

**CV247 is under investigation and its safety and efficacy have not yet been demonstrated**

## **MANGANESE GLUCONATE**

### **Introduction**

In both animals and man, Mn is essential trace element for normal brain functioning and for many ubiquitous enzymatic reactions, including hexokinase, superoxide dismutase and xanthine oxidase. Both Mn and copper can supply the hard base OH<sup>-</sup>, for enzymatic reactions when they are present in the active site of enzymes. Both have advantages when redox reactions are required as a consequence of the multiple valence states that they can cycle between the activities of many enzymes. Superoxide dismutases (SODs), are part of the defence mechanism against reactive oxygen species, and altered amounts of copper/zinc SOD and MnSOD have been implicated in multistage carcinogenesis in both rodents and man (Davis, 1999). Manganese has several additional important functions and other vertebrate enzymes containing or activated by Mn are:

Arginase which functions in urea formation

Pyruvate carboxylase and Phosphoenolpyruvate carboxylase which function in gluconeogenesis

Farnesyl pyrophosphate synthetase used in cholesterol synthesis

Glycosyltransferases and Xylosyltransferase which have important regulatory roles in cartilage formation

Manganese is also believed to be protective against lipid peroxidation. Rats injected ip with 20mgMnCl<sub>2</sub>/kg/day for 30 days, had Mn accumulation in several tissues including liver, spleen, and adrenals, in which decreased lipid peroxidation was associated (Chen, 2000).

The total amount of Mn in the human body is 12 to 20 mg, up to 25% of which is located in the skeleton. Within the cell, Mn is concentrated in the mitochondria, and hence tends to be found in highest concentrations in tissues rich in mitochondria such as bone, liver and pancreas. Whole blood has the lowest concentration, about 200nM/L and serum < 20 nM/L.

Along with copper, zinc, calcium and iron, Mn also has a specific role in skin morphogenesis and function and though all may cause skin allergies, the correct balance of trace metals is critical for normal skin and repair mechanisms following injury (Lansdown, 1995).

### **Manganese deficiency**

Animals deficient in Mn can be characterised by impaired insulin production, alterations in lipoprotein metabolism, an impaired oxidant defence system, perturbations in growth factor metabolism, and, in early development, pronounced skeletal abnormalities (Keen, 1999).

## **Manganese deficiency and carcinogenesis**

The catalytic reaction of SOD in detoxifying superoxide involves a redox reaction that utilises either copper (cytosolic and extracellular Cu/Zn SODs) or Mn (mitochondrial MnSOD). In the active site of the SOD enzymes, copper or Mn is alternatively reduced or oxidised by superoxide to produce hydrogen peroxide (which is then further metabolised by either catalase or glutathione peroxidase). As a consequence SOD activity is completely inhibited in the absence of copper and Mn (Stipanuk, 2000). MnSOD within animal tumours has been reported to be low (Markland, 1982), and since both low Cu/ZnSOD and MnSOD have been reported to be associated with cancer susceptibility (Finley and Davis, 1999), the need to maintain adequate levels of Mn is important.

Altered amounts of both copper-zinc SOD and MnSOD have been implicated in multistage carcinogenesis in rodents and man. One study investigated the interactive effects of dietary Cu, Mn and Fe on 3,2-dimethyl-4-aminobiphenyl (DMABP) induced aberrant crypt foci (ACF), which are preneoplastic lesions for colon cancer, and SOD activities in weaning rats. The rats were fed diets containing, either 0.8 or 5.1 microgFe/g diet, 0.6, or 17 microgMn/g diet, and 37 or 140 microgFe/g diet for 3.5 weeks prior to administration of DMABP. The low Cu diet induced over 100% more ACF, the low Mn diet 23% more ACF, and the low Fe diet 18% higher formation of ACF. The results were a clear indication that altered intake of these metals, that effect SOD, may affect cancer susceptibility (Davis, 1999).

Mn ions block apoptosis of phagocytes induced by various agents. The prevention of apoptosis is attributed to the activation of Mn SOD and to the anti-oxidant function of free Mn<sup>2+</sup> cations. The effect of Mn<sup>2+</sup> ions on the apoptotic process in human B lymphocyte cells has been investigated and has found that Mn<sup>2+</sup> inhibited cell growth and induced apoptosis of activated tonsillar B cells, Epstein Barr virus (EBV) negative Burkitts lymphoma cell lines and EBV transformed B cell lines. This induction of B cell apoptosis was both time and dose dependant and probably involved the activation of caspase proteases (Schrantz, 1999).

## **Dietary deficiency**

Dietary deficiency of trace metals, including Mn, among human populations was once thought to be very rare. The consumption of highly refined and heavily processed foods reduces the trace element content of the diet and is now recognised as a potential public health problem. The higher trace element requirements of pregnancy, lactation, growth, development and chronic disease further contribute to the problem of trace metal deficiency. Studies have shown that even marginal trace element deprivation can significantly alter immunologic function and have been shown to affect the initiation and progression of a large variety of neoplasia. Studies on the interaction of trace metals and cancer have so far failed, and are therefore required, to quantitate the trace element requirements in response to a neoplastic challenge (Beach 1982).

Interactions between Mn and iron and other divalent elements are known to occur and impact the toxicokinetics of Mn especially following oral exposure. Mn exposure alters iron homeostasis in blood and cerebrospinal fluid (CSF) possibly by acting on iron transport mechanisms localised at the blood/CSF barrier (Li, 2005).

## **Recommended daily Allowance**

Normal daily intake of manganese for healthy dogs is considered to be around 2.3 mg for every pound of dog food eaten (on a dry matter basis). The Feeding Stuffs Regulations lists maximum content for manganese in mg/kg in complete feeding stuffs. For manganese carbonate, chloride, oxide and sulphate 250 mg/kg are allowed. This is considered higher than in the context of the amount of manganese proposed per daily dose of CV 247.

*The Salt Institute website provides information on manganese requirements for a range of animals. In particular references suggest a levels of 50 ppm of total diet have been routinely supplied to dogs.*

Insufficient evidence is available to support establishing a concrete Recommended Daily Allowance (RDA) in man. The estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Mn in adults has been reported to be 2 to 5 mg/day, though conversely the lowest observable adverse effect level for Mn in water has been reported to be 0.06mg/kg/day, ie 4.2mgMn/day in a 70kg adult (Greger, 1998). Mn intake can vary greatly with food choices, water composition and supplement use. Rates of absorption of Mn might differ, but the percentage absorbed and the biological half life does not vary with the plant source of Mn (Johnson, 1991), though from studies in rats may be affected if the source is of animal origin, particularly beef, following consumption of which, absorption was least and half life the longest when compared to tuna, chicken and soyabean (Johnson, 1990). Individuals have been reported to consume from 2mg to 9mgMn/day (Barceloux, 1999) and from <1mg to >10mgMn/day (Gregor, 1999). Mn sulphate, oxide, and chloride are very poorly absorbed. The piconolate or gluconate forms of Mn are preferred as a food supplement, but since even these salts of Mn are poorly absorbed, a patient needing 5mg/day may need as much as 300mg of Mn gluconate to attain normal blood levels (Analyst Health Report, 2006).

Mn is a common constituent in a number of nutritional and health supplements such as Caltrate plus and Endertonic. It is also used as fungicide (MANEB) and, as a known liver specific agent, Mn is used in a contrast agent (mangofodipir trisodium, MnDPDP) for magnetic resonance imaging.

## **CLINICAL PHARMACOLOGY**

### **Absorption, distribution, metabolism and excretion in man**

The oxidation state and solubility of Mn influence the ADME of the element, with disposition being influenced by the route of exposure.

#### **Absorption**

Absorption of Mn from the gastrointestinal tract, predominantly the jejunum, following oral administration, occurs in the divalent and tetravalent forms. Homeostatic

mechanisms, which primarily involves the liver, limit the availability of the Mn absorbed. Elimination occurs mainly by excretion into the bile (Barceloux, 1999). The uptake and transport of Mn has been investigated in the human intestinal Caco-2 cell line both from the absorption and exsorption side. With regard to absorption, transport versus time revealed a biphasic pattern of uptake with an initial transient phase followed by steady state conditions. Uptake versus Mn concentrations showed saturation type kinetics with a 100% increase of Mn binding capacity when measurements were made from 0.5 to 2 hr of incubation. The transport characteristics in steady state conditions exhibited 2 components, saturable and nonsaturable usually presumed to reflect transcellular (carrier mediated) and paracellular (diffusional) pathways. Because of the existence of both pathways, the efficiency of absorption falls as the dietary level of the mineral increases. From an exsorption side, the Mn fluxes, without a transient period, had an approximately 20 fold smaller rate than in the absorptive direction, and showed mainly a nonsaturable route (Leblondel, 1999).

### **Effect of iron status on absorption**

High dietary iron intake inhibits Mn absorption, possibly by inhibiting Mn uptake into mucosal cells (Davis, 1992), whereas Mn uptake is upregulated by iron deficiency and mediated by divalent metal transporter 1, an iron regulated factor known to play a role in dietary iron absorption (Heilig, 2005). These interactions between iron and Mn in the gut are well characterised, but iron status has not been shown to affect Mn absorption. However in a study in 26 healthy young women, the role of iron status as determined by serum ferritin concentrations found that it is strongly associated with the amount of Mn absorbed. In the crossover designed study, subjects consumed diets that supplied either 0.7 or 9.5 mgMn/day for 60 days. It was found that dietary Mn did not affect Mn status, but that absorption was greatest in those subjects with low ferritin concentrations (<15 microg/L) when they were consuming the low Mn diet, and was least in subjects with high ferritin concentrations (>50microg/L). The biological t<sub>1/2</sub> was longest when subjects with high ferritin concentrations consumed the low Mn diet, and was shortest in all subjects consuming the high Mn diet. The Mn balance was only affected by the amount of Mn in the diet; when greater amounts are absorbed, the body may compensate by excreting Mn more quickly (Finley, 1999).

### **Retention of Mn after diet supplementation**

The whole body retention of radiolabelled Mn (<sup>54</sup>Mn) after intake of labelled vitamin and mineral supplement was followed in 12 healthy subjects. The supplement had mineral content according to recommended dietary allowance for trace elements, including 2.5 mg Mn as the sulphate. Retention on day 14 was 5 +/- 2% (mean +/- SD) for Mn when taken when fasting and 1 +/- 0.2% when taken with food. During days 1-14, less than 0.01% of the labelled administered Mn was excreted in urine. Based upon the rate of turnover of the radionuclide the absorption of Mn from the supplement was estimated to be 9 +/- 3% in the fasting state and 2 +/- 1% in the fed state. The results indicated that when taken with food, Mn is absorbed and metabolised in the same way as are native minerals, but that in the fasting state, the absorption of Mn is substantially higher (Sandstroem, 1987). Even after long term (30 week) trace element supplementation including intake of double the normal dietary level of Mn in 10 healthy subjects, the indices of trace element status were only affected to a very limited extent.

Indeed the absorption of Mn was lower after 30 weeks supplementation (Sandstroem, 1990). Whole body Mn retention in another study following repeated administration of <sup>54</sup>Mn labelled infant formula to 6 volunteers was found to be reproducible, though interindividual variation was substantial and varied from 0.6 to 9.2% at day 10 in a separate group of 14 subjects. In addition there was a large interindividual variation in the rate of excretion (Davidsson 1989a).

### **Absorption of Mn from milk**

Mn absorption from human milk, cows milk and infant formulae has been studied in humans by using extrinsic labelling of the diets with <sup>54</sup>Mn and <sup>52</sup>Mn. The fractional Mn absorption from human milk (8.2%) was considerably higher than from cows milk (2.4%) or soy formula (0.7%) The fractional absorption from iron fortified whey preponderant cows milk formula was less than non-iron fortified, whilst the total amount of absorbed Mn was higher from the latter as compared with human milk (Davidsson, 1989). In another study, the effects of added phytate, calcium, phosphate and ascorbic acid to human milk have been evaluated in adults. It was found that only the addition of calcium resulted in a significant decrease in Mn absorption (Davidsson, 1991).

### **The effect of fat on absorption**

The effects of diets containing Mn and enriched with either saturated or unsaturated fats were studied in a group of healthy young female volunteers to assess their effects on neuropsychological and metabolic function. Each subject was fed in a crossover design diets that provided either 0.8 or 20mg Mn/day for a total of 8 weeks One half of the subjects received 15% of energy as cocoa butter, whilst the other half received 15% of energy as corn oil. After 4 weeks a single meal containing radio-labelled Mn (<sup>54</sup>Mn) was fed followed by whole body counting for the next 3 weeks. It was observed that subjects fed the low Mn containing diet absorbed a significantly higher percentage of <sup>54</sup>Mn and exhibited a considerably longer half life of the absorbed <sup>54</sup>Mn. Mn intake at either dose had no effect on neurological measures and only marginally affected psychologic variables.

It is evident that efficient mechanisms operate to maintain Mn homeostasis regardless of dose or diet and suggest that doses as high as 20mg/day do not result in any signs of toxicity in healthy adults (Finley, 2003).

### **The effect of ascorbic acid on absorption of Mn**

The effect of ascorbic acid on Mn absorption has been studied in a group of 8 healthy subjects using radiolabelled Mn in test meals of a soy formula containing phytic acid. It was found that following whole body retention measurements for 30 days after ingestion of the meal, the effect of the ascorbic acid content of the formula even when increased from 110mg/L to 220mg/L had no effect on Mn absorption (Davidsson, 1995).

### **Distribution**

Following absorption, Mn is thought to bind to  $\alpha_2$  macroglobulin for delivery to the liver. Because Mn can be oxidised to the Mn<sup>3+</sup> state it can bind to transferrin for subsequent delivery to other tissues

## Metabolism

The metabolic balance of Mn has been evaluated in a group of 5 healthy young man fed daily diets containing different levels of Mn in conventional foods. The study was divided into 5 periods of 21, 21, 38, 11 and 14 days in which the daily dietary intake of Mn was 2.89, 2.06, 1.21, 3.79 and 2.65mg respectively. The mean Mn balances for the 5 periods ranged from -0.018 to +0.657 mg/d with retentions ranging from -7.4 to +17.3%. The mean sum of all losses when intake was theoretically zero was calculated to be 392microg/d. When these total losses were combined with the mean positive retention, the theoretical mean dietary level of Mn required for a positive balance was 3.5mg/d or 50microg/kg (Freeland-Graves, 1988).

The liver is the primary organ involved in Mn homeostasis. The human hepato-carcinoma cell line (Hep G2) shows many liver specific functions and has been investigated as a possible model of hepatic metabolism of Mn. Preliminary experiments showed the concentration of Mn in the diet, or culture medium, similarly affected the retention of Mn by isolated rat hepatocytes or Hep-G2 cells. Mn uptake by Hep-G2 cells suggested that uptake was followed by release from the cell. Uptake was saturable and half-maximal at 2 micromolMn/L. The cations Fe<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> decreased Mn uptake into the cells. Mn uptake seemed to be by a facilitated process that may be related to calcium uptake, whilst release is an active, controlled process, that involve hepatic microtubules and lysosomes (Finley, 1998).

## Excretion

When Mn is transported into the liver it either rapidly enters the mitochondria, to be incorporated into mitochondrial SOD, or it is sequestered into lysosomes. Lysosomal Mn is then actively transported into the bile and is concentrated in the gallbladder to a concentration 150 fold greater than that seen in plasma (Stipanuk, 2000). Rates of excretion in man on an interindividual basis have been observed to vary considerably (Davidsson 1989a). Under normal circumstances very little Mn is lost through urine or through cutaneous losses.

### Manganese pharmacology in man summary table

Species and age	Gender and no.	Molecule and dose	Route of admin	Period of admin	Observations	Ref
Man young	Female 26	0.7 & 9.5mg Mn/d In diet	po	60d	Absorption greatest in subjects with low ferritin concs when exposed to low Mn diet. t1/2 longest in subjects with high ferritin and low Mn diet, and shortest with high	Fin-Ley 1999

					Mn diet	
Man	12	Mn sulphate 2.5mg & 54Mn on d14	po	14d	Absorption of Mn is highest in a fasting state. Retention on d14 was 5% when fasting and 1% when Mn was taken with food	Sand-Stroem 1987
Man young	Female	0.8 or 20 mgMn/d in diet & 54Mn On d28	po	4wks on each dose	Subjects fed the low dose diet absorbed more 54Mn and had a longer t1/2 for Mn. There was no toxicological effects at either dose level which suggested that Mn homeostasis is efficient	Finley 2003
Man young	Male 5	1.21 to 3.79 mg Mn/d In diet	po	5 pds of 14-38d	Calculated that the mean dietary level needed for a positive balance is 3.5mg/d (50µg/kg)	Free-Land Graves 1988

### CLINICAL EXPERIENCE

Although commonly used as a nutritional supplement, there are no reports of formal studies on the clinical use of manganese salts.