Scientific Committee on Food

Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Manganese

(expressed on 19 October 2000)
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FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Manganese can exist in a number of oxidation states, of which Mn(II) is the predominant form in biological systems. Food is the most important source of manganese exposure for the general population. The concentrations in foodstuffs vary considerably, but are mostly below 5 mg/kg. Grain, rice, and nuts, however, may have manganese levels exceeding 10 mg/kg or even 30 mg/kg in some cases. High concentrations have been found in tea. A cup of tea can contain 0.4 to 1.3 mg manganese (WHO, 1981; WHO, 1996). The dietary intake of adults has been estimated to range from 0.9 to 7 (Schlettwein-Gsell and Mommsen-Straub, 1973), 2 to 9 (WHO, 1981) and 1.2 to 9.4 mg Mn/day (Ellen et al., 1990). A Total Diet Study showed that the estimated average intake of manganese in the UK population in 1994 was 4.9 mg/day, including 2.3 mg/day from beverages (MAFF, 1997). The intake can be higher for vegetarians because higher levels of manganese occur in food of plant origin. The consumption of tea may contribute substantially.

The daily intake of manganese from the ambient air is lower. The annual average has been estimated to be less than 2 µg/day. In areas associated with ferromanganese or siliconmanganese industries the daily exposure may rise to 10 µg, and 24-h peak values may exceed 200 µg (WHO, 1981). For workers in industries using manganese, the major route of exposure might be inhalation from air rather than ingestion of food.

2. NUTRITIONAL BACKGROUND

Manganese has been shown to be essential for various species. It is a component of arginase, pyruvate carboxylase and superoxide dismutase and plays a role as co-factor of certain enzyme systems. Accordingly, manganese-deficient animals exhibit adverse effects, e.g. impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn, and defects in lipid and carbohydrate metabolism. In contrast, evidence of manganese deficiencies in man is poor. A specific deficiency syndrome has not been described in humans (SCF 1993; Freeland-Graves, 1994; WHO, 1996).
Currently, there is no formal Recommended Dietary Allowance (RDA) for manganese. However, an estimated safe and adequate dietary intake (ESADDI) of 2-5 mg/day for adults was established by the US National Research Council (Freeland-Graves, 1994), and the Scientific Committee for Food of the EU estimated 1-10 mg/day as an acceptable range of intake (SCF, 1993).

3. HAZARD IDENTIFICATION

About 3-8% of orally ingested manganese is absorbed in the gastrointestinal tract, but absorption may be greater for young animals and infants. The absorption of manganese is inversely related to the level of iron and calcium in the diet. Highest tissue concentrations of manganese are found in the liver, kidney, pancreas, and adrenals. Preferentially, it is retained in certain regions of the brain in young animals and infants. Manganese is almost entirely excreted in the faeces. In humans, elimination is biphasic, with half-lives of 13 and 34 days (WHO, 1996).

3.1. Toxic effects in laboratory animals

The acute toxicity of manganese is relatively low. The oral LD_{50} of manganese chloride is reported to be in the range of 275-450, 250-275, and 400-810 mg Mn/kg body weight (bw) in mice, rats, and guinea pigs, respectively (WHO-IPCS, 1981).

The ingestion of diets containing manganese (II) sulphate monohydrate in 13-week feeding studies at doses ranging from about 110 to 2000 mg/kg bw/day in F344/N rats and 330 to 7400 mg/kg bw/day in B6C3F1 mice, equivalent to 36 to 650 mg Mn/kg bw/day or 107 to 2400 mg Mn/kg bw/day, respectively, was associated with lower body weight gains, lower absolute and relative liver weights, and haematological changes, partly in all exposed groups. In addition, epithelial hyperplasia and hyperkeratosis of the forestomach occurred in the highest exposed male mice (NTP, 1993).

Special studies in rodents have been performed to elucidate neurochemical effects of manganese exposure. These studies demonstrated changes of neurotransmitter levels in the brains of rats given 1 mg MnCl_{2}·4H_{2}O/ml in drinking water, equivalent to 39 mg Mn/kg bw/day (Lai et al., 1981, 1982; Leung et al., 1981; Chandra et al., 1981). In addition, some behavioural effects were found in rats and mice at the same dose (Chandra et al., 1979; Ali et al., 1981). Unfortunately, only one drinking water concentration was tested in these studies. After administration of diets containing 2g Mn/kg equivalent to about 200 mg Mn/kg bw/day in the form of several manganese compounds to mice for 100 days (Komura and Sakamoto, 1991) and 12 months (Komura and Sakamoto, 1992), retarded growth, changes of biogenic amines in the brain and changes in the motor activity were observed. Another study showed effects of manganese on the biogenic amine metabolism in the regions of the rat brain following administration of 0.54 mg MnCl_{2}·5H_{2}O/ml in drinking water. The average intake was 4.5 mg Mn/day equivalent to about 20 mg Mn/kg bw/day (Subhash and Padmashree, 1991). Similarly, earlier studies revealed neurochemical alterations in the brain of neonatal male rats orally exposed to 10 and 20 mg Mn/kg bw/day (Deskin et al., 1980) and changes in motor-activity of male Sprague-Dawley rats (hyperactivity in the first month and hypoactivity from months 7 to 8) at concentrations of 0.1 and 5 mg Mn/ml drinking water (Bonilla, 1984).
The lowest dose affecting the central nervous system was found in a study with growing male rats, in which 50 µg MnCl₂·4H₂O/rat, initially equivalent to about 0.28 mg Mn/kg bw, were given by stomach tube daily for 15 to 60 days and reported to increase significantly the monoamine oxidase in the brain and to cause neuronal degeneration in the cerebral and cerebellar cortex (Chandra and Shukla, 1978). A similar dose of 0.357 mg Mn/kg bw/day was reported to decrease significantly the learning ability of female rats following intragastric administration of MnCl₂·4H₂O for a period of 15 and 30 days (Öner and Sentürk, 1995).

A study with four male rhesus monkeys who were given orally 25 mg MnCl₂·4H₂O daily for 18 months, corresponding to 6.9 mg Mn/kg bw/day, revealed muscular weakness, rigidity of the lower limbs and marked degeneration with de-pigmentation of neurons in the region of substantia nigra (Gupta et al., 1980). The same animals had increased testis weights with interstitial oedema and degeneration of seminiferous tubules (Murthy et al., 1980).

### 3.2 Genotoxicity and related effects

The results of genetic toxicity tests with manganese are dependent on the particular assay and the protocol used.

Manganese sulphate was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without exogenous metabolic activation (S9) (Mortelmans et al., 1986), although it was reported to be mutagenic in strain TA97 by Pagano and Zeiger (1992). Manganese chloride was not mutagenic in *S. typhimurium* strains TA98, TA100 and TA1535, but it was mutagenic in TA1537 (Wong, 1988). Both manganese sulphate and manganese chloride were positive on tester strain TA102 only without S9 mix. (De Méo et al., 1991). Manganese sulphate was found positive in a gene conversion/reverse mutation assay in *Saccharomyces cerevisiae* strain D7 without exogenous metabolic activation (Singh, 1984).

Manganese chloride was found positive in the mouse lymphoma assay (tk⁻) without S9 (Oberly et al., 1982). It was also reported to be able to induce DNA damage in cultured human lymphocytes using the single-cell gel assay technique (comet assay) without S9, but not with S9 (De Méo et al., 1991). Manganese sulphate was reported to be able to induce sister chromatid exchanges (SCEs) with and without S9, and chromosomal aberrations only in the absence of S9 in cultured Chinese hamster ovary (CHO) cells (Galloway et al., 1987). Manganese chloride was not clastogenic in cultured FM3A cells in the absence of S9 (Umeda & Nishimura, 1979); in contrast it was found able to induce chromosomal aberrations in the root tips of *Vicia faba* (Glass, 1956). Manganese sulphate was found able to induce SCEs and chromosomal aberrations in CHO cells without S9, and only SCEs in the presence of S9 (NTP, 1993). Magnesium chloride was reported to be able to induce cell transformation in Syrian hamster cells (Casto et al., 1979). Manganese chloride was unable to induce somatic mutations in *Drosophila melanogaster* (Rasmuson, 1985); manganese sulphate did not induce sex-linked recessive lethal mutations in *D. melanogaster* (Valencia et al., 1985). Oral doses of manganese chloride did not cause chromosomal aberrations in the bone marrow or spermatogonia of rats (Dikshith and Chandra, 1978); oral doses of manganese sulphate induced micronuclei and chromosomal aberrations in bone marrow cells and sperm-head abnormalities in mice treated for three weeks (Joardar and Sharma, 1990). In view of the known affinity of Mn²⁺ for chromosomal components, the authors suggested that the effects were mediated by these ions.
No induction of heritable translocations in mice or dominant lethal mutations in rats were observed after administration of manganese sulphate in the diet for 7 weeks (mice), or by gavage once a day for 1 to 15 days (rats) (NTP, 1993). It seems probable that the positive results reported in several short term tests are not due to intrinsic, direct genotoxicity of manganese, but to indirect mechanisms, as it occurs for other elements. The genotoxicity of manganese compounds seems to be mediated by the bivalent ion \( \text{Mn}^{2+} \) at relatively high and cytotoxic concentrations.

Based on the presently available data no overall conclusion can be made on the possible genotoxic hazard to humans.

3.3. Carcinogenic potential

Under the conditions of a 2-year feed study with F344/N rats, there was no evidence of carcinogenic activity of manganese (II) sulphate monohydrate in rats receiving 60, 200, or 615 mg/kg bw/day (males) or 70, 230, or 715 mg/kg bw/day (females), equivalent to 19.5, 65 or 200 mg Mn/kg bw/day and 23, 75 or 232 mg Mn/kg bw/day, respectively. There was, however, equivocal evidence of carcinogenic activity in a 2-year feed study with B6C3F1 mice receiving 160, 540, or 1800 mg/kg bw/day (males) or 200, 700, or 2250 mg/kg bw/day (females), equivalent to 52, 176 or 586 mg Mn/kg bw/day or 65, 228 or 732 mg Mn/kg bw/day, respectively, based on marginally increased incidences of thyroid gland follicular cell adenoma (high-dose animals) and significantly increased incidences of follicular cell hyperplasia. In addition, increased severity of nephropathy in male rats, focal squamous hyperplasia of the forestomach in male and female mice, and ulcers and inflammation of the forestomach in male mice were observed in the highest dose groups of these studies (NTP, 1993).

3.4. Reproductive toxicity

Teratogenicity studies with manganese (II) sulphate monohydrate conducted in rats, mice, hamsters and rabbits revealed no clearly discernible effects on nidation or on maternal or fetal survival. The number of abnormalities did not differ from the control animals (NTP, 1973).

Several studies in rats and mice indicate that the ingestion of manganese can delay reproductive maturation in male animals. Male rats administered an oral dose of 13 mg manganese/kg bw/day for 100-224 days had reduced testosterone levels. Delayed growth of the testes was observed in young rats ingesting 140 mg manganese/kg bw/day for 90 days. These effects do not appear to be severe enough to affect sperm morphology or male reproductive function. In rabbits, chronic parenteral administration of manganese produced marked degenerative changes in the seminiferous tubules, resulting in infertility (WHO, 1996).

3.5. Toxic effects in humans

In workers chronically exposed to manganese dusts and fumes, neurological effects of inhaled manganese have been well documented. The syndrome known as “manganism” is characterised by weakness, anorexia, muscle pain, apathy, slow speech without inflection, emotionless “mask-like” facial expression, and slow clumsy movement of the limbs. In general, these effects are irreversible. The minimal exposure level producing neurological effects is not certain but is probably in the range of 0.1-1 mg/m³ (WHO, 1996).
A study in Japan described an epidemic outbreak of an encephalitis-like disease in a six members family and ten of their neighbours having similar symptoms. It was caused by an intoxication due to manganese dissolved accidentally in drinking water. Two different chemical analysis of the well waters consumed by all patients showed a concentration of manganese close to 14 mg/l. The source of the manganese was 400 dry-cell batteries buried near a drinking-water well. Sixteen cases of poisoning were reported, with symptoms including lethargy, increased muscle tone, tremor, and mental disturbances. Two of the severe cases died and one of the moderate cases committed suicide from melancholy. The most severe instances were seen in elderly people, with only minor effects in children. Zinc was the other metal analysed quantitatively at a concentration close to 17 mg/l. However, the clinical observations in this study were typical for subacute manganese poisoning (Kawamura et al., 1941).

An epidemiological study in Greece investigated the possible correlation between manganese exposure from water and neurological effects in elderly residents. The levels of manganese were 3,6-14,6 µg/litre in the control area and 82-253 µg/litre and 1800-2300 µg/litre in the test areas. The authors concluded that progressive increases in manganese concentration in drinking-water are associated with progressively higher prevalences of neurological signs of chronic manganese poisoning and manganese concentration in the hair of older persons. However, no data were given on exposure from other sources such as food and dust, and little information was provided on nutritional status and other possible confounding variables (Kondakis et al., 1989).

In an area with sewage irrigation, where the manganese content of drinking water was high (0.241-0.346 mg/l) compared to a control area (0.03-0.04 mg/l), the neurobehaviour of pupils aged 11-13, measured by scores of a number of tests, was impaired (He et al., 1994). The available abstract of this study does not discuss that also the exposure to other chemicals might have been responsible for the children’s neurobehavioral changes.

In cohorts from rural dwellings located in northern Germany exposed to manganese in well water of either 0.3-2.16 or less than 0.05 mg/l, differences in neurological examinations including the assessment of possible Parkinsonism signs could not be detected (Vieregge et al., 1995).

In addition to these studies, there are other reports indicating that the intake of manganese by the oral route may be of concern (Velazquez and Du, 1994). Some investigators have reported an association between the elevated hair levels of manganese and learning disabilities in children (Pihl and Parkes, 1977; Barlow and Kapel, 1979; Collipp et al., 1983). Gottschalk et al. (1991) found elevated levels of manganese in jail inmates convicted of violent felonies. Banta and Markesbury (1977) raised the possibility that symptoms of classic manganese poisoning in a 59-year-old male were caused by the patient’s consumption of large doses of vitamins and minerals for 4 to 5 years.

In an area of Japan, a manganese concentration of 0.75 mg/litre in the drinking-water supply had no apparent adverse effects on the health of consumers (Suzuki, 1970).

According to a footnote without further details, no signs of toxicity were noticed in patients given 30 mg manganese citrate (9 mg manganese) per day in a mildly alcoholic tonic for many months (Schroeder et al., 1966).
A number of sub-populations has been reported to be more susceptible to manganese neurotoxicity than the general population. One group that has received special attention is the very young, because neonates retain a much higher percentage of ingested manganese, presumably as consequence of increased absorption. Other groups of potential concern are elderly people, individuals with iron-deficiency anaemia and people with liver disease (ATSDR, 1997).

4. DOSE RESPONSE ASSESSMENT

The available data clearly show that manganese can cause adverse effects, both in humans and experimental animals. The most important target is the central nervous system. There is clear evidence that exposure to relatively high concentrations of manganese by inhalation results in profound neurotoxic effects in humans.

There are also human studies reporting effects of manganese contained in drinking water. Assuming a consumption of 2 litres of drinking water/day, the cohorts showing the reported effects were exposed to at least 28 mg Mn/day (Kawamura et al., 1941), 0.16-0.5 and 3.6-4.4 mg Mn/day (Kondakis et al., 1989) and 0.48-0.69 mg Mn/day (He et al., 1994) plus the contribution from food. In another study, 0.6-4.3 mg Mn/day from drinking water plus contribution from food showed no effects (Vieregge et al., 1995). However, the limitations of these studies including the uncertainty of the contribution from food make firm conclusions difficult.

Similarly, the dose-response relationship of adverse effects in experimental animals has not been clarified sufficiently. Although the animal data are more extensive, no-observed-adverse-effect levels (NOAELs) for the critical effects cannot be derived. The lowest-adverse-effect-levels (LOAELs) following oral administration observed so far are 0.28 mg/kg bw/day in growing male rats, still producing biochemical and neurological changes in the brain (Chandra and Shukla, 1978), and 0.36 mg/kg bw/day in adult female rats, decreasing their learning ability (Öner and Sentürk, 1995). In rhesus monkeys, 6.9 mg/kg bw/day, given for 18 months to four male animals, caused muscular weakness, rigidity of the lower limbs and marked neuronal degeneration with depigmentation in the region of the substantia nigra (Gupta et al., 1980) as well as testicular changes (Murthy et al., 1980).

5. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

Exposure to manganese by inhalation is neurotoxic. Oral intake of manganese despite its poor absorption in the gastrointestinal tract has also been shown to cause neurotoxic effects. The limitations of the human data and the non-availability of NOAELs for critical endpoints from animal studies produce a considerable degree of uncertainty. Therefore, an upper level cannot be set.
6. CHARACTERISATION OF RISK

The margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit.

7. REFERENCES


