks the tissues from highest to lowest concentration of vanadium based on the median concentration of vanadium found in the tissue. Tissues can be grouped according to the amount of vanadium found in the tissue.

Tables 10a and 10b give the results of Spearman Rank correlation between the median exposure-dose (Table 7), the number of days exposed, the end point exposure dose at the time of slaughter and the concentration of vanadium in the various tissues sampled.

Table 9 Results of the Kruskal-Wallis multiple-comparison test for differences between the median concentrations of vanadium in tissue of slaughtered South African sentinel cattle (1999-2004).

Group	Count	Median	Different From Groups
Rumen	39	13.00	Cerebrum, Cerebellum, Diaphragm, Fillet, Kidney, Liver, Lung, Spleen, Mesenteric lymphnode, Rib biopsy, Rib, Tail-bone, Testes, Triceps, Whole blood
Mesenteric Lymphnode	29	1.74	Cerebellum, Cerebrum, Diaphragm, Fillet, Rumen content, Testes, Triceps, Whole blood
Spleen	23	1.58	Rumen content, Testes, Triceps, Whole blood,
Liver	42	1.34	Cerebellum, Cerebrum, Diaphragm, Fillet, Rumen content, Testes, Triceps, Whole blood
Rib	39	1.10	Cerebellum, Cerebrum, Diaphragm, Fillet, Rumen content, Testes, Triceps, Whole blood
Kidney	42	1.10	Cerebellum, Cerebrum, Diaphragm, Fillet, Rumen content, Testes, Triceps, Whole blood
Rib biopsy	22	0.89	Rumen content, Testes, Whole blood
Lung	42	0.84	Rumen content, Testes, Whole blood
Tail-bone	42	0.67	Rumen content, Testes, Triceps, Whole blood
Diaphragm	42	0.36	Cerebellum, Kidney, Liver, Mesenteric lymphnode, Rib, Rumen content
Cerebellum	34	0.30	Kidney, Liver, Mesenteric lymphnode, Rumen content
Fillet	38	0.28	Kidney, Liver, Mesenteric lymphnode, Rib, Rumen content
Cerebrum	39	0.27	Kidney, Liver, Mesenteric lymphnode, Rib, Rumen content
Tricepts	39	0.25	Kidney, Liver, Spleen, Mesenteric lymphnode, Rib, Rumen content, Tail-bone
Testes	14	0.19	Kidney, Liver, Lung, Spleen, Mesenteric lymphnode, Rib biopsy, Rib, Rumen content, Tail-bone,
Whole Blood	8	0.08	Kidney, Liver, Lung, Spleen, Mesenteric lymphnode, Rib biopsy, Rib, Rumen content, Tail-bone,

Table 10a Correlation between exposure variables and various tissue concentrations of vanadium in South African sentinel cattle (1999-2004) using the Spearman Rank Test

	Median exposure dose	Days exposed	End exposure dose	Lung	Liver	Kidney	Diaphragm	Fillet	Triceps	Rumen content
n=	42	42	42	42	42	42	42	38	39	39
Median exposure dose	1.00	-0.04	0.49	0.06	0.06	0.01	0.06	-0.09	0.00	-0.06
P-value	0.00	0.80	0.00	0.69	0.69	0.93	0.71	0.61	0.99	0.74
Days exposed	-0.04	1.00	0.14	0.29	0.43	0.25	0.00	0.16	0.21	0.21
P-value	0.80	0.00	0.39	0.06	0.00	0.11	0.98	0.33	0.20	0.21
End exposure dose	0.49	0.14	1.00	0.51	0.66	0.52	0.61	0.53	0.55	-0.08
P-value	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.62

Table 10b Correlation between exposure variables and various tissue concentrations of vanadium in South African cattle (1999-2004) using the Spearman Rank Test

	Tail-bone	Rib	Rib biopsy	Cerebellum	Cerebrum	Mesenteric Lymphnode	Spleen	Testes	Whole Blood
n=	42	39	22	34	39	29	23	14	8
Median exposure dose	0.04	0.10	0.48	0.06	-0.05	-0.05	-0.45	0.58	0.25
P-value	0.81	0.53	0.02	0.73	0.76	0.80	0.03	0.03	0.55
Days exposed	0.26	0.01	-0.18	0.25	0.15	0.32	0.43	0.30	0.24
P-value	0.09	0.95	0.42	0.15	0.36	0.09	0.04	0.30	0.57
End exposure dose	0.69	0.58	0.13	0.52	0.51	0.53	0.47	0.14	0.25
P-value	0.00	0.00	0.56	0.00	0.00	0.00	0.02	0.64	0.55

Table 11 gives the results of the Spearman Rank the correlations between various bone samples, correlations between muscle groups and correlations between brain tissues.

Table 11 Correlations between muscle groups, between bone samples and between brain tissues of South African sentinel cattle (1999-2004) using the Spearman Rank Test

	Fillet muscle	Triceps muscle	Diaphragm muscle	Tail-bone	Rib-bone	Rib biopsy	Cerebellum	Cerebrum
n=	38	39	42	22	39	22	34	39
Fillet muscle	1.00	0.85	0.87					
P-value	0.00	0.00	0.00					
Triceps muscle	0.85	1.00	0.87					
P-value	0.00	0.00	0.00					
Diaphragm muscle	0.87	0.87	1.00					
P-value	0.00	0.00	0.00					
Tail-bone				1.00	0.76	0.38		
P-value				0.00	0.00	0.08		
Rib-bone				0.76	1.00	0.67		
P-value				0.00	0.00	0.00		
Rib biopsy				0.38	0.67	1.00		
P-value				0.08	0.00	0.00		
Cerebellum							1.00	0.81
P-value							0.00	0.00
Cerebrum							0.81	1.00
P-value							0.00	0.00

4. Discussion

4.1. Biomarkers in live cattle

The TSP changes and those of its alb and glob fractions were similar to what was described previously in field outbreaks of chronic vanadium poisoning and further discussion of the chemical pathology and haematological changes is given in a previous paper (Gummow et. al., 1994). It has been postulated that the lowering of serum Alb levels may be related to a malabsorption syndrome that develops in calves exposed to vanadium. Whether the malabsorption is due to purely physical small intestinal changes or due to biochemical changes or both is uncertain. This work has been able to show that an inverse relationship exists between exposure dose and serum Alb (Table 5), and therefore Alb could be used as a measure of exposure. The best fitting model was without the interaction of age group, indicating that the dose : response relationship was the same for all age groups. The disadvantage of using Alb is that it requires an invasive process and levels of albumin can be influenced by other disease syndromes such as helminth infestation, protein loosing enteropathies, malabsorption, malnutrition and chronic liver disease, and it is thus not specific for vanadium. The elevated β-glob fraction is thought to be a result of increased transferrin, which is one of the components of β-glob (Duncan et al., 1986). Both albumin and transferrin play a role in transporting the vanadate form of vanadium in plasma (Nriagu, 1998). A low Alb and high β-glob fraction is consistent with vanadium exposure in cattle.

Although the monocyte counts were within the normal range, the difference in monocyte numbers between the HE and LE cattle is interesting, as increased monocyte counts have been reported previously with chronic vanadium poisoning (Gummow et al., 1994). They are derived from bone-marrow and circulate briefly in the blood before being transformed into macrophages. Their increase in this case may be related to phagocytosis and digestion of foreign particulate matter, like dust entering the lungs. However, independent studies at the mine showed that dust levels in the LE camp were higher than the HE camp (Burger & Watson, 2003) and were classified by these authors as "slight" (i.e. <250 mg/m^2/d) for both camps. Dust levels therefore, do not support the monocyte difference between the HE and LE cattle. The other possibility is their relationship to macrophage production of transferrin (Duncan et al., 1986), which ties in with the concurrent increase in β-glob's, and is thus the more likely explanation. From these findings it therefore seems that a closer look at transferrin

concentrations should be made to see what its relationship to vanadium exposure is. Transferrin may prove to be a better and more sensitive biomarker for vanadium exposure than β-glob fractions or monocyte counts. A linear mixed-effects model for monocytes is given in Table 5, but because raised monocyte counts are not specific for vanadium it is doubtful if the model could be used on its own as a predictor of exposure.

AG ratios were not considered for a linear mixed-effects model because the ratio is directly related to Alb concentrations, which was already being modelled. No difference could be shown between groups with respect to Glob concentrations (Table 1).

The raised CK levels are also a consistent finding with vanadium exposure and may be related to its role in catalyzing the transfer of a high energy phosphate bond from adenosine triphosphate (ATP) to creatine in resting muscle and the reverse when muscle contracts (Duncan et al., 1986). Vanadium is known to interfere with energy transduction mechanisms, which suggest an effective participation of vanadium in the energetic coupling and transport of ATPases (Nriagu, 1998).

The higher than normal WCC, seen in these clinically healthy cattle, was also a consistent finding for chronic vanadium poisoning in calves (Gummow et al., 1994). In vanadium poisoning, this was related to an increase in lymphocytes and immature neutrophils (Gummow et al., 1994). No increase in neutrophils was seen in this study, although lymphocyte counts were in the upper normal range. It is thought that this response may be evidence of a chronic inflammatory or immune stimulation taking place.

Why Thr C's were higher in the HE animals is unknown and the link to vanadium cannot be explained. Since other confounding factors, such as splenic contraction, can increase Thr C's, it was decided not to consider Thr C's as a useful biomarker for the time being. Further work needs to be done to explain a possible link between thrombocyte production and vanadium.

It can be argued that Alb, β-glob and monocyte counts are too non-specific to be used as biomarkers, however, Aldrich and Griffith (1993) advocate employing a suite or battery of appropriate biomarkers, particularly for long term exposures. It may therefore be possible to increase the specificity for detecting vanadium exposure by using the biomarker responses as a battery of tests in parallel. In the same way, the accuracy of predicting exposure doses can be potentially increased by combining the results of the regression models, given in Table 5 for these biomarkers, rather than use them on an individual basis.

The difference in vanadium concentrations of hair and faeces between the HE and LE groups (Table 3) and the relationship between vanadium concentrations in hair and faeces, and exposure doses (Table 5), highlights these tissues as potential biomarkers of vanadium exposure for cattle. These tissues are also easy samples to obtain and require non-invasive techniques. However, a lag phase probably exists between vanadium exposure and hair concentration that is related to the rate of hair growth and further work needs to be done to try and model this. The use of hair as a biomarker has long been recognised (Reilly, 1991) and there have been a number of studies in animals that have shown that hair can be used as indicators of mineral status or as biomarkers for metal air-pollutants (Combs et al., 1982; Ronneau et al., 1983; Fisher et al., 1985). However, few studies appear to have been done with respect to vanadium (US Department of Health and Human Services, 1992). Users of hair as biomarkers need to be aware of some of the issues surrounding the collection of samples and the analytic techniques used for the determination of vanadium (Seidel et al., 2001; Steindel et al., 2001) as well as take into account confounding factors such as contaminants on the hair, hair growth stage and age of cattle when comparing results (Ronneau et al., 1983) with other studies.

The use of faeces as a biomarker is also not a new idea but no other faecal biomarker studies for vanadium appear to have been done in ruminants. It appears that some vanadium, in the form of soil may accumulate in the rumen of exposed cattle due to normal animal behaviour, and this could take some time to be excreted (Gummow, unpublished data), how this affects the relationship between exposure and faecal vanadium concentrations is yet to be determined, but there could be a lag phase between ingestion and faecal excretion. The other interesting finding that came out of the mixed-effect model was that age group played a role in predicting the exposure dose (Table 5); it is uncertain why this should be the case but it may be related to rumen development.

4.2. Concentration of vanadium in tissue

The concentrations of vanadium shown in Table 8 are for cattle that were clinically normal. All their carcasses passed meat inspection, were considered fit for human consumption and showed no obvious macroscopic pathology of any significance. These concentrations far exceed what is considered normal by Puls (1988) and also exceeds what others consider as the range of vanadium concentrations that is typical for foodstuffs (Reilly, 1991; Toxicological profile for

vanadium, 1992; Nriagu, 1998). In fact without a history, the concentration of vanadium in these tissues would be consistent with what is reported for ruminants that are suffering from clinical signs of vanadium poisoning (Gummow et al. 1994; Frank et al. 1996). Of concern therefore, is the assumption that is often made by veterinarians or environmentalists that a particular concentration of vanadium in tissue is consistent with vanadium poisoning. What becomes apparent when looking at the results of this study and what is reflected in the tissue lognormal models (Table 8) is the variability that is seen with respect to vanadium tissue concentrations. Also apparent is the fact that adult cattle can have relatively high concentrations of vanadium in their tissues without apparent ill-effect. Using tissue levels as a diagnostic tool for toxicity therefore, needs to be done with caution. This study has however confirmed that higher than normal tissues levels are consistent with vanadium exposure and can be used as a type 1 category biomarker (Aldrich et al., 1993). Frank et al. (1996) reported similar findings in Swedish cattle suffering from vanadium poisoning after grazing on pastures fertilized with basic slag containing vanadium,. The public health implications of high tissue levels have been discussed in a previous paper (Gummow et al., 2005b).

According to Nriagu (1998), experiments with animals support a retention order for vanadium of: bone> kidney> liver> spleen> bowel> stomach> blood> lung> brain, with another retention site being the testes. Examination of Table 8 shows a retention order for the cattle in this trial, based on predicted median (fifty percentile) concentrations of vanadium, to be rumen>mesenteric lymphnode>spleen>liver> rib-bone>kidney>lung>tail-bone> diaphragm> cerebellum>fillet>cerebrum>triceps> testes>whole blood.

For the retention order to be meaningful it was necessary to see if there was a significant difference in vanadium concentration between tissues. It was possible from the results (Table 9) to group the retention of vanadium in tissues into three categories, high, middle and low. The first category is the rumen content, which was significantly higher in vanadium than any of the other tissues monitored. This is understandable given that the bulk of the vanadium is taken in by cattle via the oral route and only a relatively small percentage of this vanadium is thought to be available for absorption. In this study vanadium concentrations in the rumen were 7.5 times greater than in the mesenteric lymphnodes, which had the highest tissue concentrations (Table 8 model median values).

The second category comprises the mesenteric lymphnodes, liver, bone, kidney, lung and spleen. Vanadium may target the immune system (Gummow et al., 1994), which could explain the high concentrations in lymphnodes, bone (marrow) and spleen. In addition, vanadium ions probably have a profound influence on metabolic processes involving calcium, and recent studies suggest bone and connective tissue as target sites for vanadium biological actions (Nriagu, 1998), hence the high levels found in bone. The levels of vanadium in bone are not significantly different from other tissues in this category (Table 9) and despite the long duration of exposure, there is no evidence that vanadium accumulates in bone, as has often been reported (CICAD, 2001). The lung, like the rumen is a route for vanadium absorption and the liver and kidneys are excretory organs, which may explain why they are high in vanadium. The high concentrations in the liver are also partly explained by the fact that vanadium is thought to be specially stored in organs rich in ferritin, which is found in high concentrations in the liver (Nriagu, 1998). It is interesting that Nriagu (1998) placed lungs far lower in his order of retention and this may be a function of exposure rather than retention.

The third category comprises brain, muscle, testes and blood. The relevance of vanadium concentrations in the brain is related to the nervous symptoms that can occur with vanadium exposure (Frank, 1996). There is increasing evidence to suggest that these symptoms are related to biochemical synapsal changes rather than physical damage, yet there does not appear to be any accumulation of vanadium in the central nervous system. Similarily, there do not appear to be high levels in the testes despite some authors referring to the testes as a retention site (Nriagu, 1998).

Given the variability of concentrations of vanadium in tissues (Table 8), it is unlikely that one can be more precise in the retention order of vanadium than these three categories. What distinguishes this study from other studies is the long duration over which these cattle were exposed.

4.3. The relationship between tissue concentrations and exposure

One of the primary purposes of the cattle trial was to study the merits of using cattle as sentinels within the vanadium mining industry. The question that therefore arose was what was the value of tissue levels of vanadium in assessing environmental exposure to vanadium? Particularly because tissues can be easily obtained from cattle sent to abattoirs for slaughter.

Diaphragm was sampled because it was a tissue that could be easily obtained on the slaughter line without reducing the value of the carcass. Rib-biopsies and tail-bones were taken because they were alternative bone tissues that could be sampled on farm from living animals if necessary. Tables 10a and 10b show that there was no correlation between the time an animal was in the trial and the tissue concentrations. This supports evidence that vanadium is non accumulative and has a fairly rapid excretion rate (Patterson et al., 1986). This is further supported by the fact that there is also no correlation between the median exposure dose and tissue concentrations. The relationship between the end-dose and tissue concentrations is more difficult to interpret. Tables 10a and 10b show that there is a significant (α=0.05) and moderate correlation between the end-dose and concentrations of vanadium in the tail-bone, diaphragm, liver and rib-bone in descending order of magnitude. It must be noted however that although there are positive correlations between exposure and vanadium concentrations in these tissues, the relationship is not necessarily linear, which would explain why similar concentrations of vanadium can be found in organs from poisoned animals as well as healthy animals farmed in areas just below the no-adverse-effect-level. For most other tissues, correlations with the end-dose were statistically significant but weak. Rumen content, testes, whole blood and rib-biopsies had no correlation to end-dose. The lack of correlation to whole blood and testes may be due to the smaller number of samples taken for these tissues. What was surprising was the lack of correlation between exposure dose and rumen content, which could suggest the accumulation of vanadium in the rumen over time. There was a good correlation between the tail-bone and rib-bone (Table 11), making the tail-bone a reliable indicator of bone concentrations. However, the correlation between the rib-biopsies and rib-bone and tail-bone were poor, making rib-biopsies a questionable means of assessing bone concentrations of vanadium. This is probably due to the small weight of bone sample obtained with a biopsy. The correlation of vanadium concentrations between the diaphragm and the fillet and triceps muscles was good, thus making the diaphragm a reliable substitute for more expensive muscle cuts when sampling a carcass for public health reasons.

5. Conclusions

Despite the wide range of potential biomarkers screened in this study, no ideal biomarker has emerged. Urine is not a good biomarker. Hair and faeces are better non-invasive biomarkers for

cattle. Serum albumin levels and monocyte counts are correlated to vanadium exposure and a concurrent decrease in albumin and increase in monocyte counts may be a useful tool for monitoring changes in exposure over time. Raised monocyte counts and β-glob may be linked to transferrin production, which could prove to be a better biomarker. Tissue levels of vanadium in healthy cattle include a much wider range than is currently reflected in the literature. The best tissue from slaughter animals for assessing vanadium exposure is probably the liver, which had concentrations that were moderately correlated to exposure doses of vanadium.

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Chapter 6

THE PUBLIC HEALTH IMPLICATIONS OF FARMING CATTLE IN AREAS WITH HIGH BACKGROUND CONCENTRATIONS OF VANADIUM

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Abstract

Forty-two adult Brahman-cross cattle farmed extensively in two groups, immediately adjacent to and 2 km from a vanadium processing plant respectively, were slaughtered over a five year period at a nearby abattoir. Cattle were being exposed to vanadium at close to no-adverse-effect levels. The dose of vanadium that cattle were taking in prior to slaughter was calculated for each animal from environmental and physiological data using a stochastic risk assessment model. The median exposure doses in the month prior to slaughter ranged from 0.55 mg vanadium/kg bwt/d to 2.73 mg vanadium/kg bwt/d. A range of tissues was taken from the cattle at slaughter for vanadium determination and tissue levels of vanadium in muscle, liver and kidney are reported. The concentrations of vanadium in the milk of cattle from the same farm sampled over five years are also reported. Concentrations were further modelled using a lognormal distribution function to look at possible extreme values that are likely to occur. The concentrations of vanadium in commonly consumed tissues ranged from <0.05 to 11.51 mg/kg (wet-mass basis). The median concentration of vanadium in milk was 0.23 mg vanadium/kg. People drinking milk were at highest risk. The potential oral daily intake of vanadium for people consuming these foodstuffs was modelled using a stochastic model. The model predicted that there is less than a 5% chance that the potential daily intake of vanadium from milk will be >0.44 μg/kg/d for adults. Based on this upper limit it was concluded from current knowledge of toxicity in humans that the tissue and milk residues from cattle should pose no health risk to the consumer.

Keywords: Environmental epidemiology, vanadium, cattle, meat, milk, tissue concentrations, safety

1. Introduction

Vanadium (V) and its biological role and toxicity have been the topic of much research since its discovery in 1869 (reviews: Faulker Hudson, 1964; Toxicological profile for Vanadium, 1992; CICAD, 2001; Nriagu, 1998). Yet despite the international interest in vanadium and the amount of research that is being conducted on it, very little work has been done on its effect in ruminants or the effects of long-term exposure in any domestic species or man.

Vanadium is widely distributed in nature (Faulkner Hudson, 1964; Richie, 1985; Waters, 1977) and the prevalence of vanadium exceeds that of such well-known metals as copper and

lead (Nriagu, 1998), and equals that of zinc and tin (Byerrum et al., 1974; Windholz, 1983; Grayson, 1983). So, the potential for it to enter the food chain is real. This is enhanced because many fossil fuels contain vanadium - particularly coal (at concentrations of between 19-126 ppm in ash) and crude oil (at concentrations of between 3-257 ppm) (Nriagu, 1998).

In man, acute vanadium poisoning results in weakness, nausea, vomiting, anorexia, tinnitus, headache, dizziness, green discolourisation of the tongue, palpitations, transient coronary insufficiency, bradycardia with extra systoles, dermatitis, anaemia, leucopaenia, leukocyte granulation and lowering of cholesterol levels (Faulkner Hudson, 1964; Hunter, 1975; Friberg, 1979; Reilly, 1991). People with vanadium concentrations around 14.2 µg/l in urine showed reduced neurobehavioral abilities - particularly so for visuospatial abilities and attention (Toxicological Profile for Vanadium, 1992; SIMRAC, 2000, Barth et al., 2002).

This paper looks at commonly consumed tissues and milk concentrations of vanadium in cattle that were extensively farmed over a five-year period in an area adjacent to a vanadium processing plant that was known to have higher-than-normal background levels of vanadium. We examined the safety of these concentrations in the light of what is currently known about vanadium toxicity in humans.

2. Materials and methods

2.1. Animal system

The cattle used in this trial were from a sentinel herd and experimental trial managed by a vanadium mining company and independently monitored by the Section of Epidemiology, Faculty of Veterinary Science, University of Pretoria and the Institute for Soil, Climate and Water (ISCW), Agricultural Research Council, Pretoria. The animal model system comprised 30 Brahman-cross females with a 20% annual replacement, farmed extensively in a 200 ha area adjacent to a vanadium mine. The cattle were purchased in 1999 as pregnant heifers and randomly divided by lottery into two groups: a High-Exposure (HE) Group of 10 and a Low-Exposure (LE) Group of 20 cattle. The HE Group was farmed adjacent to the mine where they were exposed to high background concentrations of vanadium ($\bar{x}$=1229 mg V/d). The LE Group was farmed approximately 2-3 km from the first group in an area where exposure was roughly half that of the HE Group ($\bar{x}$=532 mg V/d). The herd size was limited by the amount of available grazing; stocking density of 1 animal per 5 ha was used as a guideline. The area where

cattle grazed was bushveld and comprised a number of grass species; the predominant ones were a *Urochloa species, Aristida congesta, Panicum maximum, Themeda triandra, Heteropogon contortus* and *Eragrostis rigidior*. The only supplemental feeds provided were a commercial winter lick and baled lucern (fed at 3% body weight/d), which was fed for approximately 2-3 months during mid-winter (July-September) when the grazing was insufficient to sustain the animals.

2.2. Observations & analytical procedures

Cattle in the trial were monitored over a five-year period and each year non-productive cows or bull calves were slaughtered at a nearby abattoir. Six cohorts of animals were slaughtered over the five-year period on the following dates; 25-Aug-99, 09-Feb-01, 06-Mar-01, 31-May-02, 20-Jun-03, 07-May-04. A total of 42 cattle were slaughtered (Table 1). All animals underwent the same slaughter process that was routinely carried out at the abattoir for slaughter of cattle, with the exception that tissue samples were taken from the carcasses as they moved along the slaughter line. Each carcass was inspected by two veterinarians from the Faculty of Veterinary Science, University of Pretoria and the abattoir's meat inspector for lesions or abnormalities. Specific tissues were taken for analysis of vanadium concentrations. Tissues taken for vanadium analysis were approximately 5 cm x 5 cm in size where possible and were stored in a deep freezer at –18 °C prior to analysis. Analysis was carried out within 3 months of the collection of samples.

Vanadium analysis of tissues was done by the ISCW using atomic emission spectrometry (ICP-AES) according to standard internationally accepted methods and quality control procedures (USEPA Standard Methods, 1996). The detection limit for vanadium using the EPA 3052 method was 0.052 mg/kg. The following tissues were taken for vanadium analysis from animals slaughtered: a caudal coccygeal vertebra, rib bone, rib-bone biopsy (1 cm diameter x 0.5cm), *Musculus iliopsoas* (fillet muscle), *Musculus triceps brachii* (triceps muscle), *pars costalis* (diaphragmatic muscle), cerebellum, cerebrum, lung (ventral lobe), liver (caudal lobe), kidney, mesenteric lymphnode, spleen, rumen content, testes and whole blood. This paper only reports the findings of muscle, kidney and liver, which are the major components of most diets that include tissues of bovine origin. They are also the benchmark tissues used by most toxicologists for assessing dietary intake of metals.

Eighteen deposit-samplers were placed at strategic points along transections within the camps of the HE and LE groups to capture airborne particulate matter. Each transect began near the processing plant and extended out to the boundary of the camps. Soil and grass samples were collected once every 3-4 months at the same points as the deposit samples. At the same time, water samples were taken from the drinking troughs. The soil, grass and water samples were analysed by the ISCW, Pretoria, South Africa, for concentrations of heavy metals and in particular vanadium, using standard internationally accepted methods and quality control procedures (USEPA Standard Methods, 1986, 1996; Handbook of standard soil testing methods for advisory purposes, 1990). The results of the environmental sampling were used as inputs in a stochastic risk-analysis model described by Gummow et al. (2005), to calculate the exposure doses for the cattle at the time of slaughter.

At the same time the soil and grass samples were taken during the year, milk samples from individual cows were collected for determination of vanadium concentrations in the milk using the same methods as those described above for analysing tissue concentrations of vanadium.

2.3. Data analysis

Data was analysed in Microsoft Excel (version 2000) to establish the concentrations of vanadium in commonly consumed bovine tissues and milk taken from clinically healthy cattle, using descriptive statistical methods. The data was then modelled using a lognormal distribution function defined by the data fitting programme BestFit version 4.5.2 (Palisade Corporation, USA) and run 10000 times using Latin Hypercube sampling (@Risk version 4.5.2, Palisade Corporation, USA) to obtain a range of possible values that could be expected for each tissue and for milk, given the sample size and variability in the data. Ten thousand iterations were run to obtain a smooth distribution curve and the number of iterations exceeded the minimum number required for stability and convergence. A lognormal distribution function was used because it is a distribution function commonly used to describe environmental data (Vose, 2004) and because it consistently fitted the raw data well when the data-fitting programme BestFit version 4.5.2 (Palisade Corporation, USA) was used.

The potential oral daily intake (PDI) of vanadium by humans consuming animal tissue or milk from cattle farmed in areas high in vanadium was calculated as:

$$PDI = \frac{R \times FC}{BM}$$

where R is the concentration of vanadium in tissue, FC is the quantity of food (tissue or milk) consumed daily by an adult human with an average body mass (BM) of 60 kg. The FC was based on standard masses of food consumed per day by advantaged people on a western type of diet and is the accepted standard for working out tolerance levels of food residues (Booth & McDonald, 1982). These were 300 g/d meat, 100 g/d liver, 50 g/d kidney and 1.5 l/d milk. Instead of using only the mean tissue concentration of vanadium for R, the lognormal distribution function representing the variability in the concentrations was used. This allowed the PDI to be computed as a distribution function of possible amounts of vanadium that could be consumed per day by the average adult.

3. Results

Table 1 gives a summary of when the cattle were slaughtered, the number of cattle slaughtered, their sex and how many of these came from the high, or low exposure area of the trial.

Table 1 Slaughter dates, exposure group, number and sex of sentinel cattle slaughtered in South Africa on dates shown

Date of slaughter	From High Exposure Camp	From Low Exposure Camp	Male	Female
25-Aug-99	0	3	0	3
09-Feb-01	3	2	3	2
06-Mar-01	2	10	3	9
31-May-02	2	6	7	1
20-Jun-03	0	8	4	4
07-May-04	3	3	6	0
Total	10	32	23	19

Table 2 gives a summary of the model outputs for the calculated exposure doses at the time of slaughter.

Table 2 Model outputs for the dose of vanadium each group of South African sentinel cattle was taking in prior to slaughter

	Jul-99 LE	Mar-01 HE	Mar-01 LE	Apr-02 HE	Apr-02 LE	Apr-03 LE	Apr-04 HE	Apr-04 LE
Total V per day (mg/d)	552	1634	579	984	488	405	722	302
Range (mg V/kg bwt/d)	6.14	35.7	4.55	12.35	4.19	6.8	6.11	2.08
Min (mg V/kg bwt/d)	0.22	0.17	0.17	0.14	0.16	0.12	0.19	0.1
5 Percentile (mg V/kg bwt/d)	0.53	0.84	0.55	0.51	0.41	0.31	0.52	0.25
50 Percentile (mg V/kg bwt/d)	1.18	2.73	1.2	1.44	0.88	0.75	1.19	0.55
95 Percentile (mg V/kg bwt/d)	2.45	8.55	2.34	4.08	1.77	1.76	2.64	1.12
Max (mg V/kg bwt/d)	6.36	35.87	4.72	12.49	4.35	6.92	6.3	2.18
Kurtosis	5.36	13.84	4.28	9.5	5.38	9.19	5.94	4.62
Skewness	1.17	2.5	0.96	2.02	1.17	1.66	1.36	1.04

LE = Low Exposure Group; HE = High Exposure Group

Table 3 summarises the concentration of vanadium found in milk and commonly consumed tissues and the predictions of the lognormal distribution function simulation used to describe each set of data.

Table 3 Concentrations of vanadium in tissues and milk (mg/kg wet-mass basis) of South African cattle (1999-2004)

Tissue	Measured			Lognormal-model prediction				
	Min	Median	Max	Min	5^{th} %tile	50^{th} %tile	95^{th} %tile	Max
Liver	0.33	1.34	11.51	0.04	0.36	1.72	8.29	68.64
Kidney	0.22	1.09	5.37	0.07	0.39	1.42	5.19	28.31
Fillet	<0.05	0.28	2.55	0	0.04	0.37	3.59	68.38
Triceps	0.06	0.25	1.77	0.01	0.06	0.35	2	18.23
Milk	<0.05	0.23	1.92	0	0.02	0.18	1.75	45.79

Table 4 summarises the distribution functions for PDI of vanadium based on fillet and triceps muscle samples from 39 animals, liver and kidney samples from 42 animals and 139 milk samples taken over a five-year period.

Table 4 Model outputs for the potential-daily-intake of vanadium in the diet of humans when eating beef or drinking milk (µg vanadium/kg body mass/d).

	Fillet	Triceps	Liver	Kidney	Milk
Min	0	0	0.1	0.1	0
5 Percentile	0.2	0.3	0.6	0.3	0.5
50 Percentile	1.9	1.8	2.9	1.2	4.6
95 Percentile	18	10	13.8	4.3	43.8
Max	404.5	95	102.4	26.3	1639.5

4. Discussion

4.1. Concentration of vanadium in tissue

Authors vary in what they regard as normal levels of vanadium in individual foods and diets. Nriagu (1998) reports that the range of vanadium in food is between 0.1-10 µg/kg, with a typical concentration of 1 µg/kg. Reilly (1991) on the other hand puts mean levels in a variety of foods to be between 1 and 30 µg/kg. While others report that the general foodstuffs do not exceed 1 mg/kg (Toxicological Profile for Vanadium, 1992). Reilly (1991) goes on to say that meat and fish have a mean concentration of 10 µg/kg with a range of 0-120 µg/kg and milk has a mean concentration of 1 µg/kg with a range of 0-6 µg/kg. Puls (1988), gives a summary of vanadium levels found in cattle, sheep, dogs, pigs, chickens and ducks; normal liver concentrations for cattle are reported as 6-7 µg/kg (wet-mass). These appear to be much lower than the liver concentrations reported for sheep (100-220 µg/kg), dogs (30-50 µg/kg) and chickens (18-38 µg/kg) but of the same order as ducks (0.7-2 µg/kg). Hansard et al. (1982a) found that sheep on a basal diet with 2 ppm vanadium contained 0.08 mg/kg (dry matter basis) vanadium in the muscle, 0.42 mg/kg in the liver and 0.68 mg/kg in the kidney.

Gummow et al. (1994) found tissue concentrations of 0.7 mg/kg (wet-mass) (sd=0.76) in the liver and 1.15 mg/kg (sd=1.35) in the kidney of cattle (mainly calves) that had shown signs or died of chronic vanadium poisoning. The levels of vanadium in the liver and kidneys of these cattle were generally lower than those described by others for acute and sub-acute vanadium toxicity in sheep and cattle (Faulkner Hudson 1964; Hansard et al. 1978; Hansard et al. 1982a; Hansard et al. 1982b; Nechay et al. 1986, Puls, 1988).

The concentrations of vanadium shown in Table 3 are for cattle that were clinically healthy. All their carcasses passed meat inspection, were considered fit for human consumption and showed no obvious macroscopic pathology of any significance. It immediately becomes apparent when looking at the data in Table 3 that these concentrations far exceed what is considered normal by Puls (1988) and also exceed what others consider as the range of concentrations that is typical for foodstuffs. In fact without a history, the concentration of vanadium in these tissues would be consistent with what is reported for ruminants that have clinical signs of vanadium poisoning. Yet, these cattle did not exhibit any clinical signs and ended up in the food chain. Adult cattle in areas high in background vanadium can therefore have much higher concentrations of vanadium in their tissues than was previously thought.

4.2 Potential daily intake and public health implications

Estimated oral daily intakes of vanadium by humans are given as 10-30 µg/d (IPCS, 1988; Reilly, 1991). A UK total diet-study found an intake of 13 µg/d in adults (Reilly, 1991) and an Italian study of five towns found the dietary intake to range from 8-12 µg (Toxicological Profile for Vanadium, 1992); while values of up to 230 µg/d have been reported in Japan (Reilly, 1991). If it is assumed that these values do not take body mass into account, a 60 kg person would therefore consume between 0.17-0.5 µg/kg/d (using European 10-30 µg/d norm).

Table 4 shows the daily intake of vanadium when consuming meat, kidney, liver and milk from cattle coming from an area with a high background level of vanadium. The values are roughly ten times greater than what is considered normal ranges and do not take into account residues in other foodstuffs that might be consumed simultaneously. The chronic dose of vanadium that these cattle were exposed to is close to the no-observed-adverse-effect-level (NOAEL) for cattle (Gummow et al., 2005) and therefore constitutes the threshold of a worst-case scenario with respect to healthy cattle slaughtered for human consumption. The simulation model predicts possible extreme values based on the variability of the inputs, which are quite unlikely. The 5th and 95th percentiles therefore, give a better idea of the range within which most values could be expected to occur. From a safety point of view the 95th percentile values would give the best indication of the upper limits of vanadium that could be potentially ingested when consuming tissues from cattle found in areas high in vanadium or when consuming milk from cattle in these areas. From Table 4 it can be seen that the consumption of milk from these areas poses the greatest risk, with a 95th percentile PDI of 44 µg/kg/d. The question that then needs to be answered is whether humans are likely to suffer any adverse effects when consuming vanadium at these concentrations.

A number of oral dose studies have been done in humans over the years. In 1959 five male medical students were dosed 100-125 mg diammonium oxytartratovanadate/d or the equivalent to 1.7 mg/kg/d (using an average body weight of 70 kg), for 6 weeks (Curran et al., 1959). They reportedly showed no adverse clinical signs or haematological changes. In 1962 a group of 12 volunteers received 75 mg diammonium vanadotartrate/d orally for 2 weeks, followed by 125 mg/d for the remaining 5.5 months (Somerville & Davies, 1962). Two subjects withdrew due to "toxic gastrointestinal effects" and five patients had persistent upper abdominal pain, anorexia,

nausea and weight loss. Five men developed "green tongue" and one had pharyngitis with marginal ulceration of the tongue. At approximately the same time in another trial, a group of six subjects received 50-125 mg ammonium vanadyl tartrate/d orally for 45-94 days (Dimond et al., 1963) without apparent harmful effects. One of the most recent studies was a double-blind trial by Fawcett et al. (1996, 1997), which included 11 males and 4 females in the experimental group and 12 males and 4 females in the control placebo group. The experimental group received 0.5 mg vanadyl sulfate/kg/d for 12 weeks. No significant differences between the groups in terms of body weight, haematology indices, blood viscosity or standard biochemistry measurements were found. From these studies it is probably safe to assume that an oral dose of <500 μg/kg/d is likely to have no adverse effects in humans, which is well within the NOAEL of 4.1 mg V_2O_5/kg/d reported for mice after 2 years of feed exposure (Schroeder & Balassa, 1967). The levels in meat, liver, kidney and milk in this study are therefore well within this margin.

The USA Environmental Protection Agency (EPA) provides a reference dose (Rfd) of 9 μg/kg/d for oral intake of vanadium pentoxide (Toxicological Profile for Vanadium, 1992). A Rfd was defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without deleterious effects during a lifetime". Given this definition, the Rfd for vanadium pentoxide intake could be defined as a range of 0.9-90 μg/kg/d. This means that the meat, liver, kidney and milk levels all fall within the Rfd given by the EPA as being safe for humans. The conclusion therefore, has to be that edible tissues and milk from clinically healthy cattle farmed in areas with high background levels of vanadium (as presented here) are likely to have no adverse effect on human health given the current state of knowledge of vanadium toxicity.

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Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

B. Gummow

"Don't let us quarrel," the White Queen said in an anxious tone. "What is the cause of lightning?"
"The cause of lightning, "Alice said very decidedly, for she felt certain about this, "is the thunder-oh, no" she hastily corrected herself. "I meant the other way."
"It's too late to correct it," said the Red Queen: "when you've once said a thing, that fixes it, and you must take the consequences." (Carroll, 1865)

1. Introduction

"In less than 100 years, vanadium has gone from being a rare and obscure metal to become one of strategic military importance and a pillar of modern technology. As scientific and technological developments expand its horizon, vanadium is clearly poised to become the element for the twenty-first century" – Jerome Nriagu (1998). With this in mind, vanadium is also poised to potentially have a significant environmental impact within the twenty-first century, particularly in countries like South Africa, China, Russia and the USA, which are the main producers of vanadium (Chapter 1). The work in this thesis set out to study the consequences of this impact on cattle and cattle farming (Chapter 2). It went further and looked at the possibility of prophylactic measures that would allow farming to continue in the face of a polluted environment (Chapter 3) and progressed from the concept of treatment to the concept of early detection and prevention as the long term sustainable solution for the twenty-first century (Chapter 4 and 5). Finally, having accepted that vanadium will enter the food chain of twenty-first century man some of the consequences of this were examined in Chapter 6. The thesis provides a spring board for future research into the twenty-first century and a basis for future monitoring and legislation, which are discussed further in this Chapter.

In Chapter 4 it was mentioned that a structured approach was required in order to cope with the amount of data being generated by the project. This approach was lacking initially and there was a real danger of the sentinel aspect of the project becoming an exercise in collecting data for the sake of collecting data. Because of the author's experience with import risk assessment methodology (Murry et al., 2004) it was decided to use this framework for focusing the information in the sentinel project and for ensuring its sustainability. This framework has been used for the general discussion (Chapter 7) as a way of structuring and focusing the discussion on the different aspects of the work carried out over the last thirteen years. The initial step in the work for this thesis essentially began with hazard identification.

2. Hazard identification

Hazard identification, in the context of this thesis, involves identifying harmful substances associated with the mining operation that could potentially produce adverse consequences to people, plants or animals.

In the disease outbreak as presented in Chapter 2, this began by recognising that a potential source of hazards could be the adjacent alloy processing unit (APU). Initially all that was known about the APU was that it was involved in steel production and so the hazard identification involved identifying components that went into the manufacture of steel, one of which was vanadium. It was this first step that resulted in the identification of vanadium as the potential cause of the disease syndrome, although at the time we did not call this step hazard identification. This approach was refined further during the sentinel study (Chapter 4). The study revealed that key to successful hazard identification within the mining context was communication (discussed under risk communication) and involvement of the correct experts. Veterinarians and most environmentalists do not have the chemical engineering background necessary to fully understand the subtleties of the manufacturing process and the potential hazards associated with this. Their involvement in the sentinel study distinguished the level of hazard identification in the sentinel study from that of the disease outbreak investigation (Chapter 2).

Hazards associated with the vanadium mining industry are outlined in Chapter 4. The need for thorough hazard identification seems obvious now, but at the time the researchers frame of thinking was very much along the lines of an experimental study and the focus was on a single hazard, vanadium. The need for more lateral thinking was not realised until unexplained symptoms began to develop in the calves (discussed under consequence assessment). Just as with epidemiology, the concept of multifactorial determinants of disease required a shift in thinking, so too in sentinel studies, particularly of metals, a shift in thinking is required from that of a single hazard to that of a causal web involving multiple potential hazards interacting with one another. Complicating the picture further is the range of different vanadium compounds that can occur. As pointed out by Mendz (1998) “The hundreds of studies on the effects of vanadate and vanadyl (see Chapter 1) ions on living systems that have appeared in the last few years reflect the complexity of these interactions..” One of the weaknesses of the work in this thesis was that not enough attention was given to the different forms of vanadium.

An aspect that is linked to hazard identification is pollution control monitoring. Authorities should be monitoring for relevant hazards around mining industries and these need to be identified by means of a hazard identification process. Yet this is seldom done. In 1993, pollution control monitoring by authorities was not specific enough for the industry concerned.

At that time there were no emission guidelines for vanadium within the South African Atmospheric Pollution Prevention Act, No. 45 of 1965. The emission guidelines issued by the Department of National Health and Population Development covered only dust, NH_3 and SO_2 emissions (Environmental Options cc., 1993). The hazard identification process and outbreak investigation therefore succeeded in alerting authorities to potentially dangerous pollution problems and in putting pressure on the mine to reduce their pollution outputs. It also alerted authorities to problems and gaps in the pollution control legislation at that time and resulted in a government commission of enquiry to probe the extent of the pollution around the mine (Wates, 1996).

Unfortunately, the results of the outbreak investigation (Chapter 2) ended up being used in a court battle between the mine and farmer, which lasted several years before the farmer went bankrupt and had to sell his farm. From this, it was realised that these issues cannot be easily resolved in a court of law and a better approach was needed if the country was to move forward in controlling pollution from the vanadium industry. One of the problems in achieving this was the suspicion between the mining industry and agricultural industry, largely as a result of lack of communication and will to cooperate between these two economic sectors.

In summary then, the lesson learnt was that hazard identification involves communication and should be the first step in any future sentinel studies. It should not only focus on the obvious or primary hazard, and must involve someone with an intimate knowledge of the operation of the mine and its various processes. Future research should include methodology that can be used in all the levels of a sentinel study (i.e. from environmental to tissue level) to identify the different vanadium compounds playing a role, and their interaction with other chemicals in the area.

3. Risk Communication/Communication

As mentioned under hazard identification, communication was one of the key factors that determined its success. Risk communication is the process by which information and opinions regarding hazards and risks are gathered from potentially affected and interested parties during a risk analysis, and by which the results of the risk assessment and proposed risk management measures are communicated to the decision-makers and interested parties (Murry et al., 2004). It is a multidimensional and iterative process and should ideally begin at the start of the sentinel/risk analysis process and continue throughout. From our experience it is necessary to

include in this definition the gathering of information and opinion from relevant experts as well. Key to the success of the sentinel project (Chapters 4, 5 and 6) has been risk communication.

From the outset it was agreed that if the University of Pretoria was involved in the project there would be no restrictions on publishing the findings. Both parties at the onset of the project were apprehensive about this policy. The mine was afraid the findings may be sensationalised and published in the lay-press with the express purpose of damaging the credibility of the mine, while the veterinary researchers involved were concerned that the mine would not be willing to disclose relevant information that would be of importance to the project. A policy of transparency was adopted by all parties concerned, which was completely opposite to the policy adopted by the first mine during the 1991 outbreak. As part of this policy an interested parties meeting was hosted annually by the mine. Leading members of the local community, pollution control authorities, and environmental consultants to the mine were invited to the meeting, which was also attended by mine management and any other person interested in activities on the mine. The annual report of the sentinel project was presented to all interested parties at these meetings and any questions or concerns by persons attending the meeting were addressed. The value of the sentinel project at these meetings was firstly, that it acted as common ground between the local community and mine management and secondly, it provided the community with tangible evidence that the mine was taking their concerns seriously. The mine was also alerted as to perceptions or problems that the community may have had. The evidence of the project's success as a risk communication tool was quantified by the number of complaints and claims the mine received prior to the implementation of the project, compared to those while the project was in progress. An interview with the company's environmental manager revealed that between 1994 and 1999, when the sentinel project began, the mine received complaints from at least 4 farmers, who claimed losses of between 15 and 21 cattle (Table 1). In most cases animal carcasses were too autolysed to be necropsied and the cause of death and exact number of cattle affected could not be verified. In contrast, during the first five years of the sentinel project, only one farmer brought a complaint against the mine and this was for a bull that was found dead in a trench, but which an autopsy found had died of Cerebral Theileriosis. The difference in settlement costs to the mine for the 1994-1999 and 1999-2004 periods are also reflected in Table 1.

Table 1 Farmer complaints against a South African mining company before 1999 and after the sentinel cattle herd was established (1999-2004) on the mines property

Period	**Alleged no. of deaths**	**Probable cause of death**	**Compensation Paid**
August 1994 and August 1997	5 + 1 injured	Unknown	R7 882,90
November 1997	7	Arsenic	R16 275,00
May 1998	6 to 12	Vanadium spill	R8 500.00 for 3 cattle
April 1999-April 2004	1	Trauma	R 3000

During the first two years of the project vandalism occurred to locks on the gates of the cattle paddocks and the same farmer whose bull died made accusations in the press against the mine and asked the Directorate of Animal Health to do an independent investigation. The transparency of the project was further illustrated when a TV crew from the national broadcaster was allowed to film the cattle and interview workers in the project. Both the Directorate and the media found no substance to the farmers' complaints. The project therefore also proved its value as a means by which the mine could provide tangible and visual evidence to interested parties that would allow them to evaluate possible animal health problems. The value of transparency and communication between the mine and surrounding farmers has now been recognised by all parties concerned. This is further illustrated by the continued lack of complaints even though interested parties were made aware during the course of the project that some of the mine's calves in the HE group had died of vanadium poisoning.

Another aspect that became apparent when doing environmental studies is the number of role-players that are involved. These included engineers, mine managers, soil scientists, chemists, physicists, environmentalists, farmers, pollution monitoring experts and authorities, politicians, lawyers, laboratory technicians and various veterinary specialists and authorities. This illustrates the complexity of the studies and is reflected in the number of co-authors for the various chapters in this thesis. Management and communication are therefore an integral part of the success of such a project.

In summary; the lesson learnt was that a policy of transparency and risk communication is integral to the sustainability and success of a sentinel project. Future research needs to be conducted into ways of improving communication between all role players.

4. Risk Assessment

4.1. Release assessment

Release assessment consists of determining the likelihood of an animal or person being affected or contaminated by a hazard and describing the pathway(s) necessary for that hazard to be introduced into a particular environment. The aspect of release assessment is a complex one within the mining industry. It is coupled to the mines manufacturing process and physical layout, as well as environmental factors such as wind direction. The exposure model, discussed in Chapter 4, uses inputs from the point of exposure and thus removes the need for a complex release assessment model used by many environmentalists. This is one of the strengths of the model. Its value in the context of this sentinel study was as a means of understanding the complex chemical interactions that could potentially occur and the consequences of these. Because of the complexity of the release assessment in a mining industry, a structured approach needs to be followed.

Chapter 4 describes a qualitative method, using expert opinion, as a means of doing a release assessment. The problem that was difficult to address in the release assessment was the likelihood of an animal becoming affected. This was partially addressed by weighting of the sources of hazards, which then gave an indication of the likelihood of exposure to particular vanadium compounds. This distinguished this approach from other release assessment models for the vanadium industry, which tend to focus only on V_2O_5 and fail to take into account the contribution of other vanadium compounds released during the manufacture of V_2O_5 (Burger et al., 2003). Such an approach could result in an underestimation of exposure if conventional plume dispersion models are used.

There are various ways of eliciting expert opinion for risk assessments and of modelling those inputs to obtain a more quantitative assessment (Vose, 2000). The release assessment done in Chapter 4 provides a foundation that can be used for a more quantitative approach, which may give a better estimation of the likelihood of exposure. Future research needs to be done into better ways of eliciting and combining expert opinion for release assessment purposes.

4.2. Exposure assessment

Exposure assessment describes the biological pathway(s) necessary for exposure of animals and humans to the hazards identified and estimating the likelihood of those exposure(s) occurring (Murry et al., 2004). Several investigators have suggested that the failure to

accurately assess exposure has reduced the effectiveness of epidemiological research (Clarkson et al., 1983; Heath, 1983) and is the Achilles heel of traditional epidemiology (Perera and Weinstein, 1982). Poor exposure assessment promotes misclassification among exposed and comparison cohorts (Aldrich and Griffith, 1993). This proved to be the Achilles heel of the 1991 outbreak investigation (Chapter 2), since existing exposure assessment methodology failed to account for all the biological pathways and underestimated the likelihood of exposure. The model given in Chapter 4 was therefore the missing component, without which the consequences of exposure could not be easily or accurately assessed. The necessity of obtaining an accurate assessment of exposure and the strengths of the model is discussed further in Chapter 4. The important outcome of that research was that a method is now available that enables regular evaluation of exposure as the results of the environmental inputs become available. This in itself is a useful tool in alerting mine management of potential problems.

One of the other interesting spin offs of the project has been the peer review process that was inadvertently introduced when environmental samples were collected to assess exposure. Most of the mine's consultants on pollution monitoring produced work that was not peer reviewed and which was difficult to verify. The sentinel project introduced a different way of monitoring pollution (Chapter 4) to the conventional stack emission and dispersion plume models used by many mines. In the course of the project the results and methodology of the mine's consultants were cross checked with those of the sentinel project and errors in the consultants work were identified and rectified. The sentinel project therefore acted as a means of quality control for existing pollution monitoring procedures required by law.

4.3 Consequence assessment

Consequence assessment consists of describing the relationship between exposures to a hazard, the consequences of those exposures and their likelihood (Murry et al., 2004). Biomarkers can serve to mark exposure (dependent variable), thereby providing the potential for intervention, or as a predictor (independent variable) of subsequent impairment or disease (Aldrich et al., 1993). Biomarkers can therefore be used as a measure of consequence within a sentinel study and have a dual purpose.

4.3.1. The role of biomarkers

When the investigation began in 1991 (Chapter 2), sick calves were acting as biomarkers of exposure. Chapter 2 describes in some detail the consequences of that exposure in terms of the clinical signs, pathology and clinical blood chemistry changes that can occur in the presence of vanadium. The study was to a large extent descriptive and the relationship between exposure and consequence was simplified into: prolonged exposure to higher than normal background concentrations of vanadium result in the consequence of sick calves. Although simple, this was a necessary first step for future studies, as we learnt that calves are sensitive biomarkers to vanadium aerial pollution and had the potential to be used as sentinels for detecting vanadium exposure. This was supported later by Frank et al's. (1996) findings in cattle exposed to vanadium-rich slag contaminated pastures in Sweden, where heifers appeared to be more at risk than older cows.

We also began to understand how the calf biomarker responded under these circumstances, but we did not know how sensitive they were as a biomarker because we had no accurate exposure assessment, and hence no dose response relationship could be assessed. We also learnt that calves manifested signs of poisoning before the point is reached where humans become ill. In Chapter 1, it was put forward that the ideal sentinel responds to toxic insults long before clinical manifestations of disease in humans and at environmentally relevant doses and mixtures, thus making calves an ideal sentinel and biomarker for vanadium. Under these circumstances calves and the other chemical pathological parameters and pathology findings evaluated in Chapter 2 acted as dependent biomarkers and served to mark exposure. They could not however be used to quantify exposure.

With the design of the sentinel study there was a shift in focus from using biomarkers to mark exposure to finding biomarkers that would predict exposure (Chapter 5).

In 1999, when the sentinel study was designed, the risks to cattle were based on soil and grass vanadium concentrations from samples taken by independent consultants in the area where the high exposure (HE) cattle were to graze. Based on these concentrations, and the results of the 1991 outbreak, it was anticipated that it would be highly unlikely that cattle would develop any clinical signs of vanadium poisoning. This was important from an ethical perspective and also meant that a means of marking exposure other than clinical signs was needed. It was for this reason that the wide range of samples discussed in Chapter 5 were collected in the hope that one

of them would prove to be a useful biomarker. It was also thought that the exposure to cattle farmed in the low exposure (LE) area would be so low that the group could act as a control group, thus allowing the comparison of biomarker responses between an exposed and "non-exposed" group and the possibility of finding biomarkers that could predict exposure. Only after the project started, and monitoring began, was it realised that concentrations in the environment were much higher than anticipated and the cattle in the LE ("control") camp were also being exposed to relatively high levels of vanadium, and the experimental design had to be modified accordingly.

The search to find a predictive biomarker was however futile until an accurate exposure model was worked out. This having been done (Chapter 4), attention could then be spent evaluating the biomarkers as independent variables rather than dependent variables. The strength of having two or more groups of sentinel animals at varying exposures when trawling for biomarkers is obvious and necessary if biomarkers are to be evaluated as predictors rather than markers. The regression models put forward in Chapter 5 for predicting exposure doses are the first step in this process of finding predictive biomarkers. These models are not yet perfect, nor have they been validated to the point where we can say if they are reliable. They do however provide the opportunity to move away from using animals as markers of exposure to predictors of exposure and thus provide the tools that were previously lacking for more accurately assessing the relationship between exposure and hazard. For the biomarkers (Chapter 5) to be used as predictors of risk, an accurate assessment of no-adverse-effect-levels is necessary (Chapter 4 and 6) and this is discussed further below. Further research needs to be done into the use of predictive biomarker models for the mining industry.

4.3.2. Additional consequences

While the purpose of the general discussion chapter is to focus on the work published in the thesis, an aspect that has not been covered in the thesis regards the animal health parameters that were being monitored during the five year period of the work. These serve as additional consequences of exposure. They included monitoring body weights as an indicator of possible effects on weight gain, calving percentages and pregnancy rates as indicators of cow fertility and general cattle health status. The latter aspect ended up generating unanticipated necropsy results, and new clinical findings and methods of treatment. The volume of these results

warrants at least an additional two publications and so will not be covered in any depth within the framework of this thesis. However, it was felt that some of these findings should be reflected in the thesis as they were an integral part of the study. These findings also impact on the risk estimation and the discussion surrounding risk estimation.

The other consequences not covered in the thesis but which were examined as part of the study was the impact of vanadium on grazing and soils, which will be covered in a separate publication. This will not be discussed further in the thesis.

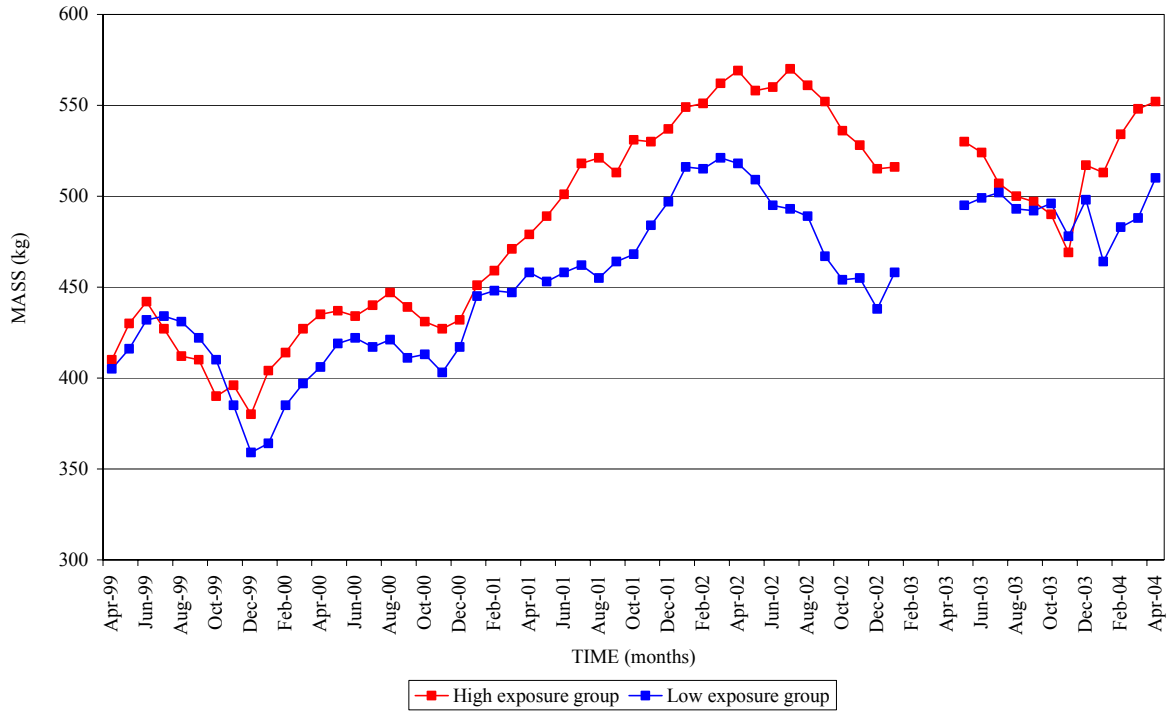

Fig. 1. Average weight of two groups of South African adult Brahman-cross sentinel cattle exposed to vanadium between 1999 and 2004

4.3.2.1 Cattle health

Throughout the five years of the study, adult cattle showed few clinical signs or evidence of adverse effect. Production in terms of weight (Fig. 1) and calving percentages (70-80% for HE group; 50-75% for LE group) were within expected parameters for a herd of this nature. Clinical pathology parameters (BUN, AST, GGT – Table 1, Chapter 5) gave no indication of liver or kidney malfunction. Apart from conditions routinely seen in herds of this nature, the only abnormal clinical finding was occasional evidence of unexplained ulcers on the lips and

nostrils with related nasal discharge and salivation in the high exposure herd. These lesions were also seen in calves, where they were usually a lot more severe (Fig. 2 and 3). There was no evidence of an increased rate of abortion or still births as reported in previous outbreaks (Chapter 2).

During 1999 the first batch of calves were born and began showing clinical signs. One calf died and two were euthanased for necropsy out of ten calves born in the HE camp. The signs were atypical and it was uncertain if they had died of vanadium poisoning. Signs not seen in the 1991 outbreak were ulcers on the muzzle, nostrils (Fig. 2) and in a few cases vulva, as well as dyspnoea as a result of upper respiratory tract congestion and swelling and a profuse muco-purrulent nasal discharge (Fig. 3). It was thought the lesions might be viral in origin but electron microscopy done later in the study failed to reveal any viral particles in the lesions. Post mortems were non-specific and it was still uncertain that the cause of death was vanadium as these signs had not been described before. Since adult cattle showed no clinical signs or adverse effects and the cause of the signs was uncertain it was decided to continue with the project. As the project continued, a trend developed whereby a proportion of calves in the HE camps would become ill, while those in the LE camp rarely showed signs. This is illustrated in the Kaplan-Meier survival analysis curves worked out for the two groups using all mortalities regardless of clinical signs (Fig. 4). The mortalities in the LE group of calves appeared, from necropsy, not to be directly related to vanadium.

Fig. 2. Lesions on the lips of South African sentinel calves exposed to chemicals emitted by the vanadium mine (1999-2004)

Fig. 3. Signs of nasal discharge, salivation and dyspnoea seen in South African sentinel calves (1999-2004)

Other signs that became apparent were signs of facial paralysis as reported by Frank et al. (1990) and visio-spatial problems as seen in humans (Barth et al., 2002), but not blindness. These nervous signs were not noticed in the 1991 outbreak (Chapter 2). Because this does not form the scope of this thesis, a more detailed description and discussion of the clinical signs and postulated pathogenesis will be published elsewhere. By 2003 it appeared that when calves in the HE camp were born within 200 m of the vanadium processing unit (VPU), they developed signs and often died but if born further away survived. This theory was tested in 2004 when the calving area for the HE calves was moved beyond this 200 m radius and all calves born during that year survived. Confounding this was evidence that the pollution levels from the mine had also been decreasing over the years of the study.

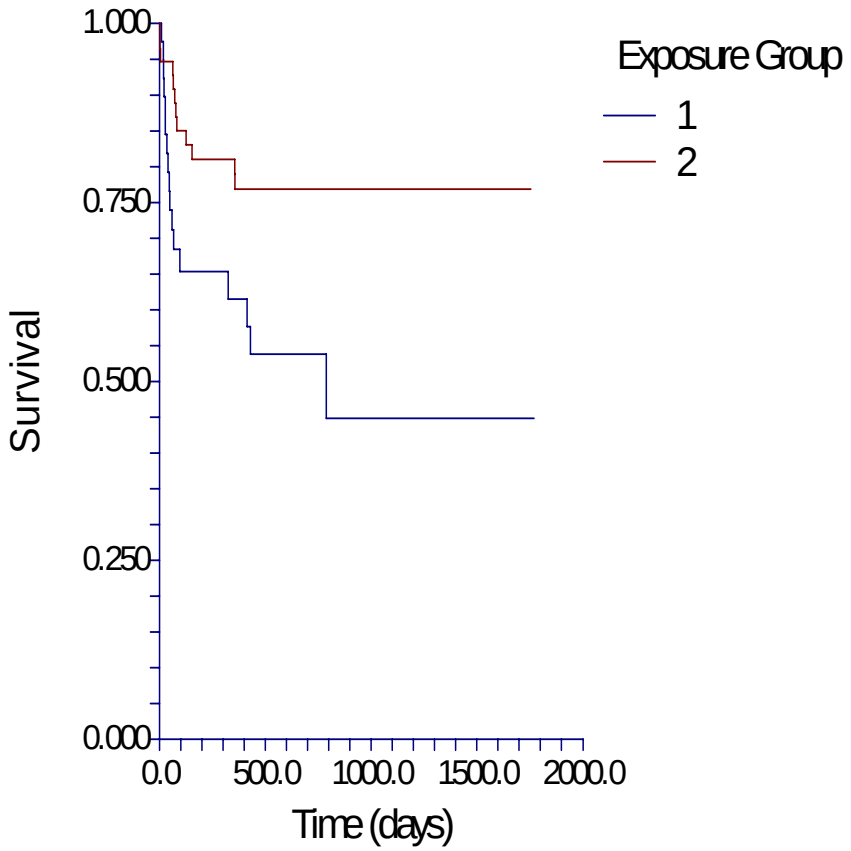

Fig. 4. Kaplan-Meier survival analysis for sentinel calves in the HE group (1) and the LE group (2), South Africa 1999-2004

The relevance of these findings to this thesis is that a high percentage of calves in the HE areas invariably became sick and died or were euthanased, while calves in the LE areas did not (Fig. 4). Hence the NOAEL must lie somewhere between the two exposure doses given in Chapter 4.

4.4. Risk estimation

Risk estimation consists of integrating the results from the release assessment, exposure assessment, and consequence assessment to produce summary measures of the risks associated with the identified hazards.

4.4.1. Risk to cattle health

Biomarkers are a way of estimating risk. The study has shown that calves act as good sentinels and markers of increased pollution. At the concentrations of vanadium found in the

HE camp ($\bar{x}$=1229 mg V/d - Chapter 4 and 5) there is a definite risk to calf health, with related production and economic impact to farmers. Viable extensive cattle farming cannot take place at these levels of exposure. At concentrations of vanadium found in the LE camp ($\bar{x}$=532 mg V/d -Chapter 4 and 5), there is minimal impact on cattle health, and extensive cattle farming should be sustainable at these levels of exposure. The Kaplan-Meier survival curves provide an estimate of survival amongst calves at these exposure doses and allow the quantification of the impact of chronic vanadium poisoning (Fig. 4).

The study has shown that the composite NOAEL for cattle farmed in the vicinity of a VPU, with its related chemical mixes, lies somewhere between 1.2 mg V/kg/d and 2.6 mg V/kg/d. This finding allows accurate risk estimation for areas with similar identified hazards.

The role of predictive biomarkers has been discussed and the regression models given in Chapter 5 may provide a means in future of estimating exposure without the necessity of detailed environmental data, thus providing a new tool for risk estimation.

A question that remained unanswered throughout the years of work was why do suckling calves become sick and not adult cattle? Finding the answer to this question is the subject of continued work at the Faculty of Veterinary Science, University of Pretoria, and is mentioned here since it forms a part of risk estimation. Recent work, using radiographs of the rumens of affected calves has shown that calves ingest a lot more soil than was previously anticipated (Fig. 5) and that it remains in the rumen for a period of weeks. Chemical analysis of the rumen gravel in these calves has shown this to be high in vanadium. How much is soluble vanadium is still to be determined. This behaviour of eating soil may now explain the lesions on the lips and nostrils of these animals and why calves are more susceptible than adult cattle. It also means that soil concentrations of vanadium and other metals, may play a much more important part of risk estimation for calves than was previously thought.

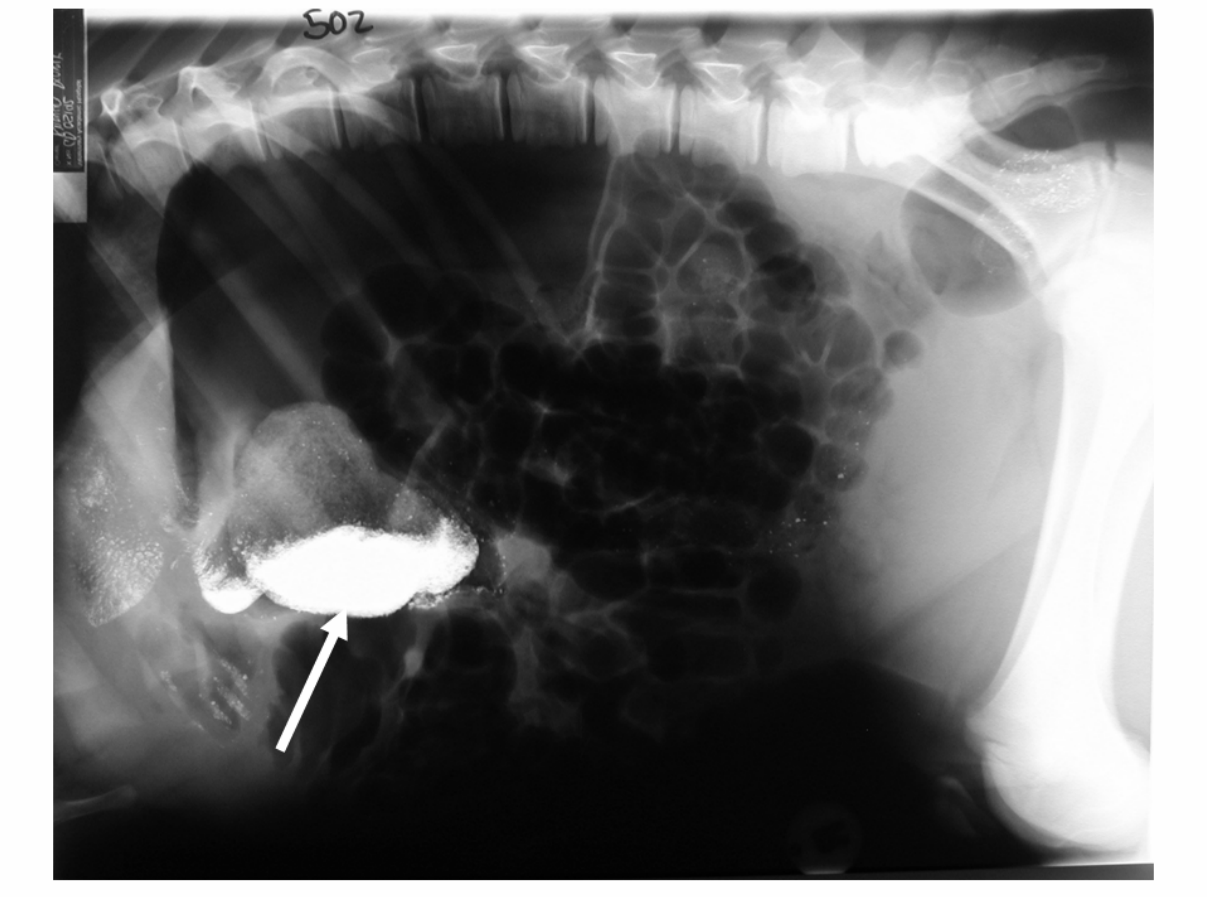

Fig. 5. Radiograph of soil (arrow) containing metals found in the rumen of a 6 w old affected sentinel calf, South Africa, 2005.

4.4.2. Risk to human health

Chapter 6 describes risk estimation with respect to vanadium residues found in the meat and milk of exposed cattle and need not be discussed further here other than to say the risks to the consumer appear to be negligible, even in the presence of sick calves in the HE camp. It however forms an important segment of the risk estimation and is a useful component of any sentinel project using cattle.

In estimating direct risks to humans, the calf biomarkers should be alerting us to potential human health problems. The question is how much can be extrapolated between cattle and human health. This question remains difficult to answer because of fundamental differences in anatomy and physiology and probably kinetics (Chapter 1). The large oral component of exposure in cattle is also a fundamental difference between risk estimation for cattle compared

to humans. This is therefore an area that requires further research if cattle are to be used as sentinels for human health.

The project has however proved useful in screening for potential biomarkers that could be used to monitor human exposure in the future. It seems that the current use of urine as a biomarker by the vanadium industry is a waste of money and time. Hair vanadium concentrations and possibly albumin, β-globulins and monocyte counts need to be investigated more closely in humans as a means of risk estimation. This is recommended for future research within the vanadium industry.

5. Risk management

Risk management is the process of determining and implementing measures to achieve the region's and/or country's and/or global "acceptable risk". Four components are identified: Risk evaluation, option evaluation, implementation and monitoring and review. Risk management is an on-going process and can be seen at various levels. In the context of this thesis, the discussion will be confined to that of cattle health. However, risk management in reality involves the mine's entire environmental programme, aspects of occupational health, various regulatory authorities, feed-back from interested parties, aspects of production methods and waste disposal and more.

5.1. Risk evaluation

Risk evaluation is where the estimated risk is compared with the country's appropriate level of protection.

International vanadium ambient air quality and work-place air quality standards for humans are given by a number of agencies and authors (Toxicological Profile for Vanadium, 1992; Environmental Options cc., 1993; Wates, 1996). These appear to have changed little in the last decade and a summary is given in Table 2.

The study found that the average ambient air vanadium levels measured in the HE camp (2.96 $\mu g/m^3/d$) exceeded the WHO air-quality guidelines for the time-weighted-average (TWA) for vanadium of less than 1 $\mu g/m^3/d$. While the average levels measured in the LE camp (0.71 $\mu g/m^3/d$) were within the guidelines (Chapter 4).

Table 2 Summary of international vanadium ambient air quality and work-place air quality standards

Ambient limit (V_2O_5)*	**Comment**	**Work place limit (V_2O_5)***	**Comment**	**Reference dose (V_2O_5)**	**Comment**
1 µg/m^3	TWA (24 h)	50 µg/m^3	Respirable dust (TLV/TWA - 8 h) – some countries accept 500 µg/m^3	9 µg/kg/d	Lifetime daily limit (humans)
0.14 µg/m^3	TWA (8 h)	50 µg/m^3	Fume (TLV/TWA – 8 h) – some countries accept 100 µg/m^3.		
*= most commonly applied international norms, including South Africa and USA; TLV = threshold limit value; TWA = time weighted average.					

The final output of the exposure assessment model was an average intake dose of 2.6 mg V/kg body weight/d for the HE animals over the five years of the study and 1.2 mg V/kg body weight/d for the LE animals (Chapter 4). The vanadium intake ranged from 0.57 to 5.44 mg vanadium/kg bwt/d in the HE group and 0.41 to 2.61 mg vanadium/kg bwt/d in the LE group over a five-year period of monitoring (Chapter 5). There are no published reference doses (Rfd) for cattle and hence this study serves as the basis for a Rfd for cattle. Based on the definition that a Rfd is "an estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the cattle population to a potential hazard that is likely to be without deleterious effects during a lifetime", a Rfd of 0.1 mg/kg/d (100 µg/kg/d) is proposed, giving a range of 0.01 to 1 mg/kg/d. This range remains below the average exposure dose of the LE camp and only slightly exceeds the minimum measured dose for the HE camp. It is approximately 10 times greater than that given for humans (Table 2).

5.2. Option evaluation

Option evaluation is where measures are identified, evaluated and selected to effectively manage the risks in line with the country's appropriate level of protection.

As pointed out under hazard identification, when the project began in the early 1990's, there were no guidelines with respect to what was considered safe for cattle, and existing pollution control legislation was non-specific for vanadium. This is largely still the case in South Africa.

The only feasible option for managing risk on contaminated farms was to try and find a prophylactic treatment. This principle had been successfully used by the author in a previous outbreak investigation involving grazing contaminated by copper from a nearby copper smelter (Gummow et al., 1991), where licks containing sulphur and zinc-sulphate were used to limit copper absorption. Chapter 3 discusses a therapeutic approach for vanadium as an option for risk management. Because the use of $CaNa_2EDTA$ was not entirely successful, the treatment study shifted our thinking away from a therapeutic approach for managing risk, to the alternative method of using sentinel animals as an early warning system. The trick has always been getting the mine to react to the early warning and look for events that may have increased pollution outputs. Having said that, the study of therapeutic measures for risk management has by no means been exhaustive and more work could be done in this respect. What we have established in this study is that early removal of cattle from a contaminated site to an unexposed area, will invariably result in reversal of signs, and is a viable short-term risk management option that can be used to save a herd in an outbreak situation. We have also established that once calves become so sick that they reach a state of cachexia, the disease remains progressive despite removal from the contaminated source, and oral force feeding seldom reverses the clinical signs. Full parental feeding is an expensive option that has however been used with some success in treating these calves (Gummow - unpublished data, 2005).

We have also established that calves are a lot more sensitive than adult cattle and in larger farming enterprises it may be viable to ensure that rearing and calving takes place in areas of low exposure, while still utilising grazing in more heavily contaminated areas for adult cattle. Alternatively, the farm management system could be changed to one of buying in weaned calves >6m old and finishing them off for market, rather than using the area to breed cattle. Supplying uncontaminated supplementary feed is likely to be a poor option as the primary problem is related to suckling calves.

The ethics of using calves as markers for pollution has always been a problem with this project and was frequently reviewed and debated from the time the first calves exhibited clinical signs. It was never the intention of the project to use calves as primary markers and sick calves were always viewed as being the exception rather than the rule, with the express purpose that, should they occur, action would be taken. In the early years of the project it was not certain if the signs were a result of vanadium or other causes and the mine was given the benefit of the

doubt until more substantial proof could be obtained. There were also indications that the mine was taking the matter seriously and introducing new processing methods that should reduce exposure and increasing stack heights to improve dispersion of pollutants. Even now we cannot be certain that the ulcers seen in cattle are a direct result of vanadium, since they don't appear to have ever been reported by others for vanadium poisoning. However, the project has now shown quite clearly that calves are being affected at the exposure doses within the HE camps and whatever the cause we cannot justify keeping calves that close to the VPU. The calves born outside the 200m radius of the VPU in 2004 were healthy and it was hoped this was the solution to the problem. A no-go-zone for calves around vanadium mining enterprises is a definite risk management option and recommendation.

Another risk management option from a pollution detection point of view is to increase the calving season to twice a year rather than once a year (as in this project), so that calves can be used as markers to cover a longer period of the year, with the intention of detecting rare events.

5.3. Implementation

Implementation is where selected measures are applied.

The measures recommended in 2004 at the conclusion of the first 5 year period of the project were implemented and no calving within 200 m of the VPU was allowed. A biannual breeding season was also adopted.

5.4. Monitoring and review

Monitoring and review is where measures are audited to ensure that they are achieving the results intended.

The monitoring of cattle and the biomarkers, hair, urine, faeces, serum proteins and milk continues with the view of validating and improving on the regression biomarker predictors. The mine has considered the project a success and has indicated they wish to continue using cattle as sentinels for at least another five years. The project has also caught the imagination of the global vanadium industry (VANITEC) and the author has been approached by the international Vanitec group to use the sentinel cattle to determine if there is an increased prevalence of pulmonary neoplasms in cattle that have been exposed to vanadium for at least five years. This study is now the longest cohort study in animals ever done for vanadium. The

question of neoplasms has arisen after a two year inhalation study done in rodents exposed to high doses of vanadium showed an increased prevalence of lung neoplasms in exposed rats (National Toxicology Programme, 2001). The implication of this finding, if verified, is far reaching and the value of long term sentinel studies becomes obvious.

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Summary

Data from animal sentinel studies can often be obtained more quickly than data from human epidemiological studies, because the ideal sentinel responds to toxic insults more rapidly than humans do (long before clinical manifestations of disease in humans) and at environmentally relevant doses. In addition, animal sentinels, like humans, are simultaneously exposed to complex and variable mixtures of chemicals and other environmental agents. Some environmental mixtures have been shown to be more toxic than would be predicted based on their principal chemical constituents. These characteristics of animal sentinel studies offer important advantages over laboratory animal studies, in which animals are usually exposed to high, constant doses of a single chemical substance that is under investigation. Thus, the use of animal sentinels constitutes an approach to identifying hazards and estimating risks in circumstances similar to those in which actual human or surrounding animal exposures occur. Chapter 2 illustrates a classic example of animals manifesting signs of poisoning before the point is reached where humans become ill.

An epidemiological investigation into an "illthrift" problem occurring on a dairy farm adjacent to an alloy-processing unit established that the probable cause of the problem was chronic vanadium poisoning. The disease manifested initially in animals 4 to 18 months old, which showed emaciation, chronic diarrhoea, and in some cases, rhinitis, conjunctivitis and recumbency followed by death. Post-mortem (n = 17) and clinical pathology findings (n = 60) indicated that malabsorption and immunosuppression were the basis of the pathogenesis in affected animals. Eight months after commencement of the investigation adult cows began showing evidence of emaciation, reduced milk production and an apparent increase in the number of abortions, stillbirths and dystocias. Over a 2-year period 134 surface soil samples, 134 sub-soil samples and 134 grass samples from the farm were analyzed for various fractions of vanadium. The levels of vanadium were found to be highest closest to the mine and surface soil levels were consistently higher than sub-soil levels suggesting aerial pollution, which was confirmed by air sampling. In addition washed grass samples were considerably lower in vanadium than the unwashed samples, indicating most of the vanadium was in the dust on the

plants. These analyses confirmed the presence of high vanadium levels in the surface soils (≤1122 ppm) and grass (≤558 ppm) on the farm and showed that the major source of vanadium was the adjacent alloy-processing unit.

To determine if calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$) could be used as a treatment for cattle on the polluted farm, or for cattle in environments high in background vanadium, an experimental study was designed. Sixteen Friesland heifer calves aged between 96 and 157 days were removed from the vanadium-polluted dairy farm and randomly allocated into two equal groups (n=8). The treatment group received 80 mg $CaNa_2EDTA$ per kg body weight intraperitonealy (IP) twice a week over a 10-week period. The control group received normal saline IP over the same period. During the trial, calves were exposed to a daily intake of vanadium in the form of contaminated tef hay derived from the farm of origin. In addition the total mixed ration was spiked with a further 20 mg V_2O_5 / kg feed to compensate for possible on-farm inhalation exposure. A stochastic model was used to estimate daily intake of vanadium as a distribution function. The model estimated that the daily intake of vanadium varied between an absolute minimum of 33 mg vanadium per day to an absolute maximum of 124 mg vanadium per day. The average intake was 71.8 mg vanadium per day per calf. Various chemical pathology parameters were measured throughout the trial as well as urine excretion rates of vanadium and lymphocyte stimulation counts. All calves were slaughtered and necropsied in cohorts of 4-6 animals at monthly intervals after completion of the trial and withdrawal of vanadium from the ration. Tissue concentrations of vanadium were determined and necropsy findings were noted. The study found that $CaNa_2EDTA$ appears to enhance the excretion of vanadium in calves, but could not prove that the treatment had a protective effect against vanadium exposure. Calves were able to tolerate the prolonged treatment with $CaNa_2EDTA$ without side effects.

By measuring average daily gains, the experimental study was able to support farmer claims that growth rates were being suppressed by vanadium. This was an important finding because it impacts on claims made by farmers to industry for damages due to vanadium pollution and countered allegations of poor farming practices made against affected farmers. The precise mechanism for poor growth remains unclear but is probably related to the lesions that have been

consistently found in the gastrointestinal tracts of calves exposed to high background levels of vanadium.

A study was then initiated to investigate whether a long-term animal sentinel system for the vanadium mining industry could act as a monitoring system for pollution problems and thus address the concerns of surrounding farmers. Such a study would also provide valuable information about the chronic effects of vanadium exposure and help answer questions relating to public health and the pathogenesis of the disease in cattle. It would provide regulatory authorities with no-adverse-effect-levels for pollution control purposes and help prevent future outbreaks from occurring. To achieve these objectives, a sentinel herd of 30 Brahman Cross cattle was run as an extensive beef commercial herd and experimental cohort on a vanadium mining property over a five year period. The cattle were farmed as two groups. A high exposure (HE) group that grazed downwind in an area immediately adjacent to the processing plant and a low exposure (LE) group whose grazing began approximately 2 km upwind of the processing plant. Various potential biomarkers and production and health parameters were monitored in the cattle over the five-year period. However, before the consequences of exposure and the value of the biomarkers could be assessed it was necessary to produce a method for quantifying how much vanadium the cattle had been taking in over the duration of the trial and what forms of vanadium were playing a role. The method had to account for chronic field exposures where there is a fluctuation of exposure over time and account for individual animal variability.

A quantitative stochastic model was developed, which dealt with uncertainty and variability within the available data. The primary inputs were direct measurements at the point of exposure and accounted for oral (grass, soil and water) and inhalation exposure (aerial fall out). This distinguished it from Gaussian plume dispersion models. The primary inputs were combined with the physiological parameters of the species concerned to provide an accurate estimate of the dose of vanadium intake. The final output of the model is a distribution curve of probable vanadium intake values based on the variability within the inputs. The exposure model estimated a composite external dose over the five-year period of between 0.05 and 23.96 mg vanadium/kg body weight/day ($\bar{x}$=2.6) for the HE group and between 0.01 and 12.72 mg/kg/d

($\bar{x}$=1.2) for the LE group. There was only a 5% probability of values being <0.56 or >6.58 mg/kg/d for the HE group and <0.33 or >2.73 mg/kg/d for the LE group.

Having developed and validated the exposure model, an exposure dose could then be modelled for each of the sampling periods throughout the trial. The vanadium intake ranged from 0.57 to 5.44 mg vanadium/kg bwt/d in the HE group and 0.41 to 2.61 mg vanadium/kg bwt/d in the LE group over a five-year period of monitoring. Samples collected from live sentinel animals over the five-year period included caudal coccygeal vertebrae, tail hair, milk, urine, faeces, rib-bone biopsies and a wide range of blood clinical pathology and haematological parameters. The data was analysed for differences in response between the HE and LE Groups. Where differences were found, a linear mixed-effects regression model was fitted to the data to model the relationship between the exposure dose and the response variable. The model included the effects of age, duration of exposure and response, and allowed the prediction of the exposure dose given these inputs. In addition to the sampling of live animals, forty-two adult cattle were slaughtered over the five years at a nearby abattoir. A wide range of tissue samples, rumen content and whole blood were taken from the cattle at slaughter for vanadium determination.

In live animals, a difference in response was found between the HE group and LE group with respect to serum albumin (n=36), monocyte (n=36) and thrombocyte (n=36) counts, and hair (n=12) and faeces (n=34) vanadium concentrations. No difference in vanadium concentrations could be shown for urine (n=36), the traditional occupational health biomarker. None of the other potential biomarkers examined proved to be of much value in determining or predicting vanadium exposure in *Bos indicus* cattle. Regression models are described for serum albumin, monocyte counts, faeces and hair, which showed the most promise as biomarkers.

Average concentrations of vanadium in the tissues of slaughtered cattle ranged from 0.08 to 2.94 mg/kg (wet-weight basis) and rumen content contained 16.67 mg/kg. No correlation could be shown between tissue concentrations and the median exposure dose for the period an animal was in the trial or the length of time exposed. Significant correlations were found between the exposure dose (end-dose) just prior to slaughter and the concentrations of vanadium in the coccygeal vertebrae, liver, diaphragm and rib-bone in descending order of magnitude. Other tissues showed poor correlation to the end-dose. Tissue levels of vanadium in healthy cattle

include a much wider range than is currently put forward in the literature. The best tissue from slaughter animals for assessing chronic vanadium exposure is probably the liver.

Since the sentinel cattle were being exposed to vanadium concentrations close to no-adverse-effect levels, it was possible to evaluate the public health risks of consuming meat and milk from cattle originating from areas high in vanadium. Using a stochastic model and lognormal distribution function to look at possible extreme tissue values that are likely to occur, the modelled concentrations of vanadium in commonly consumed tissues (meat, liver and kidney) ranged from <0.05 to 11.51 mg/kg (wet mass basis), while the median concentration of vanadium in milk was 0.23 mg vanadium/kg. People drinking milk were found to be at highest risk because of the volume of milk likely to be consumed in a western diet. The potential oral daily intake of vanadium for people consuming these foodstuffs was modelled using a stochastic model, which predicted that there is less than a 5% chance that the potential daily intake of vanadium from milk will be greater than 0.44 μg/kg/d for adults. Based on this upper limit it was concluded from current knowledge of toxicity in humans that the tissue and milk residues from cattle should pose no health risk to the consumer.

From the study we have been able to describe chronic vanadium poisoning in cattle and explain some of its pathogenesis. We have shown that vanadium mines can pose a real risk to surrounding farmers and that an ideal prophylactic treatment is still not available. We have successfully used cattle in a mining environment as a means of monitoring health risks to both cattle and humans, as a risk communication tool for interested parties and as a way of studying the chronic effects of pollutants.

Samenvatting

Gegevens afkomstig van dierlijke sentinel studies kunnen meestal sneller worden verkregen dan die uit humaan-epidemiologische studies omdat de ideale sentinel sneller dan de mens reageert op toxische noxen (lang voordat een ziekte klinisch manifest bij de mens wordt) en met blootstellingsdoses die relevant zijn voor het milieu, terwijl sectie-bevindingen ook sneller en makkelijker beschikbaar komen. Voorts worden dierlijke sentinels, net zoals de mens, simultaan blootgesteld aan een complexe en variabele mengeling van chemische stoffen en andere agentia uit de omgeving. Sommige omgevingsmengsels bleken meer toxisch te zijn dan op grond van hun afzonderlijke belangrijke componenten zou kunnen worden voorspeld. Deze karakteristieken van dierlijke sentinel studies bieden aanzienlijke voordelen ten opzichte van studies met laboratoriumdieren waarbij dieren gewoonlijk worden blootgesteld aan hoge, constante doses van een enkele chemische stof die in onderzoek is genomen. Aldus vertegenwoordigen dierlijke sentinels een benadering om gevaren (hazards) te identificeren en de daaraan verbonden risico-condities te schatten onder omstandigheden die vergelijkbaar zijn met die welke optreden bij blootstelling van mens of dier. Een klassiek voorbeeld van dieren die verschijnselen van vergiftiging laten zien nog voordat mensen ziek worden wordt in dit proefschrift gegeven, toegespitst op vanadium.

Een epidemiologisch onderzoek naar een vaag ziektebeeld op een melkveebedrijf, vlak in de buurt van een metaalerts verwerkend bedrijf wees erop dat de waarschijnlijke oorzaak van het probleem te vinden was in een chronische vanadium vergiftiging. De ziekte manifesteerde zich aanvankelijk bij dieren van 4 tot 8 maanden oud, die sterke vermagering vertoonden, chronische diarree, en in sommige gevallen, rhinitis, conjunctivitis en soporeusiteit gevolgd door de dood. Sectierapporten (n = 17) en klinisch-pathologische bevindingen (n = 60) wezen erop dat malabsorptie en immuunsuppressie de basis vormden voor de pathogenese bij de aangetaste dieren. Acht maanden na het begin van het onderzoek begonnen ook de volwassen koeien tekenen te vertonen van sterke vermagering, terugvallende melkproductie en duidelijke toename in het aantal abortusgevallen, doodgeboorten en dystocia. Gedurende een 2-jarige periode werden 134 oppervlakte-grondmonsters, 134 grondmonsters van onder de oppervlakte en 134 grasmonsters van het bedrijf onderzocht op verschillende fracties vanadium. De concentraties

vanadium bleken het hoogst te zijn dicht bij de mijn en oppervlakte-grondmonsters bleken consistent hogere gehalten te bevatten dan andere grondmonsters, hetgeen wijst op een aerogene verontreiniging die bevestigd werd middels bemonstering van de lucht. Bovendien bevatten gewassen grasmonsters aanzienlijk lagere gehalten aan vanadium dan ongewassen monsters, hetgeen erop wijst dat het meeste vanadium zich via stofdeeltjes op de plant verspreidde. Deze analyses bevestigden de aanwezigheid van hoge vanadiumgehalten aan de grondoppervlakte (1122 ppm) en in het gras (558 ppm) op het bedrijf en toonden aan dat de bron van het vanadium de ertsverwerkende industrie vlak in de buurt was.

Teneinde vast te stellen of calcium dinatrium ethyleendiamine tetra-acetaat ($CaNa_2$ EDTA) zou kunnen worden gebruikt ter behandeling van de dieren op het hiervoor genoemde probleembedrijf, of voor die gevallen dat er hoge gehalten vanadium in de omgeving zouden zijn vastgesteld, werd een experimentele studie opgezet. Zestien HF vaarskalveren—tussen de 96 en 157 dagen oud—werden van het vanadium-verontreinigde veebedrijf betrokken en random verdeeld over twee gelijke groepen (n = 8). De behandelingsgroep kreeg 80 mg $CaNa_2$ EDTA per kg lichaamsgewicht intraperitoneaal (IP) tweemaal per week gedurende 10 weken; de referentiegroep kreeg gewone fysiologische zoutoplossing IP gedurende dezelfde periode. Gedurende het onderzoek werden de kalveren blootgesteld aan een dagelijkse dosis vanadium in de vorm van gecontamineerd hooi afkomstig van het veebedrijf van herkomst. Voorts werd het gemengde rantsoen (TMR) nog voorzien met extra 20 mg V_2O_5 per kg voer om te compenseren voor mogelijke blootstelling op het bedrijf ten gevolge van inhalatie. Een stochastisch model werd gebruikt om de dagelijkse opname van vanadium als een verdelingsfunctie te schatten. Het model schatte dat de dagelijkse opname van vanadium varieerde tussen een absoluut minimum van 33 mg vanadium per dag tot een absoluut maximum van 124 mg vanadium per dag. De gemiddelde opname was 71.8 mg vanadium per dier per dag. Verschillende chemische pathologische parameters werden bepaald gedurende het onderzoek, evenals urine-excretie snelheden van vanadium en lymfocytenstimulerings tellingen. Alle dieren werden geslacht en er werd sectie op verricht in cohorten van 4 tot 6 dieren op maandelijkse intervallen na afronding van het onderzoek en eliminatie van vanadium uit het rantsoen. Weefselconcentraties aan vanadium werden bepaald en sectiebevindingen vastgelegd. Het onderzoek toonde aan dat $CaNa_2$ EDTA in staat bleek om de excretie van vanadium te

bevorderen in de kalveren, maar niet dat de behandeling een beschermend effect had tegen blootstelling aan vanadium. Kalveren waren in staat om de langdurige behandeling met $CaNa_2$ EDTA te ondergaan zonder waarneembare neveneffecten.

Door de dagelijkse groei te meten was het onderzoek ook in staat om claims van veehouders te ondersteunen dat groei van dieren door vanadium werd onderdrukt. Dit was een belangrijke bevinding omdat het effect heeft op claims van veehouders op de vanadiumindustrie vanwege vanadiumverontreiniging en evenzeer aantijgingen van de industrie inzake slecht management op veehouderijbedrijven als oorzaak van vermeende problemen kon weerleggen. Het precieze mechanisme waardoor de groei wordt onderdrukt is onduidelijk, maar het is waarschijnlijk gelieerd aan de laesies die consistent worden gevonden in de maag-darmtractus van aangetaste kalveren die zijn blootgesteld aan hoge achtergrondgehalten van vanadium.

Vervolgens werd een onderzoek opgezet om na te gaan of een lange termijn dierlijk sentinel-systeem voor de vanadiumindustrie zou kunnen fungeren als monitoringssysteem voor verontreinigingsproblemen en aldus de veehouders in de omgeving te nutte zou kunnen zijn. Zo'n onderzoek zou ook waardevolle informatie kunnen opleveren over de chronische effecten van blootstelling aan vanadium en behulpzaam kunnen zijn bij de beantwoording van vragen op het gebied van de volksgezondheid en de pathogenese van ziekten bij rundvee. Het zou de autoriteiten kunnen voorzien van "no-adverse-effect"concentraties ter vaststelling van beleid ter beheersing van verontreiniging en behulpzaam kunnen zijn om toekomstige uitbraken te voorkomen. Om deze doelstellingen te bereiken werd een sentinel-koppel van 30 Brahman kruislingen als een commercieel extensief vleesveebedrijf en een experimenteel cohort gehouden op het terrein van de vanadium-industrie voor een periode van 5 jaar. Het rundvee werd in twee groepen gehouden. Een hoge blootstellingsgroep (HE) graasde benedenwinds in een gebied direct grenzend aan de mijn en een lage blootstellingsgroep (LE) die op 2 km afstand van de mijn bovenwinds graasde. Verschillende potentiele biomarkers, en productie- en gezondheidsparameters werden bij het rundvee verzameld in deze 5-jaar periode. Voordat de gevolgen van blootstelling en de waarde van biomarkers konden worden vastgesteld was het noodzakelijk om een methode op te stellen waarmee kon worden gekwantificeerd hoeveel vanadium de dieren hadden opgenomen gedurende het gehele onderzoek en welke vormen van

vanadium hierbij een rol speelden. Zo'n methode zou rekening moeten houden met chronische blootstellingen in het veld, waar fluctuaties kunnen worden gevonden in de tijd, terwijl er ook rekening moest worden gehouden met individuele dier-variaties.

Een kwantitatief stochastisch model werd ontwikkeld dat met onzekerheid en variabiliteit binnen de data rekening hield. De primaire input bestond uit directe meetwaarden op het punt van blootstelling en hield rekening met orale blootstelling (gras; grond; water) en inhalatie-blootstelling (deeltjes in de lucht). Dit onderscheidde het model van Gaussische wind-dispersie modellen. De primaire input werd gecombineerd met de fysiologische parameters van de betreffende dieren om een nauwkeurige schatting te geven van de hoeveelheid opgenomen vanadium. De uiteindelijke output van het model is een verdelingscurve van waarschijnlijke vanadiumopnamewaarden gebaseerd op de variabiliteit in de inputwaarden. Het blootstellingsmodel schatte een samengestelde externe dosis over de 5-jaar periode vast van tussen de 0.05 en 23.96 mg vanadium per kg lichaamsgewicht per dag ($\bar{x}$ = 2.6) voor de HE groep en tussen de 0.01 en 12.72 mg per kg lichaamsgewicht per dag ($\bar{x}$ = 1.2) voor de LE groep. Er was slechts een 5% waarschijnlijkheid dat de waarden < 0.56 of > 6.58 mg/kg/dag zouden liggen in de HE groep, en < 0.33 of > 2.73 mg/kg/dag in de LE groep.

Na de ontwikkeling en validatie van het model van blootstelling, kon een blootstellingsdosis worden gemodelleeerd voor elk van de bemonsteringsperioden uit het onderzoek. De vanadiumopname varieerde van 0.57 tot 5.44 mg vanadium/kg lichaamsgewicht/dag in de HE groep en 0.41-2.61 mg/kg lichaamsgewicht/dag in de LE groep gedurende een periode van vijf jaar monitoring. Monsters genomen van levende sentinel-dieren gedurende de 5-jaar periode betroffen caudale staartwervels, staarthaar, melk, urine, faeces, rib-biopsieën, en een uitgebreid pakket van bloedmonsters voor haematochemische bepalingen en klinisch pathologische bepalingen. De data werden geanalyseerd op verschillen in respons tussen de HE en de LE groepen. Wanneer er verschillen werden gevonden, werd een lineair regressiemodel gefit op de data om het verband tussen blootstellingsdosis en responsvariabele te modelleren. Het model bevatte de effecten van leeftijd, blootstellingsduur, en responsduur, en maakte een voorspelling mogelijk van de blootstellingsdosis gegeven de eerder genoemde inputvariabelen. In vervolg op de bemonstering van levende dieren, werden er gedurende deze vijf jaar 42 volwassen runderen

geslacht op een slachthuis in de buurt. Een breed spectrum aan weefselmonsters werd genomen, evenals monsters van pensvloeistof en volbloed ter bepaling van de vanadiumconcentraties.

Bij de levende dieren werden verschillen in vanadiumconcentratie gevonden tussen de HE en LE groepen in het serumalbumine (n = 36), de monocyten (n = 36) en thrombocyten (n = 36), alsmede in haar (n = 12) en faeces (n = 34). Geen verschillen werden gevonden in de urineconcentraties in beide groepen, terwijl urine de klassieke biomarker is voor beroepsziekten. Geen van de andere potentiële biomarkers die waren onderzocht bleek van enige waarde voor de bepaling of voorspelling van vanadiumblootstelling in *Bos indicus* rundvee. Regressiemodellen zijn beschreven voor serumalbumine, monocyten-tellingen, faeces en haar die het meest belovend bleken als biomarkers.

Gemiddelde concentraties vanadium in de weefsels van geslachte runderen varieerden van 0.08 tot 2.94 mg/kg nat gewicht, terwijl de pensvloeistof 16.67 mg/kg bevatte. Er kon geen correlatie worden vastgesteld tussen weefselconcentraties en de mediane blootstellingsdosis voor de periode dat een dier in het onderzoek verbleef, noch met de duur van de blootstelling. Significante correlaties werden gevonden tussen blootstellingsdosis (= einddosis) net voor de slacht en de concentraties van vanadium in de staartwervels, lever, diafragma en rib in een dalende volgorde van belang. Concentraties in andere weefsels bleken slecht gecorreleerd met de einddosis. Weefselconcentraties van vanadium in gezond rundvee besloegen een ruimer spectrum dan de huidige literatuur laat zien. Het weefsel dat van geslachte runderen het meest geschikt lijkt om vanadiumblootstelling te schatten is waarschijnlijk de lever.

Omdat sentinel-runderen werden blootgesteld aan vanadiumconcentraties die dicht bij de “no-adverse-effect” concentraties lagen, was het mogelijk om de risico’s voor de volksgezondheid van het consumeren van vlees en melk van rundvee uit gebieden met hoge vanadiumgehalten te evalueren. Met behulp van een stochastisch model en een lognormale verdelingsfunctie, om naar mogelijke extreme weefselwaarden die zouden kunnen optreden te kijken, bleken de gemodelleerde waarden van vanadium in consumptieproducten zoals vlees, lever en nieren, uiteen te lopen van < 0.05 tot 11.51 mg/kg nat gewicht, terwijl de mediane vanadiumconcentratie in melk 0.23 mg/kg was. Mensen die melk dronken bleken het meest “at

risk" vanwege het volume melk dat normaliter deel uitmaakt van een westers dieet. De mogelijke dagelijkse orale opname aan vanadium door mensen die genoemde voedingsmiddelen consumeerden werd geschat middels een stochastisch model, dat voorspelde dat er minder dan 5% kans is dat de dagelijkse opname van vanadium via melk groter is dan 0.44 Og/kg/dag voor volwassen mensen. Gebaseerd op deze bovengrens concludeerden wij uit de huidige kennis over toxiciteit bij de mens dat de weefsel- en melk-residuen van runderen geen daadwerkelijk risico vertegenwoordigen voor de consument.

Door middel van het gehele onderzoek zijn we in staat gebleken om chronische vanadiumvergiftiging in rundvee te beschrijven, en –in mindere mate- de pathogenese. We hebben aangetoond dat de vanadiumindustrie inderdaad een reëel risico kan vormen voor omliggende veehouderijbedrijven en dat vooralsnog een profylactische behandeling niet voorhanden is. Met succes hebben wij rundvee in een mijn-omgeving ingezet als een medium om gezondheidsrisico's voor zowel mens als dier te monitoren, als een risico-communicatie instrument voor belangstellende groepen, en als een manier om chronische effecten van verontreinigende stoffen te bestuderen.

Related Publications

CONFERENCE PROCEEDINGS

Gummow, B., Bastianello, S.S., Botha, C.J., Basson, A.T., 1992. Vanadium air pollution: A possible cause of "Ill-thrift" in dairy cattle. *Proceedings of XVII World Buiatrics Congress and XXV American Association of Bovine Practitioners Conference*. St. Paul, Minnesota, USA, volume 3:299-305.

Gummow, B., Bastianello, S.S., Botha, C.J., Basson, A.T., 1992. Vanadium air pollution: A possible cause of "Ill-thrift" in dairy cattle. *Proceedings of the 9 th Faculty Day Congress*. Faculty of Veterinary Science, University of Pretoria. 1 October, paper no.2.

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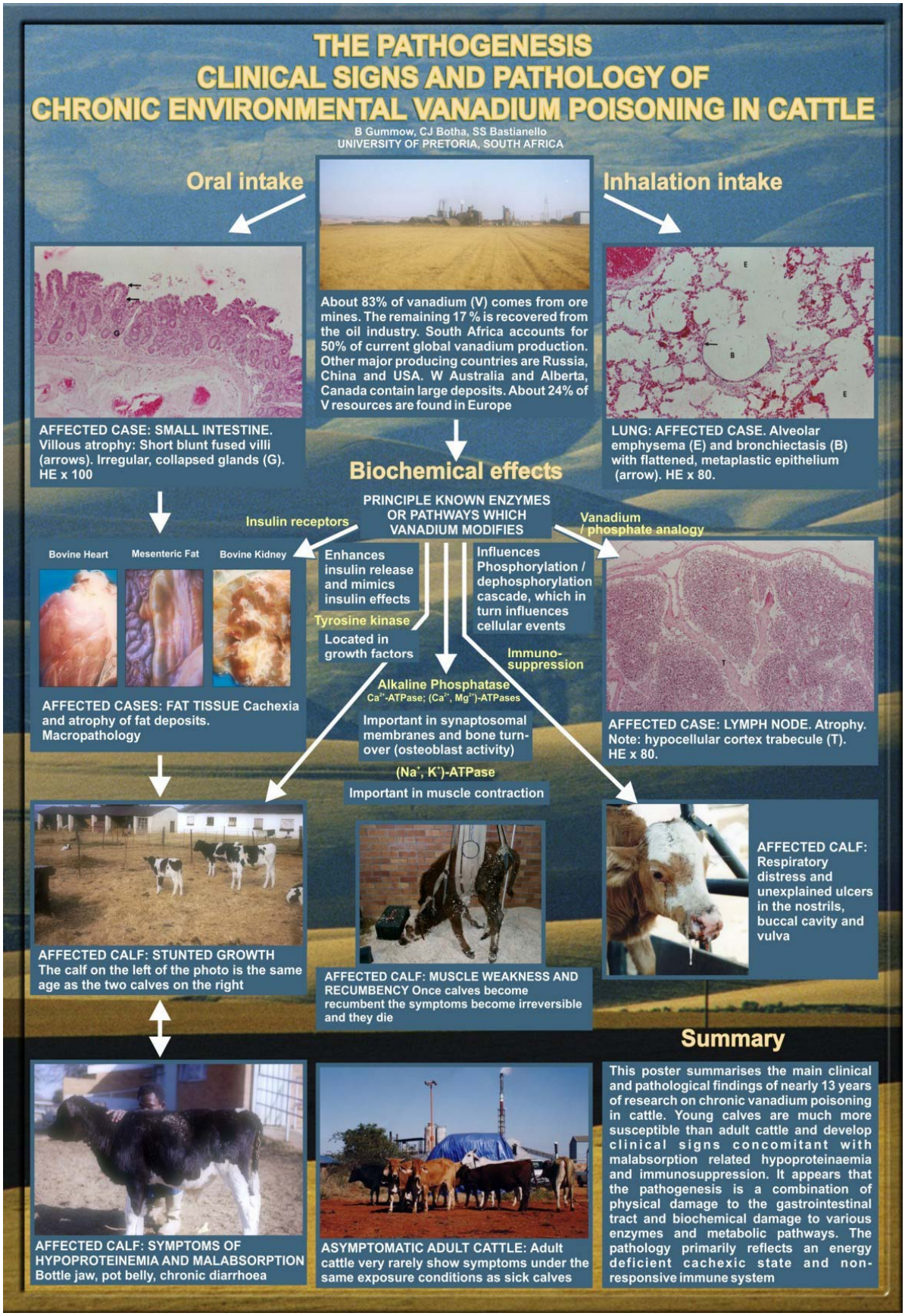
Gummow, B., Kirsten, W, Heesterbeek, J.A.P., Noordhuizen, J.P.T.M., 2004. The use of beef cattle within the vanadium mining industry for in situ monitoring of complex metal exposures. *Environmental and Health Aspects of Mining, Refining and Related Industries* Mowana Safari Lodge, Kasane, Botswana, 28th June to 2nd July

Gummow, B., Kirsten, W., Heesterbeek, J.A.P. & Gummow, R.J., 2004. A stochastic model for determining exposure doses for beef cattle used for *in situ* monitoring of complex metal exposures within the vanadium mining industry *Proceedings of the Southern African Society of Veterinary Epidemiology and Preventive Medicine*, Pretoria, 25-27 August, 69-78

CONFERENCE POSTERS

Gummow, B., Botha, C.J., Bastianello, S.S. 2004. The pathogenesis, clinical signs and pathology of chronic environmental vanadium poisoning in cattle. Annual Congress of the Society for Veterinary Epidemiology and Preventive Medicine, Martigny, Switzerland, 24-26 March

Gummow, B., Botha, C.J., Bastianello, S.S. 2004. The clinical signs, pathology and pathogenesis of chronic environmental vanadium poisoning in cattle. *Proceedings of the Southern African Society of Veterinary Epidemiology and Preventive Medicine*, Pretoria, 25-27 August, 91-92


THE PATHOGENESIS
CLINICAL SIGNS AND PATHOLOGY OF
CHRONIC ENVIRONMENTAL VANADIUM POISONING IN CATTLE
B Gummow, CJ Botha, SS Bastianello
UNIVERSITY OF PRETORIA, SOUTH AFRICA
Oral intake
Inhalation intake
About 83% of vanadium (V) comes from ore mines. The remaining 17 % is recovered from the oil industry. South Africa accounts for 50% of current global vanadium production. Other major producing countries are Russia, China and USA. W Australia and Alberta, Canada contain large deposits. About 24% of V resources are found in Europe
AFFECTED CASE: SMALL INTESTINE. Villous atrophy: Short blunt fused villi (arrows). Irregular, collapsed glands (G). HE x 100
LUNG: AFFECTED CASE. Alveolar emphysema (E) and bronchiectasis (B) with flattened, metaplastic epithelium (arrow). HE x 80.
Biochemical effects
PRINCIPLE KNOWN ENZYMES OR PATHWAYS WHICH VANADIUM MODIFIES
Insulin receptors
Vanadium / phosphate analogy
Bovine Heart
Mesenteric Fat
Bovine Kidney
Enhances insulin release and mimics insulin effects
Tyrosine kinase
Located in growth factors
Influences Phosphorylation / dephosphorylation cascade, which in turn influences cellular events
Immuno-suppression
Alkaline Phosphatase
Ca2+-ATPase; (Ca2+, Mg2+)-ATPases
Important in synaptosomal membranes and bone turn-over (osteoblast activity)
(Na+, K+)-ATPase
Important in muscle contraction
AFFECTED CASES: FAT TISSUE Cachexia and atrophy of fat deposits. Macropathology
AFFECTED CASE: LYMPH NODE. Atrophy. Note: hypocellular cortex trabecule (T). HE x 80.
AFFECTED CALF: STUNTED GROWTH The calf on the left of the photo is the same age as the two calves on the right
AFFECTED CALF: MUSCLE WEAKNESS AND RECUMBENCY Once calves become recumbent the symptoms become irreversible and they die
AFFECTED CALF: Respiratory distress and unexplained ulcers in the nostrils, buccal cavity and vulva
Summary
AFFECTED CALF: SYMPTOMS OF HYPOPROTEINEMIA AND MALABSORPTION Bottle jaw, pot belly, chronic diarrhoea
ASYMPTOMATIC ADULT CATTLE: Adult cattle very rarely show symptoms under the same exposure conditions as sick calves
This poster summarises the main clinical and pathological findings of nearly 13 years of research on chronic vanadium poisoning in cattle. Young calves are much more susceptible than adult cattle and develop clinical signs concomitant with malabsorption related hypoproteinaemia and immunosuppression. It appears that the pathogenesis is a combination of physical damage to the gastrointestinal tract and biochemical damage to various enzymes and metabolic pathways. The pathology primarily reflects an energy deficient cachexic state and non-responsive immune system

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The cattle sentinel project received the approval of both the Research and Ethics Committees of the Faculty of Veterinary Science, University of Pretoria and was registered as project no. 36.5.381

Curriculum Vitae

Qualifications

1976	Natal Senior Certificate	Westville Boys High School, Natal
1983	BVSc	University of Pretoria
1988	BVSc(Hons) (Clinical Pathology 700; Statistics 101, 102, 103, 104 and Toxicology 700)	University of Pretoria
1989	Physiology 700; Pharmacology 700	University of Pretoria
1993	Pharmacology 800 (distinction)	University of Pretoria
1993	MMedVet (Pharmacology)	University of Pretoria
2004	Dipl.ECVPH	European College of Veterinary Public Health

Employment record

2000 - Head of the Section on Veterinary Epidemiology, Economics and Risk Assessment within the Department of Production Animal Studies, Faculty of Veterinary Science, Onderstepoort

1996-1999 - Functional unit head of half the Department of Animal and Community Health, Faculty of Veterinary Science, Onderstepoort.

1995 - Associate Professor, Department of Animal and Community Health, Faculty of Veterinary Science, Onderstepoort.

1993-1995 - Senior Lecturer, Department of Veterinary Public Health

1991-1993 - Senior Lecturer, Department of Infectious Diseases

1988-1991 - Researcher, Section of Toxicology, Veterinary Research Institute, Onderstepoort

1987-1988 - Researcher, Section of Technical Statutory Advice, Veterinary Research Institute, Onderstepoort

1985-1987 -Researcher, Section of Bacteriology and Reproductive Diseases, Veterinary Research Institute, Onderstepoort

1983-1985 -National Service Veterinarian in the South African Medical Services.

Publications and presentations

- 25 refereed research papers
- 6 posters
- 4 non-referred scientific papers
- 32 presentations at local and international conferences
- 8 major contract reports for industry
- 8 interactive veterinary epidemiology workshops / courses
- 4 television interviews
- 9 dissertations promoter/co-promoter

- MMedVet Thesis: *A bioequivalence evaluation of two commercial diminazene aceturate formulations administered intramuscularly to cattle.* (External Examiner: Prof. C. R. Short, Department of Veterinary Physiology, Pharmacology & Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, USA.)