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Full Length Article

A two-generation inhalation reproductive toxicity study upon the exposure to manganese chloride

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ABSTRACT

A number of published studies have suggested that high levels of exposure to manganese, especially those found in occupational settings, can adversely affect the reproductive system. The objective of this study was therefore to investigate if these findings can be replicated using the Sprague Dawley rat and, if so, to identify those parts of the reproductive system are more susceptible.

Male and female rats were exposed to manganese dichloride ($MnCl_2$) via inhalation at concentrations of 0 (air-control); 5, 10 and 20 μg/L air over 10 weeks (F0) and over 11 weeks (F1) prior to mating, and then throughout mating, gestation and lactation until termination after the F1 and F2 generation had reached Day 21 of lactation respectively.

Animals were monitored for clinical signs of toxicity and for effects on body weight, food consumption, effects on the entire reproductive system including maternal care. The offspring were monitored for survival and growth up to weaning. Blood samples were taken from all adult animals for bioanalytical of manganese analysis prior to dosing, prior to mating and prior to weaning/necropsy.

There were no deaths related to treatment, though respiratory tract effects were observed in F0 animals in the mid and high dose animals. Body weight and food consumption were affected at high dose in both generation. There were no treatment-related effects on the oestrous cycles, mating performance, sexual maturity, fertility or duration of gestation or litter size, the sperm motility, count of morphology (sperm) or the ovary follicle scoring in either generation.

The No Observed Effect Level (NOEL) for reproductive performance was considered to be the target dose level of 20 μg/L.

Based on these findings, manganese chloride could not be considered a reprotoxicant under these conditions of exposure. Therefore, soluble and insoluble forms of inorganic manganese compounds by extrapolation cannot be considered as reprotoxicants.

1. Introduction

Manganese (Mn) is a naturally occurring and abundant element that is essential in biological systems but deficiency or excess of this mineral can lead to adverse effects. From a toxicity perspective, toxicity driven by excess has primarily been reported by exposure via the inhalation route, mainly in workplace studies (Ellingsen et al., 2003a,b; Wirth and Mijal, 2010; Egorova, 2009). Several publications have indicated that manganese ions cross the placenta reaching embryos, as the manganese content of embryonic tissues was elevated following intravenous administration of radiolabelled $MnCl_2$ to pregnant maternal animals (Koshida et al., 1965; Onoda et al., 1978). Placental transfer was also demonstrated after inhalation exposure of maternal animals (Dorman et al., 2000). Inhalation of MnSO4 in pregnant rats resulted in a clear elevation in manganese levels in the maternal animal, but only a small increase in livers of the embryos (Dorman et al., 2005). Although $MnCl_2$ and $MnSO_4$ are very soluble compounds, both exhibiting a valency of +2, marked differences in reaction to treatment have been seen when different manganese compounds were administered: while studies with MnCl2 demonstrated effects on fertility and juvenile development (Elbetieha et al., 2001; Treinen et al., 1995) no effects were observed with MnSO4 administered at considerably higher doses in some studies, a noteworthy study is a 2-year study in rats and mice, indicated no change in testes weight or in the seminiferous

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tubules upon the exposure to manganese sulphate at concentrations up to 15,000 ppm (NTP, 1993a,b).

There are reports that young animals may take up more manganese than adults, but the situation is not completely clear and may just reflect a need of the neonate for more manganese to synthesize enzymes (Fechter, 1999). The importance of considering the essentiality of manganese on reproduction has been stressed by several authors as manganese deficiency in humans' results in significant adverse consequences including growth impairment, reduced fertility and risk of birth defects, i.e. some of the very effects that have been suggested to associate with manganese excess.

Overall, the quality of published studies on the effects of manganese on reproductive toxicity varies. Most of these studies are neither good quality nor conducted in compliance with any established regulatory guidelines; with several confounders in some cases. Some report reproductive effects mainly in doses that cause maternal toxicity without making the link and others show effects with exposure via intravenous, subcutaneous or intraperitoneal routes, which are not relevant means of exposure in the workplace or to the general population. This is reflected in the current assessments by authoritative bodies (SCOEL, 2013; ATSDR, 2012) which concluded that, the evidence on the effects of manganese on reproductive functions is conflicting and inconclusive.

Therefore, a well-designed animal study by the most relevant route of exposure in the workplace with adequate doses (specifically a guideline compliant inhalation two generation reproduction study) was necessary to draw a robust conclusion. $MnCl_2$ was chosen as the test substance as it is both soluble and the most toxic as demonstrated by several experimental rodent studies.

2. Materials and methods

2.1. Introduction

This study is designed to fulfil the requirements of OECD Guideline 416 and US EPA Guideline OPPTS 870.3800 while investigating any possible reproductive effects upon exposure to manganese especially in the workplace where oral and dermal exposure are negligible.

Manganese dichloride ($MnCl_2$) was selected as an appropriate test material for this study because it is very water soluble and seen to produce greater general toxicity compared to $MnSO_4$ according to available data as discussed in the introduction.

Doses were selected after a nose-only inhalation exposure dose range finder study. Ten females and 10 males per group were exposure to manganese chloride at 5, 20 and 30 μg/L. The study was scheduled for 9 weeks however; exposure was stopped at 3 weeks for the high dose as adverse clinical signs included crackling/gasping respiration resulted in the premature sacrifice of 3 animals. Necropsy findings included distended intestines, froth filled trachea, discoloured lungs. Exposure continued to the end of the study for the low and mid dose animals with minimal effects. The high dose for the main study was therefore reduced to 20 μg/L.

In the main study, F0 animals were randomised into 3 test groups and one control group, each containing 28 males and 28 females (see Table 1). These animals were dosed for 10 weeks (6 h daily) prior to mating, and then throughout mating, gestation and lactation until termination after the F1 generation had reached Day 21 of lactation.

From each treatment group, at least 24 males and 24 females were retained for post weaning assessments. These animals continued on the study (F1) and were dosed for approximately 11 weeks (6 h daily) after weaning, and throughout mating, gestation and lactation until termination after the F2 generation had reached Day 21 of lactation.

Animals were monitored for clinical signs of toxicity and for effects on body weight, food consumption, effects on oestrous cycles, mating performance, pregnancy performance, difficulty or prolongation of parturition, and for deficiencies in maternal care. The offspring were monitored for survival and growth up to weaning. In addition, the following endpoints were evaluated: gross necropsy findings, organ weights, histopathology evaluation, qualitative examination of testes and examination of the ovaries and sperms.

2.2. Animal husbandry

One hundred and fourteen male and 114 female Sprague-Dawley rats (Crl:CD®(SD)) were housed in cages. Cages were racked by treatment group with males and females racked separately. The F0 animals were allowed to acclimate for 13 days before the commencement of dosing.

For at least 7 days prior to the commencement of dosing, all animals were conditioned to the restraint procedures (to adapt to the inhalation chamber) used for nose-only exposure by placing the animals in the restraint tubes for gradually increasing periods of restraint time up to the maximum expected duration to be used on the study—6 h daily.

SDS Rat and Mouse (modified) No. 3 Diet SQC Expanded and tap water was provided *ad libitum* to test and control animals throughout the study, except during designated procedures. Each batch of diet was routinely analysed by the supplier (supplied by SDS Special Diets Services) for various nutritional components and chemical and microbiological contaminants, including manganese, which is a known essential element in the SDS Rat and Mouse diet and the levels of manganese were (84–94 mg/kg) in each batch of diet. The drinking water was periodically analysed for dissolved materials, heavy metals, pesticide residues, pH, nitrates, nitrites

Table 1
Experimental design.

Dose Group/ Treatment	Target Concentration (μg/L Air)— Nose only	F0 Actual Concentration (μg/L Air)— Nose only	F1 Actual Concentration (μg/L Air)— Nose only	Number of Animals			
				F0 Males	F0 Females	F1 Males	F1 Females
1[a] Air Control	0	0	0	28	28	26	26
2 Low Dose	5	6	4	28	28	24	24
3 Intermediate Dose	10	15	10	28	28	24	24
4 High Dose	20	25	17	28	28	25	25

[a] For Group 1, Manganese (II) Chloride was non-detectable or non quantifiable for all samples collected over the course of the study.

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