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Carcinogenicity, mutagenicity and teratogenicity of manganese compounds

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Abstract

Manganese, an essential trace element, is one of the most used metals in the industry. Recently, several new manganese compounds have been introduced as fungicide, as antiknock agent in petrol and as contrasting agent in nuclear magnetic resonance tomography. Manganese displays a somewhat unique behaviour with regard to its toxicity. It is relatively non-toxic to the adult organism except to the brain where it causes Parkinson-like symptoms when inhaled even at moderate amounts over longer periods of time. Relatively high doses of manganese affect DNA replication and repair in bacteria and causes mutations in microorganism and mammalian cells although the Ames test does not appear to be particularly responsive to manganese. In mammalian cells, manganese causes DNA damage and chromosome aberrations. Information on organic manganese derivatives is still insufficient. Large amounts of manganese affect fertility in mammals and are toxic to the embryo and foetus. The fungicide MANEB and the contrasting agent MnDPDP also can be embryotoxic, but the latter only at doses much higher than those clinically employed. Information on the anti-knock agent MMT is inadequate. On the other hand, manganese deficiency can also affect fertility and be teratogenic. Information on cancer due to manganese is scanty but the results available do not indicate that inorganic manganese is carcinogenic. More information is desirable with regard to the organic manganese derivatives. It may surprise that an agent that causes mutations is not also carcinogenic. The experience with manganese shows that conclusions with regard to carcinogenicity of an agent based on the observation of mutations are subject to uncertainties. Altogether, it appears

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that, because of the very high doses at which positive effects have been found, manganese would not represent a significant carcinogenic risk to the population and workers. Care must, however, be exercised with respect to central-nervous symptoms after chronic exposure and with respect to effects on the embryo. Pregnant women should not be exposed to manganese at the work place.

Keywords: Manganese; Carcinogenicity; Mutagenicity; Teratogenicity

1. Introduction

Manganese has been used extensively in metallurgy, dry-cell batteries, glass, ceramics, dyes, pigments, soil and food supplements, and medicine for 100 years or more; however, public concern about environmental pollution as well as new applications of manganese compounds for magnetic resonance imaging, as anti-knock agent and fungicide have directed attention to the possible involvement of manganese compounds in causing cancer or malformations. On the other hand, manganese, an essential trace element, plays an important role in antioxidant defences and forms part of a superoxide dismutase (MnSOD) which often is characteristically modified in cancer cells.

Manganese was first recognized by C. W. Scheele and others and was first isolated as free metal by J. G. Gahn in 1774 by the reduction of the dioxide with carbon. It was named from the magnetic stone in which it was first found. It is the 12th most abundant element in the Earth's crust and constitutes about 0.1% of it. Manganese does not occur as the free metal and is found in more than 100 minerals of which the most common ones are pyrolusite [MnO_2], psilomelane [$BaMnMn_8O_{16}(OH)_4$], rhodochrosite [$MnCO_3$], rhodonite ($MnSiO_3$), braunite [$3Mn_2O_3.MnSiO_3$]. In addition, manganese is found at concentrations of 7–27% in nodules at the bottom of the sea. More than 80% of the high grade manganese ore ($>35\%$ manganese) is mined in South Africa, and the former USSR. Other producing countries are: Australia, Brazil, Chile, Gabon and Cost Rica and, more recently, the Ukraine and China. Reserves of high grade ore are about $500–600 \times 10^6$ tons, those of low grade ore are estimated at several billion tons.

Manganese has an atomic mass of 54.94 and a density of 7.21–7.44 depending on the allotropic form. It melts at 1244 °C, boils at 1962 °C. It belongs to GroupVIIB in the system of elements and occurs in 11 oxidation states of which 2, 4, and 7 are the most important ones. Divalent manganese salts are most stable, four-valent manganese (MnO_2) predominates in nature.

Manganese is the fourth most used metal in terms of tonnage, and demand for manganese ferro-alloys is forecast to increase by 1% per year through 2000, giving an increase in world consumption from 6.7 in 1995 to 7.0 million metric tons in 2000 [1]. Most manganese ($>90\%$) is used for iron and steel making and in ferro- and silicomanganese alloys. Other uses are in alloys with Cu or Al to reduce corrosion, in batteries as MnO_2 ($\sim 2 \times 10^5$ tons/y), for colouring glasses and ceramics and, as permanganate, for oxidation reactions in chemical industries. Permanganate is employed to treat skin diseases. MANEB (ethylene-1,2-bisdithiocarbamate [$(C_4HMnN_2S_4) \times$] is widely used alone or in mixture with ZINEB ($CH_6ZnN_2S_4$) as agricultural fungicide to protect against many foliage diseases. Methylcyclopentadienyl manganese tricarbonyl (MMT) improves combustion in boilers and motors and can substitute lead in petrol as an anti-knock agent but, for the time being, is added to only a small fraction of petrol although this may increase in the future [2]. This use has recently raised much concern and even resulted in litigations in the USA and Canada [3,4]. Manganese compounds were also recommended to replace gadolinium complexes as tissue specific contrast agents in nuclear magnetic resonance tomography e.g. manganese dipyridoxyl diphosphate (Mn-DPDP for liver and pancreas scans) [5].

Emission of manganese into the environment occurs from metallurgic and chemical industries, from burning coal and petrol with the additive MMT, and also from dust emitted by volcanic activity. Inhalation exposure of workers occurs during mining, refining, production of ferroalloys, and manufacturing of dry-cell batteries.

Atmospheric concentrations of manganese in the general environment vary widely from less than 0.1 $\mu g/m^3$ up to 10 $\mu g/m^3$ or more near iron, steel or alloy plants [6]. In soil, manganese occurs in oxidation states 2, 3 and 4 at average concentrations of 500–900 mg/kg [7], but certain soils can contain only one tenth of this value and a few soils contain much more. Manganese in soil is readily assimilated by plants, and MnO_2 is used as a fertilizer supplement in manganese-deficient regions. Soil can be depleted of manganese—and other minerals—due to runoff, binding to lime added to soil and high-tech farming. Manganese levels in fresh water vary between $<1–100$ μg/l. Average manganese levels in drinking water are 4 μg/l (review [8]). In sea water concentrations range from 0.03–0.8 μg/l with lower concentrations at deeper sea levels [9].

In the workplace, the current TLV-TWA (Threshold Limit Values of the Time Weighted Average concentration for a normal 8 h working day and a 40 h working week to which all workers may be repeatedly exposed

without adverse effects) of the ACGIH (American Conference of Industrial Hygienists) for the inhalable aerosol fraction of elemental and inorganic manganese compounds is 0.2 mg Mn/m^3, and 0.1 mg Mn/m^3 for the petrol additive MMT [10]. Maximal permissible air concentration recommended by the WHO (World Health Organization) is 0.3 mg/m^3 for respirable dust [11], and the permissible exposure level (TLV-ceiling) of the OSHA (Occupational Safety and Health Organization) is still set at 5 mg/m^3 [12]. Since 1994, the EPA (US Environmental Agency) inhalation reference concentration (RfC) is 0.05 μg Mn/m^3, the standard for drinking water is 60 μg Mn/kg/d, and for food it is 140 μg Mn/kg/d [13,14]. The potential of central nervous toxicity of manganese is at the basis of these values.

Man's daily intake of manganese with food varies between 5.4 and 12.4 mg with a daily intake of 2–3 mg considered to be adequate [15]. mg. Levels in human food products range from 0.02–25 μg/g. Principal food sources are vegetables, especially green ones (e.g. peas), nuts, whole-grain cereals and tea. Plants contain from 1–700 mg/kg manganese, sea fish 0.3–4.6 mg/kg and meat 0.2–0.3 mg/kg [16]. Refinement of wheat and rice can result in the loss of nearly 90% of manganese. Nevertheless, the diet of most people in developed countries contains sufficient manganese, and supplements seem unnecessary.

Only a few percent and only divalent manganese is absorbed by man [17], more by juveniles, and people suffering from iron deficiency [18,19]. About 40–70% of inhaled manganese is taken up depending on its physicochemical characteristics.

The human body contains about 10–20 mg of manganese of which 5–8 mg are turned over daily [5,20]. About half of manganese in the body occurs in bones, the remainder is found in the active metabolic organs, liver, pancreas, pituitary gland, adrenal glands, and kidneys. [21]. Although brain takes up less manganese, it retains it tenaciously (half-life 34 d in rats) [6].

Blood levels of manganese are < 1 μg/100 ml, most of it is present in erythrocytes; urine contains < 2 μg per g of creatinine, more after treatment with chelating agents [22]; however, almost 80% of it is excreted via the bile and pancreatic juices and appears in the feces. Half-life of slow elimination manganese is about 37–39 days in control humans and shorter in people exposed to manganese [23,24].

2. Biochemical aspects

Manganese is an **essential trace element**. It is a component of the mitochondrial enzymes pyruvate carboxylase, certain superoxide dismutases, glutamine synthetase, alkaline phosphatase and arginase and activates a large spectrum of enzymes. Manganese-containing superoxide dismutase has found particular attention because it intervenes in antioxidant activity and tumor defences and may be increased or decreased in cancer cells. It is impossible to review even briefly the large number of publications that have appeared on this topic [25]. Manganese is an antagonist of iron and can replace $Mg2^+$ in certain enzymes and because of its similar ionic radius can interfere with the metabolism of calcium [26]. Manganese is also essential for normal bone structure and the formation of mucopolysaccharides. [27]. In its latter action, it intervenes in proteoglycan synthesis via glycosyl transferase, and this could explain some of the skeletal and cartilage damage reported.

Manganese deficiency has not been observed in man [28], but it has been suggested that infant mortality rates are lower when the blood manganese levels are maintained at a certain level. In animals, experimental manganese deficiency results in a variety of symptoms of which those during pregnancy and early postnatal development (disturbed reproductive functions, intrauterine and neonatal mortality, skeletal abnormalities, brain damage) are most prominent [29]. Manganese deficiency also causes abnormal carbohydrate, bone and lipid metabolism and is most likely to arise in non-grazing animals (poultry, pigs) where "slipped tendon" and nutritional chondrodystrophy in poultry can have some economic impact [30].

3. General toxicology

Metals may be divided into four groups [31]: (a) those with greatest toxicological significance that are wide-spread in the human environment (arsenic, cadmium, lead, mercury, and uranium), (b) essential trace metals (chromium, cobalt, manganese, selenium, and zinc), (c) other metals with evident biological interest (nickel and vanadium), and (d) metals of pharmacological interest (aluminum, gallium, and lithium). Indeed, manganese seems to be one of the least toxic minerals from a nutritional point of view. There is no known natural toxicity from manganese in food or from taking reasonable amounts in supplements. From a review by the US National Academy of Sciences, it appears that manganese in the environment is no threat to man or the environment [8] even under conditions where the soil contains elevated levels as long as increased concentrations of manganese are not inhaled or ingested with contaminated drinking water.

Acute effects with severe consequences have been seen after administration of potassium permanganate as abortifacient or for suicidal purposes [32]. Hyperacute toxicity is associated with cardiovascular symptoms and later with liver damage. Inhalation of manganese fumes, as those of other metals, can cause "metal fume

fever'' in some persons characterized by acute pneumonitis, tracheobronchitis and pulmonary oedema [33].

Studies in experimental animals confirm the relative low toxicity of inorganic manganese salts in general and also indicate that orally administered bivalent manganese compounds are more toxic than tri-valent ones because they are better absorbed. Median lethal doses for injected $MnCl_2$ are in the order of 300 μmol/kg for rodents but are higher for dogs (in the order of 3,000 μmol/kg). Medium lethal doses for orally administered manganese salts range from 7–15 mmole/kg.

Cyclopentadienyl manganese tricarbonyl (CMT) produces convulsions and pulmonary edema in Sprague-Dawley rats. The ED50s (dose where 50% of the animals display the effect) for convulsion is 32 mg/kg for an oral administration and 20 mg/kg for an intraperitoneal one. The LD50s for an oral and intraperitoneal administration are 22 mg/kg and 14 mg/kg respectively [34]. The toxic effects of methylcyclopentadienyl manganese tricarbonyl (MMT) are similar as those of CMT and appear mainly in lung, liver and kidney [35].

The **contrast agent mangafodipir trisodium** (MnDPDP, Teslascan) is tolerated by dogs at doses of approximately 2000 μmol/kg, approximately 400 times a single imaging dose of 5 μmol/kg [36] and thus at about 10 times higher doses than $MnCl_2$. It also caused no added effects after repeated application (3 weeks) in which the no-observed-adverse-effect level for the rat, monkey and dog was 116, 29 and 10 μmol/kg, respectively with respect to several tests such as testis weight, hematology, facial flushing.

Chronic toxicity from inhaled manganese, especially to the central nervous system (CNS), is much more important than acute toxicity [37–40]; in some cases, symptoms have been observed after exposure of workers to less than 1 mg/m^3. Couper (see in [41]) was first to describe toxic CNS symptoms of chronic manganese exposure in 1837. In Chile, where large amounts of manganese are mined, workers were found to develop *locura manganica*, or ''manganese madness''. Several studies in people exposed at work to relatively low levels of manganese (and usually simultaneously to other metals) confirm the long-term toxic action of manganese on the central nervous system and on fertility (human studies those dealing with possible carcinogenic effects are mentioned below). Thus, long-term low-level exposure to manganese (0.19–1.39 mg/m^3 for 1–45 years) in 30 men (aged 20–64 years) from two steel smelting works compared to 60 unexposed persons (aged 22–65 years) [42] yielded no changes in ECG or psychiatric tests but suggested some early (subclinical) signs of disturbances of the same type as Parkinsonism. Performance (reaction time, digit span, finger tapping, verbal comprehension) of 60 manganese-exposed workers [43] was slightly deteriorated compared to that of a matched control group, but there was no correlation between performance and present manganese exposure levels or the number of years employed in manganese work. The authors considered, in 1990, these results as an indication that the exposure standards for manganese—in Sweden 2.5 mg/m^3 and in most other countries 5 mg/m^3—might be insufficient to protect workers. Workers (118 persons) exposed to manganese dioxide dust in a Polish factory making piles and batteries [44] showed signs of pyramidal tract lesions in 16.9%, extrapyramidal syndromes in 6.8%, peripheral nervous system involvement in 13.5% and neurotic complaints in 42.4%. The frequency of neurological manifestations was directly proportional to the duration of work under exposure situations. Manganese-exposed workers [45] had significantly poorer postural stability compared to a reference group possibly due to a subclinical effect of manganese on the basal ganglia (pallidus) resulting in postural instability when the visual input is cut off.

The first symptoms of severe manganism are anorexia, weakness, and apathy. Following an initial manic phase, characterized by inappropriate laughter, increased sexuality, insomnia, even delusions or hallucinations, a period of depression, disturbances in equilibrium, impotence, and excessive sleeping can ensue. Parkinson-symptoms such as tremors and muscle rigidity appear in the later stages. These observations are confirmed in animal studies: repeated intravenous manganese applications to monkeys [46] produces a Parkinson-like syndrome characterized by bradykinesia, rigidity, and facial grimacing. In addition, chronic manganism also affects carbohydrate metabolism, and patients with chronic manganism often have hypoglycemia following a glucose load. It has been suggested that manganism like Parkinson's disease is related to a loss of dopamine in the brain cells. In many countries, manganese poisoning is considered an occupational disease of which authorities must be informed.

4. Mutagenicity

The development of cancer diseases involves a variety of successive steps of which the first one, the malignant transformation of a cell,—but probably not the following ones, promotion and progression of the tumor—is related to changes in the genome. In order to rapidly screen a variety of agents for a possible carcinogenic action and, thereby, to avoid lengthy and expensive animal experiments, more than 100 short-term assays have been developed to evaluate the mutagenic/carcinogenic potential of chemicals. These tests observe several different types of changes in the genetic material such as the sequence or integrity of nuclear DNA, the production of gene mutations (forward and reverse), cyto-

genetic changes of the structure of the chromosome etc. Such tests can be performed in vitro on prokaryotic or eukaryotic cells or in vivo on different organisms (plants, insects, mammals, etc) but they would, obviously, fail for agents that cause cancer disease by promoting an already transformed cells or by modifying the progression of a tumor. Moreover, it must be recognized that the biokinetics and metabolism as well as individual susceptibility of a cell in a given tissue and the likelihood that it can be promoted and may progress will greatly influence the risk of cancer from an agent.

Potassium permanganate ($KMnO_4$)can cause damage to the **integrity of the DNA chain** as shown by the result of the single- cell gel assay (SCGA, or comet test) in human peripheral blood lymphocytes [47]. **Fidelity of DNA replication** decreases substantially in the presence of Mn^{2+} by way of modifying the activity of DNA polymerase [48,49]. Mn^{2+} does, however, not seem to interfere with the repair of chemically induced DNA damage [50]. Mn^{2+} and, to a lesser extent, Mn^{7+}, can induce a pleiotropic cellular response called SOS repair when the normal progression of the replication fork is impeded [51]. Divalent manganese may also mediate in vitro mispairing of ethylating agents or aliphatic epoxides [52].

Manganese sulfate ($MnSO_4$) induces **mutations** in T4 phage growing on Escherichia coli [53,54] or enhance UV mutagenesis in Escherichia coli [55]; $KMnO_4$ is less effective than $MnSO_4$ in these respects.

Three principal assays are used to assess the production of mutations by manganese in bacteria: the Rec-assay in Bacillus subtilis, the reversion assay in Salmonella typhimurium (Ames test) and the formation of mutations in the lac I gene of Escherichia coli. $MnCl_2$, $Mn(NO_3)_2$, $MnSO_4$, $Mn(CH_3COO)_2$ at concentrations of 0.05 M gave slightly positive results in the Rec-assay in the Bacillus subtilis strains H17 (Rec^+, arg^-, and trp^-) and M45 (Rec^-, arg^- and trp^-) [56]. Positive results in the Rec-assay usually indicate a covalent binding to DNA or a chemical breakage of DNA, but the mode of action is obscure and perhaps unrelated to genetic damage. The conventional Ames test does not appear very suitable for detecting the genotoxicity of metal salts [57]. It is, therefore, not surprising that studies performed to assess the ability of manganese compounds such as $MnSO_4$ and $KMnO_4$ yielded negative results in this assay [58,59]. Manganese chloride was the only compound to give positive results with the TA102 strain but, conversely, appears to display a dose-dependent anti-mutagenic activity towards aflatoxin B1 [60] and quercetin [61]. However, a microtechnique adapted from the classical Ames test suggests that $MnSO_4$ and Mn Cl_2 might behave as directly-acting mutagens requiring no metabolic activation to induce reverse mutations [47]. Mn^{2+} is also mutagenic in the lac I gene of Eschericchia coli but the mutations observed involve changes that do not yield nonsense mutations [62].

Manganese acts as an **error-producing factor** during mit-DNA replication in Saccharomyces cerevisiae. This fact is in agreement with the finding that Mn^{2+} induces efficiently erythromycine-resistant mitochondrial mutations [63–65], and that it cannot induce such mutations in the presence of hydoxyurea which inhibits nuclear and mitochondrial DNA synthesis [66].

Manganese chloride given orally to larvae trans-heterozygous for the wing-hair mutations mwh and flr during the third instar stage was clearly effective in inducing spots with one or two mutant hairs (small spots) in the wing spot test of **Drosophila melanogaster** [67]. The ability of $MnCl_2$ to induce forward mutations was confirmed in vitro in eukaryotic cells in Chinese hamster V79 mammalian cells (mutants resistant to 8-azaguanine), in CHO-K1-BH4 cells and in L5178Y mouse lymphoma cells (mutants resistant to 6-thioguanine) [68,69]. The in vitro transformation ability by the simian adenovirus SA7 in Syrian hamster embryo cells was enhanced by $MnCl_2$ [70], but the relevance of this test for an evaluation of the mutagenic potential of chemicals is questionable.

The carcinogenic potential of chemicals has often been considered as associated to their ability to cause cytogenetic changes. In this respect, it is of interest to note that Vicia faba roots cultured in the presence of $Mn(NO_3)_2$ display aberrations in mitotic cells [71,72], and that $MnCl_2$ also causes disturbances in spindle formation in Allium cepa [73]. Regarding mammalian cells in vitro, Mn^{2+} and to a lesser extent $KMnO_4$ induce chromosome damage in human fibroblasts, Chinese hamster ovary cells and FM3A cells isolated from a C3H mouse mammary carcinoma [74,75]. Yet, no strand breaks or cross links were observed by McLean et al [76] in human leucocytes treated with Mn^{2+}. The clastogenic activity of manganese salts has been confirmed in vivo in mice given orally daily doses of $KMnO_4$ but not for $MnSO_4$. [77]. Sheep fed 0.75 or 1.5 g daily for one year of a metal mixture from an aluminium processing plants containing in addition to 0.063% Mn also Al, As, Cd displayed an increase in SCE in cultured lymphocytes [78]. In view of the complex composition of this material, the positive results can hardly be attributed to manganese. It should be added that MnDPDP was reported not to be genotoxic in a battery of several different tests [36].

In summary, some manganese compounds in the order $MnCl_2 > KMnO_4, > MnSO_4$ gave positive results in several short-term tests which might be related to the small carcinogenic activity shown in some long-term studies. It should be pointed out that even where manganese gave positive responses, they were always lower than those from other metals such as nickel and

chromium, metals with pronounced carcinogenic activity.

5. Carcinogenicity

Cancer development after manganese exposure were studied in only a few **long-term animal studies**, all of them indicating that manganese, although a proven mutagen, does no present a significant carcinogenic risk. No report has appeared where cancer could be definitely attributed to manganese exposure in man. Thus, the US National Toxicology Program [79] found no evidence for a carcinogenic effect of manganese compounds in rats and equivocal evidence in mice fed manganese sulfate in the diet for a period of 2 years. Based on such evidence USA-EPA [13] classified manganese as a group D carcinogen, i.e. one not classifiable as to human carcinogenicity.

F344/N rats fed 15 000 mg/kg of $MnSO_4$ during 2 years showed reduced survival in males, but not in females, mainly due to nephropathy but only a small decrease in body weight and no increase in neoplasms. Lower doses had no effect. Mice or rats fed 15 mg/kg of $MnSO_4$ for 2 years displayed a reduced body weight and increased focal hyperplasia of the forestomach epithelium as well as a slight increase in follicular cell adenomas [80]. In both species, clinical chemistry and haematology were normal except for a reduction in hepatic iron.

When manganese dioxide and manganese powder suspended in trioctanoin were injected into inbred F344 rats and Swiss albino mice [81], or when manganese powder was administered orally to the rats, no difference in tumor incidence could be noted between treated and control animals.However, manganese acetylacetonate injected into the muscle of rats produced a statistically significant number of fibrosarcomas at the sites of injection.

Multiple i.p. injections of 13 metallic compounds in strain A mice caused a significant increase in the average number of lung adenomas per mouse following the administration of 19 injections over 30 weeks of a total 660 mg/kg of manganese sulfate, but this did not occur at lower doses [82]. An abstract mentions that lymphosarcoma appear earlier in mice treated with manganese chloride [83] but these data were never published in detail. It has also been reported that an intramuscular injection of manganese dust into rats did not cause sarcomas and reduced the tumours induced by Ni subsulfide [84].

Mancozeb [85] a polymeric complex of ethylene bis (dithiocarbamate) manganese with a zinc salt used as a protective fungicide caused benign tumors 31 weeks after an topical application to female Swiss albino mice at a dose of 100 mg/kg body weight dissolved in 100 μl of dimethyl sulfoxide 3 times per week. A percutaneous exposure to 5 mg/l solution of the fungicide MANEB 4 days a week for 37 weeks to two strains of adult crested newt with different prevalences of spontaneous melanoma [86] yielded no significant differences in melanoma incidence between control and MANEB-treated animals of either population. Melanoma incidence in the one population was 94% in controls and 98% in MANEB-treated newts whereas, in the other population, it was 7% and 2%, respectively.

Epidemiological studies on manganese exposed populations are fraught with the usual problems of confounding factors, in particular the presence of other contaminants and smoking. Mortality rates for all cancers, respiratory system cancer, respiratory disease, and external causes were studied in US cities from 1969 to 1971 [87]. Certain socioeconomic variables considered, ie an index of cigarette smoking (by state), had a highly significant influence on cancer incidence whereas air pollution variables did not with the notable exception of the trace metal manganese, whose exposure was associated with cancers and respiratory disease. Because of the low ambient concentrations in this study, it is likely that manganese is only a surrogate for some other effect, such as occupational influences. In another study, a significant clustering of prostatic cancer in and around Kyoto was detected associated with locations where manganese ores were distributed in the town [88].

6. Teratogenicity

The conclusions of a very complete evaluation of the **embryotoxic aspects of the nutritional manganese deficiency** published by Hurley in 1981 [89] remain largely valid 20 years later. On the basis of her own studies and of the literature reviewed starting with the first observations in 1931 by Greny and McCollum ([90]) on rats and of Kemmerer et al. [91] on mice, Hurley concluded that manganese deficiency during the prenatal period can result in skeletal abnormalities, ataxia, reduced litter size, increase of stillborns and premature birth, modification of brain function, and that the genetic constitution affects the response. Similar observations were made in other species such as chicks, rats, mice, guinea-pigs. No information on such effects in man appears to be available.

Several years ago, Mottet and Ferm [92] had concluded that ''The significance of **manganese excess** in producing toxicity to the developing embryo and fetus remains largely unknown''. Since that time, a large number of studies on chick embryos or pregnant rodents treated with manganese chloride ($MnCl_2$), manganese dioxide (MnO_2), manganese tetroxide (Mn_3O_4), manganese sulfate ($MnSO_4$) and more recently with MnDPDP and MANEB has appeared and indicate that

large amounts of manganese not only affect fertility as mentioned above but also cause toxic effects to mother and foetus and may be teratogenic. This toxicity is made possible by the fact that, in contrast to many other metal salts, most manganese salts penetrate readily the placenta [93].

An injection of 100, 400, 700 or 1000 μg/egg of $MnCl_2$ into the air sacs of chick eggs on day 2 of incubation causes toxic and teratogenic effects [94], and exposure of cultured mouse embryos in vitro to 9.85 ng/ml–39.4 μg/ml of $MnCl_2$ interfered with development at the higher doses [95]. On the other hand, concentrations of 5 or 10 μg/ml of manganese protect embryo from the toxic effects of 5 μg/ml of $CdCl_2$ in a similar way as does zinc, an effect probably caused by competition for sensitive binding sites [96].

A study [97] conducted to obtain more information on the ingestion of diets containing high levels of manganese, and thus, to elucidate its toxic effects on performance under field conditions demonstrated that rats fed diets with basal manganese levels of 50 mg/kg (0.91 mM) plus the addition of 0, 500 and 1000 mg/kg manganese (on a dry basis) for up to 7.5 months of treatment showed a failure in reproductive performance as well as in growth and survival of neonates. Histological studies of the testes and ovaries disclosed a reduction of spermatogenesis, epithelial alterations, atretic follicles, and persistence of corpus luteum, indicative of a disfunction of the sexual glands.

An **intraperitoneal injection** of 12.5–50 mg/kg of $MnSO_4$s/CH mice on day 8, 9 or 10 resulted in fetal death and embryo resorption as well as in some cases of exencephaly after 12.5 and 25 mg/kg on day 8; 50 mg/kg were lethal at all times [98]. This study can, however, not be considered a proof that manganese is truly teratogenic rather than simply toxic to the embryo because not only exencephaly can occur spontaneously in mice but also because this effect was observed at all stages of pregnancy and not at any particular one as expected for a true teratogenic action. Subcutaneous daily injection of Swiss mice with doses of 0, 2, 4, 8, and 16 mg/kg of $MnCl_2{\cdot}4H_2O$ from day 6 to day 15 of gestation did not reduce the number of total implants, early resorptions, dead foetuses or changed sex ratio, but increased significantly late resorptions in the groups receiving 4, 8, and 16 mg/kg/day; it also reduced foetal weight and caused morphological defects in the group receiving 8 and 16 mg/kg/day [99]. Swiss mice given 50 mg $MnCl_2$ on day 9, 10, 11, 12, and killed on day 18 showed reduced foetal body weight postimplantation loss and skeletal defects, especially if the treatment was made on day 9, but these changes cannot be considered to be a veritable teratogenic damage [100].

An **iv daily injection** of 1 mg/kg of $MnCl_2$ to rats—but not of 0.2 mg/kg—from days 6–17 of gestation is toxic to the embryos [101], and a single s.c. higher dose (50 mg/kg) to Swiss mice on day 9, 10, 11, or 12 of gestation increased the percentage of resorbed embryos, post-implantation loss and skeletal defects, and reduced foetal body weight [100]. Similar results were reported ([102]) on rats given i.v. injections of 1, 4, 8, or 80 mg/kg of $MnCl_2$ on days 6–17 of gestation, In golden hamster, manganese was found to cause foetal death but no malformations [103].

Female mice exposed to MnO_2 dust (7 hours/day, 5 days/week) beginning 16 weeks prior to gestation until day 17 of pregnancy had significantly larger litter sizes than the controls in the exposed, but their offspring show reduced neonatal activity scores and retarded growth. No malformations were observed in this study [104].

Possible **embryotoxic effects of** the magnetic resonance contrast agent MnDPDP have become of particular concern because of the relative high concentrations given as parenteral administration with a single clinical dose in the order of 5 μmole/kg. Intravenous injection of 0.4, 1, or 4 mg/kg MnDPDP to Sprague Dawley rats from 6–17 days of gestation did not induce maternal toxicity or affect the numbers of foetuses, foetal viability, numbers of resorptions, implantations and corpora lutea, or the percent of pre- and post-implantation losses. However, 4 mg/kg caused a significant decrease in foetal body weight and increased skeletal malformations ([102]). Injections of 4, 8, or 16 mg/kg on days 6–8, 9–11, 12–14, or 15–17 confirmed the induction of similar skeletal malformations (angulated or irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia, and/or ulna) in a dose-dependent manner. Similar results were reported for rats ([101]) after repeated i.v. injections of 2 or 8 mg/kg (10, 40 μmol/kg) Mn DPDP from days 6–17 of gestation. No skeletal abnormalities but embryotoxicity was observed [105] in rabbits given 5, 20, 40, 60 μmole/kg except at the highest dose. The no-observed adverse effect (NOEAL) is 60 μmole/kg for maternal and 40 μmole/kg for developmental enbryotoxicity.

The **fungicide MANEB** has teratogenic properties as has been demonstrated in chick ([106]) after immersion of non-incubated eggs (157–207 per treatment group) in 0.5, 1.5, 4.5, or 13.5 g/litre for 30 s causing mainly unilateral lower limb deformities at all concentrations. Percutaneous exposure to 5 mg/kg of MANEB80 did not affect the regeneration of amputated limbs in the adult crested newt, Trichinus cristatus carnifex within 10–12 weeks after the operation ([107,108]). Similar results were obtained in Xenopus laevis ([109]).

7. Conclusions

Manganese displays a somewhat unique behaviour with regard to its toxicity. It is relatively non-toxic to

the adult organism except to the brain where it causes Parkinson-like symptoms when inhaled at moderate concentration over longer periods of time.

Relatively high doses of manganese affect DNA replication and repair in bacteria and causes mutations in microorganism and mammalian cells although the Ames test does not appear to be particularly responsive to manganese. In mammalian cells, manganese causes DNA damage and chromosome aberrations. Information on organic manganese derivatives is still insufficient.

Information on cancer due to manganese is scanty but the results available do not indicate that inorganic manganese is carcinogenic in agreement with the conclusions of Proctor et al [110]. More information is desirable with regard to the organic manganese derivatives.

It may surprise that an agent that causes mutations is not also carcinogenic. However, mutagenicity is only one aspect of cancer induction. The experience with manganese shows that conclusions with regard to carcinogenicity of an agent based on the observation of mutations are subject to uncertainties.

Large amounts of manganese affect fertility in mammals and are toxic to the embryo and foetus. The fungicid MANEB and the contrasting agent MnDPDP also can be embryotoxic, but the latter only at doses much higher than those clinically employed. Information on the anti-knock agent MMT is inadequate. On the other hand, manganese deficiency can also affect fertility and be teratogenic.

Altogether, it appears that, because of the very high doses at which positive effects have been found, manganese would not represent a significant carcinogenic risk to the population and workers. Care must, however, be exercised with respect to central-nervous symptoms after chronic exposure and with respect to effects on the embryo. Pregnant women should not be exposed to manganese at the work place.

References

[1] Roskill Reports on Metals and Minerals: Manganese. 2000, Quoted in:http://www.roskill.co.uk/mangan.html.

[2] Frumkin H, Solomon G. Manganese in the U.S. gasoline supply. Am J Ind Med 1997;31:107–15.

[3] Forum: Environmental Health Perspectives,106, No 2, February 1998, Quoted in: http:/ehpnet1.niehs.nih.gov/docs/1998/106-2/forum.html.

[4] Davis JM. Methylcyclopentadienyl manganese tricarbonyl: health risk uncertainties and research directions. Environ Health Perspect 1998;106(Suppl. 1):191–201.

[5] Misselwitz B, Muhler A, Weinmann HJ. A toxicologic risk for using manganese complexes? A literature survey of existing data through several medical specialties 1995;30:611–20.

[6] Piscator M. Manganese. In: Friberg L, Nordberg GF, Vouk VB, editors. Handbook on the Toxicology of Metals. Amsterdam: Elsevier-North Holland Biomedical Press, 1979:485–501.

[7] Bingham FT, Peryea FJ, Jarell WM. Metal toxicity to agricultural crops. In: Sigel H, editor. Metal Ions in Biological Systems, vol. 20. New York and Basel: Marcel Dekker, Inc, 1986:119–56.

[8] National Academy of Sciences. Manganese. In: Medical and Biological Effects of Environmental Pollutants. Washington DC: National Research Council, 1973 3–50, 101–131.

[9] Schiele R. Manganese. In: Merian E, editor. Metals and their Compounds in the Environment. Weinheim: VCH, 1991:1035–44.

[10] American Conference of Governmental Industrial Hygienists ACGIH. Threshold Limit Values for Chemical Substances and Physical Agents — Biological Exposure Indices. Cincinnati, 2001.

[11] World Health Organization Study Group. Recommended Health-based Limits in Occupational Exposure to Heavy Metals. WHO Technical Report No 647, 1980.

[12] Occupational Safety and Health Administration (OSHA). OSHA Safety and Health Standards. US Dep. Labor OSHA 2206 Sect. 1910 p. 634.

[13] US Environmental Protection Agency. Integrated Risk Assessment Systemat. Retrieval for metals, 9/14/92. Denver CO: Micromedeex Inc, 1992.

[14] EPA. IRIS (Innovative Risk Information System) Manganese. Quoted in: www.epa.gov/ngispgm3/iris/subst/0373.htm.

[15] WHO World Health Organization. Trace Elements in Human Nutrition. Series 532 Ed. Genève: WHO, 1973.

[16] Bowen HJM. Environmental Chemistry of the Elements. London: Academic Press, 1979.

[17] Mena I. Manganese. In: Bronner F, Coburn JW, editors. Disorders of Mineral Metabolism, vol. 1. New York: Academic Press, 1981:233–70.

[18] Ewers V, Schlipköter HW. Intake, distribution and excretion of metals and metal compounds in humans and animals. In: Merian E, editor. Metals and their Compounds in the Environment. Weinheim, New York, Basel, Cambridge: VCH, 1991:571–83.

[19] World Health Organization. Environmental Health Criteria: Manganese. Vol. 17. Geneva: WHO, 1981.

[20] Cotzias JC. Manganese in health and disease. Physiol Rev 1958;38:503–32.

[21] Beliles RP. The lesser metals. In: Oehme FW, editor. Toxicity of Heavy Metals in the Environment, part 2. New York and Basel: Marcel Dekker Inc, 1979:547–616.

[22] Lauwerys RR. Toxicologie Industrielle et Intoxications Professionelles, 4th. Paris: Masson, 1999.

[23] Cotzias GC, Miller ST, Edwards J. Neutron activation analysis: The stability of manganese concentrations in human blood and serum. J Lab Clin Med 1966;67:839–49.

[24] Mahoney JP, Small WJ. Studies on manganese iii. The biological half-life of radiomanganese in man and factors which affect this halflife. J Clin Invest 1968;47:643–53.

[25] StClair DK. Tumor suppression by manganese superoxide dismutaseL A novel function of MnSOD? A commentary. Oncol Rep 1996;3:429–32.

[26] Oehme FW. Toxicity of Heavy Metals in the Environment, vol. 2. New York and Basel: Marcel Dekker, 1978.

[27] Keen CL, Leach RM. Manganese. In: Seiler HG, Sigel H, Sigel A, editors. Handbook on Toxicity of Inorganic Compounds. New York, Basel: Marcel Dekker Inc, 1988:405–14.

[28] National Research Council (NRC). Recommended dietary allowances, 10th. Washington DC: National Academic Press, 1989.

[29] ATSRD USPHS Agency for Toxic Substances and Disease Registry. Toxicological Profile for Manganese. Atlanta GA: Report TP-91/19, 1992.

[30] Shamberger RJ. Beneficial effects of trace elements. In: Oehme FW, editor. Toxicity of Heavy Elements in the Environment, vol. 2. New York, Basel: Marcel Dekker Inc, 1979:689–796.
[31] Domingo JL. Metal-induced developmental toxicity in mammals: a review. J Toxicol Environ Health 1994;42:123–41.
[32] Henderson S, Watt JD. Potassium'permanganate as abortifacient. Brit Med Journ 1951;1:759–60.
[33] Nemery B. Metal toxicity and the respiratory tract. Europ Respirat J 1990;3:202–19.
[34] Penney DA, Hogberg K, Traiger GJ, Hanzlik RP. The acute toxicity of cyclopentadienyl manganese tricarbonyl in the rat. Toxic 1985;34:341–7.
[35] Hinderer RK. Toxicity studies of methylcyclopentadienyl manganese tricarbonyl (MMT). Am Ind Hyg Assoc J 1979;40:164.
[36] Larsen LE, Grant D. General toxicology of MnDPDP. Acta Radiol 1997;38(4 Pt.2):770–9.
[37] Huang CC, Chu NS, Lu CS, Wang JD, Tsai JL, Tzeng JL, Wolters EC, Calne DB. Chronic manganes intoxication. Arch Neurol 1989;46:1104–6.
[38] Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, De Cooman S. Fertility of male workers to mercury vapor or manganese dust: A questionnaire study. Am J Ind Med 1985;7:171–6.
[39] Roels H, Lauwerys R, Buchet JP, Genet P, Sarhan MJ, Hanotiau I, de Fays M, Bernard A, Stanescu D. Epidemiological survey among workers exposed to manganese: Effects on lung, central nervous system and some biological indices. Am J Ind Med 1987;11:307–27.
[40] Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, De Coomans S. Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am J Industr Med 1985;7:171.
[41] Aschner M, Aschner JL. Manganese neurotoxicity: cellular effects and blood-brain barrier transport. Neurosci Biobehav Rev 1991;15:333–40.
[42] Wennberg A, Iregren A, Struwe G, Cizinsky G, Hagman M, Johansson L. Manganese exposure in steel smelters a health hazard to the nervous system. Scand J Work Environ Health 1991;17:255–62.
[43] Iregren A. Psychological test performance in foundry workers exposed to low levels of manganese. Neurotoxicol Teratol 1990;12:673–5.
[44] Wisniewska-Hejka Z, Cempel M, Nyka WM. Neurological examinations of workers with chronic exposure to manganese dioxide during the production of piles and batteries]. Neurol Neurochir Pol 1978;12:435–41.
[45] Chia SE, Gan SL, Chua LH, Foo SC, Jeyaratnam J. Postural stability among manganese exposed workers. Neurotoxicol 1995;16:519–26.
[46] Olanow CW, Good PF, Shinotoh H, Hewitt KA, Vingerhoets F, Snow BJ, Beal MF, Calne DB, Perl DP. Manganese intoxication in the rhesus monkey: a clinical, imaging, pathologic, and biochemical study. Neurol 1996;46:492–8.
[47] De Meo M, Laget M, Castegnaro M, Duménil G. Genotoxic activity of potassium permanganate in acidic solutions. Mutat Res 1991;260:295–306.
[48] Van de Sande JH, McIntosh IP, Jovin TN. Mn^{2+} and other transition metals at low concentrations at low concentration induce the right-to-left helical transformation of poly d(G–C). EMBO J 1982;1:777–82.
[49] Beckman RA, Mildvan AS, Loeb LA. On the fidelity of DNA replication: manganese mutagenesis in vitro. Biochem 1985;24:5810–7.
[50] Scicchitano DA, Pegg AE. Inhibition of O6-alkylguanine-DNA-alkyltransferase by metals. Mutat Res 1987;192:207–10.
[51] Olivier P, Marzin D. Study of the genotoxic potential of 48 inorganic derivatives with SOS chromotest. Mutat Res 1987;189:263–9.
[52] Bhanot OS, Solomon JJ. The role of mutagenic metal ions in mediating in vitro mispairing by alkylpyrimidines. Environ Health Perspect 1994;102(Suppl. 3):81–90.
[53] Orgel A, Orgel LE. Induction of mutations in bacteriophage T4 with divalent manganese. J Mol Biol 1965;14:453–7.
[54] Rossman T.G., Molina M., Meyer L.W. The genetic toxicology of metal compounds: I. Induction of lambda prophage in *E coli* WP2s(lambda), 1984;6:59–69.
[55] Rossman T.G., Molina M. The genetic toxicology of metal compounds: II. Enhancement of ultraviolet light-induced mutagenesis in *Escherichia coli* WP2, 1986;8:263–271.
[56] Nishioka H. Mutagenic activities of metal compounds in bacteria. Mutat Res 1975;31:185–9.
[57] Léonard A. Mechanisms in metal genotoxicity: the significance of in vitro approaches. Mutat Res 1988;198:321–6.
[58] De Flora S. Study of 106 organic and inorganic compounds in the *Salmonella microsome* test. Carcinogenesis 1981;2:283–98.
[59] Marzin DR, Vo Phi H. Study of the mutagenicity of metal derivatives with *Salmonella typhimurium* TA102. Mutat Res 1985;155:49–51.
[60] Francis AR, Shetty TK, Bhattacharya RK. Modifying role of dietary factors on the mutagenicity of aflatoxin B1: in vitro effect of trace elements. Mutat Res 1988;199:85–93.
[61] Hatcher JF, Bryan GT. Factors affecting the mutagenic activity of quercetin for *Salmonella typhimurium* TA98 metal ions, antioxidants and pH. Mutat Res 1985;148:13–23.
[62] Zakour RA, Glickman BW. Metal-induced mutagenesis in the lacI gene of *Escherichia coli*. Mutat Res 1984;126:9–18.
[63] Putrament A, Baranowska H, Prazmo W. Induction by manganese of mitochondrial antibiotic resistance mutations in yeast 1973;126:357–66.
[64] Prazmo W, Balbin E, Baranowska H, Ejchart A, Putrament A. Manganese mutagenesis in yeast. II. Conditions of induction and characteristics of mitochondrial respiratory deficient *Saccharomyces cerevisiae* mutants induced with manganese and cobalt. Genet Res 1975;26:21–9.
[65] Putrament A, Bazanowska H, Eychart A, Jachymezyk W. Manganese mutagenesis in yeast VI Mn^2 uptake, mitDNA replication and E^R induction. Comparison with other divalent cations 1977;18:69–76.
[66] Slater ML. Effect of reversible inhibition of deoxyribonucleic acid synthesis on the yeast cell cycle. J Bacteriol 1973;113:263–70.
[67] Ogawa HI, Shibahara T, Iwata H, Okada T, Tsuruta S, Kakimoto K, Sakata K, Kato Y, Ryo H, Itoh T, et al. Genotoxic activities in vivo of cobaltous chloride and other metal chlorides as assayed in the Drosophila wing spot test. Mutat Res 1994;320:133–40.
[68] Miyaki M, Akamatsu N, Ono T, Koyama H. Mutagenicity of metal cations in cultured cells from Chinese hamsters. Mutat Res 1979;68:259–63.
[69] Oberly TJ, Piper CE, McDonald DS. Mutagenicity of metal salts in the L5178 Y mouse lymphoma assay. J Toxicol Environ Health 1982;9:367–76.
[70] Casto BC, Meyers J, DiPaolo JA. Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. Cancer Res 1979;39:193–8.
[71] Gläss E. Untersuchungen über die Einwirkung von Schwermetallsalzen auf die Wurzelspitzenmitose von Vicia faba. Ztschrft Botan 1955;43:359–403.
[72] Gläss E. Untersuchungen über die Einwirkung von Schwermetallsalzen auf die Wurzelspitzenmitose von Vicia faba. Ztschrft Botan 1956;44:1–58.
[73] Singh O.P. Effects of certain chemical polluants on plant chromosomes [Dissertation]. University of Calcutta, 1979.
[74] Snyder RD. Role of active oxygen species in metal-induced DNA strand breakage in human diploid fibroblasts. Mutat Res 1988;193:237–46.

[75] Tan EL, Williams MW, Schenkley RL, Perdue SW, Hayden TL, Turner JE, Hsie AW. The toxicity of sixteen metallic compounds in Chinese hamster ovary cells. Toxic Appl Pharmacol 1984;74:330–6.
[76] McLean JR, McWilliams RS, Kaplan JG. Rapid detection of DNA strand breaks in human peripheral blood cells and animal organs following treatment with physical and chemical agents. Prog Mutat Res 1982;3:137–41.
[77] Joardar M, Sharma A. Comparison of clastogenicity of inorganic Mn administered in cationic and anionic forms in vivo. Mutat Res 1990;240:159–63.
[78] Sivikova K, Dianovsky J. Sister-chromatid exchanges after exposure to metal-containing emissions. Mutat Res 1995;347:17–22.
[79] National Toxicology Program (NTP). Management Status Report, Research. Triangle Park, NC, 1992.
[80] TR-428 Internet. Toxicology and carcinogenesis studies manganese (II) sulfate monohydrate (CAS No.10034–96–5) in F344/N rats and B6C # F1 mice (feed studies). 1993. Quoted in: http://ehis.niehs.nih.gov/ntp/docs/tr428/428abs.pdf.
[81] Furst A. Tumorigenic effect of an organomanganese compound on F344 rats and Swiss albino mice. J Natl Cancer Inst 1978;60:1171–3.
[82] Stoner GD, Shimkin MB, Troxell MC, Thompson TL, Terry LS. Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. Cancer Res 1976;36:1744–7.
[83] DiPaolo JA. The potentiation of lymphosarcomas in mice by manganous chloride. Fed Proc 1964;23:393.
[84] Sunderman FW Jr., Lau TJ, Cralley LJ. Inhibitory effect of manganese upon muscle tumorigenesis by nickel subsulfide. Cancer Res 1974;34:92–5.
[85] Shukla Y, Antony M, Kumar S, Mehrotra NK. Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. Cancer Lett 1990;53:191–5.
[86] Zavanelli T, Zaffaroni NP, Arias E. Testing of the fungicide maneb for carcinogenicity in two populations of the European crested newt. Cancer Lett 1980;10:109–16.
[87] Lipfert FW. Statistical studies of mortality and air pollution. Multiple regression analyses by cause of death. Sci Total Environ 1980;16:165–83.
[88] Watanabe H, Mishina T, Ohe H, Araki H, Nakao M. A clustering of prostatic cancer in an area with many manganese mines. Tohoku J Exp Med 1981;135:441–2.
[89] Hurley LS. Teratogenic aspects of manganese, zinc, and copper nutrition. Physiol Rev 1981;61:249–95.
[90] Greny ER, McCollum EV. Effects of deprivation of manganese in the rat. J Biol Chem 1931;92:651.
[91] Kemmerer AR, Elvehjem CA, Hart EB. Studies on the relation of manganese to the nutrition of the mouse. J Biol Chem 1931;92:623–30.
[92] Mottet NK, Ferm VH. The congenital teratogenicity and perinatal toxicity of metals. In: Clarkson TW, Nordberg GF, Sager PR, editors. Reproductive Toxicivity of Metals. New York, London: Plenum Press, 1983:93–126.
[93] Saric M. Manganese. In: Friberg L, Nordberg GF, editors. Handbook on TOxicology of Metals. Specific Metals, vol. II. Amsterdam: Elsevier, 1986:354–77.
[94] Gilani SH, Alibhai Y. Teratogenicity of metals to chick embryos. J Toxicol Environ Health 1990;30:23–31.
[95] Hanna LA, Peeters JM, Wiley LM, Clegg MS, Keen CL. Comparative effects of essential and nonessential metals on preimplantation mouse embryo development in vitro. Toxicol 1997;116:123–31.
[96] Yu HS, Chan ST. Cadmium toxicity on mouse preimplantation zygotes in vitro: interactions of cadmium with manganese, zinc and calcium ions. Toxicol 1988;48:261–72.
[97] Corella-Vargas R. High levels of manganese in the diet of rats (Rattus norvegicus albinicus). I. Effect on reproduction]. Arch Latinoam Nutr 1984;34:457–65.
[98] Webster WS, Valois AA. Reproductive toxicology of manganese in rodents, including exposure during the postnatal period. Neurotoxicol 1987;8:437–44.
[99] Sanchez DJ, Domingo JL, Llobet JM, Keen CL. Maternal and developmental toxicity of manganese in the mouse. Toxicol Lett 1993;69:45–52.
[100] Colomina MT, Domingo JL, Llobet JM, Corbella J. Effect of day of exposure on the developmental toxicity of manganese in mice. Vet Hum Toxicol 1996;38:7–9.
[101] Grant D, Blazak WF, Brown GL. The reproductive toxicology of intravenously administered MnDPDP in the rat and rabbit. Acta Radiol 1997;38(Pt. 2):759–69.
[102] Treinen KA, Gray TJ, Blazak WF. Developmental toxicity of mangafodipir trisodium and manganese chloride in Sprague-Dawley rats. Teratol 1995;52:109–15.
[103] Ferm VH. The teratogenic effects of metals on mammalian development. In: Woollam DHM, editor. Advances in Teratology, vol. 5. New York: Academic Press, 1972:51–5.
[104] Lown BA, Morganti JB, D'Agostino R, Stineman CH, Massaro EJ. Effects on the postnatal development of the mouse of preconception, postconception and/or suckling exposure to manganese via maternal inhalation exposure to MnO_2 dust. Neurotoxicol 1984;5:119–29.
[105] Blazak WF, Brown GL, Gray TJB, Treinen KA, Denny KH. Developmental toxicity study of mangafodipir trisodium injection (MnDPDP) in New Zealand white rabbits. Fundam Appl Toxicol 1996;33:11–5.
[106] Maci R, Arias E. Teratogenic effects of the fungicide maneb on chick embryos. Ecotoxicol Environ Safe 1987;13:169–73.
[107] Arias E, Zavanelli T. Teratogenic effects of manganese ethylenebisdithiocarbamate (MANEB) on forelimb regeneration in the adult newt, *Triturus cristatus carnifex*. Bull Environ Contam Toxicol 1979;22:297–304.
[108] Zavanelli T, Zaffaroni NP, Arias E. Abnormal limb regeneration in adult newts exposed to the fungicide Maneb 80. A histological study. J Toxicol Environ Health 1984;13:735–45.
[109] Bancroft R, Prahlad KV. Effect of ethylenebis (dithiocarbamic acid) disodium salt (nabam) and ethylenebis (dithiocarbamato) manganese (maneb) on *Xenopus laevis* development. Teratol 1973;7:143–50.
[110] Proctor NH, Highes JP, Fischman ML. Chemical Hazards at the Workplace, 2nd. Philadelphia PA: JB Lippincott Co, 1988.

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