This report contains the collective views of an international group of experts on available literature. The generation of this report followed a clearly defined process: literature search>evaluation of data and writing of the draft by a specialist >review of the draft by a group of scientists>peer-reviewed by an expert (s) in the said field > and finalisation.

A Toxicokinetic Assessment for the Registration, Evaluation and Authorisation of Chemicals, Regulation (EC) No. 1907/2006 (REACH)

MANGANESE AND ITS INORGANIC COMPOUNDS:

1. TOXICOKINETIC ASPECTS

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This report was written under the sponsorship of Manganese REACH Administration (MARA) having regard to Regulation (EC) No 1907/2006 of the European Parliament and of the Council, of 18 December 2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.

August 2009

In accordance with the requirements of Regulation (EC) No. 1907/2006 (REACH), a literature search is required in order to assess relevant information available to the registrant, in order to include where appropriate the information into the technical dossier required for registration. One of the endpoints requiring information is toxicokinetics (TK). This report is written with a view to assisting the registrant with this requirement in mind.

The literature search strategy is described in the body of the report. Briefly, 2 searches were made:

Literature search using Datastar:

Medline (1966+), Embase (1974+) and Toxfile (1966+) in December 2001, with a further update search being performed in August 2002.

A second literature search using STN:

The databases Medline, Embase, Biosis, HCAPLUS and Toxcenter were searched from 1959 to May 2009

In addition various reference lists from publicly available review documents were also assessed and additional literature deemed relevant was obtained for review.

SVBB 31 August 2009

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1 SUMMARY AND DISCUSSION

A toxicokinetics (TK) assessment is required under REACH (Annex VIII level section 8.8) and is necessary to confirm the manganese substance grouping for read-across for the purposes of classification and labelling and risk assessment. This report aims to critically evaluate the TK of manganese and inorganic manganese compounds by evaluating the results of published TK studies taking into account the relevance of the studies, the quality of the methodologies used and the reporting, and the reliability of the results and conclusions. The heterogeneity of individual solubilities within the "manganese compounds" make a general conclusion on toxicokinetics impossible, and care must be taken to clearly define the compound when discussing the fate and behaviour in the body. In this report, insoluble and soluble compounds are discussed separately where possible.

- Soluble compounds: $MnSO_4$, $Mn(NO_3)_2$, $MnCl_2$.
- Insoluble compounds: manganese metal, insoluble salts (MnS, $MnCO_3$), manganese oxides (Mn_3O_4, MnO and MnO_2), slags, ores and alloys.

Whilst there was a considerable volume of published studies performed in both humans and animals with manganese chloride and manganese sulphate, and to a lesser extent manganese oxide(s), there was either no or very little data on the other forms of manganese identified in the above list.

The potential routes of exposure in humans in an occupational setting are dermal, inhalation and oral. However, if any dermal exposure occurs, subsequent systemic exposure is deemed unlikely due to low absorption of inorganic compounds, especially insoluble ones. There is virtually no published data on the dermal uptake of inorganic manganese, possibly reflecting the general opinion of the low relevance of this route of exposure in this instance. Direct oral exposure should be easily controlled by good hygiene practice. However, indirect oral exposure, via mucocilliary transport of inhaled particles followed by swallowing could occur. Inhalation exposure is considered the most likely route of systemic exposure in an occupational setting due to the handling of dusts and powders. Consequently, for risk assessment, as a result of inhalation, consideration is required for both direct systemic (lungs and the potential olfactory transport mechanism) and also possible absorption via the alimentary canal as a result of mucocilliary transport. Whilst protective measures can minimise human exposure to manganese, inhalation is seen as the most critical route of exposure in an occupational setting.

Manganese is an essential trace element for humans and the oral uptake and excretion are thus subjected to body-controlled homeostasis. The presence of a homeostatic control for manganese is one of the main complicating factors for interpretation of absorption-, retention- and elimination-data from traditional mass-balance-investigations. The body appears to be very adaptive towards moderately increased loads of manganese by such measures as reduced gastrointestinal (GI) absorption, enhanced hepatic elimination and increased biliary and pancreatic excretion of manganese (Roth, 2006). However, the uptake of manganese from inhalation can initially bypass the body-controlled homeostasis and first-pass effect seen with oral exposure and thus inhalation is of the greatest concern for occupational exposure as extrapolation from oral to inhalation exposure is not likely to be appropriate for manganese.

The data used for this TK assessment has been taken from published data that were not conducted to GLP or to regulatory guidelines. As such, unless studies were conducted by the same group of workers, there was very little consistency in study design and reporting, which meant that the ability to directly compare/contrast data between studies was hindered. The assessment and evaluation of the published data for its reliability and relevance was performed based upon the Klimisch system (Klimisch et al., 1997) and Klimisch Code ratings (1-5) have been assigned to all studies. Much of the human data were taken from relatively old publications, which documented manganese exposure in occupational settings. Whilst these publications were old and generally not as thorough in their methodology descriptions as would be expected today, their relevance is very high.

1.1 ABSORPTION

The absorption efficiency of manganese from soluble salts after oral uptake is low and comparable in animals and healthy human individuals (3-13%). It was shown in animal experiments that absorption is mediated by active transport in the upper gastrointestinal tract, whilst no active transport is present in the colon. The rate of manganese absorption is rapid and reaches maximal blood concentration after a very short time. The human absorption of manganese from the soluble sulphate or acetate salts is likely to be lower than for the soluble chloride salt (Bales et al., 1987).

The following factors influence the oral uptake of manganese (shown in humans and animals):

- iron status (heme and non-heme iron, anaemia, low ferritin; transport of manganese and iron is obviously in both cases via transferrin and, as such, an antagonistic relationship is likely),
- dietary matrix (especially the presence of transitional metal ions can influence uptake in the upper GI-tract) and the ionic form and source of manganese, e.g. as a $MnCl_2$ solution (higher) or as in food (lower),
- bioavailability from manganese-rich matrices is decreased, while manganese-poor matrices and fasting lead to increased bioavailability,
- a high existing body burden of manganese will decrease future absorption, whilst a low body burden (inadequate for normal bodily functioning) will increase absorption.

It was shown in animal experiments that oral and inhalation bioavailability of insoluble manganese compounds is less efficient compared to soluble compounds. Oral absorption from insoluble compounds is delayed in time, most likely due to incomplete absorption due to excretion before dissolution is complete. No data in humans on the absorption of insoluble manganese compounds including the metal is available.

The monitoring of manganese in urine as a marker of recent manganese exposure seems to be possible as shown in humans, but is not fully validated; blood levels seem to be less predictive of recent exposure and appear to better reflect the overall body-burden of manganese (cumulative exposure and retention). However, whilst both urine- and blood-manganese monitoring as markers of manganese exposure show a good correlation on a group basis, there is limited correlation on an individual basis.

The three potential routes of entry for manganese by inhalation are:

- Through the nasal mucosa.
- Transport across the pulmonary epithelial lining and subsequent distribution in lymph/blood.
- Clearance from the lung by mucocilliary elevator and subsequent ingestion from the GI tract.

However, the relative proportion absorbed by each process is not accurately known although it is well established that the absorption of particles via the lungs depends upon the particle size and solubility as well as the geometry of the respiratory tract (Schlesinger, 1996). The absorption of ultrafine particles (UFPs) of an insoluble form of manganese in rats has been shown to be of the same magnitude as that of a soluble form of manganese (Elder et al., 2006). Following the intratracheal instillation to rats of either soluble manganese chloride or insoluble manganese tetraoxide (90% of particles <1 µm diameter), the manganese uptake, distribution and excretion was far more rapid from the soluble form (Drown et al., 1986). Maximal brain manganese concentrations were of a similar magnitude and whilst the absorption from the lungs of the two forms of manganese proceeded at different rates: hours for the soluble manganese chloride and days for the insoluble manganese tetraoxide, the overall exposure was very similar. The variation in manganese levels in the intestinal contents with time showed a similar profile as many other tissues, with the soluble chloride group having a higher and earlier C_{max}. As such, the clearance of the manganese tetraoxide from the lungs by the mucocilliary elevator followed by absorption from the GI tract did not seem to be the most likely process if the subsequent absorption from the GI tract of manganese was inefficient. However, there is no information on the efficiency of the uptake of manganese from the GI tract following elimination by the mucocilliary elevator. The actual mechanism involved in the clearance of insoluble manganese from the lungs does not appear to have been investigated either within this publication (Drown et al., 1986) or the wider literature.

Clarification of this mechanism would aid the interpretation of inhalation data on insoluble manganese compounds, as well as help to assess whether any read-across from soluble to insoluble manganese inhalation exposure is feasible.

The absorption of manganese from inhaled soluble salts in rats is partly mediated by direct transport along the olfactory neurons into the brain. This route of entry is postulated for humans as well, but no study in humans exists to prove this hypothesis. It has been proposed that the percentage of air flow that reaches the rats' olfactory mucosa is comparable to humans, suggesting that potential absorption of airborne metals by rats and humans would be similar (Thompson et al., 2007). However, this is in contrast to the work of other researchers (Kimbell, 2006) who reported computer models that predict a larger portion of inspired air passed through olfactory-lined regions in the rat than in the monkey or human during resting breathing. The same workers also reported that with particles that are 5 µm in aerodynamic diameter, preliminary simulations at minute volume flow rates predicted nasal deposition efficiencies of 92%, 11% and 25% in the rat, monkey, and human, respectively, with more vestibular deposition in the rat than in the monkey or human. As such, direct comparison between rats and humans is complicated by interspecies differences in nasal and brain anatomy and physiology. Differences in the relative size of the rat olfactory mucosa and olfactory bulb likely predispose rats, more so than humans, to nasal deposition and olfactory transport of manganese. Although the rat is a good animal model for olfactory transport, it is a poor model for manganese neurotoxicity in humans. The rat fails to selectively accumulate manganese in the striatum and does not demonstrate the behavioural and pathological changes characteristic of manganism in human and nonhuman primates (Brenneman et al., 1999).

When groups of monkeys were exposed to either air or $MnSO_4$ (up to 1.5 mg Mn/m^3, MMAD 1.72-2.12 µm) for 65 exposure days before tissue analysis, monkeys at the lowest dose level developed increased manganese concentrations in the olfactory epithelium, olfactory bulb, olfactory cortex, globus pallidus, putamen, and cerebellum. A greater than 3- to 5-fold increase in mean tissue concentration was observed in the globus pallidus, putamen and caudate of monkeys exposed at the highest dose level. Results from this work were combined with MRI, pallidal index (PI), and T(1) relaxation rate (R1) to establish a direct association between MRI changes and pallidal manganese concentrations (Dorman et al., 2006c). The authors stated that their results indicated that the R1 can be used to estimate regional brain manganese concentrations and may be a reliable biomarker of occupational manganese exposure. Further, they suggested that this study was the first to provide indirect evidence of direct olfactory transport of an inhaled metal in a nonhuman primate. However, they also stated that to their knowledge significant neuronal connections between the monkey olfactory bulb and the globus pallidus do not exist. As such the transport of manganese was not likely to go beyond the olfactory bulb into deeper brain tissues like the globus pallidus, a situation that was also likely to be operable in humans. Instead, they concluded that the pallidal delivery of manganese was likely to have arisen primarily from systemic delivery and not directly from olfactory transport.

1.2 DISTRIBUTION

There appears to be a lack of reliable comprehensive data on the complete tissue distribution of manganese in rodents following oral administration. Many more studies report the distribution following ip administration; however, as there appears to be an important first-pass effect with manganese following oral absorption, this will be bypassed following other administration routes.

The key points arising from the distribution of manganese in rodents following oral, ip and iv administration are:

- Manganese is widely distributed to all tissues.
- Manganese potentially crosses placental and blood–brain barriers.
- Chronic exposure can lead to accumulation of manganese in particular regions of the brain.
- Manganese accumulation in tissues is also associated with increases in blood levels of manganese.

From studies in rats, *in vivo* and *in vitro*, it has been shown that manganese can enter the brain via carrier-mediated transport and can leave the brain via diffusion only, a much slower process than carrier-mediated transport (Yokel and Crossgrove, 2004). This suggests that no mechanism exists to protect the brain from accumulating manganese and this has important implications for neurotoxicity resulting from chronic manganese exposure. Although Yokel and Crossgrove studied manganese transport rates in rats, their observations may have some relevance to humans because transport mechanisms at the blood–brain barrier (BBB) are similar in rodents and humans. However, humans have a more localised pattern of manganese delivery to the brain (vs. rat) and the mechanisms involved in this difference are poorly understood. Even if similar proteins are found in the rat and human BBB the relative rates could still differ (e.g., due to the relative efficiency of the transporters and quantity).

The key points arising from the distribution of manganese in rodents following inhalation exposures are:

- Inhalation of insoluble forms of manganese can lead to higher lung manganese concentrations (slower clearance than soluble forms).
- Inhalation of soluble forms of manganese leads to higher concentrations in other tissues (readily absorbed and distributed).
- Inhalation of manganese appears to result in a more extensive distribution within the brain than following oral administration.
- The olfactory bulb and brain tissues show accumulation of manganese without concurrent increases in the blood manganese.

This last point differs considerably from oral and ip administration where the femur showed the most accumulation and significant accumulation in the brain and other tissues appeared to be related to an overall increased body burden as evidenced by increased blood manganese concentrations. However, following inhalation, increases in brain manganese levels were seen without corresponding increases in blood manganese levels, and only moderate increases in femur manganese levels. This implies that either the manganese tendency to cross the BBB was enhanced following inhalation, or there was an additional route of delivery to the brain following inhalation that is not seen after oral exposure, or both.

Investigations into manganese uptake to the brain via the olfactory route in rats have shown that manganese moves relatively freely from the olfactory bulb to the olfactory cortex at an amount dependent on the level of influx into the bulb. The transport to the rest of the brain is related to the amounts in the olfactory bulb and the olfactory cortex, but the relative proportion reaching this area increases with increasing doses. A nose-only inhalation with either one nostril plugged or both nostrils patent, found that the olfactory route contributes the majority (up to >90%) of the ^{54}Mn found in the olfactory pathway, but not in the striatum of the rat brain up to 8 days following a single inhalation exposure to $^{54}MnCl_2$ (Brenneman et al., 2000). Using the same study exposure conditions, inhalation exposure of the relatively poorly soluble $^{4}MnHPO_4$ resulted in a slower clearance of manganese from the olfactory epithelia than for the soluble $^{54}MnCl_2$ (Dorman et al., 2002). However, overall the results were qualitatively similar to those obtained with the more soluble form of manganese including that this route of administration did not significantly contribute to increases in striatal concentrations of manganese. A different group of workers showed that solid ultrafine particles (UFPs) of manganese oxide were also translocated via the rat olfactory neuronal pathway to the CNS system of rats and that this was not due to soluble manganese from the manganese oxide UFP but was from solid particles of manganese oxide UFP being transported directly (Elder et al., 2006).

The influence of the route of administration on the absorption and cerebral distribution of manganese in rats following dosing with either $MnCl_2$ solutions or MnO_2 suspensions showed that higher elevations of blood manganese concentrations following intratracheal instillation of $MnCl_2$ were seen compared to oral administration (Roels et al., 1997). The authors suggested that the high blood manganese concentration, produced during a short period after pulmonary absorption, transiently overwhelmed the mechanisms controlling the uptake of manganese into the brain. The elective distribution of manganese in the striatum compared to the other areas of the brain may be explained by the presence of different manganese uptake mechanisms in the various cerebral regions.

The use of the intranasal and intratracheal instillation dose routes for rodents has shown that:

- The olfactory route does provide a direct method of transport of manganese to the brain.
- Pulmonary absorption of manganese can result in elevated blood manganese levels compared to oral absorption.

Inhalation studies in monkeys have shown that the olfactory route of absorption of inhaled manganese is also operative in primates, although as with rats the direct delivery of manganese to the basal ganglia was not evident from MRI investigations (Dorman et al., 2006b).

1.3 METABOLISM

It is likely that manganese is metabolised by the body in the form of converting manganese from the Mn^{2+} valence state to the Mn^{3+} valence state, and that ingested manganese is absorbed as Mn^{2+} possibly bound to alpha2-macroglobulin or albumin. In transversing the liver it is removed nearly quantitatively, but a small proportion is oxidised to the Mn^{3+} valence state, bound to transferrin and enters the systemic circulation to be transported to tissues. More recently, it has been proposed that the oxidation state of manganese exposure may be an important determinant of the toxicokinetics of manganese and tissue toxicodynamics and subsequently neurotoxicity (Reaney et al., 2006).

The kinetic processes for the uptake and elimination of orally absorbed manganese are likely to be different following iv or inhalation administration whereby the manganese enters the systemic circulation without passing through the liver. The influence of the absorption process followed by direct delivery to the liver (first-pass effect) can considerably reduce the bioavailability of a material compared to iv administration. Manganese from inorganic sources is almost entirely excreted in the bile where it undergoes enterohepatic recirculation.

1.4 ELIMINATION

The elimination of absorbed manganese is primarily through the bile (>95%) and, as such, the main excretion route for manganese is ultimately in the faeces; however this route can also include unabsorbed dose following oral dosing, which is one of the complicating factors when trying to measure proportions of dose absorbed. Very little manganese is excreted in the urine or other potential routes of excretion such as milk or sweat. The biological half-life of manganese in human urine is estimated to be less than 30 hours following cessation of exposure to manganese (Roels et al., 1987b). Chelation therapy with EDTA leads to forced excretion of stored manganese via the urine. An increased uptake of manganese results in faster elimination through the body's natural homeostatic regulation.

When interpreting data on the absorption of manganese or manganese elimination, the body's natural homeostatic control of manganese must be taken into account, particularly so if this is following oral absorption. Notwithstanding this a number of early studies focused on the elimination of manganese using ^{54}Mn as a tracer usually as a test meal. The typical whole-body terminal half-lives for manganese in healthy volunteers are around 30-40 days (Mena et al., 1969; Johnson et al., 1991; Finley et al., 1994; Finley et al., 2003). As with the absorption of manganese, factors such as the pre-body burden of manganese and dietary levels of manganese, considerably affect its rate of elimination.

Following the iv dosing of a ^{54}Mn tracer it was established that the elimination of manganese is at least biphasic with an initial fast elimination phase, typically around 3-4 days in healthy human subjects (Mahoney and Small, 1968). As with the slower terminal elimination phase, this initial fast phase can be modulated by manganese body burden.

The elimination of absorbed manganese is dependent on bile production and flow into the small intestine. The efficiency of this process is delayed in the rat neonate following birth and may lead to manganese retention following high doses of manganese. However, in the human neonate, efficient

bile flow normally occurs within the first three days after birth, therefore the accumulation of manganese in human neonates may not be as much of a potential problem as with rat neonates (Inoue et al., 1997). The manganese eliminated in the bile usually then undergoes enterohepatic recirculation. There is evidence from a study in rats that auxiliary GI routes of elimination to that of the bile are also in operation (Wieczorek and Oberdorster, 1989), namely through the intestinal wall and with the pancreatic juice. However this was from an inhalation study and so this could have been due to clearance of manganese from the lung by mucocilliary elevator and subsequent direct excretion into the GI tract.

As discussed earlier, it has been shown that manganese can enter the brain via carrier-mediated transport and can leave the brain via the slower process of diffusion only, and thus the elimination of manganese from the brain has been observed to be slower than for other tissues.

1.5 PBPK

PBPK modelling of the fate of manganese in the body is not trivial and is complicated by the body's natural homeostatic regulation of manganese. Due to the complexity of the toxicokinetics of manganese, this is a challenging area of research, although the use of PBPK models to inform human health risk assessments is generally becoming more acceptable.

1.6 READ-ACROSS AND EXTRAPOLATION

One of the outcomes of this TK assessment of manganese is to try and assess the possibility for read-across between manganese substances for the purposes of classification, labelling and risk assessment. Whilst there was a considerable volume of published studies performed in both humans and animals with the soluble manganese chloride and manganese sulphate, and to a lesser extent the insoluble manganese oxide(s), there was either no or very little data on the other forms of manganese identified by the Manganese Consortium.

Soluble compounds – $MnCl_2$, $MnSO_4$ and $Mn(NO_3)_2$:

^{54}Mn was used in the form of the soluble manganese chloride salt for the conduct of ADME studies that utilised the radioactive tracer. This included studies whereby non-radiolabelled manganese sulphate was dosed prior to the ^{54}Mn administration. Once absorbed by the body, the ^{54}Mn is assumed to be representative of any other Mn^{2+} that was absorbed including its conversion to Mn^{3+} for binding to transferrin. It would appear that the fate of manganese in the body from soluble salts (Mn^{2+}) was generally assumed to be equivalent, irrespective of the original salt. There was no evidence from the TK review of manganese to dispute this assumption, other than one study where manganese chloride was shown to have a greater oral bioavailability in man than either manganese sulphate or acetate salts (Bales et al., 1987) and as such manganese chloride may produce greater effects due to its increased bioavailability. Consequently, the read-across of data between the soluble salts of manganese is likely to be applicable. Since no data was found on the toxicokinetics of the soluble manganese nitrate ($Mn(NO_3)_2$), the ability to read-across from the data from manganese chloride and manganese sulphate will undoubtedly be pertinent.

Insoluble compounds: manganese metal, insoluble salts (MnS, $MnCO_3$), manganese oxides (Mn_3O_4, MnO and MnO_2), slags, ores and alloys:

The opportunity to use read-across for the insoluble compounds of manganese is not so straightforward as with the soluble salts as the influence of particle size is likely to be the major factor affecting the TK of insoluble manganese compounds. Additional factors such as the degree of insolubility and the speciation (Mn^{2+}, Mn^{3+} or Mn^{4+}) also need to be taken into consideration.

It is well established that the absorption of inhaled particles via the lungs depends upon the particle size and solubility as well as the geometry of the respiratory tract (EN481, 1993; Schlesinger, 1996). In humans, particles with an aerodynamic diameter between 5 and 30 μm are mostly deposited in the nasopharyngeal region and the ciliary-mucosal cleaning system transports such particles to the throat where they are swallowed. Particles between 1 and 5 μm are deposited in the tracheobronchial regions while particles less than 1 μm are deposited in the alveolar region as well as being deposited in the nasal region. Particles deposited in the alveoli can be cleared by the mucocilliary escalator, by alveolar macrophage phagocytosis or by direct uptake. The European Standard EN 481 defines that only 1% of inhalable particles with an aerodynamic diameter of 10 μm would be in a respirable fraction, however this rises to 97% of inhalable particles with a aerodynamic diameter of 1 μm (EN481, 1993). The solubility of manganese tetraoxide and manganese phosphate has been shown to be dramatically increased in artificial lung fluid containing proteins (Vitarella et al., 2000a). The olfactory neuronal pathway was shown to be efficient for translocating inhaled manganese oxide as solid ultrafine particles (UFPs) to the central nervous system of groups of rats, with similar manganese burdens (~8.2% dose) found in the olfactory bulbs of rats compared to manganese chloride inhalation (Elder et al., 2006).

A study that compared the kinetics of ^{54}Mn following the intratracheal instillation of either soluble $^{54}MnCl_2$ or insoluble $^{54}Mn_3O_4$ to rats, found that whilst the absorption from the lungs of the two forms of manganese proceeded at different rates due to the slower clearance from the lungs of the less soluble forms of manganese, $MnCl_2$ (hours), Mn_3O_4 (days), the overall exposure was very similar (Drown et al., 1986). However, the initial C_{max} was considerably higher and earlier in most tissues following dosing with the soluble manganese form, although after this initial peak the depletion curves for most tissues were very similar. The concentration of manganese in the lungs from rats following 2 weeks inhalation exposure to 3 different forms of manganese, were significantly higher following exposure to the insoluble manganese tetraoxide and manganese phosphate compared to the soluble manganese chloride groups (Vitarella et al., 2000a; Dorman et al., 2001a). With the exception of the lungs, generally the tissue concentrations following the soluble manganese exposure were greater than following the insoluble manganese exposures. As such, this confirms that the direct read-across of TK data from soluble forms of manganese to insoluble forms of manganese is not appropriate.

The absorption of manganese metal in rats following inhalation appeared to behave quite differently to either manganese phosphate or a manganese phosphate/sulphate mixture despite all dose and other parameters being equivalent (Normandin et al., 2004). In particular, the concentration of manganese in the lungs from the metallic manganese group were less than 2-fold greater than controls whereas the concentration of manganese in the lungs following dosing with the other manganese compounds were 30- to 50-fold greater than controls. This data suggests that the particles were likely cleared rapidly by the mucocilliary escalator and were therefore unavailable for absorption via the lung. The mucocilliary clearance of this particle could be relatively efficient versus the other forms where their solubility is sufficient to allow for pulmonary absorption. Whether read-across for manganese can be used for the insoluble compounds of manganese needs to be carefully considered on a case-by-case basis, with particular emphasis on equivalent particle size as well as speciation and the level of insolubility. It seems likely that the bioavailability following inhalation of manganese from SiMn is lower than from the other inhalable manganese constituents found in the atmosphere of workers in manganese alloy-producing plants (Ellingsen et al., 2003a).

Route-to-route extrapolation

The kinetic processes for the uptake and elimination of orally absorbed manganese are likely to be different following iv or inhalation administration whereby the manganese enters the systemic circulation without passing through the liver. The influence of the absorption process followed by direct delivery to the liver (first-pass effect) can considerably reduce the bioavailability of a material compared to other routes of administration. Inhalation studies in animals have shown that either the manganese tendency to cross the BBB was enhanced following inhalation, or there was an additional route of delivery to the brain following inhalation that was not seen after oral exposure, or both. The use of the intranasal and intratracheal instillation dose routes for rodents has shown that the olfactory route does provide a direct method of transport of manganese to the brain in rodents. Animal studies

have also shown that pulmonary absorption of manganese can result in elevated blood manganese levels compared to oral absorption. These differences in the kinetics of manganese via different routes of administration were also highlighted in the PBPK modelling work. As a consequence, the route-to-route extrapolation of manganese data (e.g. oral to inhalation) does not appear to be appropriate.

Species extrapolation

Whilst the use of the rat for assessing the oral absorption of manganese may appear to be suitable, albeit the rat is likely to overestimate oral absorption (Keen et al., 1986; Finley et al., 1994; Zheng et al., 2000), the use of the rat as a model for human inhalation and distribution of manganese is less so. The interspecies differences in nasal and brain anatomy and physiology mean that the appropriateness of the rat is questionable. Both of these have been discussed previously (see Section 1.1). A better model for humans would appear to be the nonhuman primate, where monkeys exposed to manganese have shown similar neurobehavioral clinical signs to human symptoms. Indirect evidence for the direct olfactory transport of inhaled manganese in a nonhuman primate has also been shown, although the authors concluded that the pallidal delivery of manganese, however, likely arises primarily from systemic delivery and not directly from olfactory transport (Dorman et al., 2006c). The brain distribution of manganese in the monkey also appears to be a better model for that in the human than the rat.

1.7 Summary Tables

1.7.1 Manganese Absorption

Table 1.7.1.1 Summary of the oral absorption of manganese (net amount reaching systemic circulation)

		Human	Rat
		Oral	**Oral**
Soluble Manganese Salts	$MnCl_2$	~5%[a]	13.2%[e]
	$MnSO_4$	2±1%[b]	ND[c]
	$Mn(NO_3)_2$	ND[c]	ND[c]
Insoluble Salts	MnS	ND[d]	ND[d]
	$MnCO_3$	ND[d]	ND[d]
Manganese Oxides	MnO	ND[d]	ND[d]
	MnO_2	ND[d]	ND[d]
	Mn_3O_4	ND[d]	ND[d]
Manganese metal, sinter ore and slags	Mn	ND[d]	ND[d]
	FeMn	ND[d]	ND[d]
	SiMn	ND[d]	ND[d]

Factors that decrease oral absorption	**Factors that increase oral absorption**
Repeat dosing	Overnight fasting before dosing
High existing manganese body burden	Low existing manganese body burden
High dietary levels of manganese	Low dietary levels of manganese
High dose levels of manganese	Low dose levels of manganese
Age (older)	Age (younger)
High starch diet	High sucrose diet
Diet rich in iron	Low iron status (anaemic)
Administered with food	Administered as solution
Less soluble salts	More soluble salts
	Manganese previously excreted in bile

Key:
a- Individuals with iron deficiency (low serum ferritin) are likely to absorb greater amounts
b- For non-fasted group, whereas 9±3% for fasted groups (Sandstrom et al., 1987)
NDc- No data found, however expected to be similar or lower than other soluble salts
NDd – No data found, however expected to be lower than soluble salts
e - (Zheng et al., 2000), however depending on age of rats and existing levels of manganese, values up to 66% absorption have been reported (Keen et al., 1986)

1.7.2 Manganese Distribution

Table 1.7.2.1 Manganese distribution in rats (µg/g wet weight) after oral and inhalation exposures

Tissue	**20 mg Mn/mL (Lifetime)[a]**	**75 mg Mn/day (28 days)[b]**	**3 mg Mn/m^3 (14 days)[c]**	**3 mg Mn/m^3 (14 days)[c]**	**3 mg Mn/m^3 (14 days)[d]**	**4 mg Mn/m^3 (91 days)[e]**	**0.1 mg Mn/m^3 (12 days)[f]**
Manganese Form (Dose Route)	$MnCl_2$ (Drinking Water)	$MnCl_2$ (Oral)	Mn_3O_4 (Inhalation) (MMAD 1.8 µm)	$MnSO_4$ (Inhalation) (MMAD 2.1 µm)	Manganese Phosphate – Hureaulite (Inhalation) (MMAD 1.60 µm)	Metallic Manganese (Inhalation) (90%< 1.55 µm)	MnO/Mn_2O_3 (61/39) UFPs (Inhalation) (3-8 nm -aerosol agglomerates ~30 nm)
Femur		4.8±1.9*	0.68±0.05*	1.28±0.06*	1.19±0.14*		
Liver		2.0±0.2	3.48±0.31	3.64±0.46*	3.00±0.21	2.44±0.19	
Bile			0.95±0.13*	1.51±0.17*			
Spinal cord		0.49±0.05*					
Adrenal glands		3.7±0.5*					
Heart		0.53±0.04*					
Pancreas		1.5±0.2*					
Lung		11.6±9.1**	14.73±2.57*	7.33±0.37*	20.31±0.95*	0.29±0.07*	*0.45**
Testes		0.49±0.02*	0.46±0.03	0.79±0.18*		0.30±0.03	
Olfactory bulb			3.09±0.29*	4.42±0.23*	1.89±0.13*		*1.75**
Brain		0.79±0.27*					
Striatum	1.39±0.24*		1.48±0.12*	3.18±0.59*	0.90±0.06*	0.86±0.04*	*0.7**
Cerebellum	1.04±0.10*		NS	NS	0.74±0.09*	0.63±0.04	*0.6**
Pons and Medulla	1.12±0.10*						
Midbrain	1.33±0.20*						NS
Cerebral cortex	0.83±0.16*						
Hypothalamus	1.89±0.12*						
Globus pallidus						0.93±0.06*	
Red blood cells					0.10±0.01		
Serum/Plasma		NS	0.10±0.02	0.31±0.20	0.12±0.01		

Key:
a - (Lai et al., 1992) b- (Missy et al., 2000), c - (Dorman et al., 2001a), d - (Vitarella et al., 2000b) e - (Normandin et al., 2004)
f - (Elder et al., 2006)
Values in *italics* have been estimated from graphical data. MMAD – Mass Median Aerodynamic Diameter
* - data statistically significantly different from respective controls, NS – not statistically significantly different from controls.
** - High results and variability following oral administration may have been due to the repeat dosing technique (force-feeding and possible regurgitation).
UFPs – Ultra Fine Particles - particles of 3-8 nm in diameter forming an aerosol with agglomerates of approximately 30 nm (simulated welding fumes).

Table 1.7.2.2 Manganese tissue distribution in male rhesus monkeys (µg/g wet weight) after subchronic inhalation exposure to manganese sulphate (Dorman et al., 2006b)

Tissue	**Control**	**0.06 mg Mn/m^3 (65 days) (MMAD 1.73 µm)**	**0.3 mg Mn/m^3 (65 days) (MMAD 1.89 µm)**	**1.5 mg Mn/m^3 (65 days) (MMAD 2.12 µm)**	**1.5 mg Mn/m^3 (65 days) 45 days post-exposure[a] (MMAD 2.12 µm)**
Organs:					
Femur	0.13±0.02	0.11±0.01	0.13±0.03	0.20±0.03	0.12±0.02
Heart	0.16±0.03	0.33±0.03*	0.49±0.03*	0.62±0.05*	0.23±0.03
Kidney	1.14±0.12	1.43±0.05	1.86±0.14*	2.61±0.30*	1.38±0.13
Liver	2.49±0.09	2.91±0.18	3.17±0.20	3.52±0.45*	2.88±0.27
Lung	0.15±0.03	0.18±0.01	0.25±0.02*	0.33±0.04*	0.09±0.01
Pancreas	1.59±0.11	1.72±0.09	2.34±0.11*	2.95±0.24*	1.14±0.24
Muscle	0.15±0.03	0.12±0.03	0.18±0.02	0.58±0.19*	0.19±0.02
Bone	0.08±0.04	0.05±0.02	0.13±0.06	0.25±0.04*	0.17±0.03
Testis	0.26±0.03	0.35±0.03	0.40±0.05	0.39±0.07	0.36±0.04
Olfactory tissues:					
Olfactory epithelium	0.42±0.01	1.22±0.15*	2.96±0.46*	7.10±2.01*	0.65±0.04
Olfactory bulb	0.31±0.01	0.77±0.04*	1.36±0.15*	2.40±0.18*	0.35±0.02
Olfactory tract	0.30±0.06	0.43±0.02	0.61±0.05*	1.12±0.08*	0.18±0.02
Olfactory cortex	0.19±0.004	0.27±0.02*	0.31±0.01*	0.42±0.01*	0.26±0.01*
Brain:					
Globus pallidus	0.48±0.04	0.80±0.04*	1.28±0.15*	2.94±0.23*	1.09±0.03*
Putamen	0.36±0.01	0.58±0.04*	0.75±0.05*	1.81±0.14*	0.58±0.03*
Caudate	0.34±0.02	0.47±0.04	0.69±0.03*	1.72±0.10*	0.57±0.03
Frontal Cortex	0.25±0.03	0.29±0.02	0.29±0.01	0.47±0.02*	0.26±0.01
Cerebellum	0.44±0.01	0.62±0.02*	0.70±0.04*	1.10±0.11*	0.66±0.04
Pituitary	0.84±0.12	1.53±0.25	2.43±0.13*	6.19±0.61*	3.01±0.91*
Trigeminal nerve	0.17±0.05	0.17±0.01	0.21±0.01	0.42±0.08*	0.18±0.01
Fluids:					
Bile	0.89±0.22	1.65±0.31	3.78±0.34*	7.60±1.68*	1.17±0.28
Blood	0.010±0.001	0.015±0.002	0.022±0.003*	0.026±0.003*	0.021±0.002*

Key:
* - data statistically significantly different from respective controls
a – all tissue concentrations had fallen to control tissue levels by 90 days post-exposure

1.7.3 Manganese Elimination

Table 1.7.3.1 Human whole body elimination of manganese

Subject group	Sex	Terminal phase (days)	Reference	Klimisch Code
High manganese diet	F	14 (13-16)	(Finley et al., 2003)	2
Healthy miners	M	15±2	(Mena et al., 1969)	4
Anaemic patients	M+F	23±3	(Mena et al., 1969)	4
High manganese loading	NS	33	(Mahoney and Small, 1968)	2
Controls	M+F	37±7	(Mena et al., 1969)	4
Controls	M	48±21	(Finley et al., 1994)	2
Controls	F	34±16	(Finley et al., 1994)	2
Controls	M+F	36.5-41	(Mahoney and Small, 1968)	2
Manganism patients	M	34±5	(Mena et al., 1969)	4
Manganism patients	M	28±8	(Cotzias et al., 1968)	2
Low manganese diet	F	30 (28-33)	(Finley et al., 2003)	2
Calorie restricted	M	92	(Mahoney and Small, 1968)	2

Key:
NS – Not specified

It is apparent that the body's natural homeostatic regulation will increase the rate of elimination of manganese if there is a high existing body burden and/or a continued high uptake of manganese.

Table 1.7.3.2 The elimination of manganese in animals

Species	Manganese Treatment Regimen	Elimination measurement	Initial elimination rate (days)	Terminal elimination rate (days)	Reference:	Klimisch Code
Mice	80 (control) mg/kg diet	Whole body following an ip injection of a ^{54}Mn tracer	2.3±0.2	17.5±1.0	(Sato et al., 1996)	2
	240 mg/kg diet		2.3±0.2	11.9±0.3		
	800 mg/kg diet		1.9±0.1	11.8±0.4		
	2400 mg/kg diet		1.3±0.1	9.5±1.4		
	8000 mg/kg diet		0.9±0.1	8.5±0.7		
Rats	~4 mg/kg diet	Whole body following an ip injection of a ^{54}Mn tracer		31.8-32.6	(Lee and Johnson, 1988)	2
	~45 mg/kg diet			10.9-12.5		
	~82 mg/kg diet			10.3		
Rats	Controls	Whole body following an iv injection of a $^{54}MnCl_2$ tracer	3.8-5.7	31-34	(Vitarella et al., 2000b; Dorman et al., 2001a)	2
	Short-term inhalation of manganese sulphate		2.4±0.24	27.1±1.4		
	Short-term inhalation of manganese tetraoxide		2.9±0.28	32.6±0.45		
	Short-term inhalation of manganese phosphate		3.3±1.0	32.0±5.2		
Dogs	Inhalation of $^{54}MnO_2$	Lower respiratory tract		34	(Morrow et al., 1964)	4
Monkeys	Controls	Whole body following an ip injection of a ^{54}Mn maleate tracer	6	95	(Dastur et al., 1971)	4
Monkeys	Subchronic inhalation of manganese sulphate	Olfactory bulb		4.9	(Dorman et al., 2006b)	2
		Globus pallidus, putamen, caudate		15.7-16.7		
		Olfactory cortex		19.4		
		Pituitary		23.6		
		Cerebellum		32.3		

2 INTRODUCTION

Manganese is an essential trace element for living organisms and is needed to maintain normal metabolic functioning. The major human low-level exposure to manganese is generally from the diet, whereby soluble manganese is absorbed through the GI tract. This is in contrast to high-level occupational exposure, whereby there is a risk of inhaling manganese (typically in an insoluble form), which has historically led to manganese intoxication (manganism).

Manganese is widely distributed in the environment constituting approximately 0.1% of the earth's crust. It is a ubiquitous element, forming numerous mineral complexes such as rhodochrosite and manganite with its most prevalent chemical form being manganese dioxide (MnO_2). Manganese is essential to the manufacturing of steel and is one of the most widely used alloy elements in the world. It is used to form alloys with iron, aluminium, nickel-silver, nickel-chromium and bronze and is also used in a wide range of products such as paints, fungicides, fertilizers, wood preservatives, dry cell batteries and as a fuel additive.

The World Health Organization (WHO) estimates that adults consume between 0.7 and 10.9 mg of manganese per day in the diet, with higher intakes for vegetarians who may consume a larger proportion of manganese-rich nuts, grains, and legumes in their diet as compared to non-vegetarians in the general population (World_Health_Organization, 2004). Typical drinking water concentrations of manganese are below 10 µg/L although may commonly range from 1 to 100 µg/L (Keen and Zidenberg-Cherr, 1994).

2.1 Scope

A toxicokinetics (TK) assessment is required under REACH (Annex VIII level section 8.8) and is necessary to confirm the manganese substance grouping for read-across for the purposes of classification and labelling and risk assessment. The following manganese substances were considered in the preparation of this TK report:

- Manganese metal: Mn
- Soluble salts: $MnSO_4$, $Mn(NO_3)_2$, $MnCl_2$
- Insoluble salts: MnS, $MnCO_3$
- Manganese oxides: Mn_3O_4, MnO and MnO_2
- FeMn, SiMn, FeMn and SiMn slags and manganese sinter ore

Although the organometallic compound methylcyclopentadienyl manganese tricarbonyl (MMT) is widely used as a fuel additive, it was not specifically considered within the scope of this report as a requirement under REACH. However, the combustion products of MMT emitted from automobiles equipped with catalytic converters are primarily the manganese phosphate and sulphate forms with smaller amounts of manganese oxides (Dorman et al., 2006a). As such, there will be a degree of overlap within this report to data that primarily focuses on MMT toxicokinetics.

The aims of this report are to critically evaluate the TK of manganese and inorganic manganese compounds by evaluating the results of published TK studies taking into account the relevance of the studies, the quality of the methodologies used and the reporting, and the reliability of the results and conclusions (see Data Quality). Where available, abstracts from all the publications considered have been included in the bibliography section at the end of this report to improve the readability of the main text of the report whilst maintaining the authors' original wording and detail.

Literature Search: Harlan Laboratories supplied 164 publications to be considered for this report selected as being relevant to the TK of manganese and its compounds. Additionally, in December 2008 the library services of the Royal Society of Chemistry were used to search the 11-15th Collective Indexes of Chemical Abstracts using the keywords "Mammalian Toxicokinetics (TK),

absorption (uptake), distribution, metabolism and excretion (elimination) (ADME)" of manganese and its compounds. Further publications were considered from the citations contained within references identified from the search strategy. In addition to publications reporting the results from studies actually performed, several review articles were also identified from the above search strategy (Table 2.1). These review articles make excellent further reading for in-depth understanding of concepts such as the mechanisms involved in the homeostatic regulation of manganese and the transport of manganese in the CNS and across the blood-brain barrier.

Table 2.1 Review articles on kinetic data of manganese

Title	**Authors**
Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese	Andersen et al., 1999
Manganese uptake and distribution in the central nervous system (CNS)	Aschner et al., 1999
Manganese: pharmacokinetics and molecular mechanisms of brain uptake	Aschner and Dorman, 2006
The transport of manganese across the blood-brain barrier	Aschner, 2006
Manganese in Health and Disease	Aschner et al., 2002
Manganese dosimetry: species differences and implications for neurotoxicity	Aschner et al., 2005
Application of pharmacokinetic data to the risk assessment of inhaled manganese	Dorman et al., 2006a
Pharmacokinetic factors that influence manganese delivery to the brain	Dorman et al., 2001c
Speciation and toxicological relevance of manganese in humans	Michalke et al., 2007
The speciation of metals in mammals influences their toxicokinetics and toxicodynamics and therefore human health risk assessment	Yokel et al., 2006
Manganese neurotoxicity: A bioinorganic chemist's perspective	Quintanar, 2008
Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination	Roth, 2006
Pharmacokinetic modeling of manganese	Teeguarden et al., 2007a; Teeguarden et al., 2007b; Teeguarden et al., 2007c; Nong et al., 2008
Manganese toxicokinetics at the blood-brain barrier	Yokel and Crossgrove, 2004

Data from the following references have not been included in this report as the data contained were assessed as Klimisch Codes 3, 4 or 5 (see Data Quality).
(Akoume et al., 2003), (Altstatt et al., 1967), (Banta and Markesbery, 1977), (Baly et al., 1985), (Bast-Pettersen and Ellingsen, 2005), (Bast-Pettersen et al., 2000), (Bertinchamps and Cotzias, 1958), (Bertinchamps et al., 1966), (Bird et al., 1984), (Bonilla and Diez-Ewald, 1974), (Bonilla, 1985), (Burnett et al., 1952), (Cahill et al., 1980), (Casalino et al., 2004), (Cikrt, 1972), (Cikrt, 1973), (Crossgrove and Yokel, 2005), (Dastur et al., 1969), (Deimling and Schnell, 1984), (Davis and Greger, 1992), (Dorner et al., 1989), (Dorman and Wong, 2006), (Ellingsen et al., 2000), (Elsner and Spangler, 2005), (Eriksson et al., 1992), (Eriksson et al., 1987b), (Eriksson et al., 1987a), (Fechter, 1999), (Fechter et al., 2002), (Finley et al., 1997), (Freeland-Graves et al., 1988), (Freeland-Graves and Lin, 1991), (Furst, 1978), (Gwiazda et al., 2007), (Gupta et al., 1980), (Hughes and Cotzias, 1960), (Hughes and Cotzias, 1961), (Hughes et al., 1966), (Garcia-Aranda et al., 1983), (Gianutsos et al., 1985), (Greger and Snedeker, 1980), (Greenberg and Campbell, 1940), (Greenberg et al., 1943), (Gruden, 1984), (Hanlon et al., 1975), (Heilig et al., 2006), (Hobbesland et al., 1999), (Hurley et al., 1984), (Hussain et al., 1997), (Kitagawa and Wada, 1990), (Kimura et al., 1978), (Kobayashi et al., 2007), (Kojima et al., 1983), (Komura and Sakamoto, 1993), (Koshida et al., 1963), (Koshida et al., 1965), (Kostial et al., 1978b), (Kostial et al., 1989), (Laitung and Mercer, 1983), (Lewis et al., 2005), (Newland et al., 1987), (Malik and Srivastava, 1987), (Matrone et al., 1959), (Maynard and Cotzias, 1955), (Mena et al., 1974), (Miller et al., 1975), (Moore et al., 1974), (Morganti et al., 1985), (Murthy et al., 1980), (Norwegian-Labour-Inspectorate, 2007), (Panic, 1967), (Ponzoni et al., 2002), (Salehi et al., 2003), (Sanchez et al., 1995), (Sandstrom et al., 1990), (Sandstrom, 1992), (Schafer et al., 1974),(Scheuhammer and Cherian, 1981) , (Singh et al., 1977), (Schwartz et al., 1986), (Shukla and Chandra, 1981), (Shukla and Chandra, 1982), (Shukla and Chandra, 1987), (Singh and Junnarkar, 1991), (Slikker et al., 2004), (Smialowicz et al., 1985), (Smialowicz et al., 1984), (Smialowicz et al., 1987), (Smialowicz et al., 1988), (Spencer et al., 1979), (St-Pierre et al., 2001), (Tipton et al., 1969), (Wassermann and Wassermann, 1977), (Witzleben et al., 1968; Witzleben, 1969), (Yoshikawa, 1974), (Zhang et al., 2003; Zhang et al., 2005), (Yamaguchi et al., 1986), (Yu et al., 2003),

2.1.1 Manganese as an essential element (EE)

Manganese is an essential element (EE) for living organisms and is needed to maintain normal metabolic functioning and a deficiency results in a reduction of biological function (Mertz, 1974). Most species have the ability to homeostatically regulate the internal manganese concentrations at an optimal concentration range (Van Assche et al., 1997); it has been established that absorption as well as excretion play an important role in its homeostasis (Abrams et al., 1976). Manganese is involved in the metabolism of lipids, proteins and carbohydrates and serves as a co-factor for many enzymes (Wedler, 1993). It is also essential for the development and functioning of the brain, with human adult brains containing approximately 0.25 µg/g manganese (wet weight).

When the manganese body level becomes too high or low, toxicity or deficiency can occur. Chronic exposure to manganese at low levels is nutritionally essential in humans. The recommended daily intake of manganese is 2 to 5 mg/day for adults and adolescents (FNB/IOM, 2001). Whilst the potential for manganese toxicity is obviously key to any risk assessment, manganese deficiency and the subsequent consequences are not considered further in this report.

2.1.2 Manganism

A rare CNS disorder that results from chronic high-dose exposure to manganese is known as manganism. This has similarities to idiopathic Parkinson's disease largely because a similar part of the brain is affected (Crossgrove and Zheng, 2004). Manganism causes dystonia, a neurological sign associated with damage to the globus pallidus. Although manganism can exhibit symptoms similar to parkinsonism, it can also manifest additional symptoms distinct from those common to parkinsonism. Treatments of manganism patients with dopamine analogues (used for the treatment of Parkinson's disease) do not result in any improvement, whereas treatment with manganese chelators at an early stage can reverse the condition. Parkinson's disease results from target destruction of dopaminergic neurons, whereas manganism results from the deposition of manganese in particular brain regions. As the basal ganglia region is the main site of neurodegeneration in both diseases, there is some debate as to whether excessive manganese exposure may further undermine the functionality of the basal ganglia and exacerbate the onset of parkinsonism (Witholt et al., 2000; Hudson et al., 2001; Gitler et al., 2009). Detailed review of the mechanisms involved in manganism and the differences and similarities between manganism and parkinsonism are not included in the scope of this report.

2.1.3 Biomarkers of manganese exposure

The elimination of absorbed manganese is primarily through the bile and, as such, the main excretion route for manganese is ultimately in the faeces; however this route can also include unabsorbed dose following oral absorption. The very low proportion of manganese dose excreted in the urine (<1%) means that analysing the urine from workers potentially exposed to manganese is not straightforward. Although the manganese concentration in the urine from groups of workers exposed to manganese was typically 5 to 10-fold higher than control groups, this correlation did not extend to measurements made on an individual basis or to their blood manganese levels (Roels et al., 1987a; Roels et al., 1987b; Gan et al., 1988; Ellingsen et al., 2003b). On a group basis, urine manganese concentration appeared to reflect very recent exposure, whilst blood manganese concentration was to some extent a reflection of the body burden of manganese. The biological half-life of manganese in urine is estimated to be less than 30 hours following cessation of exposure to manganese.

A negative association was found between the concentration of serum soluble transferrin receptor and the concentration of manganese in whole blood in manganese alloy-production workers (Ellingsen et al., 2003a). The results suggested that manganese-exposed workers have higher intracellular iron concentration in the erythrocyte precursors than the controls, resulting in a down-regulation of transferrin receptors on the surface of these cells.

Despite a significant difference in respirable manganese levels in human populations (5 ng Mn/m^3 rural and 25 ng Mn/m^3 urban), no significant differences were measured in the blood concentrations of manganese. The authors concluded that manganese blood concentrations were not a reliable biomarker for the low-level manganese exposure by the respiratory route (Bolte et al., 2004). The exceedingly fast initial clearance of manganese from blood means that the measurement of blood manganese levels is not suitable for the estimation of very recent exposure. In order to produce consistent plasma manganese responses from fasted human subjects (Bales et al., 1987), it was necessary to provide oral doses of 40-50 mg manganese (as $MnCl_2$ salt in capsule), this being 10-fold greater than estimated dietary intake. A T_{max} of 1 hour was observed with blood manganese levels of 1.29 µg/L, approximately twice the background level.

The level of manganese in scalp hair has shown to be useful in determining a population's overall exposure to manganese (Stauber et al., 1987). However the analytical procedure and the interpretation of the data are fraught with problems because of the need to distinguish between endogenous manganese (from the body) and exogenous manganese from the environment. In which case this method is generally unsuitable in a workplace where the potential exposure is from airborne manganese. The hair samples of >50 year old males and females who had over 1000-fold different concentrations of manganese in their drinking water (low level <15 ug/L, high level >1800 ug/L) showed significant differences in the concentration of manganese in their hair (Kondakis et al., 1989). Blood manganese concentrations were around 15-18 ug/L and did not show any significant differences between the drinking water groups.

The use of toenail clippings as a marker of manganese exposure was investigated with workers exposed to manganese during Mn-alloy production (Zaprianov et al., 1985). However, this publication contained relatively brief details (Klimisch Code 4) and this approach does not appear to have been followed up by other workers.

The paramagnetism of manganese using magnetic resonance images (MRI), pallidal index (PI), and T(1) relaxation rate (R1) in concert with chemical analysis was used to establish a direct association between MRI changes and pallidal manganese concentration in rhesus monkeys following subchronic inhalation of manganese sulfate (Dorman et al., 2006c). The authors stated that their results indicated that the R1 can be used to estimate regional brain manganese concentrations and may be a reliable biomarker of occupational manganese exposure.

2.1.4 Important factors in the toxicokinetic assessment of manganese

The presence of a homeostatic control for manganese is the main complicating factor when assessing toxicokinetic behaviour by traditional mass balance technique (measuring the absorption, retention and elimination of manganese). The body appears to be very adaptive towards moderately increased loads of manganese by such measures as reduced gastrointestinal (GI) absorption, enhanced hepatic elimination and increased biliary and pancreatic excretion of manganese (Roth, 2006). All these biological systems are presumably to help maintain normal manganese tissue concentrations under the natural day-to-day variation in manganese dietary uptake.

Whilst the homeostatic regulation of manganese by the body seems to be very efficient, this does appear to be overwhelmed by chronic high doses leading to toxicity.

The main factors that have to be considered when interpreting toxicokinetic data on manganese are summarised as follows:

- the ubiquitous nature of manganese in the environment, food, drinking water, which results in a background level of manganese in the body;
- the body's ability to modulate the absorption and excretion of manganese in order to maintain homeostasis (for example in response to fasting prior to administration or to iron status due to the antagonistic relationship existing between iron and manganese);
- the route of exposure, e.g. oral and/or inhalation;
- the physical form of the manganese presented e.g. as a soluble salt, insoluble salt, dust (particle size) and the dose vehicle;
- enterohepatic recirculation of manganese excreted in bile and reabsorbed;
- the very rapid initial half-life of manganese in blood;
- the very low proportion of dose excreted in the urine;
- the health of the subject (liver function) and the age of subject;
- natural turnover of manganese as it is utilised in many essential bodily functions such as amino acid, protein, lipid and carbohydrate metabolism.

An additional factor that exacerbated the compilation of summary tables and the ability to compare and contrast data was the non-uniformity of the study designs. Since the data used for the toxicokinetic assessment of manganese were taken from publications as opposed to studies conducted within a GLP environment using standard study designs (based upon regulatory requirements) even simple comparisons were hampered unless the studies had been conducted by the same group of researchers. For example, dosing regimens for repeat dosing included: every day, 5 days/week, or 3 times/week; similarly the time-points selected for tissue sampling after the last dose varied considerably. The selection of tissue samples to be analysed was inconsistent, as well as how the results were presented and units quoted; for example µg/g to mg% to % increases over controls or % dose. Even comparison of particle sizes for the inhalation studies was confounded by the range of ways used to present this data, for example: mass median aerodynamic diameters (MMAD), geometric mean diameters (GMD) and also just quoting a percentage of particles smaller than a given value. If the publications used figures to present data, then very often this data would not also be presented in tables and as such estimations of data values from the figures were attempted.

2.1.5 Data Quality

The evaluation of data quality included assessment of adequacy of the information from published literature for hazard/risk assessment and classification and labelling purposes and furthermore the two basic elements of relevance and reliability[1]. The system to describe the reliability was the system introduced by Klimisch (Klimisch et al., 1997):

Code	Category
1	Reliable without restriction
2	Reliable with restrictions
3	Not reliable
4	Not assignable
5	Not evaluated

In assigning a Code 1, reliable without restriction, the study would have typically been conducted and reported according to international accepted guidelines (EU, EPA, OECD etc.) and preferably in compliance with the principles of Good Laboratory Practice (GLP).

Although a multitude of studies has been performed for manganese and its compounds that can be considered for this TK expert report, most of these do not claim to have been conducted to international guidelines or under GLP conditions. As such, studies performed for manganese and its compounds that have been assigned a Code 2, 'reliable with restrictions', will form the weight of evidence considered for this report provided they are well documented and scientifically acceptable and published in peer-reviewed journals. Data from studies that were rated a Klimisch Code 3 have not been used in the main body of the report although their assessment has been included in the bibliography appendix. There were also a considerable number of older publications that contained insufficient details to be able to properly assess the reliability and as such were assigned a Klimisch Code 4. These are not discussed in the main report unless their relevance was considered very high and there was no more recent reliable data.

The data quality assessment of adequacy of the information from published literature is the personal opinion of the author based upon his interpretation of the REACH requirements for the purpose of this report and, as such, is only intended to be used for this purpose.

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[1] These terms are defined as follows **Klimisch HJ, Andreae M and Tillmann U (1997)** A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol* **25:**1-5.

Relevance - covering the extent to which data and/or tests are appropriate for a particular hazard identification or risk characterisation. In consideration of the multitude of published studies on manganese that could be considered to contain information relating to the toxicokinetics of manganese and its compounds, a further refinement on their relevance was needed. The Klimisch code 5 **"not-evaluated"** was assigned to studies that were not considered to uniquely and unequivocally contain data that could inform the toxicokinetic assessment of manganese and its compounds for this report. No further assessment of the reliability or adequacy of studies falling into this category has been performed. This should not be seen as any judgement of the scientific integrity of the studies, but merely that the information they contained was not considered sufficiently relevant and critical for this review. This Klimisch code 5 was also used for publications that have been referenced in this report, but were not directly assessed for the toxicokinetics of manganese, for example the publication that describes the Klimisch codes or review articles.
Reliability - evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. Reliability of data is closely linked to the reliability of the test method used to generate the data.
Adequacy - defining the usefulness of data for hazard/risk assessment purposes. Where there is more than one study/data set for each endpoint, the greatest weight is attached to the studies that are the most relevant and reliable.

2.1.6 Symbols and their definitions

Acronyms have been described in full the first time they are used in the text of this report where possible. The data units used publications have been converted to standard units where possible to enable comparisons of data between studies.

Symbol	Definition or explanation (units)
AAS	Atomic Absorption Spectroscopy
ADME	Absorption, Distribution, Metabolism and Excretion
AMMD	Aerodynamic Mass Median Diameter
AUC	Area Under the Curve. The timepoints, if included, will be shown as a subscript
BBB	Blood Brain Barrier
bw	Bodyweight
CD	Caesarean Derived
CL	Clearance. The timepoint, if included, will be shown as a subscript
C_{max}	Maximum concentration
CMnP or CMP	Chronic Manganese Poisoning
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DMT1	Divalent Metal Transporter-1
EDTA	Ethylene Diamine Tetraacetic Acid
EE	Essential Element
EPA	Environmental Protection Agency
EU	European Union
FPLC	Fast Protein Liquid Chromatography
GI	Gastrointestinal
GLP	Good Laboratory Practice
GMD	Geometric Mean Diameter
ip	Intraperitoneal
iv	Intravenous
Kbeq or kBq	Kilobecquerel
M	Molar
mg	Milligram
MMA-SS	Manual Metal Arc-Stainless Steel
MMAD	Mass Median Aerodynamic Diameter
MMD	Mass Mean Diameter
MMT	Methylcyclopentadienyl Manganese Tricarbonyl
^{52}Mn	Manganese isotope with an atomic weight of 52
^{54}Mn	Manganese isotope with an atomic weight of 54
MRI	Magnetic Resonance Imaging
MW	Molecular Weight
nm	Nanometre
NS	Not Statistically Significant or Not Specified
OECD	Organisation for Economic Co-operation and Development
PI	Pallidal Index
PBPK	Physiologically-Based PharmacoKinetic modelling
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
PK	Pharmacokinetics
sc	Subcutaneous
R1	Relaxation rate
SD	Standard Deviation
TK	Toxicokinetics
TLV	Threshold Limit Value
T_{max}	Time of maximum concentration
TPN	Total Parental Nutrition
Tf	Transferrin
T1	Spin-lattice relaxation time
$t_{½}$	Half life
UFP	Ultra Fine Particles
µL	Microlitre
µm	Micrometer
WHO	World Health Organisation

3 THE ABSORPTION OF MANGANESE

The primary route of absorption of manganese in humans is either by ingestion or by inhalation and the majority of this section focuses on these routes.

There are several methods that are used to study inhalation; the first is inhalation chambers for whole body exposure. These chambers can create atmospheres with exposure conditions that can be related to human exposure using a known particle size, atmospheric concentration and exposure time. However, this method is not suitable for the use of radiolabelled material due to the excessive quantities that would be needed and wasted (ventilation of non-absorbed dose) and, as such, it is not possible to establish an actual dose and only measure the exposure. Whilst this method obviously covers all possible routes of absorption following inhalation (through the nasal mucosa, transport across the pulmonary epithelial lining and clearance from the lung by mucocilliary elevator and subsequent ingestion from the GI tract), non-restrained animals can also lick their fur and so introduce oral absorption as an additional route. A nose-only inhalation technique gets over this particular issue and uses less material. In order to examine whether uptake is via the lungs or through the nasal mucosa, intratracheal or intranasal techniques are used. These techniques also have the advantage that smaller amounts of test material are needed and, as such, the administered dose can be accurately measured and radiolabelled material can also be used if required. However, avoiding damage to the tissues that come into direct contact with the dosing applicator is a concern. Direct dose application by intratracheal administration also bypasses any earlier deposition or clearance of particles in the nasopharyngeal region that may have occurred naturally following inhalation.

Consideration has been further divided into the soluble salts of manganese and the remaining insoluble salts, oxides and other inorganic forms of manganese.

The dermal absorption of manganese is not likely to be an important route of absorption for inorganic manganese and as such there is virtually no published data on this. One study does report that a human was burnt with a hot acid solution containing manganese (Laitung and Mercer, 1983). However this is not considered relevant for this report since it is probable that the hot acid would have compromised the barrier properties of the skin. It is generally expected that the dermal absorption of manganese would be very low.

Intravenous (iv) administration, intraperitoneal injection (ip) and subcutaneous injection (sc) are also common routes of administration primarily used in animal studies and are not directly relevant to the human exposure to manganese. However, these routes are primarily used as a means to bypass the absorption process and thus to study the systemic behaviour of manganese in order to test specific mechanisms and hypotheses and, as such, can provide valuable information on the TK of manganese.

Humans can also receive manganese through total parental nutrition (TPN), whereby manganese is included as an essential trace element. Total absorption is achieved through this route and as such will not be discussed further in this section.

3.1 Manganese soluble salts: manganese chloride ($MnCl_2$), manganese sulphate ($MnSO_4$) and manganese nitrate ($Mn(NO_3)_2$)

3.1.1 Humans

3.1.1.1 Absorption by the oral route

In order to measure the specific uptake of manganese from a single dose above the ubiquitous manganese already in our bodies and diet, the use of radiolabelled ^{54}Mn is usually employed. Once administered, the radiolabelled manganese becomes indistinguishable (to the body) from the unlabelled manganese already present in the system and thus studying the fate of the ^{54}Mn tracer is considered representative of the fate of the unlabelled material. The amount of radiolabelled dose administered and subsequently retained is measured using whole body counting. Obviously this methodology does not distinguish between dose that has been absorbed from the gastrointestinal tract (GI tract) and is systemically available compared to dose that remains unabsorbed in the GI tract or dose that has been absorbed and subsequently excreted into the GI tract via the bile. Whole-body retention curves of ^{54}Mn show an initial steeply declining portion and then appear linear after around Day 7. As such, estimates of absorption are often made by back-extrapolating data from Days 10-30 (Davidsson et al., 1989b) back to the ordinate in order to obtain an initial estimate of absorption.

Estimating manganese absorption using this method, particularly if combined with a crossover approach, whereby subjects serve as their own controls, is very useful for comparative purposes (between studies) and investigating factors affecting absorption (within studies). However, a potential weakness with this methodology is that any ^{54}Mn absorbed and rapidly excreted into the gut by the way of bile within the first few days will not be accounted for (Finley, 1999). As such, estimates of absorption calculated using this methodology can be considered to be on the conservative side and absolute absorption is likely to be higher.

An early study compared the absorption of a ^{54}Mn tracer (as $^{54}MnCl_2$) to fasted human subjects (Mena et al., 1969), although this was an old study (Klimisch Code 4) it contains very relevant human data. An estimation of the intestinal absorption was around 3% for groups of healthy individuals and patients with manganism, whilst manganese absorption was significantly higher in anaemic patients (about 7.5%).

Sex differences in manganese absorption and excretion has been observed (Finley et al., 1994); however, the interpretation of this study may have been confounded by iron deficiency in 11 out of 20 female subjects, while only 1 out of 20 males had iron deficiencies (determined by serum ferritin values < 20 ug/L). The influence of iron status (serum ferritin concentration) on manganese absorption and retention by young women was investigated further by the same author (Finley, 1999). The author concluded that iron status, as measured by serum ferritin concentration, is strongly associated with the amount of manganese absorbed from a meal by young women. A thorough investigation of all the results using correlation analysis led the author to propose that when greater amounts of manganese are absorbed, the body may compensate by excreting manganese more quickly. If this were the case, then this would not have been included with the estimates of absorption using a back extrapolation method.

The interaction of dietary manganese, heme iron, and nonheme iron in women was found to influence the bioavailability of manganese (Davis et al., 1992a). Increasing dietary intake of nonheme iron was found to have negative effects on the nutritional status in regard to manganese. Unfortunately this study did not utilise ^{54}Mn to assess retention and so comparative absorption data is not reported.

The influence of varying iron stores upon the rate of absorption of manganese in patients with a duodenal perfusion showed that the rate of absorption of manganese (from manganese chloride) was increased in iron deficient patients and that enhanced absorption of manganese could be inhibited by increasing dietary iron levels (Thomson et al., 1971).

A dual-radioisotope method was used to simultaneously study whole-body manganese retention from a chicken liver based meal intrinsically labelled with ^{54}Mn and extrinsically labelled with ^{52}Mn (Davidsson et al., 1988). Both radioisotopes were retained to a similar degree and excreted at identical rates; however there was a considerable variation between subjects. The authors did not back extrapolate the retention data in order to obtain an estimate of initial absorption. Instead they quoted retention data on Days 5 and 10, with the absorption at Day 5 estimated as approximately 3-fold greater than at Day 10. A further study by the same laboratory (Davidsson et al., 1989b) addressed the issue of the methodology for estimating absorption by back-extrapolating retention data, where a single exponential function, $r^2>0.95$ was fitted for all subjects (data from Days 10-30). The authors concluded that this method gave a reasonable assessment for manganese absorption, although it may represent an underestimate in some subjects.

In a further study of the same workers, the effect of dietary components on manganese absorption was investigated (Davidsson et al., 1991). By using paired observations, with subjects acting as their own control, these workers were able to eliminate interindividual variation in manganese absorption. Addition of calcium to human milk resulted in a significant decrease in manganese absorption, whereas none of the other components tested did. No significant differences were found in a comparison of manganese absorption from human milk at a 7-fold difference in manganese dose levels.

The influence of overnight fasting upon the uptake of ^{54}Mn as a tracer together with a vitamin supplement containing 2.5 mg manganese (as manganese sulphate) found that absorption of manganese was substantially higher (Sandstrom et al., 1987). Estimates of absorption, using Day 7-20 retention data, were 9±3% and 2±1% for the fasted and non-fasted groups respectively.

The validity of using extrinsic ^{54}Mn tracers for the study of manganese absorption from foods of plant origin confirmed that there was no difference in absorption or biological half-life for any of the intrinsic/extrinsic food pairs; however differences in percentage absorption between the different food types were found (Johnson et al., 1991).

In an investigation into whether dietary manganese and dietary fats affected clinical or neuropsychological measures in healthy young women using a crossover study design, the absorption of manganese was estimated from a ^{54}Mn tracer administered in orange juice (Finley et al., 2003). The percentage of manganese absorption, calculated by linear regression, was unaffected by dietary fat, but was almost 40% lower when subjects consumed a high manganese diet (20 mg Mn/day) compared to a low manganese diet (0.8 mg Mn/day).

In an early study, a subject with an iron deficiency anaemia, Hb 109 g/L, was reported to have absorbed 45.5% manganese (Sandstrom et al., 1986) utilising a ^{54}Mn tracer. This was an old study (Klimisch Code 4), however it contains very relevant human data. Although the absolute data from this study may not be adequate for hazard/risk assessment and classification and labelling purposes, as defined earlier, it adds weight to the evidence supporting the following conclusions:

- Human oral absorption of manganese from levels typically in the range encountered in our diet, are likely to be low, below 5%, in healthy subjects where their natural homeostatic regulation of manganese is operating normally.
- Individuals with iron deficiency (low serum ferritin) are likely to absorb greater amounts.
- The source of manganese, i.e. as a $MnCl_2$ solution or as in food/drink, is likely to influence the level of absorption.
- The levels of other dietary constituents, particularly other transitional mineral nutrients such as iron (heme and nonheme), zinc as well as calcium affect manganese absorption.

Table 3.1.1 Human oral absorption of manganese at basal levels

Mn vehicle	**% dose absorbed**	**Factors affecting absorption**	**Reference**	**Klimisch Code**
Fruit juice	1.35±0.51% (Day 10-20, males) 3.55±2.11% (Day 10-20, females, >50% with low ferritin values)	A low iron status increased manganese absorption based upon ferritin values	(Finley et al., 1994)	2
Chicken liver	5.0 ± 3.1% (Day 10, females)	No differences between intrinsically and extrinsically labelled uptake	(Davidsson et al., 1988)	2
Infant formula	5.9±4.8% (Day 10-30, males and females)	Method of calculation	(Davidsson et al., 1989b)	2
Milk	8.2±2.9% (human milk), 2.4±1.7% (cow's milk)	Source of milk and whether manganese was bound to lactoferrin.	(Davidsson et al., 1989a)	2
Milk	4.9±2.3% (human milk), 3.0 ±1.6% (human milk with added calcium),	Adding calcium significantly reduced manganese absorption.	(Davidsson et al., 1991)	2
Light meal or fasting	2±1% (light meal) 9±3% (fasting)	Fasting state gave substantially higher absorption	(Sandstrom et al., 1987)	2
Foods of plant origin and $MnCl_2$	$MnCl_2$ control 8.90%, lettuce 5.20%, spinach 3.81%, wheat 2.16%, sunflower seeds 1.71%	Dose vehicle	(Johnson et al., 1991)	2
Infant formula, multi-element preparation	8.4±4.7% (formula) 8.9±3.2% (preparation) 45.5% (iron deficient subject)	Iron deficiency increases manganese absorption	(Sandstrom et al., 1986) (Thomson et al., 1971)	4 and 2
$MnCl_2$	4.86±0.58% LF, LM 0.97±0.68% HF, LM 2.3±0.58% LF, HM 1.03±0.63% HF, HM	Serum ferritin concentration Key: LF – Low ferritin subject HF – High ferritin subject LM – Low manganese diet HM – High manganese diet	(Finley, 1999)	2
Fruit juice	3.2±0.4% CO, LM 1.8±0.4% CO, HM 3.7±0.5% CB, LM 2.6±0.5% CB, HM	Key: CO – Diet enriched with Corn Oil CB – Diet enriched with Cocoa Butter LM – Low manganese diet HM – High manganese diet	(Finley et al., 2003)	2

In order to produce consistent plasma manganese responses from fasted human subjects (Bales et al., 1987), it was necessary to provide oral doses of 40-50 mg manganese (as $MnCl_2$ salt in capsule), this being 10-fold greater than estimated dietary intake. A T_{max} of 1 hour was observed with blood manganese levels of 1.29 µg/L, approximately twice the background level. Blood manganese responses were better with manganese delivered as the chloride salt than as the sulphate or acetate salts. Addition of three dietary components, cellulose, pectin and phytate, were all found to reduce the plasma uptake of manganese.

There are further threads of research on manganese absorption that investigate the affect of co-administered mineral elements and iron as well as dietary composition (carbohydrates, protein etc.) in addition to the health status of the subjects. One of the main drivers behind this research is the concern over the potential inadequate intake of manganese in humans, despite the lack of documented cases of human manganese deficiency. Whilst these lines of investigation will help to further explain the body's excellent natural homeostatic regulation of manganese absorption and excretion, their influence and relevance to the toxicokinetics of manganese required for this report are far less consequential. In the context of this report, studies documenting the toxicokinetics of manganese at dose levels closer to those that produce toxic effects or other measurable end points are far more relevant for hazard/risk assessment purposes.

3.1.1.2 Absorption by the inhalation route

The average levels of airborne manganese in ambient air are 5 and 33 ng Mn/m^3 in rural and urban air respectively (Aschner et al., 2005). Higher airborne levels are encountered near manganese mines or manganese-emitting industries. Even higher airborne concentrations of manganese have been encountered by workers in the mining industry, for example levels of up to 8.6 mg Mn/m^3 were recorded in a plant producing manganese oxides and salts (Roels et al., 1987b). Workers in other occupations such as welding can also be exposed to high airborne concentrations of manganese. Chronic exposure to high levels of manganese may produce adverse neurological, reproductive and respiratory effects (Crossgrove and Zheng, 2004). In humans, clinical manganese-induced neurotoxicity is associated with a motor dysfunction syndrome, commonly referred to as manganism (see Section 2.1.2).

Workers in the manganese alloy-producing industry are exposed to aerosols containing a variety of manganese compounds: MnO, MnO_2, Mn_2O_3, Mn_3O_4, FeMn and SiMn. Chemical analysis of personal air sampling equipment from such workers revealed that four distinct manganese components of the aerosol could be identified (Thomassen et al., 2001). This technique was then used to assess the speciation of manganese in workroom aerosols from one hundred workers from Norwegian manganese alloy-producing plants (Ellingsen et al., 2003b). Separate inhalable fractions (the fraction of airborne material drawn into the mouth and nose during breathing) and respirable fractions (particulate matter with the largest potential to reach the gas exchange region of the lung, <4 µm) were collected using personal samplers. Sequential chemical leaching of the sampler filters was performed in order to separate out four distinct fractions; heat (75˚C in autoclave with microwave-assisted digestion) was used in the processing of fractions 2-4. The speciation of manganese in the inhalable and respirable aerosol fractions using chemical leaching showed that little (approximately 10%) manganese was present as the ''water soluble'' Components 1. The highest amounts (approximately 50%) of manganese were found as Components 2 (Mn^0 and Mn^{2+}) for both the respirable and the inhalable aerosol fractions. Around 25% of the manganese was recovered as an ''insoluble'' fraction (Components 4). Workers in the furnace house had on average the highest proportions of respirable manganese, which was mainly related to the substantially higher proportion among the crane operators. Only approximately 10% of the inhalable manganese was found in the respirable fraction, which suggests that a low percentage of the inhaled manganese penetrates into the deeper compartments of the lungs. Consequently the majority of the inhaled manganese is likely to pass into the GI tract, where the absorption efficiency of manganese is also low as discussed in the previous Section (3.1.1.1). The strongest association between the air measures of exposure and biological samples was found between urinary manganese levels and ''soluble'' respirable

manganese. This could suggest that the ‘‘soluble’’ compounds in the respirable aerosol fraction may be the most relevant with respect to uptake into the body.

Table 3.1.1 Concentrations (µg/m^3) of manganese in the inhalable and respirable aerosol fractions measured in the personal samples from workers in manganese alloy-producing plants (Ellingsen et al., 2003b)

Component fraction:	**Inhalable (n=199)**		**Respirable (n=150)**	
	AM[a]	GM[b]	AM[a]	GM[b]
1. “water soluble” – manganese dissolved in ammonium acetate	26	14	3	2
2. Mn^0 and Mn^{2+} - dissolved in 25% acetic acid (Mn metal, FeMn, MnO and the Mn^{2+} part of MnO_2, Mn_2O_3, Mn_3O_4)	367	113	33	13
3. Mn^{3+} and Mn^{4+} - dissolved in 0.5% hydroxylamine hydrochloride in 25% acetic acid (The non-dissolved Mn^{3+} part of MnO_2, Mn_2O_3, Mn_3O_4)	216	31	12	2
4. “insoluble” – required strong acids in the presence of hydrofluoric acid in order to be solubilised (residual silicate-bound manganese or insoluble SiMn alloy)	183	49	11	4
Total of all components	**791**	**258**	**60**	**26**

a – AM: arithmetic mean
b – GM: geometric mean

It is well established that the absorption of particles via the lungs depends upon the particle size and solubility, as well as the geometry of the respiratory tract (Schlesinger, 1996). Figure 3.1 shows the mechanics of pulmonary deposition, absorption, and clearance can influence systemic delivery of inhaled manganese (Dorman et al., 2006a).

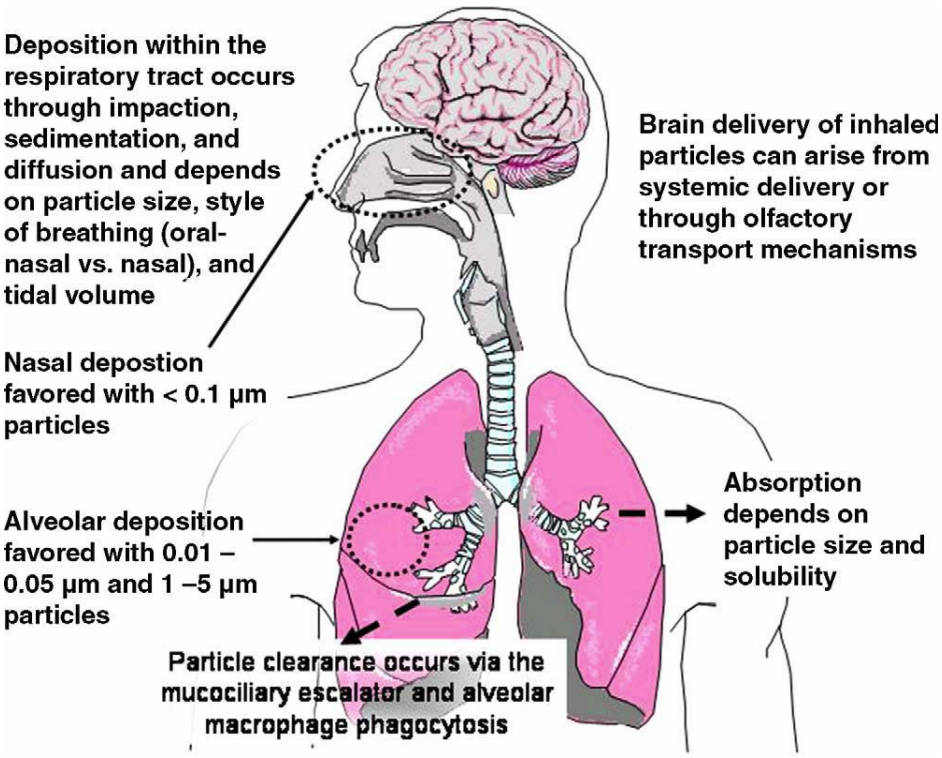


Figure 3.1 Pulmonary deposition, absorption, and clearance can influence systemic delivery of inhaled manganese (Dorman et al., 2006a)

The three potential routes of entry for manganese by inhalation are (Roth, 2006):

- Through the nasal mucosa
- Transport across the pulmonary epithelial lining and subsequent disposition in lymph/blood
- Clearance from the lung by mucocilliary elevator and subsequent ingestion from the GI tract

An early study reported the GI tract was the main absorption site of manganese from human inhalation of either a nebulised solution of $^{54}MnCl_2$ or a nebulised suspension of $^{54}MnO_3$ (Mena et al., 1969).

Despite a significant difference in respirable manganese levels in human populations (5 ng Mn/m^3 rural and 25 ng Mn/m^3 urban), no significant differences were measured in the blood concentrations of manganese. The authors concluded that manganese blood concentrations were not a reliable biomarker for the low-level manganese exposure by the respiratory route (Bolte et al., 2004). Furthermore this finding shows that the body can adapt well to inhalation exposure to elevated manganese-levels, as long as certain limits are not crossed during exposure to overloading the compensatory mechanisms.

There appears to be no reported studies detailing the percentage absorption of manganese in humans via inhalation, although it is expected that absorption of manganese from the lungs would be higher for the more soluble forms of manganese (Aschner et al., 2005).

3.1.2 Animals

3.1.2.1 Absorption by the oral route

An early *in vitro* investigation into the absorption of manganese ($MnCl_2$ carrier) through the rat intestinal wall showed that there was an active transport system through the walls of the duodenum, jejunum and ileum, but only simple diffusion through the colon wall (Cikrt and Vostal, 1969).

The effect of dietary intake of manganese from manganese sulphate (12-14 days) on the levels of absorption of an oral dose of ^{54}Mn ($^{54}MnCl_2$) in rats demonstrated that ^{54}Mn absorption decreased with increasing dietary manganese consumption (Abrams et al., 1976). In a study where rats were fed 6 different levels of dietary manganese (1.5 ppm to 100 ppm manganese chloride), the percentage absorption was found to decrease with increasing dietary levels of manganese (Weigand et al., 1986). When the actual intake (mass, µg) of manganese was calculated it was found that this reached a plateau of approximately 25 µg /day at 35 ppm dietary manganese. Increased turnover (excretion) of manganese was also noted in response to the increased dietary manganese.

The toxicokinetics of manganese was investigated in male rats following either a single intravenous (iv) or oral dose of $MnCl_2$ (6.0 mg Mn/kg) (Zheng et al., 2000). Following oral administration of $MnCl_2$ manganese rapidly entered the systemic circulation with a C_{max} of 0.30±0.11 µg/mL (T_{max} = 0.25 h). Since an iv administration was included in the study, the absolute oral bioavailability of Mn from a $MnCl_2$ solution was calculated to be 13.2%.

A model was developed with rats to quantitate endogenous gut losses of manganese in which the parenterally administered isotope was distributed like fed isotope. Young, growing rats fed 45 µg Mn/g diet were calculated to absorb 8.2% of their manganese intake (Davis et al., 1993). The authors reported a problem of coprophagy, which may have compromised the results.

An investigation into the intestinal transport system for both manganese and iron using open-ended loops of rat duodenum, jejunum and ileum found that in iron overload, a similar amount of manganese was absorbed from all 3 sites (Thomson et al., 1971). However, in iron deficiency, manganese absorption was increased from the duodenum and jejunum. In iron deficiency, when iron

was added to the manganese perfusate, it competitively inhibited manganese absorption. Greater amounts of iron than manganese were absorbed from an equimolar mixture. Overall, the authors suggested that in iron overload manganese is absorbed largely by diffusion, whereas in iron deficiency, manganese absorption in the proximal intestine is increased by the enhanced activity of a system that is dose-saturable and can be competitively inhibited by iron.

There are several publications that report the level of manganese absorption from milk in neonatal rats; however the percentage absorbed ranged from 31% to 66% of dose. A comparison of selected data from these studies is given in Table 3.1.2. Unfortunately the level of detail in the methodology reported was very poor in two of the publications, (Keen et al., 1986) and (Kostial et al., 1978a), and even in the third publication it was still relatively brief (Chan et al., 1987). This meant that details such as the levels of background manganese in the human milk and the dams' diets cannot be compared and as such, all three publications have a Klimisch Code of 4. In addition, the study designs vary in some key features such as length of time after dosing before measuring manganese absorption, all of which makes direct comparisons difficult. However, where investigated the common consensus was that:

- The absorption of manganese from human milk by neonatal rats is relatively high, >30%
- The percentage dose absorbed decreased in stages as the age of the rats used increased
- This was reported as a gradual decrease between 10 and 13 days old, a steeper decline between 13 and 20 days old and a drastic decline above 20 days old (Chan et al., 1987).

This increased bioavailability of manganese from milk may be due to two processes: neonatal rats have a high need for manganese due to the increased enzyme production triggered by growth and development, and the amount of manganese in dams milk is low.

Key study design features	**(Keen et al., 1986)**	**(Chan et al., 1987)**	**(Kostial et al., 1978a)**
Klimisch Code	4	4	4
Age of pups at dosing	12 days	9 days	7 days
Pups fasted overnight	Yes (18 hours)	Yes (14 hours)	Not stated
Level of manganese in diet fed dams	Not stated, although it was a purified diet	Regular chow	Stock laboratory diet
Details of ^{54}Mn mixing with human milk	1 hour, temperature not stated	2 hours at room temperature, buffered at pH7.4	Not stated
Level of manganese in human milk	Not stated.	8 µg/L	Not stated
Volume of milk dosed	0.5mL	0.1-0.2 mL	Not stated
Time of sacrifice for manganese measurement	24 hours after dosing	3 hours after dosing	6 days after dosing
How administered dose was calculated and total recoveries	Not stated.	Whole body counting where possible, >98% recovery.	Not stated
Handling of GI tract	Small intestine flushed and perfusate analysed separately.	GI tract removed and analysed separately	Not stated
% Dose absorbed (retained) excluding GI tract	66.3%	31±7%	39.9±1.5% Not stated whether GI Tract was included/excluded.

Table 3.1.2. The level of manganese absorption from milk in neonatal rats (Kostial et al., 1978a; Keen et al., 1986; Chan et al., 1987).

The effects of different levels of dietary manganese on the absorption of a ^{54}Mn tracer in rats showed that the percentage absorption was inversely proportional to level of manganese in diet (Lee and Johnson, 1988). In this extensive and well-designed study, the authors also reported that an oral dose of the ^{54}Mn tracer was absorbed four times higher by fasted rats than in un-fasted rats or in fasted rats fed a test meal. In addition, it was found that sucrose-fed rats absorbed more ^{54}Mn tracer than starch-fed rats. In a further study (Lee and Johnson, 1989) the authors confirmed the trend that the

percentage absorption of manganese was inversely proportional to the level of manganese in diet in rats fed either a soy protein-rich or casein-rich diet.

An investigation into how varying levels of manganese (added to the diet as manganese carbonate, an insoluble form of manganese) and iron in diet affected manganese absorption ($^{54}MnCl_2$) in the rat found that manganese deficient animals retained more of a ^{54}Mn tracer and high iron intake inhibited manganese absorption (Davis et al., 1992b). The rats were fed three levels of manganese (0.9, 48 or 188 µg Mn/g diet) and two levels of iron (19 or 276 µg Fe/g diet) for 7 weeks before being fed ^{54}Mn as a tracer.

Following a subchronic treatment of $MnCl_2$ administration to rats by either ip (6 mg Mn/kg/day) or oral (75 mg Mn/kg/day) for 4 weeks followed by a 2-week rest period, greater concentrations of manganese were found in tissues following ip dosing compared to oral dosing (Missy et al., 2000). If ip dosing is considered to be 100% absorbed, then the average absorption by the oral route was less than 8%.

The effect of the manganese source on manganese absorption by the intestine of broiler chickens found that the ileum was the main site of manganese absorption (Ji et al., 2006). The authors also reported that organic manganese (amino-acid chelates of glycine and methionine) was more efficiently absorbed, 2 to 3-fold, than inorganic manganese ($MnSO_4$), which might be due to different absorption modes for organic and inorganic manganese.

Table 3.1.3. A summary of the oral absorption of ^{54}Mn in rats

Mn vehicle	% dose absorbed	Factors affecting absorption	Reference	Klimisch Code
$MnCl_2$ solution (6 mg Mn/kg b.w.)	13.2% (calculated from iv dose)		(Zheng et al., 2000)	2
45 µg Mn/g diet (from $MnCl_2$)	8.2%	coprophagy, animals fasted for 16 hours prior to isotope dosing	(Davis et al., 1993)	2
$^{54}MnCl_2$ solution whilst on a diet containing $MnSO_4$ at levels from 4-2000 ppm Mn	decreased with increasing Mn levels in diet	Mn level in diet	(Abrams et al., 1976).	2
$^{54}MnCl_2$ from milk	At 3 hours after dosing: 30% (9-day old) 15% (15-day old) 5% (30-day old)	Age – neonatal rats used	(Chan et al., 1987)	2
$^{54}MnCl_2$ tracer in a glycine/saline solution after 2 weeks on controlled dietary manganese to non-fasted rats.	7.8% (2.8 mg/kg diet) 5.0% (6.6 mg/kg diet) 3.3% (12.3 mg/kg diet) 3.4% (19.4 mg/kg diet) 2.2% (23.9 mg/kg diet) 1.1% (43.8 mg/kg diet) 0.5% (82.4 mg/kg diet)	% absorption was inversely proportional to level of manganese in diet.	(Lee and Johnson, 1988)	2
$^{54}MnCl_2$ tracer in a glycine/saline solution after 2 weeks on controlled dietary manganese.	6.8% (2.8 mg/kg diet) NF 24.3% (2.8 mg/kg diet) F 6.9% (2.8 mg/kg diet) FM 2.7% (49.2 mg/kg diet) NF 8.1% (49.2 mg/kg diet) F 2.0% (49.2 mg/kg diet) FM	NF – Non fasted F – fasted overnight FM – fasted overnight but dosed with test meal	(Lee and Johnson, 1988)	2
$^{54}MnCl_2$ tracer in test meal after 2 weeks on controlled manganese and starch or sucrose diet	2.2% (4.1 mg/kg diet) ST 5.3% (4.1 mg/kg diet) SU 0.9% (45.3 mg/kg diet) ST 1.9% (45.3 mg/kg diet) SU	ST – a 65% starch diet SU – a 65% sucrose diet	(Lee and Johnson, 1988)	2
$^{54}MnCl_2$ tracer in test meal after 1 week on controlled manganese and soy protein or casein diet	3.8% (2.8 mg/kg diet) SP 2.6% (2.8 mg/kg diet) CA 1.0% (52.8 mg/kg diet) SP 2.3% (52.8 mg/kg diet) CA	SP – a soy protein-rich diet CA – a casein-rich diet	(Lee and Johnson, 1989)	2
Tracer level of $^{54}MnCl_2$ in diets with varied levels of manganese and iron.	57.3% LMnMFe 27.3% LMnHFe 21.3% AMnMFe 17.8% AMnHFe 20.3% HMnMFe 6.4% HMnHFe	Manganese and iron level in diet, Key: LMn = Low level of manganese AMn = Adequate level of manganese HMn = High level of manganese MFe = Marginal level of iron HFe = High level of iron	(Davis et al., 1992b)	2
$MnCl_2$ solution (75 mg Mn/kg b.w./day)	Less than 8% average.		(Missy et al., 2000)	2

3.1.2.2 Absorption by the inhalation route

The distribution of manganese in the olfactory bulb, olfactory tubercle and striatum of rats was measured after an intranasal injection of manganese chloride (Gianutsos et al., 1997). Significantly elevated levels of manganese in the tissues tested were found for dose administrations above 200 µg manganese. The authors attributed this to the transport of manganese along olfactory neurons as a result of this method of administration.

Following dosing to the olfactory chambers of pikes of $^{54}MnCl_2$, $^{54}Mn^{2+}$ was taken up in the olfactory receptor cells and was transported at a constant rate along the primary olfactory neurones into the brain (Tjalve et al., 1995). The authors concluded that it appeared that manganese has the ability to pass the synaptic junctions between the primary and the secondary olfactory neurons in the olfactory bulbs and to migrate along secondary olfactory pathways into the telencephalon and the diencephalons in pike. Therefore, it was concluded that the olfactory route might be a crucial pathway by which manganese gains access to the brain.

Further studies by the same workers focused on the uptake of manganese ($^{54}MnCl_2$ used for dosing) from the nasal mucosa into the central nervous system via olfactory pathways in rats (Tjalve et al., 1996; Henriksson et al., 1999). By comparison with an equivalent ip dose of $^{54}MnCl_2$, the authors concluded that the results appeared to show that the uptake of manganese in the olfactory bulbs after nasal instillation in all probability occurred via the primary olfactory neurons and not via the circulation.

The absorption of intranasally instilled ^{54}Mn from a tracer dose of $^{54}MnCl_2$ was significantly reduced in Belgrade rats and was enhanced in iron-deficient rats compared to iron-sufficient controls (Thompson et al., 2007). The Belgrade rat is anaemic and displays significant defects in both iron and manganese metabolism due to a glycine-to-arginine substitution (G185R) in their divalent metal transporter-1 (DMT1) gene product. This work suggested that the DMT1 is involved in uptake of inhaled manganese.

Rats were exposed (inhalation) for 6 h/day for 7 days/week (14 exposures) to manganese sulphate ($MnSO_4$) at 3 different dose levels (Dorman et al., 2001a). The mass median aerodynamic diameter (MMAD) of the aerosols generated were approximately 1.5-2.0 µm with a standard deviation of <2 µm. Significant increases in the manganese concentration in the lungs were found following this repeated short-term exposure at the 0.3 and 3 mg Mn/m^3 exposure levels but not at the 0.03 mg Mn/m^3 compared to controls.

Following a subchronic (13-week) inhalation exposure of young male rats to manganese sulfate (MMAD 1.85-2.03 µm) and manganese phosphate (MMAD 1.47 µm) some nasal toxicity (irritation) was seen (Dorman et al., 2004b). An estimation of the actual quantity of manganese delivered to the olfactory epithelium following 6 hour/day inhalation exposures to 0.5 Mn/m^3 atmosphere was 2.3 µg/day or a total of 0.15 mg over the total exposure period.

The ^{54}Mn absorption from the lungs to the blood after an intratracheal instillation of $^{54}MnCl_2$ tracer was lower in rats fed a high iron diet compared to age-matched controls (Thompson et al., 2006). The authors concluded that diminished transport of manganese across the air-blood barrier indicates that this metal is taken up by an iron-responsive mechanism and clearly demonstrated that the pathway of pulmonary manganese absorption can be down-regulated under iron-loading conditions.

There were several other publications that looked at the inhalation of manganese sulphate and manganese tetraoxide and manganese phosphate in rats (Vitarella et al., 2000b; Dorman et al., 2001a). However these studies were more focused on the delivery and distribution of manganese than the absolute absorption and as such, there was no quantitative data to extract.

3.2 Manganese oxides, other insoluble manganese salts and manganese metal and ore

3.2.1 Humans

3.2.1.1 Oral

There was no data found detailing the human oral absorption of manganese from manganese oxides, other insoluble manganese salts or manganese metal and ore.

3.2.1.2 Inhalation

An early study reported the GI tract was the main port of entry for absorption of manganese from a human inhalation of either a nebulised solution of $^{54}MnCl_2$ or a nebulised suspension of $^{54}Mn_2O_3$ (Mena et al., 1969).

A positive correlation of blood manganese concentrations to increased hand tremor related to manganese exposure in workers from manganese-alloy producing plants was shown (Bast-Pettersen et al., 2004). One hundred male manganese-alloy plant workers were age matched to referents from plants with similar working conditions to manganese-alloy plants.

Table 3.2.1 Air, blood and urine manganese concentrations of workers in industrial plants producing ferro-manganese and silico-manganese. (Bast-Pettersen et al., 2004)

	Exposed workers	**Referent workers**
Inhalable manganese	Range 0.009-11.46 mg Mn/m^3 Mean 0.753 mg Mn/m^3	
Respirable manganese	Range 0.009-0.356 mg Mn/m^3 Mean 0.064 mg Mn/m^3	
Inhalable manganese (soluble)	Range 0.009-9.00 mg Mn/m^3 Mean 0.570 mg Mn/m^3	
Respirable manganese (soluble)	Range 0.002-0.3520 mg Mn/m^3 Mean 0.049 mg Mn/m^3	
Blood manganese	Range 4.6-23.5 µg Mn/L Mean 10.4 µg Mn/L	Range 4.0-20.7 µg Mn/L Mean 9.2 µg Mn/L
Urinary manganese	Range 0.05-61.4 µg Mn/g creatinine Mean 1.90 µg Mn/g creatinine	Range 0.05-6.37 µg Mn/g creatinine Mean 0.44 µg Mn/g creatinine

When the exposed subjects were stratified into low, medium or high blood manganese levels (<8.7 ug/L, 8.7-11.2 ug/L and >11.2 ug/L), then the high blood manganese subjects showed statistically (P<0.001) more tremor than the age matched referents on the Static Steadiness test.

The correlation of blood and urine manganese concentrations to estimated inorganic manganese exposure was investigated in 141 male subjects working in a manganese oxide and other salt (manganese sulphate, nitrate and carbonate) producing plant (Roels et al., 1987a; Roels et al., 1987b). The airborne dust had between 0.07 to 8.61 mg Mn/m^3 with a geometric mean of approximately 100-fold greater than control samplings and the 95th percentile was 3.30 mg Mn/m^3. The average blood manganese concentration was twice as high in the exposed workers compared to a control group. The responses to a hand-eye eye-hand coordination test suggested the existence of a threshold for the neurological response which was associated with manganese blood concentrations of >10 µg Mn/L of whole blood. The average urine manganese concentration was ten times as high in the exposed workers compared to a control group. On a group basis, urine manganese concentration appeared to reflect very recent exposure, whilst blood manganese concentration was to some extent a reflection of the body burden of manganese. The authors also concluded that a time-weighted average exposure to airborne manganese dust (total dust) of about 1 mg/m^3 for less than 20 years might present preclinical signs of intoxication.

Table 3.2.2 Air, blood and urine manganese concentrations of workers in an industrial plant producing manganese oxides and salts (Roels et al., 1987b).

	Exposed workers (141)	**Control workers (104)**
Air-borne manganese (total dust)	Range 0.07-8.61 mg Mn/m^3 Mean 1.33±0.14 mg Mn/m^3 Median 0.97 mg Mn/m^3	Range 0.002-0.052 mg Mn/m^3 Mean 0.012 mg Mn/m^3 Median 0.007 mg Mn/m^3
Blood manganese	Range 1.0-35.9 µg Mn/L Mean 13.6±6.4 µg Mn/L	Range 0.4-13.1 µg Mn/L Mean 5.7±0.27 µg Mn/L
Urinary manganese	Range 0.06-141 µg Mn/g creatinine Mean 4.76 µg Mn/g creatinine	Range 0.01-5.04 µg Mn/g creatinine Mean 0.30 µg Mn/g creatinine

Workers from manganese ore milling and dry-cell battery manufacturing plants were studied to assess the extent of absorption and exposure to manganese dioxide (Gan et al., 1988). This was a relatively old study and lacked detailed methodology (Klimisch Code 4), however it contains very relevant human data. Although over half of the samples collected having values exceeding a TLV of 5 mg/m^3, analysis of the crushed MnO_2 revealed that the mass median diameter (MMD) was in the range 12.53-55.73 µm, which meant that although the dust was inhalable, it was likely non-respirable and would be subject to clearance by the mucocilliary elevator mechanism.

3.2.2 Animals

3.2.2.1 Oral

Rats that received a single oral dose of MnO_2 at 24.3 mg Mn/kg b.w. showed both a significant delay (96 to 120 hour) and lower concentration (27%) of the blood manganese C_{max} compared to a single oral dose of $MnCl_2$ at the same dose level (Roels et al., 1997). The authors concluded that the time course of blood manganese after oral administration of MnO_2 most likely reflected a slow and limited transfer of Mn^{2+} from the GI tract into the circulation. The authors reported a low oral bioavailability of manganese from MnO_2 that could also be due to the oxide not being resorbed by the GI tract and instead being directly excreted in the faeces.

3.2.2.2 Inhalation

Rats were exposed (inhalation) for 6 h/day for 7 days/week (14 exposures) to either manganese phosphate in the mineral form hureaulite ($Mn_5(PO)_4)_2[(PO_3)(OH)]_2 \cdot 4H_2O$) or manganese tetraoxide (Mn_3O_4) at 3 different dose levels (Vitarella et al., 2000b). The mass median aerodynamic diameter (MMAD) of the aerosols generated were approximately 1.5 µm with a standard deviation of <1.5 for the manganese phosphate and 1.5-2.0 µm with a standard deviation of <2 for the manganese tetraoxide. Significant increases in the manganese concentration in the lungs were found following this repeated short-term exposure at the 0.3 and 3 mg Mn/m^3 exposure levels but not at the 0.03 mg Mn/m^3 dose level compared to controls. The increase in the concentration of manganese in the lungs from rats exposed to the insoluble manganese phosphate and tetraoxide over rats exposed to manganese sulphate in the same study (see Section 3.1.2.2) was due to the more soluble manganese sulphate being cleared from the rat lung more rapidly that the insoluble forms.

The manganese level in the lungs from rats exposed to a metallic manganese aerosol was dramatically less than rats exposed to similar levels of manganese phosphate or manganese phosphate/sulphate aerosols (Normandin et al., 2004). The rats were exposed for 6 hour/day, 5 days a week for 13 weeks to aerosols with 80% of particles less than 1.55 µm diameter.

Table 3.2.2 Concentration of manganese in selected tissues (µg Mn/g) from rats following 13 weeks inhalation exposure to three chemical forms of manganese (Normandin et al., 2004).

	Control	**Metallic Manganese**	**Manganese Phosphate**	**Manganese Phosphate/Sulphate mixture (61:39)**
Particle size		90% <1.55 µm	80% <1.55 µm	80% <1.55 µm
Exposure concentration (µg Mn/m^3)	0.30±0.02	3750±846	3208±481	2841±529
Olfactory Bulb	0.66±0.27	Not Measured	2.32±1.22[a]	2.30±0.20[a]
Globus Pallidus	0.61±0.19	0.93±0.06[a]	1.25±0.23[a, b]	1.51±0.47[a, b]
Striatum	0.49±0.08	0.86±0.04[a]	1.06±0.14[a, b]	1.21±0.09[a, b, c]
Cerebellum	0.56±0.14	0.63±0.04	0.73±0.05[a, b]	0.84±0.08[a, b, c]
Lungs	0.18±0.03	0.29±0.07[a]	9.86±3.77[a, b]	6.21±1.85[a, b]
Testis	0.31±0.04	0.30±0.03	0.36±0.05[a, b]	0.42±0.02[a, b, c]

[a-] significant difference with the control group (P<0.05)
[b-] significant difference with the group exposed to metallic Mn (P<0.05)
[c-] significant difference with the group exposed to manganese phosphate (P<0.05)

The finding that the level in manganese in lungs from the rats exposed to metallic manganese was less than 2-fold greater than control animals compared to 30 to 50-fold for the manganese salts was not discussed by the authors. This data suggests that the particles were likely cleared rapidly by the mucocilliary escalator and were therefore unavailable for absorption via the lung. The mucocilliary clearance of this particle could be relatively efficient versus the other forms where their solubility is

sufficient to allow for pulmonary absorption. However the manganese content of the olfactory bulb for the metallic manganese exposure group was not measured (no reason given) and so this could not be substantiated. Whilst it may initially appear that exposure to manganese from metallic manganese might be substantially less than from manganese salts, the necessary evidence to verify this was unfortunately not presented in this study.

The olfactory neuronal pathway was shown to be efficient for translocating inhaled manganese oxide as solid ultrafine particles (UFPs) to the central nervous system of groups of rats (Elder et al., 2006). Similar manganese burdens (approximately 8.2% of dose) were found in the olfactory bulbs of rats 24 hours after intranasal instillation of similar quantities (5-7 mg manganese) of either a manganese chloride solution or a suspension of UFP manganese oxide. The manganese oxide UFPs were composed of particles of 3-8 nm in diameter forming an aerosol with agglomerates of approximately 30 nm.

Feral pigeons (*Columba livia*) were exposed to a low level (0.24 mg Mn/m^3) of manganese oxide dust (98% of particles < 1 μm diameter) for 7 hours/day, 5 days/week for up to 13 weeks (Sierra et al., 1998). Significant increases ($P \leq 0.05$) in tissue manganese concentrations in the brain (0.59±0.02), lung (0.58±0.16) and bone (3.02±0.29) in the exposed birds compared to controls were seen after 13 weeks (0.46±0.07, 0.19±0.05 and 2.72±0.44 respectively).

3.2.3 Summary and discussion of absorption data

The oral absorption of manganese is considerably influenced by the body's natural homeostatic regulation of manganese together with a number of factors that affect the GI tract absorption of manganese. Taking these into consideration the following summary points can be made:

- Human oral absorption of manganese from levels typically in the range encountered in our diet, are likely to be low, below 5%, in healthy subjects where their natural homeostatic regulation of manganese is operating normally.
- Individuals with iron deficiency (low serum ferritin) are likely to absorb greater amounts.
- The source of manganese, i.e. as a $MnCl_2$ solution (higher) or as in food (lower), whether the subject has been fasting or on a low manganese diet (both higher) are all likely to influence the level of absorption.
- The levels of other dietary constituents, particularly other transitional mineral nutrients such as iron (heme and nonheme), zinc as well as calcium affect manganese absorption levels. Since manganese and iron apparently share common bonding sites in the intestinal mucosa and are both transported by transferrin in the blood, it is likely that an antagonistic relationship exists between iron and manganese affecting their bioavailability.
- The absorption of manganese from the sulphate or acetate salts are likely to be lower than for the chloride salt (Bales et al., 1987)

Data showing the human absorption of manganese from very high doses are not available. However animal studies showed the proportion of dose absorbed decreased with increasing dose. Therefore, it can reasonably be expected that this would also apply to humans.

No data were found detailing the human oral absorption of manganese from manganese oxides, other insoluble manganese salts or manganese metal and ore. As such, an extrapolation to the human situation would be that manganese absorption from manganese oxides, other insoluble manganese salts or manganese metal and ore are likely to be equivalent or lower than from the soluble salts.

The oral absorption of manganese in rats is very rapid and a wide range of absorption percentages has been reported. In addition to the various homeostatic and other factors discussed for humans, the age of the rats also makes a substantial difference to the absorption of manganese. In particular, the oral absorption of manganese from milk in neonatal rats is relatively high, >30% (Kostial et al., 1978a; Keen et al., 1986; Chan et al., 1987). This high oral absorption for young rats does appear to decrease with age which may be a result of several factors, such as, very young rats having a too low body burden of manganese for the body's natural growth and processes (milk is very low in manganese compared to diet) or the GI tract of the very young rats may not be fully functional. Although it is possible that human infants may also be susceptible to higher absorption levels for manganese, other than one study examining longitudinal manganese and copper balances (Dorner et al., 1989) there is very little information available. The absolute oral bioavailability of manganese in adult rats from a manganese chloride solution (6.0 mg Mn/kg) was calculated to be 13.2% by comparison with an iv dose (Zheng et al., 2000).

The three potential routes of entry for manganese by inhalation are (Roth, 2006):

- Through the nasal mucosa
- Transport across the pulmonary epithelial lining and subsequent disposition in lymph/blood
- Clearance from the lung by mucocilliary elevator and subsequent ingestion from the GI tract

However, the relative proportion absorbed by each process is not accurately known.

There appears to be no reported studies detailing the percentage absorption of manganese in humans via inhalation, although it is expected that absorption of manganese from the lungs would be higher for the more soluble forms of manganese (Aschner et al., 2005). However, since the majority of toxic effects seen in humans are linked to inhalation through occupational exposure, typically workers involved in steel production or in the mining and processing of manganese ores, then the importance of manganese exposure by inhalation cannot be understated.

Although it may not be possible to directly measure the proportions of dose absorbed by inhalation, it is possible to compare the relative levels of absorption from different sources of manganese by measurement of the resulting tissue levels (Roels et al., 1997; Dorman et al., 2001a; Dorman et al., 2004a). The manganese tissue levels are covered in more detail in Section 4. Overall the many studies confirm the following:

- soluble forms of manganese are more readily absorbed than insoluble ones
- nasal absorption of manganese by rats is also an important pathway of absorption
- the absorption of ultrafine particles (UFPs) of an insoluble form of manganese can be of the same magnitude to a soluble form of manganese (Elder et al., 2006)

It is possible that the absorption of manganese from a metallic manganese aerosol by inhalation in rats may be substantially less than for manganese salts, however the data is unsubstantiated.

An estimation of the actual quantity of manganese delivered to the olfactory epithelium of a rat following a 6 hour/day inhalation exposure to 0.5 mg Mn/m^3 atmosphere was 2.3 µg/day or a total of 0.15 mg over the total 13-week exposure period (Dorman et al., 2004b).

Table 3.2.3 Summary of the absorption of manganese

		Human	Rat
		Oral	Oral
Soluble Manganese Salts	$MnCl_2$	~5%[a]	13.2%[e]
	$MnSO_4$	2±1%[b]	ND[c]
	$Mn(NO_3)_2$	ND[c]	ND[c]
Insoluble Salts	MnS	ND[d]	ND[d]
	$MnCO_3$	ND[d]	ND[d]
Manganese Oxides	MnO	ND[d]	ND[d]
	MnO_2	ND[d]	ND[d]
	Mn_3O_4	ND[d]	ND[d]
Manganese metal, sinter ore and slags	Mn	ND[d]	ND[d]
	FeMn	ND[d]	ND[d]
	SiMn	ND[d]	ND[d]

Factors that decrease oral absorption	**Factors that increase oral absorption**
Repeat dosing	Overnight fasting before dosing
High existing manganese body burden	Low existing manganese body burden
High dietary levels of manganese	Low dietary levels of manganese
High dose levels of manganese	Low dose levels of manganese
Age (older)	Age (younger)
High starch diet	High sucrose diet
Diet rich in iron	Low iron status (anaemic)
Administered with food	Administered as solution
Less soluble salts	More soluble salts
	Manganese previously excreted in bile

Key:
a- Individuals with iron deficiency (low serum ferritin) are likely to absorb greater amounts
b- For non-fasted group, whereas 9±3% for fasted groups (Sandstrom et al., 1987)
ND[c]- No data found, however expected to be similar or lower than other soluble salts
ND[d] – No data found, however expected to be lower than soluble salts
e - (Zheng et al., 2000), however depending on age of rats and existing levels of manganese, values up to 66% absorption have been reported (Keen et al., 1986)

4 DISTRIBUTION, METABOLISM AND TRANSPORTATION OF MANGANESE

4.1 Manganese soluble and insoluble salts

In order to measure the distribution of manganese in the body there are several common methods employed. The use of radiolabelled ^{54}Mn for humans has been discussed previously in the absorption section of this report and relies on whole body counting. It is not possible to pinpoint the exact location of radioactivity using this technique, for example to an individual tissue, but it can identify regions within the body. However, this short-coming is overcome in animal experiments using radiolabelled ^{54}Mn, whereby tissues and organs can be separated at necropsy and sub-samples of the tissues and organs can be processed for measurement of the radioactivity.

The concentration of manganese (non-radiolabelled) in samples of blood, serum, plasma, hair and excreta in humans and animals can be readily measured after sample processing using atomic absorption spectrometry or neutron activation. Additionally the manganese concentration of animal tissues taken at necropsy can be analysed. Interpretation of these results rely on the assessment of background levels of manganese; these may be obtained from historical data, control groups or assessment of manganese levels in the same subjects pre-administration or re-assessment at time periods after the cessation of treatment.

The third method for assessing manganese distribution is a qualitative method that exploits the paramagnetism of manganese using magnetic resonance imaging (MRI). This is a non-invasive technique and can produce images of the soft tissue *in vivo* in both humans and animals. A T1-weighted pulse sequence utilises manganese's reduction of proton spin-lattice relaxation times, a measure of the rate at which hydrogen nuclei (protons) return to thermal equilibrium after being perturbed in a strong magnetic field. This technique can clearly distinguish between several separate and specific regions of the brain since the spin-lattice relaxation time, T1, is strongly influenced by tissue characteristics such as water and fats content. Manganese is not the only trace metal that could be responsible for these observations (shortening of T1); other paramagnetic metals such as zinc, iron, copper and magnesium can also show the same effects. This technique has recently been expanded to use MRI, pallidal index (PI), and T(1) relaxation rate (R1) in concert with chemical analysis to establish a direct association between MRI changes and pallidal manganese concentration in rhesus monkeys following subchronic inhalation of manganese sulfate (Dorman et al., 2006c).

It is likely that manganese is metabolised by the body in the form of converting manganese from the Mn^{2+} valence state to the Mn^{3+} valence state (Gibbons et al., 1976). The authors proposed that ingested manganese is absorbed as Mn^{2+} possibly bound to alpha2-macroglobulin or albumin. In transversing the liver it is removed nearly quantitatively. However, a small proportion is oxidised to the Mn^{3+} valence state, bound to transferrin and enters the systemic circulation to be transported to tissues. More recently, it has been proposed that the oxidation state of manganese exposure may be an important determinant of the toxicokinetics of manganese and tissue toxicodynamics and subsequently neurotoxicity (Reaney et al., 2006).

4.1.1 Humans

Six members of one family were all reported as having encephalitis-like symptoms; however when investigated further encephalitis was discounted and intoxication with manganese was found to be the cause (Kawamura et al., 1941). Although a very old study, Klimisch Code 4, the descriptions and investigations seem adequate. Old dry battery cells (over 300) from bicycle lamps had been buried near a well starting in August and by the November intoxication cases of local inhabitants were manifest. Drinking from the suspected well waters was prohibited in the latter part of December and the intoxication ceased to appear in the beginning of January. Prior to the removal of the dry batteries the well waters had contained approximately 14 mg/L manganese. Autopsy tissues taken showed

raised levels of manganese in liver (about three-fold compared to control) and blood (about three-fold compared to control), and slight elevations in kidneys and urine. Raised levels of zinc were also found, although the authors concluded that the intoxication symptoms were more consistent with manganese poisoning rather than zinc poisoning. The use of water contaminated with manganese at approximately 14 mg/L for drinking and cooking appears to have been at a sufficiently high level to produce a sub-acute dose, which must have overwhelmed the homeostatic regulation of manganese absorption. Removal of the source of contamination appeared to prevent further immediate cases of intoxication; however, longer-term monitoring is not recorded.

The hair samples of >50 year old males and females who had over 1000-fold different concentrations of manganese in their drinking water (low level <15 ug/L, high level >1800 ug/L) showed significant differences in the concentration of manganese in their hair (Kondakis et al., 1989). Blood manganese concentrations were around 15-18 ug/L and did not show any significant differences between the drinking water groups. Unfortunately there was no detail on actual consumption levels of manganese from combined water and food. As such, it is impossible to actually estimate total oral consumption of manganese and thus correlate with study findings. However, the authors concluded that these results indicated that progressive increases of manganese concentration in drinking water are associated with a progressively higher prevalence of neurological signs of chronic manganese poisoning (CMnP) and manganese concentration in hair of older people. Whilst it may not be possible to relate the hair manganese concentrations to absorbed dose, the analysis of hair samples for manganese may be a useful marker for manganese exposure. It has to be kept in context that an elevated manganese content of hair needs to be adequately distinguished above the variable baseline levels of manganese in the hair of a suitable control group.

When interpreting the manganese levels in hair, sufficient care must be given to distinguish between exogenous (from the environment) and endogenous (systemic circulation) sources. This was investigated with the hair from Aboriginals and Caucasians from a small township on the Australian island of Groote Eyandt (Stauber et al., 1987). Although an old study, Klimisch Code 4, the descriptions and investigations seem adequate. Manganese ore is mined through open-cut strip mining on Groote Eyandt and at this time there were high environmental levels of manganese, e.g. road-side dust (4% manganese), air dust (1.7% manganese), local water (2 mg/L manganese) (Cawte and Florence, 1987). Analysis indicated that the high manganese levels in the hair from Caucasians were from exogenous sources and the high manganese levels in the hair from Aboriginals were from both exogenous and endogenous sources. This finding correlated with the Groote Eyandt Aboriginal population having a high incidence of neurological disturbances referred to as the Groote Eyandt Syndromes (Cawte, 1984). The use of toenail clippings as a marker of manganese exposure was investigated with workers exposed to manganese during Mn-alloy production (Zaprianov et al., 1985). However, this publication contained relatively brief details (Klimisch Code 4) and this approach does not appear to have been followed up by other workers.

A 66-year-old male patient who ingested 125 ml of a 8% solution of potassium permanganate (10 g) within 4 weeks developed psychological alterations and neurological examination revealed disturbances of many subsystems of the CNS (Holzgraefe et al., 1986). An ingestion of 10g potassium permanganate equates to 3.5 g of manganese, which over 4 weeks would be equivalent to 124 mg Mn/day (~1.8 mg Mn/kg/day). Blood and hair manganese levels were determined upon administration into hospital and again 14 years later, although the publication was only a brief report and therefore a Klimisch Code of 4 was assigned (Bleich et al., 1999). However, it was interesting to note that although the blood manganese levels had returned to normal levels the hair scalp levels were increased.

Table 4.1.1 Blood and scalp hair manganese levels after chronic manganese ingestion, approximately 124 mg Mn/day for 4 weeks (Holzgraefe et al., 1986; Bleich et al., 1999).

	Reference level	**After 4-weeks administration**	**14 Years later**
Blood (µg/L)	7.1-10.5	150	4.8
Scalp hair (µg/g)	0.07-1.00	1.6	2.79

An autopsy on a patient who had suffered from chronic manganese poisoning (CMP) showed no elevation in average concentration of manganese in the brain, although there were some changes in its distribution (Yamada et al., 1986). However, the patient had retired from working in a manganese ore crushing plant some 5 years previously, and had been admitted to hospital the following year where he was diagnosed with CMP. His manganese levels were elevated in urine 104 ug/L (normal <20 ug/L) and blood 34 ug/L (normal 4-20 ug/L) and, as such, he was started on an iv infusion of ethylene diamine tetraacetic acid (EDTA). Although this didn't improve the symptoms, it resulted in a marked urinary excretion of manganese (564 ug/L). The patient died of gastric cancer 4 years later, whereupon an autopsy was performed. Although the authors used samples from 4 other control cases and one of a Parkinson's disease sufferer, none of these would have undergone chelation with EDTA and, as such, direct comparisons should be treated with caution.

A 3 year old child was admitted to a Paediatric Neuropsychiatry Unit due to epileptic seizures which were eventually linked to manganese poisoning (Hernandez et al., 2003). The publication was only a case report and therefore a Klimisch Code of 4 was assigned. The boy's father had been electrode-welding a long balcony banister over a period of 1 month and the boy had stayed beside the father during the welding. As such, the authors deduced that the manganese poisoning was due to welding fumes inhalation. High blood manganese levels (15 to 20 ug/L) were recorded and also high urinary manganese levels (>10-fold above the normal range). After several treatments with a chelating agent ($CaNa_2EDTA$), the seizure frequency dramatically decreased and the blood manganese levels reduced. Although Hernandez et al. linked the child's symptoms and high manganese blood and urine levels to exposure from welding, a recent review of whether electric arc welding was linked to manganism found that welders were not at high risk of apparent clinical damage from exposure to manganese (McMillan, 2005). This review (Klimisch Code 5) appears to have only studied adult welders and perhaps the age of the child in the Hernandez report may have been significant and, as such, it is possible that young children may be at greater risk of manganese intoxication than adults.

An adult patient undergoing haemodialysis for chronic renal failure secondary to adult polycystic kidney disease, received an excessive iv dose of manganese from a dialysate solution that had been contaminated with manganese (Taylor and Price, 1982). The patient's serum manganese levels were 250, 94 and 36 ug/L at 2, 3 and 6 days after administration, compared to a background level of 5-10 ug/L.

An adult patient receiving long-term total parenteral nutrition (TPN), which included the addition of manganese as an essential trace element, was admitted to hospital with gait disturbance (Ejima et al., 1992). The publication was only a letter of a case report and therefore a Klimisch Code of 4 was assigned. The dosage of manganese was 2.2 mg/day for a total of 23 months (total dosage was estimated to be 1.5g). The publication does not state the patient's bodyweight, however, if a bodyweight of between 50 and 80 kg is assumed for an adult, then this equates to a dosage of between 28-44 ug/kg b.w./day. His whole blood manganese was 30-56 ug/L (compared to a normal range quoted as 4-20 ug/L) although his serum and plasma manganese concentrations had never been above the normal range. The authors concluded that he had parkinsonism and performed an MRI scan which revealed symmetrical high-signal intensity on T1-weighted images in the basal ganglia which is interpreted to demonstrate presence of manganese, especially globus pallidus, tectum, and tegmentum of midbrain and pons. These regions were not enhanced by gadopentetate dimeglumine; not did they show changes on T2-weighted images. At 15 weeks after the cessation of the manganese supplementation the whole blood manganese concentration had fallen to 5 ug/L and the high intensity of basal ganglia and brainstem on MRI had decreased and was much decreased by 22 weeks, although the globus pallidus retained some hyperintensity.

The T1-weighted MRI scans from the brain of an adult female patient receiving long-term TPN for 3 years were compared to scans 1 year after removal of manganese from the TPN regimen (Mirowitz and Westrich, 1992). The publication was only a case report and therefore a Klimisch Code of 4 was assigned. The basal ganglial signal intensity alterations showed a complete reversal after discontinuation of parenteral manganese administration.

Short-term administration of manganese by TPN to adults from a trace element preparation which contained manganese at approximately 1.1 mg per 2 mL of daily dose showed increased blood manganese concentrations (after 15 days) and elevated signal intensities on T1-weighted MRI scans (Orimo and Ozawa, 2001). The publication was only a short communication and therefore a Klimisch Code of 4 was assigned. The MRI lesions, in particular in the bilateral globus pallidus, were rated as severe in some cases. The degree of the high signal-intensities appeared to be related to blood manganese concentration.

The long-term administration of manganese by TPN to children has been associated with hypermanganesaemia, cholestasis and increases in signal intensities on T1-weighted MRI scans (Reynolds et al., 1994; Azaz et al., 1995; Fell et al., 1996). The publications by Reynolds et al. and Azaz et al. were only brief reports and have been assigned a Klimisch Code of 4. However, it should be noted that the cause and effect between high blood manganese concentrations and hepatic disease was difficult to distinguish in these cases – does liver disease cause hypermanganesaemia (95% of manganese excreted in bile) or vice versa (Fell et al., 1996)? When the levels on manganese administration was reduced or completely withdrawn, generally the blood manganese levels and other changes reduced. All the authors were agreed that the recommended levels of manganese in TPN should be reduced.

A 2 year old child who had been receiving TPN for over a year started to show generalised tonic seizures several times a week and also had mild psychomotor retardation. MRI revealed symmetrical areas of hyperintensity in the bilateral globus pallidus, thalamus, tegmentum of the mid-brain, pons and cerebellar white matter on T1-weighted images (Komaki et al., 1999). The publication was only a short communication and therefore a Klimisch Code of 4 was assigned. The child had received 1.1 mg of manganese daily (82 ug/kg b.w. /day) from 8 months of age and had a blood manganese level of 97 ug/L (a normal level was quoted as below 25 ug/L). Three months after withdrawal of manganese administration, the blood manganese level evolved to normal, and seizure, tremor and MRI abnormalities completely disappeared without using antiepileptic drugs.

These various examples of possible manganese poisoning following manganese administration by TPN raise a number of key points:

- Signs of CMnP were seen from around 30-80 ug/kg b.w. /day manganese by TPN.
- Blood manganese was raised, presumably reflecting total body burden.
- MRI scans showed T1-weighted images in basal ganglia especially globus pallidus.
- After cessation of manganese administration, whole blood manganese decreased back to normal range and high intensity of basal ganglia decreased.

In addition, it should be remembered that when a patient needs to receive TPN,

- the patient is also likely to have an impaired liver function which may well decrease the effectiveness of biliary excretion of manganese,
- this combined with a route of administration which bypasses the manganese absorption process (from the GI tract),

means that the body's natural homeostatic regulation of manganese is likely to be considerably compromised, particularly if the level of manganese presented is far greater than is needed.

4.1.2 Animals

4.1.2.1 Distribution following oral, ip and iv routes

In an early study investigating the excretion (turnover) rate of a ^{54}Mn tracer administered ip ($^{54}MnCl_2$) to groups of mice, the authors proposed that manganese was distributed into at least 2 sets of compartments (Britton and Cotzias, 1966). As the study was very old it only had a limited amount of detail and was assigned a Klimisch Code of 4. At 2 hours after dosing they proposed that the manganese was located primarily in the parenchymatous organs and was thus readily excretable. Yet at 56 days after dosing, the manganese was distributed further into "the carcass," probably the bone.

Another early study investigating the interdependence of the biliary excretion of manganese (^{54}Mn tracer) in the rat (Klaassen, 1974) reported the following key findings at 2 hours after administration:

- The highest concentration of manganese was in the liver (>60-fold higher than plasma);
- Kidney, heart and bone also had relatively high concentrations of manganese compared to that in plasma;
- Only a small decrease in manganese concentration in bone was observed between 2 hours and 5 days after administration.

However, as the study was very old it only had a limited amount of detail and was assigned a Klimisch Code of 4.

An investigation into the binding of manganese ($^{54}MnCl_2$) to bovine and caprine blood constituents (Gibbons et al., 1976) had the following key findings:

- $^{54}Mn^{2+}$ bound to transferrin after incubation with fresh serum, but would not bind to purified transferrin *in vitro* without the presence of an oxidizing agent;
- A $^{54}Mn^{3+}$-transferrin complex was removed much more slowly (half-life of about 3 hours) from cow's blood (*in vivo*) than either free Mn^{2+} or a Mn^{2+} alpha2macroglobulin complex.

The authors proposed the following hypothesis to help explain the homeostasis of manganese: "ingested manganese is absorbed as Mn^{2+} possibly bound to aplha2-macroglobulin or albumin. In transversing the liver it is removed nearly quantitatively, but a small proportion is oxidised, bound to transferrin and enters the systemic circulation to be transported to tissues."

The distribution of a ^{54}Mn maleate tracer following a single ip injection in the rat was described as arbitrarily divided into four groups on the basis of uptake and turnover (Dastur et al., 1969). The study was very old; it only had a limited amount of detail and was assigned a Klimisch Code of 4. The highest concentrations of radioactivity were in the adrenal glands, pituitary, liver and kidneys, the second group was the gastrointestinal system, heart lung and spleen and the third group which reached peak activity on the fourth day was the residual carcass (bone, muscle, skin and hair). The final group was the CNS consisting of the cerebrum, cerebellum and spinal cord, which showed a gradual increase in radioactivity from day 13 to day 34. A further study by the same workers which followed the distribution of a ^{54}Mn maleate tracer following a single ip injection in the monkey was described as arbitrarily divided into three groups on the basis of uptake and turnover (Dastur et al., 1971). The study was very old; it only had a limited amount of detail and was assigned a Klimisch Code of 4. The first group consisting of the very cellular and glandular structures showed the highest peak concentrations of radioactivity within two days of dosing. The second group consisting of the heart, lungs, bone, muscle, skin and hair showed a lower peak concentration of radioactivity. The third group consisting of the CNS showed a steady trend of increasing radioactivity from day 7 to day 278.

The distribution of manganese from an iv injection of $^{54}MnCl_2$ to pregnant rats indicated that ^{54}Mn crossed the feto-maternal barrier (Kaur et al., 1980). This was an old study; it only had a limited amount of detail and was assigned a Klimisch Code of 4. The localisation of ^{54}Mn in liver and brain of the embryo was deemed highly significant.

Another study investigating the distribution of manganese from iv injections of $^{54}MnCl_2$ to pregnant rats at different stages of gestation, confirmed that ^{54}Mn crossed the feto-maternal barrier (Onoda et al., 1977). This was an old study; it only had a limited amount of detail and was assigned a Klimisch Code of 4. The concentration of manganese in the whole foetus (approximately 70 ng Mn/g) 3 hours after a 0.5 mg Mn/kg iv dose were of a similar order to the levels found in the uterus, placenta and ovary of the dams on day 10 of gestation. Results from dosing on 13, 17 and 19 days of gestation were of a similar order for the whole foetus, maternal uterus and ovary but higher and much more varied for the placenta. Similar results were seen when the iv dose of manganese was increased 20-fold to an estimated maximum tolerated dose.

The first of a series of studies reported around the same time also looked at the tissue distribution of manganese from a sucrose suspension of Mn_3O_4 in neonatal rats (Rehnberg et al., 1980). The neonates were orally dosed daily from birth for 21 days with dose solutions containing approximately 0, 21, 71 or 214 µg Mn/µL at a dose rate of 1 µL/g bodyweight. The Mn_3O_4 particles were calculated to have an average particle diameter of 0.6 µm (0.1-2.8 µm range) with 80% of particles less than 1 µm in diameter. Significant mortally (post-day 12) and bodyweight loss were seen in the 2 higher dose groups compared to controls. Significant increases in manganese concentrations were seen in the liver, kidneys, brain and testes from the 2 higher dose groups compared to the control animals. Although the lowest treatment group (approximately 21 mg Mn/kg bodyweight) showed increases in manganese tissue concentrations compared to the control group, these were no longer statistically significant by day 18.

In a follow-on study by the same workers, neonatal rats were orally dosed daily from birth for either 12 or 27 days with a sucrose suspension of Mn_3O_4 at 71 mg Mn/kg bodyweight which produced elevated manganese concentrations in all tissues tested (Rehnberg et al., 1981). The removal of the Mn_3O_4 from the daily intake of rats at day 12 resulted in a rapid decrease in liver, kidneys and testes manganese concentrations and a slower release from the brain tissue. The rats dosed for 27 days had higher manganese tissue concentrations than the dose group exposed for only 12 days and the decrease in these higher tissue concentrations took much longer to reach control levels. The tissue manganese concentrations had already started to decrease before day 27 of the repeated dosing, which the authors proposed was due to a decrease in the intestinal absorption of manganese as the rats developed an effective homeostatic mechanism. The authors also proposed that the slower decrease in tissue manganese concentrations from the 27-day dose group was due to the daily dosing having carried on beyond the maturation of tissue barriers. In particular the slower removal of manganese from the cerebrum, hypothalamus and pituitary was due to the blood-brain barrier being established during the dosing period.

A comparison of neonatal rat manganese tissue concentrations after the same dosing regimen between these two studies showed strikingly different results. For example, the manganese concentration in the liver and kidneys from neonatal rats on day 12 were reported as 20.5±9.3 and 4.8±2.4 µg/g (Rehnberg et al., 1980) and 5.8±2.2 and 1.5±0.9 µg/g (Rehnberg et al., 1981) respectively. The authors did not offer any explanation for these differences, which would not be expected as the animals were apparently dosed with the same dose solution (71 mg Mn/kg bodyweight) for the same length of time by the same workers. As such, the data from these two studies should be viewed with caution.

In a third study by the same workers, rats were chronically exposed to varying levels of Mn_3O_4 in their diet through two generations resulting in dose-related increases in tissue accumulation of manganese (Rehnberg et al., 1982). Dietary iron deficiency in addition to chronic Mn_3O_4 exposure resulted in additional increases in tissue manganese concentrations. The highest tissue manganese concentrations were seen in weanling rats whereby the pre-weanling rats had the maximum uptake and retention of manganese. The authors concluded that rats over 40 days old were protected by biliary excretion and tissue barriers, which meant that they accumulated minimal amounts of manganese.

Table 4.1.2 Manganese concentrations (μg/g) in selected tissues of postnatal rats orally dosed daily with Mn_3O_4 sucrose solutions at 71 mg Mn/kg bodyweight for 12 or 27 days (Rehnberg et al., 1981)

Tissue	Day	Control	Group 2 (dosed for 12 days)	Group 3 (dosed for 27 days)
Cerebrum	12	0.23±0.06	4.4±1.5*	-
	24	0.59±0.05	0.83±0.12	6.2±2.4*
	30	0.44±0.05	0.59±0.08	1.9±0.4*
	60	0.33±0.02	0.40±0.04	0.56±0.07*
	100	0.43±0.05	0.43±0.02	0.44±0.02
Hypothalamus	12	0.17±0.04	5.2±1.1*	-
	24	0.68±0.06	1.2±0.2	8.6±3.2*
	30	0.65±0.07	0.89±0.15	3.7±1.0*
	60	0.40±0.02	0.43±0.11	0.90±0.13*
	100	0.48±0.07	0.49±0.03	0.51±0.04
Pituitary	12	0.35±0.13	4.6±1.3*	-
	24	1.5±0.2	2.4±0.2	10.2±3.3*
	30	0.84±0.13	1.1±0.2	3.5±0.5*
	60	0.73±0.07	0.67±0.11	1.0±0.1*
	100	0.75±0.08	0.83±0.10	0.84±0.11
Testes	12	0.24±0.11	0.68±0.14*	-
	24	0.66±0.05	0.68±0.06	1.2±0.5*
	30	0.55±0.03	0.49±0.06	0.71±0.11*
	60	0.42±0.06	0.42±0.04	0.44±0.05
	100	0.37±0.03	0.43±0.07	0.45±0.14

* - statistically significantly different from control group ($p < 0.05$)

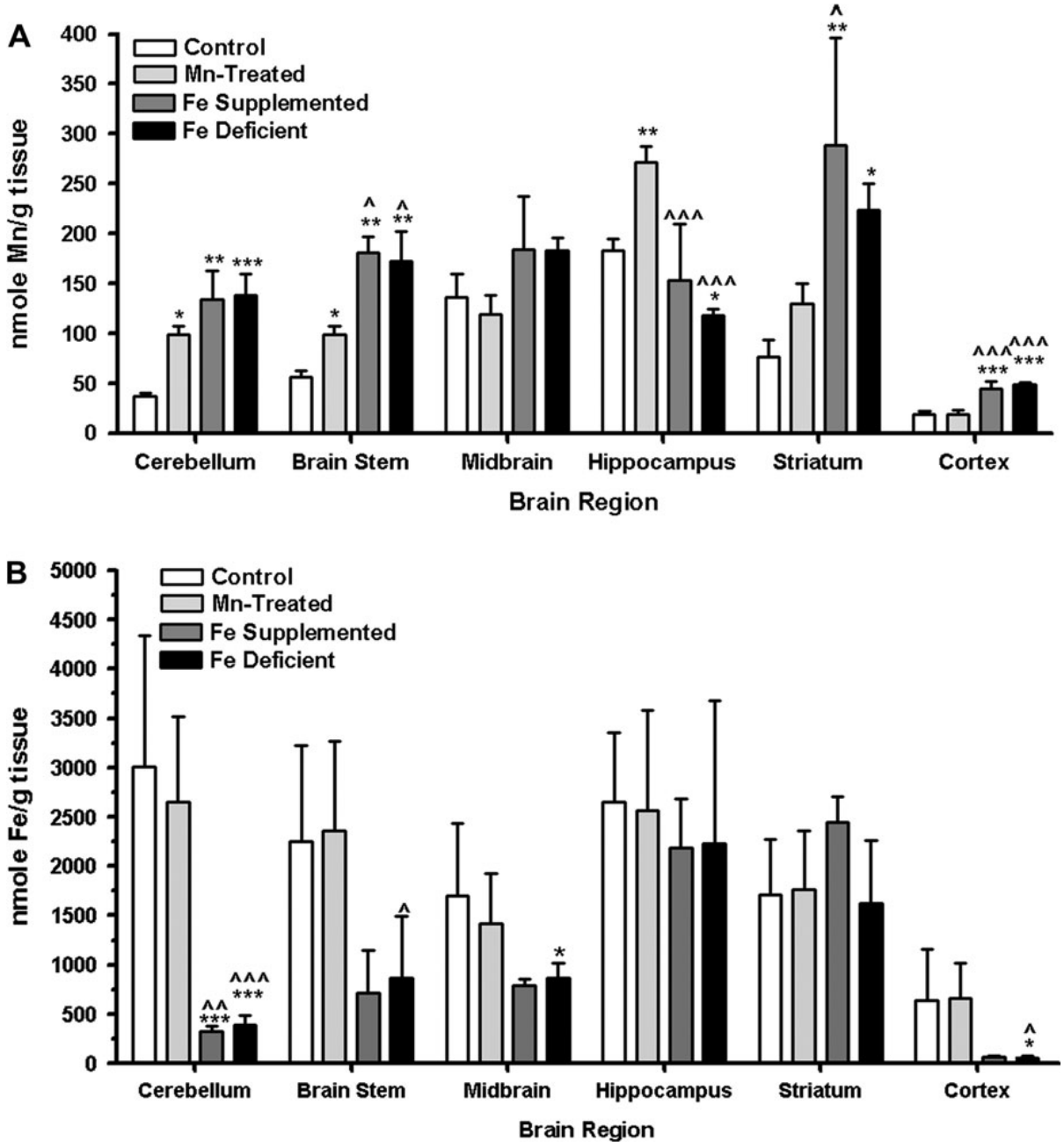
Using a combination of fast protein liquid chromatography (FPLC) with a combination of anion exchange and gel filtration columns, transferrin was identified as the major manganese-binding protein in rat plasma (Davidsson et al., 1989c). The authors concluded that this result was independent of the route of administration (oral or iv) or the length of time after administration of ^{54}Mn. In addition, when $^{54}Mn^{3+}$ was added to plasma *in vitro*, more ^{54}Mn was identified as bound to transferrin than when $^{54}Mn^{2+}$ was added, if the ^{54}Mn was added immediately before injection into the FPLC system. However, no difference was observed between $^{54}Mn^{3+}$ and $^{54}Mn^{2+}$ binding if allowed to incubate first (5 mins).

The addition of ceruloplasmin, which is associated with the oxidation of Fe^{2+} to Fe^{3+}, to the incubation of $^{54}MnCl_2$ in rat plasma significantly increased the transferrin-bound fraction of ^{54}Mn (Aschner and Aschner, 1990). The authors also described how the ^{54}Mn rat brain uptake levels were significantly reduced when the rats were pre-treated for 6 hours with a continuous infusion of ferric-hydroxide dextran complex. The authors concluded that the manganese uptake across the blood-brain barrier (BBB) might also be modulated by plasma iron homeostasis. The rapid uptake of $^{54}Mn^{2+}$ into brain and choroid plexus from the circulation was studied using the *in situ* rat brain perfusion technique (Rabin et al., 1993). The authors concluded that the results demonstrated that $^{54}Mn^{2+}$ is readily taken up into the CNS, most likely as the free ion, and that transport is critically affected by plasma protein binding. Further the results supported the hypothesis that Mn^{2+} transport across the blood-brain barrier is facilitated by either an active or a passive mechanism. More recently the carrier-mediated influx of manganese citrate, as well as Mn^{2+} and manganese transferrin, using an *in situ* rat brain perfusion technique through the BBB has been reported (Crossgrove et al., 2003). In a companion paper, the same workers also demonstrated that the rate of manganese efflux from the brain was consistent with diffusion (Yokel et al., 2003).

Thus the relationship between manganese and iron transport is attributed to the fact that both metals can be transported via the same molecular mechanisms. Whether brain manganese distribution patterns due to increased manganese exposure compared to iron deficiency are the same, or whether iron supplementation would reverse or inhibit manganese deposition were investigated in a specific series of experiments in rats (Fitsanakis et al., 2008). Three treated groups of rats all received weekly

injections of manganese chloride (3 mg Mn/kg) for 14 weeks with one group on an iron deficient diet (FeD), another on an iron supplemented diet (FeS) and one on a standard control diet. The distribution of manganese in the brains was determined by both MRI and atomic absorption spectroscopy (AAS). An increase in manganese accumulation and a difference in distribution was seen, as expected, in the rats on the FeD diet compared to the treated rats on the control diet. However, the same accumulation and regionally specific pattern of manganese distribution was also seen in the brains of the FeS rats compared to the FeD rats.

Figure 4.1.2 Regional brain metal content—AAS was used to determine the amount of (a) Mn or (b) Fe in discrete brain regions. Both Mn and Fe levels were differentially altered in each treatment group and for each discrete brain region. For both graphs in the figure, the following symbols are used: *p < 0.05, **p < 0.01, and ***p < 0.001 compared with control (untreated); p < 0.05, p < 0.01, and p < 0.001 compared with manganese treated (Fitsanakis et al., 2008)



The authors were quite surprised to find that the FeS and FeD diets did not have opposite effects in brain manganese deposition since manganese and iron compete for the same transporter systems.

They suggested several possible reasons for this phenomenon; however it is clear that further work is needed to more closely examine the relationships between metal dysregulation or dietary iron manipulation and the transport of both manganese and iron to the brain. An additional complicating factor that was not discussed by the authors was that this study design eliminated the effect of iron deficiency or supplementation on the absorption of manganese. Iron deficiency or supplementation can considerably affect both the oral absorption of manganese as well as the uptake of manganese following inhalation (Thomson et al., 1971; Davis et al., 1992b; Brain et al., 2006; Heilig et al., 2006; Thompson et al., 2006). This has been discussed in detail elsewhere in this report.

During an investigation into how varying levels of manganese (as manganese carbonate in the diet) and iron affect manganese absorption by rats, the relative distribution of a ^{54}Mn tracer ($^{54}MnCl_2$) either administered via food, or injected intraportally as a manganese-albumin complex were compared (Davis et al., 1992b). The relative distribution of ^{54}Mn in tissues was generally similar for rats when ^{54}Mn was administered in these two ways and the authors proposed that intraportal injection of manganese complexed to albumin could be used further to study endogenous losses of manganese. Subsequently, a model was developed with rats to quantitate endogenous gut losses of manganese in which the parenterally administered isotope was distributed like fed isotope. Intraportally injected ^{54}Mn complexed to albumin distributed in tissues like the fed isotope, but carrier-free ^{54}Mn injected intraperitoneally, intravenously, or intraportally, or ^{54}Mn complexed to transferrin and injected intraportally did not. Thus, manganese appears to be complexed to albumin or an albumin-like protein when it leaves the intestine (Davis et al., 1993).

Following a single ip injection of $MnCl_2$ at either 2.5, 10 or 40 mg Mn/kg to adult male rats, the manganese content of selected tissues was measured for up to 24 hours post-dose (Keen et al., 1984). A dose-response increase in manganese concentrations was found in the plasma, liver, kidney and brain with the liver concentration of manganese returning to basal levels by 24 hours post-dose. Gel filtration chromatography of rat livers from the 10 mg Mn/kg dose level showed that manganese was initially (up to 1 hour post-dose) distributed between a protein fraction of MW ~80,000 that co-eluted with transferrin and lower molecular weight substances.

Wistar rats that had chronic administration of manganese in their drinking water ($MnCl_2.4H_2O$, 1 mg/ml tap water) from conception (parents) to adult (130 days old) showed that manganese was not uniformly distributed in the brain of the rat (Lai et al., 1981). This was a brief report and therefore has been assigned a Klimisch Code of 4. The elevated levels of manganese in the brains of the treated rats were up to 2-fold greater than in controls, these differences being significant in the hypothalamus, cerebellum, pons and medulla, striatum and hippocampus regions of the brain. No significant differences were found in the midbrain or cerebral cortex. An extension of this work (Chan et al., 1981) with rats chronically administered manganese in their drinking water ($MnCl_2.4H_2O$, 1 mg/ml tap water) for 2 years, reported that significant increases in manganese concentrations were found in the lung (+130%), liver (+45%), kidney (+33%) and brain (+31%). Again this was a brief report and therefore has been assigned a Klimisch Code of 4. In a subsequent study where the level of manganese in drinking water was increased 20-fold, larger increases (up to 6-fold greater than controls) in manganese brain concentrations were found (Lai et al., 1992). Although this was a more substantial publication, much of the details of the methodology were cross-references to other work; therefore it has also been assigned a Klimisch Code of 4. The hypothalamus and striatum regions of the brain from treated rats showed the highest significant increases (5 to 6-fold), with the cerebellum, pons and medulla, midbrain and cerebral cortex all showing an approximate 3-fold increase in manganese levels.

When adult male rats were exposed to 0.5% manganese as $MnCl_2$ in their drinking water for 1, 4, or 6 weeks, the manganese concentrations in the blood, brain, liver and kidney, were highest after one week of exposure (Hietanen et al., 1981). The authors concluded that the results suggested an adaptation to manganese absorption during continuous exposure.

Table 4.1.3 Significant increases in rat tissue manganese concentrations following exposure to 0.5% $MnCl_2$ in drinking water (Hietanen et al., 1981). Data are expressed as nmoles Mn/g (wet weight).

Exposure (weeks)	Blood	Liver	Brain	Kidney
Controls (n=12)	4.8±1.4	75±3	12±1	39±4
1 (n=6)	42±16*	225±29***	60±10***	156±18***
4 (n=6)	16±5*	121±8***	28±3***	59±6*
6 (n=6)	24±4***	190±48*	32±5**	49±7

*:2p<0.05; **: 2p<0.01; ***:2p<0.001

Tissue accumulation of manganese (from $MnCl_2$) was greater following ip administration (6 mg Mn/kg/day) than by oral administration (75 mg Mn/kg/day) for 4 weeks to groups of rats (Missy et al., 2000). After a 2-week rest period, increases in manganese concentrations were observed in most of the tissues, particularly significant in the nervous system (brain and spinal cord), the stomach, spleen and femur. Whole blood concentrations of manganese were at 191±39 µg/L, an 18.5-fold increase compared to controls following ip administration; however plasma levels were not significantly different to controls. The authors believed that the increase of manganese in whole blood but not plasma was due to manganese being associated with certain blood elements, such as haemoglobin, instead of iron. Thus the progressive disappearance of the high levels of manganese from the blood would have been closely related to the length of life and renewal of the erythrocytes. This hypothesis correlated with the increase in manganese concentrations seen in the spleen, where old erythrocytes are destroyed.

A study utilising older female rats (~8 months old) found that the tissue accumulation of manganese was significantly higher (>25%) following ip injections of Mn^{3+} than following equimolar ip injections of Mn^{2+} (Reaney et al., 2006). Both the manganese chloride solution (Mn^{2+}) and the manganese pyrophosphate solution (Mn^{3+}) were administered at nominal doses of 0, 2 or 6 mg Mn/kg via ip dose 3 times a week for 5 weeks. The Mn^{3+} exposures produced significantly higher blood and brain manganese concentrations than the Mn^{2+} exposures at both treatment levels. There was no difference in manganese concentration between the regions of the brain examined within a dose level or oxidation state. The authors concluded that these data substantiate the heightened susceptibility of the globus pallidus to manganese, and they indicate that the oxidation state of manganese exposure may be an important determinant of tissue toxicodynamics and subsequent neurotoxicity.

The brain distribution of manganese in mice 24 hours after the last of 3 daily ip administrations of $^{54}MnCl_2$, found that more manganese was distributed to the striatum, hippocampus and the remainder of the brain that contained the substantia nigra than to the cortex (Kobayashi et al., 2003). This publication had a very brief description of the methodology and so was assigned a Klimisch Code of 4. The mice from the high dose of 50 mg/kg/day exhibited catalepsy and the retention of manganese was more than twice the retention of manganese from the lower 30 mg/kg/day dosed mice, which did not exhibit catalepsy.

The changes in the concentration of manganese in regions of the brain of a non-human primate (the common marmoset, Callithrix jacchus) following four systemic injections of 30 mg/kg $MnCl_2 \cdot H_2O$ in the tail vein using MRI and ICPMS were compared these to changes in the rat following the same exposure route and dose. The doses were spaced 48 hours apart and the animals were imaged 48 hours after the final dose. The brain structures that had a significantly greater increase in enhancement in the marmoset versus the rat were the visual cortex, the striatum, the globus pallidus, the ventral pallidum, and the substantia nigra. Two of these structures are proximal to a large volume of CSF in the marmoset but not the rat. In the marmoset, the visual cortex is adjacent to the posterior horn of the lateral ventricle. In the rat, the posterior horn does not extend to this structure. As well, the caudate of the striatum in the marmoset forms the lateral wall of the anterior horn and body of the lateral ventricle. While the same is true in the rat, the CSF space is much smaller than in the marmoset. The authors suggested that these two species differences suggest that the CSF-brain route of uptake is important in the marmoset and that the stronger manganese uptake in the marmoset brain

is because of the geometry of the lateral ventricles. There was also a significantly higher accumulation of manganese in marmoset brains than rat brains even though the dose was normalised to bodyweight. When gross pathology was performed post-mortem it was found that two of the four marmosets had significant liver damage, which suggested that marmosets were more susceptible to liver damage than rodents. As such, the higher accumulation of manganese into the marmoset brains could have been due to a longer lifetime of manganese in the blood due to poor hepatobiliary clearance and thus compromising the study.

Table 4.1.4 Comparison of brain manganese concentration (µg/g wet tissue) after repeated manganese iv administration (30 mg/kg $MnCl_2 \cdot H_20$) in the rat and marmoset (Bock et al., 2008)

	Marmoset (n=4)		**Rat (n=4)**	
Region	Control	Treated	Control	Treated
Frontal cortex	0.63±0.13	2.92±0.56*+	0.52±0.07	1.52±0.30*
Striatum	1.16±0.39	4.45±2.02*+	0.58±0.05	2.38±0.32*
Thalamus	1.04±0.41	4.03±1.81*+	0.49±0.05	2.12±0.42*
Hippocampus	0.83±0.64	3.08±1.19*+	0.58±0.05	1.78±0.29*

* - significant increase over control in the same species ($p<0.05$)
+ - significant increase in the marmoset verses the rat ($p<0.05$)

The brain manganese concentrations of Cynomolgus macaques following 45 weekly injections of manganese sulphate (10-15 mg/kg, equivalent to 3.26 - 4.89 mg Mn/kg) showed a greater increase in the globus pallidus compared to the other brain regions (Guilarte et al., 2006). In addition to measuring tissue concentrations post-mortem, manganese distribution using T1 weighted MRI was performed and analysed using a pallidal index (PI) equivalent approach at 18 and 41 weeks. Only small increases (not statistically significant) in PI equivalent were seen in several brain tissues which was a lot less than the increases seen in blood manganese at these time-points and also in the increases seen in the tissues post-mortem. The authors explained that these differences were due to concurrent increases in manganese levels in the frontal white matter, which is used as the denominator for the PI equivalent ratio calculation. They also suggested that better correlation was not seen as the tissue concentrations of manganese were measured later (4 weeks) than the MRI investigations. It is also interesting to note that although the increase in blood manganese was significant at 18 weeks into the study, the level had dropped at 41 weeks and further still at 45 weeks, becoming no longer statistically significant (at $p<0.05$).

Table 4.1.5 Brain manganese concentration (µg/g wet tissue) and blood manganese levels (µg/L) after 45 weekly manganese iv administrations (10-15 mg/kg $MnSO_4$) in the monkey (Guilarte et al., 2006).

Tissue	**Control (n=3)**	**Treated (n=4)**	**Fold increase**
Globus pallidus	0.72±0.14 (0.47-0.94)	3.30±0.52* (2.59-4.83)	x4.6
Caudate	0.38±0.05 (0.31-0.47)	1.22±0.15* (0.86-1.49)	x3.2
Putamen	0.48±0.07 (0.35-0.56)	1.50±0.11* (1.24-1.75)	x3.1
Frontal white matter	0.17±0.02 (0.15-0.20)	0.57±0.02* (0.54-0.62)	x3.4
Blood (µg/L) after 45 weeks	9.2±2.7 (5.1-14.2)	42.6±9.9 (14.6-58.6)	x5.0
Blood (µg/L) after 41 weeks		55.7±10.8* (29.4-73.7)	x6.1
Blood (µg/L) after 18 weeks		67.1±13.7* (42.9-106)	x7.3

* - significant increase over control ($p<0.05$)

4.1.2.2 Inhalation / instillation

4.1.2.2.1 Distribution following nasal instillation

Following $^{54}MnCl_2$ dosing to the olfactory chambers of pikes, $^{54}Mn^{2+}$ was taken up in the olfactory receptor cells and was transported at a constant rate along the primary olfactory neurones into the brain (Tjalve et al., 1995). The $^{54}Mn^{2+}$ accumulated in the entire olfactory bulbs, although most marked in central and caudal parts. The metal was also seen to migrate into large areas of the telencephalon, apparently mainly via the secondary olfactory axons present in the medial olfactory tract. The results also showed that there was a pathway connecting the two olfactory bulbs of the pike and that this can carry the metal. The authors concluded that it appeared that manganese has the ability to pass the synaptic junctions between the primary and the secondary olfactory neurones in the olfactory bulbs and to migrate along secondary olfactory pathways into the telencephalon and the diencephalons in pike. Therefore, it was concluded that the olfactory route might be a crucial pathway by which manganese gains access to the brain.

In a further study by the same workers the uptake of manganese ($^{54}MnCl_2$) from the nasal mucosa into the central nervous system via olfactory pathways in rats was investigated (Tjalve et al., 1996). By comparison with an equivalent ip dose of $^{54}MnCl_2$, the authors concluded that the results appeared to show that the uptake of manganese in the olfactory bulbs after nasal instillation in all probability occurred via the primary olfactory neurons and not via the circulation. Following the intranasal administration of manganese the results showed that the radioactivity passed from the olfactory bulbs to the basal forebrain areas which constituted the terminal regions of the secondary olfactory neurones. Other areas of the brain, such as the cerebral cortex, hypothalamus, hippocampus and striatum slowly reached peak levels of radioactivity at about 1-3 weeks after administration. The authors added a highly speculative proposal that the neurotoxicity of inhaled manganese is related to an uptake of the metal into the brain via the olfactory pathways. In this way manganese can circumvent the blood-brain barrier and gain direct access to the central nervous system.

Table 4.1.6 Selected tissue manganese concentrations (µg Mn/g tissue wet weight) following either a single intranasal or ip administration of $^{54}MnCl_2$ to rats at 4 µg Mn/kg bodyweight (Tjalve et al., 1996).

Tissue	**Intranasal administration**			**ip administration**		
	1 day	**1 week**	**3 weeks**	**1 day**	**1 week**	**3 weeks**
Olfactory bulb	110±39	65±33	19±13	0.7±0.3	1.0±0.1	0.7±0.1
Basal forebrain	7.7±3.8	39±25	15±10	0.4±0.2	0.7±0.1	0.7±0.1
Cerebral cortex	0.5±0.2	7.0±4.7	5.5±4.5	0.4±0.2	0.7±0.1	0.5±0.1
Hypothalamus	0.2±0.0	10±6.3	8.6±6.0	0.5±0.2	0.8±0.1	0.8±0.1
Hippocampus	0.2±0.1	2.3±1.3	3.9±3.1	0.5±0.2	0.6±0.1	0.5±0.1
Striatum	0.2±0.1	5.7±3.2	7.2±5.3	0.4±0.2	0.6±0.1	0.6±0.1

The distribution of manganese following a single intranasal injection of manganese chloride into the right nostril of rats at >25 µg Mn/rat, found significantly elevated manganese levels in the right olfactory bulb and right olfactory tubercle compared to the left ones (Gianutsos et al., 1997). These elevated levels appeared within 12 hours of instillation and lasted for up to 3 days. No increases in either blood or striatum manganese levels were found for single doses up to 200 µg Mn/rat. The manganese tissue concentrations had fallen to control levels by 1 week after dosing. When a second intranasal injection of manganese chloride into the right nostril of rats at 200 µg Mn/rat was made one week after the first injection, elevated levels of manganese were also found in the right striatum. A 4-fold elevation of manganese levels was seen in the right olfactory bulb and a 2-fold increase in the

right olfactory tubercle and right striatum. Although the study did not address whether the dosing technique could have damaged the nasal lining and hence compromise the results, the authors concluded that air-borne manganese can be retrogradely transported along olfactory neurons to the CNS and can reach deeper brain structures under appropriate exposure conditions.

The dose-dependence of the uptake and subcellular distribution of the manganese in the olfactory epithelium and the brain of rats was examined after a single intranasal instillation of $^{54}MnCl_2$ (Henriksson et al., 1999). The results indicated that the manganese transport was a saturable process, both regarding the uptake into the olfactory epithelium and the transfer to the olfactory bulb. The data also indicated that manganese moves relatively freely from the olfactory bulb to the olfactory cortex at an amount dependent on the level of influx into the bulb. The transport to the rest of the brain was related to the amounts in the olfactory bulb and the olfactory cortex, but the relative proportion reaching this area increased with increasing doses. The authors concluded that their results showed that the olfactory neurons provided a pathway with a considerable capacity to transport manganese into the brain and that the neurotoxicity of inhaled manganese is related to an uptake via this route. This statement failed to take into account the difference in the relative size of the olfactory bulb in rats and humans and presumably assumed that the same pathway with the same capacity was present in humans, although unfortunately this was not discussed. Similarly it fails to address the suitability of the rat as a model for manganese neurotoxicity in humans. Additionally the study failed to provide evidence to support the proposal that the neurotoxicity of inhaled manganese is related to an uptake via this route, and thus it can only be speculation.

Brenneman and co-workers developed a very useful experimental technique for studying the role of olfactory transport in delivering inhaled toxicants to the rat brain (Brenneman et al., 2000). This unilateral nasal occlusion procedure prevents the deposition of inhaled manganese on the side ipsilateral to the occluded nostril. By preventing intranasal deposition of the inhaled chemical on one side of the nose, the contribution of olfactory transport to brain levels of the inhaled toxicant is initially eliminated on one side of the brain following inhalation. The side of the brain ipsilateral to the occluded nostril relies predominantly on systemic delivery of the inhaled toxicant to the brain, while toxicant transported to the side of the brain that is ipsilateral to the unoccluded nostril is delivered via both the blood and olfactory routes. Thus a comparison of brain levels of the inhaled toxicant on the unoccluded versus the occluded sides can be used to determine the contribution of the olfactory route, relative to the blood route, to brain levels of the toxicant (Dorman et al., 2002). A diagram of this unilateral nasal occlusion model is shown in Figure 4.1.1.

Male 8-week old CD rats underwent a single 90-min nose-only exposure to a $^{54}MnCl_2$ aerosol (0.54 mg Mn/m^3; MMAD 2.51 μm) applying this exposure model. The left and right sides of the nose and brain, including the olfactory pathway and striatum, were sampled at 0, 1, 2, 4, and 8 days post-exposure. Brain and nose samples from the side with the occluded nostril had negligible levels of ^{54}Mn activity. High levels of ^{54}Mn were observed in the olfactory bulb and tract/tubercle on the side or sides with an open nostril within 1-2 days following inhalation exposure. These results demonstrated that the olfactory route contributes the majority (up to >90%) of the ^{54}Mn found in the olfactory pathway, but not in the striatum, of the rat brain up to 8 days following a single inhalation exposure (Brenneman et al., 2000).

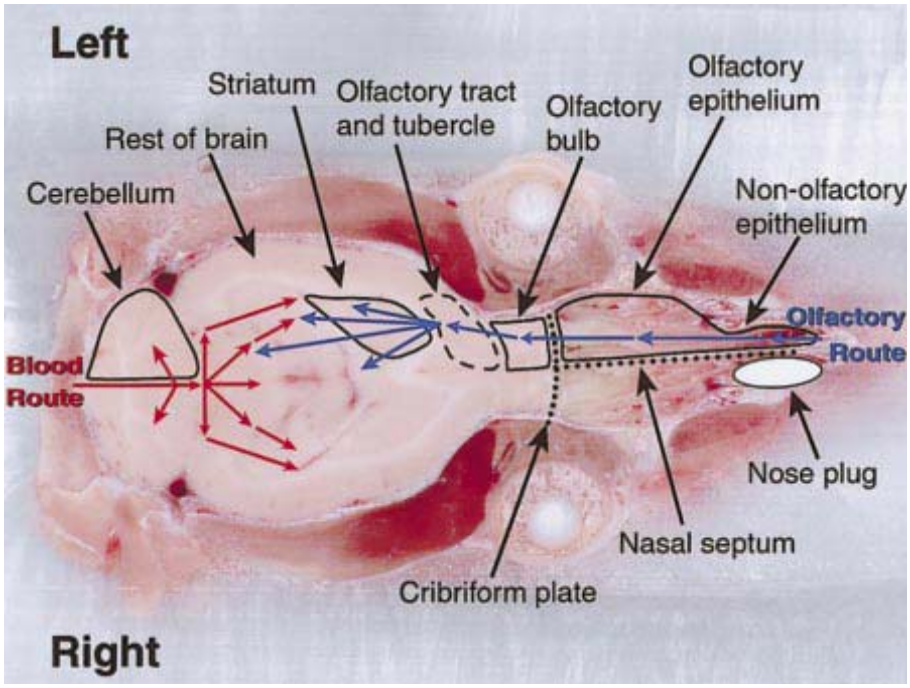


Figure 4.1.1. Diagram of the unilateral nasal occlusion model overlying a horizontally-sectioned, frozen head of an 8-week-old, male CD rat exposed via inhalation to $^{54}MnCl_2$. In this model, the right nostril is occluded with a plug to prevent nasal deposition and olfactory transport of inhaled ^{54}Mn on the right side of the nose and brain. The left nostril is patent, allowing nasal deposition and olfactory transport (blue) of inhaled ^{54}Mn on the left side of the nose and brain. The blood route (red) transports ^{54}Mn absorbed from the respiratory and gastrointestinal tracts to both sides of the head, presumably in equivalent amounts. The regions of the nose and brain evaluated for ^{54}Mn levels are demarcated (top). The difference between concentrations of ^{54}Mn found on the left (blood and olfactory routes) and right (blood route only) sides of the brain can thus be used to determine the relative contribution of the olfactory route to brain manganese levels (taken from Brenneman et al., 2000).

In a further study from the same laboratory, male 8-week old CD rats underwent a single 90-min nose-only exposure to a $^{54}MnHPO_4$ aerosol (0.39 mg Mn/m^3; MMAD 1.68 µm). The left and right sides of the nose and brain, including the olfactory pathway, cerebellum and striatum, were sampled at 0, 1, 2, 4, 8 and 21 days post-exposure (Dorman et al., 2002). The authors reported that rats with two patent nostrils had equivalent ^{54}Mn concentrations on both sides of the nose, olfactory bulb, and striatum, while asymmetrical ^{54}Mn delivery occurred in rats with one occluded nostril. High levels of ^{54}Mn activity were observed in the olfactory bulb and tubercle on the same side (i.e., ipsilateral) to the open nostril within 1–2 days following $^{54}MnHPO_4$ exposure, while brain and nose samples on the side ipsilateral to the nostril occlusion had negligible levels of ^{54}Mn activity. Overall, these results were qualitatively similar to the results obtained with the more soluble form of manganese, manganese chloride in the earlier work (Brenneman et al., 2000). The authors concluded that both these results demonstrated that the olfactory route contributes to ^{54}Mn delivery to the rat olfactory bulb and tubercle. However, this pathway did not significantly contribute to striatal ^{54}Mn concentrations following a single, short-term inhalation exposure to $^{54}MnHPO_4$.

4.1.2.2.2 Distribution following inhalation or pulmonary instillation

Differences in the time and level of maximum tissue concentrations (T_{max} and C_{max}) of radioactivity were observed following dosing by intratracheal instillation of 55 µg of either soluble $^{54}MnCl_2$ or insoluble $^{54}Mn_3O_4$ to male Sprague-Dawley rats (Drown et al., 1986). The particle size of the insoluble manganese tetraoxide was determined by scanning electron microscopy to be less than 5µm in diameter, with 90% of the material having a diameter of 1 µm or less. The T_{max} and C_{max} manganese tissue values were generally earlier and higher in the rats dosed with $^{54}MnCl_2$ than $^{54}Mn_3O_4$, with the major exception being the lungs. The faster distribution of radioactivity to tissues following the $^{54}MnCl_2$ was apparently a result of the faster pulmonary clearance. The whole blood manganese concentrations at 4 hours after dosing were 4 and 2 ng/g following $^{54}MnCl_2$ and $^{54}Mn_3O_4$ dosing respectively. Maximal brain manganese concentrations were of a similar magnitude, with peaks at 1 day and 3 days following $^{54}MnCl_2$ and $^{54}Mn_3O_4$ dosing respectively. These results imply that whilst the absorption from the lungs of the two forms of manganese proceeded at different rates: hours for the soluble manganese chloride and days for the insoluble manganese tetraoxide, the overall exposure was very similar. The variation in manganese levels in the intestinal contents with time showed a similar profile as many other tissues, with the soluble chloride group having a higher and earlier C_{max}. As such, the clearance of the manganese tetraoxide from the lungs by the mucocilliary elevator followed by absorption from the GI tract does not seem to be the most likely process, since the overall exposure would have been expected to be a magnitude lower due to the low oral absorption of manganese.

Adult male CD rats were exposed (inhalation) for 6 h/day for 7 days/week (14 exposures) to either manganese sulphate ($MnSO_4$), manganese tetraoxide (Mn_3O_4) or manganese phosphate in the mineral form hureaulite ($Mn_5(PO)_4)_2[(PO_3)(OH)]_2 \cdot 4H_2O$) at 3 different dose levels (Vitarella et al., 2000b; Dorman et al., 2001a). Significant increases in the manganese concentration in some tissues were found following this repeated short-term exposure at the 0.3 mg Mn/m^3 exposure levels and in most tissues at the 3 mg Mn/m^3 exposure levels but not at the 0.03 mg Mn/m^3 compared to controls. A summary of the manganese tissue concentrations, which were taken immediately after the last exposure following the 3 mg Mn/m^3 exposure level is presented in Table 4.1.5. Although the lungs showed the largest increases in manganese concentration, the increase in manganese sulphate group was significantly less than the other two forms of manganese salts. Since the aerosols were aerodynamically similar with equivalent exposure concentrations of manganese, these results suggested that the more soluble manganese sulphate was cleared more rapidly than the less soluble particles from the manganese tetraoxide and phosphate forms following inhalation. Conversely, the olfactory bulb and striatal levels of manganese were significantly higher following the soluble manganese sulphate exposure than the less soluble particles from the manganese tetraoxide and phosphate forms. This suggests that the more soluble forms of manganese are more readily delivered to the olfactory bulb and striatum, adding credence to the direct olfactory transport theory (Brenneman et al., 2000; Dorman et al., 2002). Overall, the authors concluded that dissolution rate could influence the pulmonary clearance of a metal and thus affect its delivery to the brain and other organs. In the earlier inhalation study (Brenneman et al., 2000) with the soluble manganese chloride, significant increases in the level of manganese in the striatum were generally not seen. However, the manganese exposure was at a much lower dose (0.54 mg Mn/m^3) and also this study was a single 90-minute exposure as opposed to repeated exposure.

Table 4.1.7. Tissue manganese concentrations (µg Mn/g tissue wet weight) following repeated short-term exposure of rats to aerosols of either manganese sulphate, manganese tetraoxide or manganese phosphate (Vitarella et al., 2000b; Dorman et al., 2001a).

Tissue	**Controls**	**3 mg Mn/m^3 (6 h/day for 14 days)**		
		Manganese Sulphate (MMAD 2.1 µm)	**Manganese Tetraoxide (MMAD 1.8 µm)**	**Manganese Phosphate (MMAD 1.6 µm)**
Serum	0.13±0.03, 0.13±0.02	0.31±0.20	0.10±0.02	
Plasma	0.13±0.04			0.12±0.01
Lung	0.45±0.04, 0.37±0.08, 0.36±0.05	7.33±0.37*[b]	14.73±2.57*	20.31±0.95*
Testes	0.32±0.04, 0.36±0.02	0.79±0.18*	0.46±0.03	-
Femur	0.52±0.05, 0.43±0.03, 0.41±0.06	1.28±0.06*	0.68±0.05*	1.19±0.14*
Liver	2.38±0.23, 3.18±0.13, 2.48±0.15	3.64±0.46*	3.48±0.31	3.00±0.21
Bile	0.58±0.08, 0.29±0.09	1.51±0.17*	0.95±0.13*	-
Skeletal muscle	0.16±0.02	-	-	0.29±0.09*
Olfactory bulb[a]	0.51±0.04	4.42±0.23*[c]	3.09±0.29*[d]	1.89±0.13*
Striatum[a]	0.48±0.04	3.18±0.59*[c]	1.48±0.12*	0.90±0.06*
Cerebellum[a]	0.43±0.06	NS	NS	0.74±0.09*

Key:
* – statistically significant increase over controls ($p<0.05$)
[a] – actual data for the manganese sulphate and tetraoxide groups were not given in publication (Dorman et al., 2001a)
[b] – statistically significantly lower lung manganese concentration compared to manganese tetraoxide or phosphate groups
[c] – statistically significantly higher olfactory bulb manganese concentrations than either the manganese tetraoxide or phosphate groups
[d] – statistically higher olfactory bulb manganese concentrations than the manganese phosphate group
NS – no statistically significant increase

In a later study that focused on the nasal toxicity of manganese sulphate (MMAD 1.85-2.03 µm) and manganese phosphate (hureaulite, MMAD 1.47 µm) following a 13-week sub-chronic inhalation exposure in rats, the manganese concentration in the olfactory bulb, striatum and cerebellum were compared (Dorman et al., 2004b). Rats exposed to $MnSO_4$ (at 0.1 mg Mn/m^3) had elevated olfactory bulb and striatum manganese concentrations when compared to hureaulite-exposed rats, thus confirming the laboratory's earlier studies (Dorman et al., 2001a) showing that particle solubility is an important determinant of manganese delivery to the brain.

In a further study in rats by the same workers, the influence of old age and gender on the pharmacokinetics of inhaled manganese sulfate (MMAD 1.85-2.03 µm) and manganese phosphate (hureaulite, MMAD 1.47 µm) was investigated (Dorman et al., 2004a). Gender and age did not affect manganese delivery to the striatum, a known target site for neurotoxicity in humans, but did influence manganese concentrations in other tissues. End-of-exposure olfactory bulb, lung, and blood manganese concentrations were higher in young male rats than in female or aged male rats and the authors concluded that this might reflect a portal-of-entry effect.

Adult male Sprague-Dawley rats were exposed (inhalation) for 6 h/day for 5 days/week for 13 consecutive weeks to manganese sulphate at 3 different dose levels in order to assess the effect of the subchronic exposure to manganese on locomotor activity, neuropathology and blood serum biochemical parameters (Tapin et al., 2006). The tissue collections were performed approximately 42 hours after the last manganese exposure to allow for locomotor activity assessments to be made.

Table 4.1.8. Tissue manganese concentrations (µg Mn/g tissue wet weight, sampled approximately 42 hours after the last exposure) following subchronic exposure of rats to aerosols of manganese sulphate at 3 dose levels (Tapin et al., 2006)

Tissue	**Controls**	**Manganese Sulphate inhalation (6 h/day, 5 days/week for 13 weeks)**		
		0.030±0.006 mg Mn/m^3 (MMAD 0.69 µm)	0.295±0.066 mg Mn/m^3 (MMAD 0.88 µm)	3.220±0.578 mg Mn/m^3 (MMAD 0.97 µm)
Blood	0.005±0.007	0.005±0.003	0.014±0.007[a,b]	0.023±0.008[a,b,c]
Lung	0.17±0.03	0.22±0.03[a]	0.25±0.02[a,b]	0.58±0.13[a,b,c]
Testis	0.32±0.09	0.32±0.04	0.34±0.03	0.45±0.07[a,b,c]
Liver	2.22±0.35	2.56±0.33[a]	2.18±0.30[a,b]	2.37±0.39[a,b,c]
Kidney	1.01±0.13	0.91±0.17	1.04±0.11[a]	1.32±0.16[a,b,c]
Olfactory bulb	0.62±0.21	0.50±0.05	0.97±0.11[a,b]	2.27±0.37[a,b,c]
Frontal cortex	0.58±0.17	0.43±0.06[a]	0.77±0.20[a,b]	1.47±0.32[a,b,c]
Cerebellum	0.55±0.14	0.46±0.03[a]	0.51±0.03[a,b]	0.80±0.06[a,b,c]
Globus pallidus	0.64±0.11	0.45±0.07[a]	0.61±0.08[b]	1.20±0.21[a,b,c]
Caudate/putamen	0.47±0.07	0.41±0.06	0.58±0.16[a,b]	0.99±0.16[a,b,c]

Key:
a – significantly different from the control group ($p<0.05$)
b - significantly different from the 0.03 mg Mn/m^3 group ($p<0.05$)
c - significantly different from the 0.3 mg Mn/m^3 group ($p<0.05$)

When the results from the highest dose group, approximately 3 mg Mn/m^3, are compared to the 14-day inhalation study where the tissues were sampled at the end of exposure (Dorman et al., 2001a) the manganese levels in the olfactory bulb, testes and lung are all around 50% of these levels. This implies that the 3-day delay in tissue sampling resulted in a steady decline in manganese tissue levels, assuming that the tissue levels at the end of exposure were higher or at least equivalent to the 14-day study. The difference in the concentration of manganese in the lungs between these two studies is quite different, with greater than 10-fold more manganese seen in the 14-day rats compared to the 90-day rats with the delayed sampling. This is likely to be due to the lungs sampled at the end of the exposure period still containing a considerable amount of manganese directly from the inhalation exposure which was still to be cleared from the lungs. Whereas, the manganese levels measured in the lungs 3-days after the end of exposure were similar or even lower than other tissue levels indicating that the clearance of the inhaled manganese from the internal surface of the lungs was likely to have finished. The main anomaly between these two studies is in the blood/serum/plasma manganese concentrations. The plasma and serum manganese levels in the control rats from the 14-day study were around 0.13 µg Mn/g, approximately 3 to 4-fold less than other control tissues. The serum manganese level was 0.31 µg Mn/g in the exposed group, approximately 3-fold greater than the control group. However, in the 90-day study the blood manganese level in the control group was 0.005 µg Mn/g and in the exposed group 0.023 µg Mn/g. Both of these values were 13 to 26-fold less than the 14-day study. The authors of the 90-day study suggested that this was due to the time of sampling after the end of exposure between the two studies, although this wouldn't explain the difference between the control animals.

When groups of rats were administered $MnCl_2$ solutions once a week for 4 weeks at a dose of 24.3 mg Mn/kg body wt. (b.w.) by oral gavage or 1.22 mg Mn/kg b.w. by intraperitoneal injection or intratracheal instillation, the resulting concentrations of manganese in blood and tissues were compared (Roels et al., 1997). The oral dose level was based on an assumption that 5% of the oral dose would be absorbed, making the effective systemic exposure level the same for all 3 dose routes. This assumption appears to be corroborated by the very similar achieved steady-state levels of manganese in blood from all dose routes. The intratracheal instillation route was used as a surrogate of inhalative exposure to airborne manganese compounds.

Table 4.1.9. Significant increases in manganese concentration of rat blood and tissues following repeated administration of $MnCl_2$ (Roels et al., 1997). Data are expressed as % increase over control animal data.

Dose route	Blood	Liver	Cerebellum	Striatum	Cortex
oral (gavage)	68%	NS	NS	NS	22%
intraperitoneal	59%	NS	NS	34%	36%
intratracheal instillation	68%	NS	27%	205%	48%

NS – no significant difference to controls

Further statistical analysis showed that there was no apparent effect of dose route upon the resulting manganese levels in the blood, liver or cortex. Despite the elevated blood manganese levels there was no corresponding increase in liver manganese levels, indicating that the liver was still effectively maintaining manganese concentrations through biliary excretion following this dose regimen. The elevated blood levels were probably due to a fraction of the dose distributed into a deeper compartment which was in equilibrium with the blood.

The most significant result was that of the increase in levels of manganese in the striatum following repeated administration by the intratracheal instillation route. The authors suggested that the normal homeostatic control was less effective in the case of pulmonary absorption of manganese. To investigate this further, additional groups of rats were given a single dose by either oral or intratracheal instillation (same dose levels as for the repeated dose) and blood samples taken for up to 240 hours. Animals dosed by intratracheal instillation had a very rapid C_{max} of over 5-fold greater than by oral dosing and also still had blood manganese levels greater than control values at 24 hours after dosing. The authors further suggested that the high blood manganese concentration, produced during a short period after pulmonary absorption, transiently overwhelmed the mechanisms controlling the uptake of manganese into the brain. The elective distribution of manganese in the striatum compared to the other brain regions may be explained by the presence of different manganese uptake mechanisms in the various cerebral regions.

When groups of rats were administered MnO_2 suspensions (mean particle size 3.7 µm) once a week for 4 weeks at a dose of 24.3 mg Mn/kg body wt. (b.w.) by oral gavage or 1.22 mg Mn/kg b.w. by intraperitoneal injection or intratracheal instillation, the resulting concentrations of manganese in blood and tissues were compared (Roels et al., 1997). This is part of the same study discussed in the soluble salts section above.

Table 4.1.10 Significant increases in manganese concentration of rat blood and tissues following repeated administration of MnO_2 (Roels et al., 1997). Data are expressed as % increase over control animal data.

Dose route	Blood	Liver	Cerebellum	Striatum	Cortex
oral (gavage)	NS	NS	NS	NS	NS
intraperitoneal	79%	NS	40%	124%	67%
intratracheal instillation	41%	NS	31%	48%	34%

NS – no significant difference to controls

The repeated oral administration of MnO_2 suspensions did not produce any significant increase in manganese concentration in the blood or tissues tested, in contrast to the oral administration of $MnCl_2$ solutions following the same regimen. This clearly shows that the oral bioavailability of manganese from a MnO_2 suspension is significantly less than from an equivalent $MnCl_2$ solution. Similarly, the raised concentrations of manganese in both blood and the striatum following repeated oral administration of MnO_2, whilst above control values, were not as elevated as oral administration of $MnCl_2$ solutions following the same regimen. Additional groups were given a single dose of an MnO_2 suspension by either oral or intratracheal instillation (same dose levels as for the repeated dose) and blood samples taken for up to 240 hours. Animals dosed by either route had very slow increases

in blood manganese concentrations compared to animals dosed with $MnCl_2$ solutions. These results also highlight the difficulty in trying to extrapolate manganese TK data between the oral and inhalation routes of exposure.

Table 4.1.11 Comparison of rat blood manganese kinetics after single administration of either a $MnCl_2$ solution or MnO_2 suspension (Roels et al., 1997).

Dose route	**Oral (gavage) (24.3 mg Mn/kg b.w.)**		**Intratracheal instillation (1.22 mg Mn/kg b.w.)**	
Manganese source	$MnCl_2$ solution	MnO_2 suspension	$MnCl_2$ solution	MnO_2 suspension
Blood T_{max}	1 hour	144 hours	0-0.5 hours	168 hours
Blood C_{max}*	16,600 ng/L	9,000 ng/L	70,500 ng/L	17,600 ng/L

* - control blood manganese concentrations were ~5,000 ng/L

An investigation into whether there was evidence for increased susceptibility to manganese loading in iron-deficient rats showed some quite significant findings (Heilig et al., 2005). The distribution of a ^{54}Mn tracer ($^{54}MnCl_2$) administered by intratracheal instillation to a group of rats that had been fed a low-iron diet (20-25 ppm) for 3 weeks showed significantly less radioactivity in the liver and kidneys and more radioactivity in the small intestine than rats fed a standard diet containing 200 ppm iron. The accumulation of the radioactivity in the small intestine was most likely due to the duodenal re-absorption of dose that had been excreted in the bile. This increase in intestinal absorption of manganese is likely to be due to the up-regulation due to iron deficiency (Thomson et al., 1971). The proportions still remaining in the lungs were not statistically different between the groups, which would indicate that the pulmonary uptake was not affected by the iron deficiency. Following iv injection the iron-deficient group also showed accumulation of the radioactivity in the small intestine. This group also showed a significant increase in the level of radioactivity in the brain compared to the control group; however this could have been in part due to the increase in manganese blood levels seen in the iron-deficient groups, although this was not discussed. At 4 hours after the intratracheal instillation the manganese levels in the blood samples from the iron-deficient group was approximately 4-fold higher than the control (standard iron diet) dosed group, although it was lower at 5 and 15 minutes post administration. The radioactivity was accumulated in the cellular fraction of blood samples from the iron-deficient rats, which could be linked to the transferrin levels being enhanced by iron-deficiency.

Table 4.1.12 Comparison of the distribution (% dose) 4 hours after the administration of a ^{54}Mn tracer either by intratracheal instillation or iv injection to rats (Heilig et al., 2005).

Dose route	**Intratracheal Instillation**		**iv**	
Dose group	Control	Iron-deficient	Control	Iron-deficient
Brain	0.07±0.00	0.06±0.01	0.21±0.02	0.40±0.04*
Liver	7.93±0.43	6.17±0.27*	21.85±1.76	20.98±1.98
Lungs	60.66±1.67	64.72±1.48	1.02±0.09	2.95±1.15
Kidneys	2.41±0.14	1.42±0.07*	8.02±0.43	7.38±0.97
Skeletal muscle	4.04±0.30	3.02±0.22	12.73±0.98	12.11±0.75
Small intestine	4.91±0.72	12.27±1.33*	19.05±0.76	28.66±1.52*
Large intestine	3.56±0.73	1.14±0.16*	5.55±0.98	6.57±0.57
Total recovered	86.15±0.61	91.81±1.33	74.03±0.63	88.92±5.58

* - significantly different ($p<0.05$) from control group

The same group of workers also looked at the effect of iron status on the transpulmonary transport and tissue distribution of manganese using rats that had either been repeatedly bled (low iron status) or exposed to iron oxide fumes (5 x 4 hour of 100 mg/m3 iron oxide, aerodynamic mass median diameter, AMMD, 0.68 μm) before a single intratracheal instillation of a $^{54}MnCl_2$ tracer (Brain et al., 2006). The rats exposed to iron oxide retained significantly more manganese in the lungs at 4 and 72 hours than the controls and showed a slower distribution of manganese to other tissues than the control group. Although the bled rats showed similar levels of manganese in the lungs at 4 hours, they showed significantly more radioactivity in the brain than the control group.

Table 4.1.13 Comparison of the distribution (% dose) at 4 and 72 hours after the administration of a ^{54}Mn tracer by intratracheal instillation to rats with differing iron status (Brain et al., 2006)

Dose group	Control	Bled (low iron status)	Iron Oxide exposed	Control	Bled (low iron status)	Iron Oxide exposed
Time	**4 hour**			**72 hour**		
Lungs	57.37±3.62	58.07±2.22	73.77±2.40*	20.02±1.90	25.03±1.81	35.99±2.72*
Brain	0.054±0.008	0.078±0.005*	0.030±0.001*	0.121±0.024	0.167±0.006	0.167±0.041
Liver	4.97±1.50	4.55±2.11	2.26±0.83	7.75±1.53	6.88±0.85	7.22±0.57
Blood	0.089±0.023	0.159±0.036	0.062±0.019	0.118±0.026	0.118±0.024	0.083±0.019
Kidneys	3.12±0.26	3.26±0.27	1.37±0.09*	2.99±0.59	3.30±0.25	2.70±0.08

* - significantly different ($p<0.05$) from control group

A separate investigation by the same group of workers using a very similar dosing regimen showed that ^{54}Mn absorption from the lungs to the blood was lower in rats fed a high iron diet (Thompson et al., 2006). The distribution of a ^{54}Mn tracer administered by intratracheal instillation to a group of rats that had been fed a high-iron diet (1% carbonyl iron) for 4 weeks showed significantly less radioactivity in the blood at 4 hours after dosing compared to rats fed a control diet (0.033% compared to 0.093% of instilled dose). There was significantly less radioactivity in the small intestine, which was probably due to a lower amount of dose that had been excreted in the bile for potential duodenal re-absorption. There was significantly more radioactivity remaining in the lungs at 4 hours post-dose from the high iron diet group, which was consistent with the reduced amount circulating in the blood. All these finding were consistent with a lower uptake of manganese from the lungs in the high iron diet group of rats dosed by intratracheal instillation. Following the iv injection the high iron diet group of rats also showed a more rapid disappearance of radioactivity from the blood compared to rats fed the control diet.

Table 4.1.14 Comparison of the distribution (% dose) 4 hours after the administration of a ^{54}Mn tracer either by intratracheal instillation or iv injection to rats (Thompson et al., 2006)

Dose route	**Intratracheal Instillation**		**iv**	
Dose group	Control	High Iron Diet	Control	High Iron Diet
Brain	0.05±0.01	0.03±0.00	0.21±0.01	0.23±0.02
Liver	4.90±0.90	5.34±0.34	22.41±1.36	22.40±1.26
Lungs	57.94±2.49	79.18±0.28*	0.92±0.03	1.14±0.06*
Kidneys	2.56±0.31	1.89±0.05	8.00±0.49	9.98±0.47*
Skeletal muscle	4.29±0.72	4.73±0.35	13.33±0.79	12.53±0.98
Small intestine	6.44±0.83	2.64±0.44*	16.13±0.69	15.89±1.40
Large intestine	2.87±0.74	2.67±0.26	6.64±0.57	6.42±0.71

* - significantly different ($p<0.05$) from control group

A study measuring manganese body burden in CD rats and foetuses following inhalation of a $MnSO_4$ aerosol during pregnancy found that the placenta partially sequesters inhaled manganese, thereby limiting exposure to the foetus (Dorman et al., 2005a).

A follow-on study by the same workers determined tissue manganese concentrations in lactating CD rats and their offspring following manganese sulfate aerosol inhalation found that neonatal tissue manganese concentrations are dependent on the $MnSO_4$ exposure concentration and the age of the animal (Dorman et al., 2005b).

The olfactory neuronal pathway was shown to be efficient for translocating inhaled manganese oxide as solid ultrafine particles (UFPs) to the central nervous system of groups of rats (Elder et al., 2006). The manganese oxide UFPs were composed of particles of 3-8 nm in diameter forming an aerosol with agglomerates of approximately 30 nm. After 12 days of exposure (6hr/day) to 465±94 µg Mn/m^3 with both nostrils patent, manganese concentrations in the olfactory bulb increased 3.5-fold, whereas lung manganese concentrations doubled; there were also significant increases in the

striatum, frontal cortex, and cerebellum. With the right nostril occluded for a 2-day exposure, manganese accumulated only in the left olfactory bulb indicating that this accumulation was from direct uptake rather than from systemic circulation. Analysis of the UFP manganese oxide showed that it composed of approximately 61% MnO (Mn^{2+}) and 39% Mn_2O_3 (Mn^{3+}) with an overall dissolution rate of up to 1.5% per day. However, acidification to pH 4.5, similar to the phagolysosomal conditions of alveolar macrophages resulted in rapid dissolution and likely contributed to the blood-borne manganese that was then distributed through the body. Given the low solubilisation of UFP manganese oxide at neutral pH, it was expected that only a small fraction that was deposited on the olfactory mucosa would be translocated to the olfactory bulbs in its soluble form. However, similar manganese burdens (approximately 8.2% of dose) were found in the olfactory bulbs of rats 24 hours after intranasal instillation of similar quantities (5-7 mg manganese) of either a manganese chloride solution or a suspension of UFP manganese oxide. The authors concluded that these data indicated that the appearance of manganese in the olfactory tissues was not due to soluble manganese from the manganese oxide UFP but was from solid particles of manganese oxide UFP being transported directly.

The distribution of manganese in rat tissues after exposure to manganese from welding-fumes (2 hours per day for 30 days of 63.5 ±2.3 mg/m^3 welding fumes, 2.76±0.06 mg Mn/m^3) showed similar distribution patterns in both the iron-deficient and iron-sufficient groups (Park et al., 2007b). The authors concluded that iron deficiency did not have any apparent effect on the transport of manganese into the brain after the inhalation of welding fumes. There was only a single dose level used in this study, which although relevant to human industrial exposure, did not appear to produce significant nor informing results to the toxicokinetic assessment of manganese. It also appears that the iron present in the welding fumes was at a sufficient level to compensate for the iron-deficiency that had been introduced into the rats' diets and, as such, both groups had similar iron serum levels by the end of the exposure period.

The distribution of manganese in tissues and brains from rhesus monkeys that had inhaled manganese dioxide dust (approximately 0.7 or 3 mg manganese dust/m^3) for 22 hours/day, seven days a week for 10 months, were compared to controls (Nishiyama et al., 1977). Approximately 80% of the manganese dioxide dust was <1 μm in diameter and all particles were <2 μm in diameter. Presumably due to the low group size, statistical evaluation of the significance of the results was not performed; however manganese levels in almost all organs and tissues increased in proportion to the exposure levels. The authors noted that the accumulation of manganese in the basal nuclei of the brain (caudate nucleus, pallidum and putamen) was most striking, with manganese concentrations of 17-20 μg/g (dry weight) in the high dose group compared to around 6 μg/g (dry weight) for the control group.

Monkeys' brains were imaged before and after manganese administration (as manganese chloride) in coronal and horizontal planes that included the basal ganglia and substantia nigra (Newland et al., 1989). One monkey was exposed to manganese chloride aerosol (20-40 mg Mn/m^3, mean particle size 1.2 μm) for 2 hr/day, 4 day/week and had its brain imaged after 3 and 5 months' exposure. Other monkeys received either 5, 10 or 20 mg Mn/kg by iv injection and had their brains imaged starting at 2 days after administration. The kinetics of manganese accumulation was important in determining the imaged intensity of these regions, but the route of administration (inhalation or iv) was not. Spin-lattice relaxation times showed that T1 was shortened at lower doses of manganese and remained shortened longer in the globus pallidus and pituitary gland while little effect appeared in grey and white matter. T1 effects in caudate and putamen effects were intermediate. These data suggested selective affinity for manganese in the globus pallidus and pituitary regions of the brain.

These findings were consistent with the tissue manganese concentrations observed in young male rhesus monkeys following subchronic manganese sulphate inhalation for 6 h/day, 5 days/week (Dorman et al., 2006b). Groups of monkeys were exposed to either air or $MnSO_4$ (0.06, 0.3, or 1.5 mg Mn/m^3, MMAD 1.72-2.12 μm) for 65 exposure days before tissue analysis. Monkeys at the lowest dose level developed increased manganese concentrations in the olfactory epithelium, olfactory bulb, olfactory cortex, globus pallidus, putamen, and cerebellum. A greater than 3 to 5-fold

increase in mean tissue concentration was observed in the globus pallidus, putamen and caudate of monkeys exposed at the highest dose level. Results from this work were combined with MRI, pallidal index (PI), and T(1) relaxation rate (R1) to establish a direct association between MRI changes and pallidal manganese concentrations (Dorman et al., 2006c). The authors stated that their results indicated that the R1 can be used to estimate regional brain manganese concentrations and may be a reliable biomarker of occupational manganese exposure. Further, they suggested that this study was the first to provide indirect evidence of direct olfactory transport of an inhaled metal in a nonhuman primate. They concluded that the pallidal delivery of manganese, however, likely arises primarily from systemic delivery and not directly from olfactory transport.

A decrease in the intensity of MRI T1 relaxation times was observed between 30 and 60 days after the start of a male cynomolgus monkey inhalation exposure study to manual metal-arc stainless steel (MMA-SS) welding fumes (Park et al., 2007a; Sung et al., 2007). These changes in MRI T1 relaxation times appeared to precede a measured increase in blood manganese levels (approximately 2 to 4-fold above control values after 90 days). The monkeys were exposed for 2 hours per day to the MMA-SS welding fumes at dose levels of either 0.95 or 1.95 mg Mn/m^3 in an inhalation chamber for a total of 230 days. The treatment related results from the low dose group, both blood and tissue manganese levels and MRI T1 relaxation times appear to higher (blood and tissue Mn) and shorter (MRI T1) than the high dose group, possibly because the two dose levels were too close together.

Table 4.1.15 Monkey tissue manganese concentrations (µg Mn/g tissue wet weight, n=2 per group) after inhalation exposure (2 hour/day for 230 days) to manual metal-arc stainless steel welding fumes (Park et al., 2007a)

Tissue	Unexposed	0.95 mg Mn/m^3	1.95 mg Mn/m^3
Spleen	0.16	0.32	0.17
Lungs	0.10	41.9	55.8
Liver	0.69	1.40	1.40
Kidneys	0.48	0.98	0.97
Epididymus	0.14	0.66	0.17
Testes	0.09	0.45	0.35
Temporal lobe	0.41	0.55	0.44
Frontal lobe	0.48	0.75	0.55
Hippocampus	0.38	0.74	0.47
Cerebellum	0.78	1.07	0.67
Substantia nigra	0.51	0.58	0.72
Caudate	0.54	0.87	0.66
Putamen	0.42	0.85	0.81
Globus pallidus	0.58	1.23	1.44

4.2 Discussion of Distribution, Transportation and Metabolism Data

Manganese is naturally distributed throughout the human body with concentrations in most tissues within the 0.3 to 3.0 µg Mn/g wet tissue weight range (Aschner et al., 2005). Manganese levels in the human liver and kidneys are slightly higher at around 4 to 5 µg Mn/g and a lot lower in whole blood with values typically in the range 4-20 µg Mn/L. Manganese tissue levels in other species are generally of a similar magnitude.

As manganese is essential to many of the body's normal metabolic functions the body is in a continuous cycle of absorption, distribution and excretion of manganese, which is controlled by a relatively efficient homeostatic regulation.

It is likely that manganese is metabolised by the body in the form of converting manganese from the Mn^{2+} valence state to the Mn^{3+} valence state (Gibbons et al., 1976). The authors proposed that ingested manganese is absorbed as Mn^{2+} possibly bound to alpha2-macroglobulin or albumin. In transversing the liver it is removed nearly quantitatively, but a small proportion is oxidised to the Mn^{3+} valence state, bound to transferrin and enters the systemic circulation to be transported to tissues. More recently, it has been proposed that the oxidation state of manganese exposure may be an important determinant of the toxicokinetics of manganese and tissue toxicodynamics and subsequently neurotoxicity (Reaney et al., 2006).

Examples of manganese poisoning in humans following manganese administration by TPN raise a number of key points:

- Signs of CMnP were seen from around 30-80 ug/kg b.w./day manganese by TPN.
- Young children are more sensitive than adults.
- Blood manganese was raised, presumably reflecting total body burden.
- MRI scans showed T1-weighted images in basal ganglia especially globus pallidus.
- After cessation of manganese administration, whole blood manganese decreased back to normal range and high intensity of basal ganglia decreased.

Based upon using a 70 kg bodyweight for an adult human, the toxic levels of manganese seen from repeated daily TPN equate to between 2.1 to 5.6 mg Mn/day being added to the systemic circulation. Based upon an estimated oral absorption of 5%, the ingestion of manganese from a potassium permanganate solution (Holzgraefe et al., 1986; Bleich et al., 1999) at approximately 124 mg Mn/day, which resulted in chronic manganese poisoning, would similarly be 6.2 mg Mn/day. These estimated daily levels of manganese added to the body are only approximately 10-fold greater than might be expected from a 10 mg/day manganese consumption (World_Health_Organization, 2004) from the diet based upon a 5% absorption rate. In a study investigating the plasma uptake of manganese, it was found that an oral daily dose of 40 mg Mn/day or greater was needed to get a consistent manganese plasma response in all subjects (Bales et al., 1987). This consistent manganese plasma response level could well be the upper limit beyond which the body's natural homeostatic regulation is exceeded. Using the estimated 5% oral absorption figure for manganese, this would equate to an actual daily systemic uptake of 2 mg Mn/day. The level of manganese in drinking water contaminated with manganese which led to manganese poisoning was approximately 14 mg Mn/L (Kawamura et al., 1941). Assuming a daily water intake of 2 litres (World_Health_Organization, 2004) this would provide a minimum of an additional 28 mg manganese per day for oral absorption over the normal dietary intake. The contaminated well water was also likely to be used for cooking and washing leading to further potential additional exposure to manganese. Added together, these could make an estimated 40 mg Mn/day or greater for a period of 3 months, which on an estimated 5% oral absorption figure for manganese would equate to actual daily systemic uptake of 3 mg Mn/day. All of these examples are only estimates, however, using a 5% oral absorption rate it appears that a chronic systemic uptake of 2 mg Mn/day or above in healthy humans could be a trigger level that can lead to manganese intoxication.

Humans with hepatobiliary disease are likely to be more at risk of manganese intoxication. The examination of human brain samples at autopsy from 12 cirrhotic patients compared to matched controls showed that manganese levels were significantly increased in the frontal cortex (by 38%), occipital cortex (by 55%), pallidum (by 186%), putamen (by 66%), and caudate (by 54%) of cirrhotic patients compared with controls (Rose et al., 1999). The same publication also reported that groups of rats with either end-to-side portacaval anastomosis or with biliary cirrhosis, showed increased manganese levels in the pallidum and caudate/putamen compared with control groups. The authors concluded that brain manganese deposition results both from portal-systemic shunting and from liver dysfunction.

Focusing on the distribution of manganese as a percentage of a ^{54}Mn dose is possibly very deceptive due to the natural homeostatic regulation of manganese and the variable factors affecting oral absorption (see Section 3.2.3).

Evidence that there may be an adaptive response to manganese absorption during continuous exposure was seen when adult male rats were exposed to 0.5% manganese as $MnCl_2$ in their drinking water (Hietanen et al., 1981). The manganese concentrations in the blood, brain, liver and kidney, were highest after one week of exposure and had reduced considerably by 6 weeks after exposure. Of the limited tissues analysed, it seemed that there was an apparent correlation between the blood and brain manganese levels during exposure. The chronic exposure of rats throughout development until 90 days of age resulted in large increases in manganese concentrations in all brain regions, although the increases were not distributed evenly (Lai et al., 1992, Klimisch Code 4). Of the tissues analysed, the hypothalamus and striatum had the lowest manganese levels in the controls and the highest in the treated group.

Table 4.2.1 Tissue distribution (µg/g wet weight) of manganese in the brains of rats after chronic lifetime exposure to 20 mg/mL $MnCl_2$ drinking water (Lai et al., 1992).

	Hypothalamus	**Cerebellum**	**Pons and Medulla**	**Striatum**	**Midbrain**	**Cerebral Cortex**
Control	0.30±0.13	0.38±0.05	0.37±0.12	0.24±0.03	0.38±0.01	0.34±0.07
Treated	1.89±0.12*	1.04±0.10*	1.12±0.10*	1.39±0.24*	1.33±0.20*	0.83±0.16*

* - statistically significant increase ($P<0.003$) over control

There appears to be a lack of reliable comprehensive data on the complete tissue distribution of manganese in rodents following oral administration. Many more studies report the distribution following ip administration whereby a known amount of manganese is administered and, as such, is assumed to be a surrogate for oral administration. However, there appears to be an important first-pass effect with manganese following oral absorption that will be bypassed following other administration routes (see Section 6).

An example comparing the distribution of manganese in rats following repeated oral and ip administration (Table 4.2.2) generally showed a good similarity with the exception of tissues that may have been physically compromised by the repeat dosing (Missy et al., 2000). Despite the ip dose level being only 8% of the oral dose level, a level estimated to try and obtain equivalent effective doses, there was still greater accumulation in most tissues following the ip dosing. The femur and the brain showed the highest accumulation following oral dosing with the spinal cord, testes, spleen and blood being additional tissues also showing higher accumulation following ip administration.

Table 4.2.2 Tissue distribution (µg/g wet weight) of manganese in rats after the cessation of a 4-week chronic administration of $MnCl_2$ either orally (75 mg Mn/kg/day) or by ip injection (6 mg Mn/kg/day) (Missy et al., 2000)

	Oral administration (75 mg Mn/kg/day)			**ip administration (6 mg/kg/day)**		
Tissue	Control	Treated	Accumulation factor	Control	Treated	Accumulation factor
Femur*	1.2±0.2	4.8±1.9	x4.0	1.2±0.10	33±8	x27.6
Stomach	0.88±0.34	2.1±0.3	x2.4	0.82±0.19	1.70±0.34	x2.1
Spinal cord	0.32±0.02	0.49±0.05	x1.5	0.32±0.02	0.74±0.16	x2.3
Skeletal muscle	0.08±0.01	0.12±0.01	x1.5	0.08±0.01	0.14±0.01	x1.7
Spleen	0.30±0.02	0.38±0.03	x1.3	0.31±0.02	1.10±0.18	x3.5
Lungs**	0.24±0.02	11.6±9.1	x48.3	0.23±0.02	0.35±0.06	x1.5
Fur, skin**	0.08±0.01	0.24±0.12	x3.0	0.08±0.02	0.12±0.02	x1.5
Oesophagus**	0.16±0.04	0.48±0.22	x3.0	0.17±0.06	0.29±0.14	NS
Brain	0.40±0.03	0.79±0.27	x2.0	0.43±0.04	0.90±0.10	x2.1
Adrenal glands	2.8±0.4	3.7±0.5	x1.3	3.12±1.00	3.58±1.01	NS
Kidneys	1.5±0.2	1.8±0.1	x1.2	1.51±0.15	2.36±0.20	x1.6
Heart	0.46±0.04	0.53±0.04	x1.1	0.43±0.03	0.60±0.04	x1.4
Duodenum***	0.52±0.09	0.69±0.09	x1.3	0.57±0.10	3.36±2.89	x5.9
Pancreas	1.2±0.1	1.5±0.2	x1.2	1.13±0.16	1.78±0.41	x1.6
Colon	1.3±0.2	1.6±0.2	x1.2	1.53±0.33	2.63±0.73	x1.7
Testes	0.43±0.04	0.49±0.02	x1.1	0.46±0.03	1.46±0.86	x3.2
Adipose tissue	0.08±0.02	0.14±0.07	NS	0.09±0.03	0.20±0.13	x2.2
Liver	1.9±0.2	2.0±0.2	NS	1.88±0.21	2.02±0.27	NS
Jejunum***	0.25±0.13	0.32±0.10	NS	0.28±0.13	2.11±2.19	x7.5
Ileum***	0.34±0.18	0.44±0.31	NS	0.34±0.17	1.63±1.15	x4.8
Bone marrow	-	-	-	0.22±0.09	1.15±0.74	x5.2
Blood****	-	-	NS	0.010±0.002	0.191±0.039	x18.5

Key:
* - µg/g dry weight
** - High results and variability following oral administration may have been due to the repeat dosing technique (force-feeding and possible regurgitation)
*** - High results and variability following ip administration may have been due to the repeat dosing technique (direct contact with ip administration)
**** - µg/mL
NS – No statistical significant accumulation

The ip route of administration was used to examine the brain distribution of manganese in mice 24 hours after the last of 3 daily ip administrations of $^{54}MnCl_2$ (Kobayashi et al., 2003, Klimisch Code 4). Although there were only 3 daily doses, the top dose level of 50 mg Mn/kg/day was high enough for the mice to show catalepsy within one hour of injection. This high dose level also showed a continued day-on-day increase in body burden of radioactivity whereas the lower 30 mg Mn/kg/day dose level reached a plateau after the first injection. This highest dose level, which was both exhibiting toxicity and non-linear kinetics, showed that more manganese was distributed to the striatum, hippocampus and the remainder of the brain that contained the substantia nigra than to the cortex. The same dose regimen of 30 mg Mn/kg/day was used in rats for 30 days repeated ip injection and thirteen brain regions analysed for manganese levels (Scheuhammer and Cherian, 1981)(Klimisch Code 4). These results concurred that under these conditions of chronic exposure, manganese was taken up by the striatal, mid-brain and the thalamic regions of the brain at a greater rate than the other areas. The mechanisms behind the transport of manganese across the blood-brain barrier (BBB) has been described in detail in many excellent review papers (Crossgrove and Zheng, 2004; Crossgrove and Yokel, 2004; Yokel and Crossgrove, 2004; Aschner, 2006). From studies in rats, *in vivo* and *in vitro*, it has been shown that manganese can enter the brain via carrier-mediated transport and can leave the brain via diffusion only, a much slower process than carrier-mediated transport (Crossgrove et al., 2003; Yokel et al., 2003; Yokel and Crossgrove, 2004). The authors concluded that it suggests that no mechanism exists to protect the brain from accumulating

manganese and this has important implications for neurotoxicity resulting from chronic manganese exposure. Although Yokel and Crossgrove studied manganese transport rates in rats, their observations may be relevant to humans because transport mechanisms at the blood–brain barrier are similar in rodents and humans.

A consistent region-specific pattern of T1-weighted hyperintensities was observed using MRI in the brains of rats treated with 14 weekly iv injections of 3 mg Mn/kg as manganese chloride solution (Finkelstein et al., 2008). Cortical hyperintensities were prominent in the hippocampus and dentate gyrus. The authors concluded that the prominent manganese depositions (determined by MRI) after the subacute manganese exposure were compatible with the clinical picture of manganism during its early stages, and thus may explain its pathophysiology.

Manganese has also been shown to cross the feto-maternal barrier in rats following iv dosing (Onoda et al., 1977; Kaur et al., 1980), although both these studies had a Klimisch Code rating of 4.

The key points arising from the distribution of manganese in rodents following oral, ip and iv administration are:

- Manganese is widely distributed to all tissues.
- Manganese apparently crosses placental and blood–brain barriers.
- Chronic exposure can lead to accumulation of manganese in particular regions of the brain.
- Manganese accumulation in tissues is also associated with increases in blood levels of manganese.

The main focus of animal distribution studies with manganese is concerned with the tissue distribution following inhalation, since the major human workplace exposure to manganese is via this route. A summary of the key results for animal manganese distribution studies following inhalation exposure are presented in Tables 4.2.3 – 4.2.6.

The key points arising from the distribution of manganese in rodents following inhalation exposures are:

- Inhalation of insoluble forms of manganese can lead to higher lung manganese concentrations due to less efficient clearance mechanisms.
- Inhalation of soluble forms of manganese leads to higher concentrations in other tissues.
- Inhalation of manganese appears to result in a more extensive distribution within the brain and olfactory tissues than following oral administration.
- The olfactory bulb and brain tissues show accumulation of manganese without concurrent increases in the blood manganese.

This last point is quite intriguing, as following oral and ip administration the femur showed the most accumulation, and significant accumulation in the brain and other tissues appeared to be related to an overall increased body burden as evidenced by increased blood manganese concentrations. However, following inhalation, increases in brain manganese levels were seen without large increases in blood manganese levels, and only moderate increases in femur manganese levels. This implies that either the manganese tendency to cross the BBB was enhanced following inhalation, or there was an additional route of delivery to the brain following inhalation that is not seen after oral exposure, or both. In order to test these hypotheses, manganese distribution and uptake utilising techniques such as nose-only inhalation with one nostril plugged, nasal instillation and intratracheal instillation have been studied.

Investigations into manganese uptake to the brain via the olfactory route in rats have shown that manganese moves relatively freely from the olfactory bulb to the olfactory cortex at an amount dependent on the level of influx into the bulb. The transport to the rest of the brain is related to the amounts in the olfactory bulb and the olfactory cortex, but the relative proportion reaching this area increases with increasing doses. A nose-only inhalation with either one nostril plugged or both nostrils patent, found that the olfactory route contributes the majority (up to >90%) of the ^{54}Mn found in the olfactory pathway, but not in the striatum, of the rat brain up to 8 days following a single

inhalation exposure to $^{54}MnCl_2$ (Brenneman et al., 2000). Using the same study exposure conditions, inhalation exposure of the relatively poorly soluble $^{4}MnHPO_4$ resulted in a slower clearance of manganese from the olfactory epithelia than for the soluble $^{54}MnCl_2$ (Dorman et al., 2002). However, overall the results were qualitatively similar to those obtained with the more soluble form of manganese including that this route of administration did not significantly contribute to increases in striatal concentrations of manganese. A different group of workers showed that solid ultrafine particles (UFPs) of manganese oxide were also translocated via the rat olfactory neuronal pathway to the CNS system of rats and that this was not due to soluble manganese from the manganese oxide UFP but was from solid particles of manganese oxide UFP being transported directly (Elder et al., 2006). Significant trans-synaptic transport of Mn^{2+} to secondary and tertiary olfactory neurons of the rat olfactory system was shown as early as 1 hour after administration of 10 µL of 1 M manganese chloride into the right nasal epithelium of rats by using MRI (Cross et al., 2004). In a later study by the same workers, it was shown that age-related decreases in axonal transport rate and bulk transport of Mn^{2+} in the olfactory tract of living rat brains (Cross et al., 2008). Longitudinally scanned, mid-age group was decreased by 58% and the aged group by 71% of young rate (neuronal transport was 4.07+/-1.24 mm/h, 1.72+/-0.89 mm/h, and 1.16+/-0.18 mm/h for young, mid-age, and aged, respectively). The neuronal transport rate decreases correlated with increased age.

A comparison of the uptake of either soluble $^{54}MnCl_2$ or insoluble $^{54}Mn_3O_4$ to rats by a single intratracheal instillation showed that radioactivity from $^{54}MnCl_2$ was cleared from the lungs faster than from $^{54}Mn_3O_4$ resulting in higher and earlier T_{max} and C_{max} values (Drown et al., 1986). The particle size of the insoluble manganese tetraoxide was determined by scanning electron microscopy to be less than 5µm in diameter, with 90% of the material having a diameter of 1 µm or less. The iron status of rats were shown to have considerable influence on the uptake and blood manganese kinetics following administration by either iv or intratracheal instillation of $^{54}MnCl_2$ (Heilig et al., 2005; Brain et al., 2006; Thompson et al., 2006). The rats on the high-iron diet showed a decrease in uptake of radioactivity from the lungs and generally lower blood manganese levels (both administration routes) as well as lower brain manganese levels than the rats on a control diet. Following iv dosing to rats on an iron-deficient diet, the blood manganese levels were much higher than controls at all time points and there was also increased brain manganese levels. A substantial increase in radioactivity in the red blood cells over controls was seen following both administration routes. The blood manganese levels following intratracheal instillation were initially lower in the iron-deficient group until about 15 minutes post-administration whereby they reached an apparent plateau, which was in contrast to the control group.

The influence of the route of administration on the absorption and cerebral distribution of manganese in rats following dosing with either $MnCl_2$ solutions or MnO_2 suspensions showed that higher elevation of blood manganese concentrations following intratracheal instillation of $MnCl_2$ were seen compared to oral administration (Roels et al., 1997). The authors suggested that the high blood manganese concentration, produced during a short period after pulmonary absorption, transiently overwhelmed the mechanisms controlling the uptake of manganese into the brain. The elective distribution of manganese in the striatum compared to the other areas may be explained by the presence of different manganese uptake mechanisms in the various cerebral regions.

The use of the intranasal and intratracheal instillation dose routes for rodents has shown that:

- The olfactory route does provide a direct method of transport of manganese to the brain.
- The likelihood of elevated blood manganese levels is greater following pulmonary absorption compared to oral absorption.

Inhalation studies in monkeys have shown that the olfactory route of absorption of inhaled manganese is also operative in primates, although the direct delivery of manganese to the basal ganglia via the olfactory pathway was not evident from MRI investigations (Dorman et al., 2006b).

In summary, it has been shown through animal studies focussing on the uptake of manganese into brain tissue that there are three routes of entry:

1. from the bloodstream through the cerebral spinal fluid (CSF) via the choroid plexus (Murphy et al., 1991)
2. from the bloodstream across the BBB at the cerebral capillaries (Rabin et al., 1993; Crossgrove et al., 2003)
3. from nasal inhalation through the olfactory nerve via the olfactory epithelium (Tjalve et al., 1995; Tjalve et al., 1996)

Whether one route dominates in the cases of manganese poisoning in humans and non-human primates has not been established.

Table 4.2.3 Tissue distribution in rats (µg/g wet weight) of manganese after inhalation exposure to manganese sulphate

Tissue	**Control**	**0.3 mg Mn/m^3 (14 days)[a] (MMAD 2.1 µm)**	**0.5 Mn/m^3 (66 days)[c] (MMAD 2.03 µm)**	**0.5 Mn/m^3 (91 days)[d] (MMAD 2.03 µm)**	**0.5 Mn/m^3 (66 days)[e] (GMD 1.05 µm)**	**1.0 Mn/m^3 (66 days)[e] (GMD 1.07 µm)**	**0.92 Mn/m^3 (14 days)[b] (MMAD 2.1 µm)**	**3 mg Mn/m^3 (14 days)[a] (MMAD 2.1 µm)**	**3 mg Mn/m^3 (91 days)[f] (80%< 1.55 µm)**
Femur	0.5-0.6	0.74±0.06*	0.86±0.05*		0.77±0.05	0.89±0.06*	0.64	1.28±0.06*	
Liver	1.8-3.2	2.78±0.15	2.04±0.09		3.37±0.15	4.28±0.76*	2.51	3.64±0.46*	2.61±0.27
Bile	0.3-0.4	0.51±0.13					0.73*	1.51±0.17*	
Pancreas	1.6-2.2		2.65±0.06*		1.29±0.28	1.91±0.23			
Lung	0.2-0.5	1.24±0.09*	0.36±0.02*		0.86±0.07*	1.05±0.06*	1.17*	7.33±0.37*	6.21±1.85*
Testes	0.3-0.4	0.40±0.02	0.35±0.02				0.43	0.79±0.18*	0.42±0.02*
Olfactory bulb	*0.6-1.1*	*2.0**	1.95±0.09*	*1.9**	1.40±0.07*	1.73±0.07*	1.38	4.42±0.23*	2.30±0.20*
Striatum	*0.4-0.6*	*0.8*	0.72±0.02*	*0.75**	0.74±0.02*	0.89±0.02*	0.88*	3.18±0.59*	1.21±0.09*
Cerebellum	0.4-0.6		0.59±0.03*	*0.6**	0.60±0.01*	0.61±0.03*	0.63		0.84±0.08*
Globus pallidus	0.6								1.51±0.47*
Blood	0.05-0.1		0.08±0.02		0.06±0.01	0.05±0.01			
Serum	0.1-0.2	0.14±0.02					0.20	0.31±0.20	
Milk	0.21				0.47±0.06	0.77±0.10*			

Key:
a - (Dorman et al., 2001a)
b - (Dorman et al., 2001b)
c - (Dorman et al., 2004a) – young male rats
d - (Dorman et al., 2004b) – young male rats
e - (Dorman et al., 2005b) – lactating female rats
f - (Normandin et al., 2004) – male rats with a manganese sulphate/manganese phosphate mixture (39:61)
Values in *italics* have been estimated from graphical data.
* - data statistically significantly different from respective controls
MMAD – Mass Median Aerodynamic Diameter
GMD – Geometric Mean Diameter

Table 4.2.4 Tissue distribution in rats (µg/g wet weight) of manganese after inhalation exposure to manganese phosphate, manganese oxides and metallic manganese.

Tissue	**Control**	**0.3 mg Mn/m^3 (14 days)[a]**	**3 mg Mn/m^3 (14 days)[a]**	**0.1 mg Mn/m^3 (66 days)[b]**	**0.1 mg Mn/m^3 (91 days)[c]**	**0.3 mg Mn/m^3 (14 days)[d]**	**3 mg Mn/m^3 (14 days)[d]**	**3 mg Mn/m^3 (91 days)[e]**	**4 mg Mn/m^3 (91 days)[e]**	**0.1 mg Mn/m^3 (12 days)[f]**
	Manganese Form	**Mn_3O_4 (MMAD 1.6 µm)**	**Mn_3O_4 (MMAD 1.8 µm)**	**Manganese Phosphate (Hureaulite) (MMAD 1.47 µm)**	**Manganese Phosphate (Hureaulite) (MMAD 1.47 µm)**	**Manganese Phosphate (Hureaulite) (MMAD 1.56 µm)**	**Manganese Phosphate (Hureaulite) (MMAD 1.60 µm)**	**Manganese Phosphate (Hureaulite) (80%< 1.55 µm)**	**Metallic Manganese (90%< 1.55 µm)**	**MnO/Mn_2O_3 (61/39) UFPs (3-8 nm -aerosol agglomerates ~30 nm)**
Femur	0.4-0.6	0.47±0.08	0.68±0.05*	0.67±0.03		0.69±0.09	1.19±0.14*			
Liver	1.8-3.2	3.46±0.15	3.48±0.31	1.94±0.06		3.06±0.23	3.00±0.21	2.66±0.39	2.44±0.19	
Bile	0.3-0.4	0.27±0.07	0.95±0.13*							
Pancreas	1.6-2.2			2.24±0.04						
Lung	0.2-0.5	1.65±0.10*	14.73±2.57*	0.64±0.05		2.54±0.31±	20.31±0.95*	9.86±3.77*	0.29±0.07*	*0.45**
Testes	0.3-0.4	0.41±0.03	0.46±0.03	0.31±0.01				0.36±0.05*	0.30±0.03	
Olfactory bulb	*0.6-1.1*	*1.3*	3.09±0.29*	0.83±0.02	*0.8*	1.13±0.11*	1.89±0.13*	2.32±1.22*		*1.75**
Striatum	*0.4-0.6*	*1.0*	1.48±0.12*	0.51±0.02	*0.55*	0.81±0.09*	0.90±0.06*	1.06±0.14*	0.86±0.04*	*0.7**
Cerebellum	0.4-0.6			0.47±0.02	*0.5*	0.60±0.03	0.74±0.09*	0.73±0.05*	0.63±0.04	*0.6**
Globus pallidus	0.6							1.25±0.23*	0.93±0.06*	
Blood	0.05-0.1			0.03±0.01						
Serum/Plasma	0.1-0.2	0.11±0.02	0.10±0.02			0.14±0.03	0.12±0.01			

Key:

a - (Dorman et al., 2001a)
b - (Dorman et al., 2004a)
c- (Dorman et al., 2004b)
d - (Vitarella et al., 2000b)
e - (Normandin et al., 2004)
f - (Elder et al., 2006)
Values in *italics* have been estimated from graphical data.
* - data statistically significantly different from respective controls
MMAD – Mass Median Aerodynamic Diameter

Table 4.2.5 Tissue distribution in rats (µg/g wet weight) of manganese after intratracheal instillation of manganese chloride and manganese oxides

Tissue	**Control**	**0.055 mg (single dose)[a]**		**0.055 mg (single dose)[a]**		**3 ng Mn/kg (single dose)[b]**	**(single trace dose)[c]**	**1.2 mg Mn/kg (28 days)[d]**	**1.2 mg Mn/kg (28 days)[d]**
	Mn Form	$MnCl_2$		Mn_3O_4 (90% of particles < 1 µm)		$MnCl_2$	$MnCl_2$	$MnCl_2$	MnO_2
	Sampling Time-point	3 Days	7 Days	3 Days	7 Days	4 Hours	4 Hours	4 Days	4 Days
Bone		*0.05*	*0.03*	*0.09*	*0.05*				
Liver		*0.25*	*0.14*	*0.50*	*0.20*	7.9%	4.9%		
Brain		*0.035*	*0.035*	*0.047*	*0.037*	0.065%	0.046%		
Lung		*1.1*	*0.7*	*5*	*2*	60.7%	57.9%		
Testes		*0.06*	*0.05*	*0.08*	*0.05*				
Cortex	*0.4*							*0.55**	*0.45**
Striatum	*0.4*							*1.2**	*0.6**
Cerebellum	*0.4-0.45*							*0.6**	*0.55**
Blood (µg/L)	*0.05-0.06*							*0.085**	*0.08**

Key:

a - (Drown et al., 1986)
b - (Heilig et al., 2005)
c - (Thompson et al., 2006)
d - (Roels et al., 1997)
Values in *italics* have been estimated from graphical data.
* - data statistically significantly different from respective controls

Table 4.2.6 Tissue distribution in rats (µg/g wet weight) of manganese after intranasal instillation of manganese chloride

Tissue	**4 µg Mn/kg[a]**		**0.8 mg Mn[b]**	
	3 Days	7 Days	1 Day	1 Day
	Single	Single	Single	2 doses, 1 week apart
Liver	1.7±0.7	1.2±0.8		
Olfactory Bulb	105±26	65±33	*x4***	*x2***
Olfactory Tubercle			*x2***	*x1***
Basal Forebrain	32±9.4	39±25		
Cerebral Cortex	3.3±1.1	7.0±4.7		
Hypothalamus	4.5±1.5	10±6.3		
Hippocampus	0.9±0.2	2.3±1.3		
Striatum	2.1±0.9	5.7±3.2	*NS*	*x1***
Midbrain	1.0±0.4	4.7±3.2		
Cerebellum	0.2±0.0	0.5±0.3		

Key:
a - (Tjalve et al., 1996)
b - (Gianutsos et al., 1997)
Values in *italics* have been estimated from graphical data.
** - accumulation compared to control
NS – No statistical significant difference from control

Table 4.2.7 Tissue distribution in male rhesus monkeys (µg/g wet weight) of manganese after subchronic inhalation exposure to manganese sulphate (Dorman et al., 2006b)

Tissue	**Control**	**0.06 mg Mn/m^3 (65 days) (MMAD 1.73 µm)**	**0.3 mg Mn/m^3 (65 days) (MMAD 1.89 µm)**	**1.5 mg Mn/m^3 (65 days) (MMAD 2.12 µm)**	**1.5 mg Mn/m^3 (65 days) 45 days post-exposure[a] (MMAD 2.12 µm)**
Organs:					
Femur	0.13±0.02	0.11±0.01	0.13±0.03	0.20±0.03	0.12±0.02
Heart	0.16±0.03	0.33±0.03*	0.49±0.03*	0.62±0.05*	0.23±0.03
Kidney	1.14±0.12	1.43±0.05	1.86±0.14*	2.61±0.30*	1.38±0.13
Liver	2.49±0.09	2.91±0.18	3.17±0.20	3.52±0.45*	2.88±0.27
Lung	0.15±0.03	0.18±0.01	0.25±0.02*	0.33±0.04*	0.09±0.01
Pancreas	1.59±0.11	1.72±0.09	2.34±0.11*	2.95±0.24*	1.14±0.24
Muscle	0.15±0.03	0.12±0.03	0.18±0.02	0.58±0.19*	0.19±0.02
Bone	0.08±0.04	0.05±0.02	0.13±0.06	0.25±0.04*	0.17±0.03
Testis	0.26±0.03	0.35±0.03	0.40±0.05	0.39±0.07	0.36±0.04
Olfactory tissues:					
Olfactory epithelium	0.42±0.01	1.22±0.15*	2.96±0.46*	7.10±2.01*	0.65±0.04
Olfactory bulb	0.31±0.01	0.77±0.04*	1.36±0.15*	2.40±0.18*	0.35±0.02
Olfactory tract	0.30±0.06	0.43±0.02	0.61±0.05*	1.12±0.08*	0.18±0.02
Olfactory cortex	0.19±0.004	0.27±0.02*	0.31±0.01*	0.42±0.01*	0.26±0.01*
Brain:					
Globus pallidus	0.48±0.04	0.80±0.04*	1.28±0.15*	2.94±0.23*	1.09±0.03*
Putamen	0.36±0.01	0.58±0.04*	0.75±0.05*	1.81±0.14*	0.58±0.03*
Caudate	0.34±0.02	0.47±0.04	0.69±0.03*	1.72±0.10*	0.57±0.03
Frontal Cortex	0.25±0.03	0.29±0.02	0.29±0.01	0.47±0.02*	0.26±0.01
Cerebellum	0.44±0.01	0.62±0.02*	0.70±0.04*	1.10±0.11*	0.66±0.04
Pituitary	0.84±0.12	1.53±0.25	2.43±0.13*	6.19±0.61*	3.01±0.91*
Trigeminal nerve	0.17±0.05	0.17±0.01	0.21±0.01	0.42±0.08*	0.18±0.01
Fluids:					
Bile	0.89±0.22	1.65±0.31	3.78±0.34*	7.60±1.68*	1.17±0.28
Blood	0.010±0.001	0.015±0.002	0.022±0.003*	0.026±0.003*	0.021±0.002*

Key:
* - data statistically significantly different from respective controls
a – all tissue concentrations had fallen to control tissue levels by 90 days post-exposure

Table 4.2.8 Tissue distribution in female rhesus monkeys (µg/g wet weight) of manganese after inhalation exposure to manganese dioxide (Nishiyama et al., 1977)

Tissue	**Control**	**0.7 mg Mn/m^3 (10 months at 22 hours/day) (80% particles < 1 µm)**	**3 mg Mn/m^3 (10 months at 22 hours/day) (80% particles < 1 µm)**
Organs: (µg/g wet weight)			
Heart	*0.3*	*0.6*	*1*
Kidney	*1.5*	*2*	*2.5*
Liver	*2*	*2.5*	*2.5*
Lung	*2*	*22.3*	*24.2*
Pancreas	*2*	*3*	*4*
Muscle	*0.5*	*0.5*	*1*
Pituitary	*7*	*9.5*	*12*
Lymph nodes	*2.5*	*8*	*10*
Salivary gland	*1.5*	*4.5*	*6*
Bile	*1*	*4.5*	*8*
Brain: (µg/g dry weight)			
Cerebellum	*5.5*	*9*	*10*
Diencephalon	*8*	*13*	*12.5*
Mesencephalon	*7.5*	*8.5*	*11*
Pons	*10*	*15*	*12.5*
Medulla oblongata	*2.5*	*6.5*	*10*
Basal ganglia	*7*	*14*	*18*
White matter	*4*	*7.5*	*9*
Grey matter	*5*	*7*	*8.5*

Key:
Values in *italics* have been estimated from graphical data.

5 EXCRETION OF MANGANESE

5.1 Manganese soluble and insoluble salts

The main excretion route for manganese is in the faeces, however this route obviously also includes unabsorbed dose following oral dosing (around 95%) which is one of the complicating factors when trying to measure proportions of dose absorbed. Very little manganese is excreted in the urine or other potential routes of excretion such as milk or sweat.

5.1.1 Humans

An early study (McLeod and Robinson, 1972) measured the metabolic balance of manganese in young women over 27 days on a carefully controlled diet and almost achieved a complete mass balance, with an estimated 12% of intake unaccounted for. The authors reported that the urinary excretion of manganese was negligible compared to faecal excretion.

An early study compared the absorption and excretion of a ^{54}Mn tracer (as $^{54}MnCl_2$) to fasted subjects (Mena et al., 1969). The half-lives were estimated as 23±3, 34±5 and 37±7 days respectively for groups of anaemic patients, patients with manganism and a control group; however manganese excretion was significantly faster in healthy working manganese miners (15±2 days).

Men (n = 20) and women (n = 20) consuming a diet adequate in manganese (3-6 mg/day) were fed ^{54}Mn in a test meal. Subjects were counted in a whole-body counter during a 70-day period to determine whole-body retention of ^{54}Mn. Data from days 10 to 20 and from days 19 to 70 were analyzed by linear regression to calculate absorption and biological half-life. A failure in the pre-screening of subjects to measure serum ferritin concentrations meant that 11 out of 20 women and 1 out of 20 men were considered to be iron-deficient (serum ferritin values < 20 ug/L) when the measurements were made 2 weeks into the study. The authors acknowledged that the sex differences seen in manganese metabolism and excretion may have been related to iron status and concluded that men and women differ in manganese metabolism and that such differences may be related to iron status. As such, whilst the study provides useful information on manganese absorption and excretion in humans from diet, the sex differences seen may have been significantly influenced by the iron-deficient subjects, who were predominantly female. Mean estimates of half-life of manganese using whole body counts between days 10 and 20 were between 34 and 48 days (Finley et al., 1994).

The validity of using extrinsic ^{54}Mn tracers for the study of manganese absorption from foods of plant origin was studied (Johnson et al., 1991). Biological half-life of manganese, calculated from day 10 onwards data, was around 40 days (37 to 42 for the different groups).

The influence of overnight fasting upon the uptake of ^{54}Mn as a tracer together with a vitamin supplement containing 2.5 mg manganese (as manganese sulphate) calculated an estimated half-life of manganese using day 7-20 retention data was 17 days (Sandstrom et al., 1987). Less than 1% of the ^{54}Mn was excreted in the urine.

In an investigation into whether dietary manganese and dietary fats affected clinical or neuropsychological measures in healthy young women using a crossover study design, the absorption of manganese was estimated from a ^{54}Mn tracer administered in orange juice (Finley et al., 2003). Estimates of the biological half-lives of manganese were almost twice as short when subjects had consumed a high manganese diet (14-15 days) compared to a low manganese diet (30-34 days).

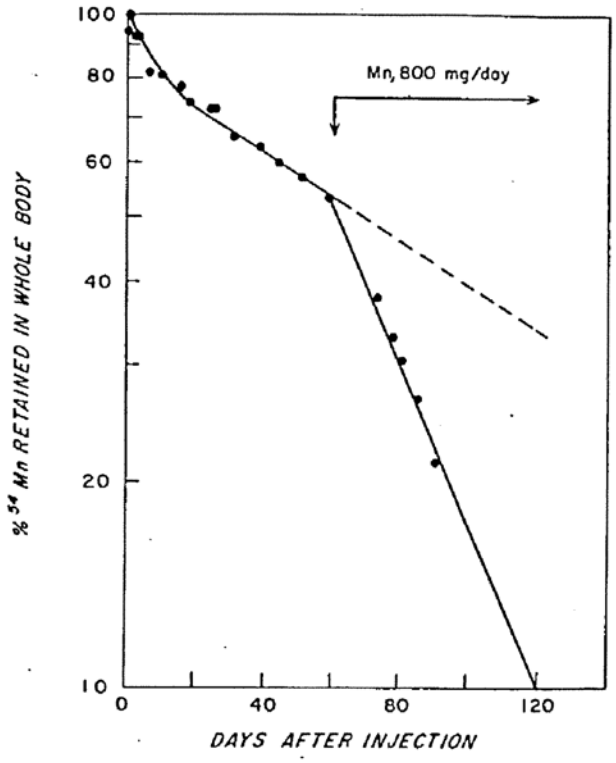


Figure 5.1 Disappearance of retained manganese in a mildly iron deficient subject on a calorie restricted diet (~800 cal/day) for 6 months prior to the study and continued on the diet during the study. Daily ingestion of 800 mg of manganese per day ($MnCl_2$ solution) was instigated at Day 50. (Mahoney and Small, 1968)

Some very interesting early work reported the disappearance of manganese from the body as a curve having two exponential components (Mahoney and Small, 1968). The half-life (measured using whole body counts following ^{54}Mn iv dosing) for the "fast" component was around 4 days followed by a slower second component with a half-life around 39 days. An additional human subject was preloaded with 300 mg of unlabelled manganese per day ($MnCl_2$ solution) for 10 days before the injection of the ^{54}Mn. The authors reported that the major effect was a 3-fold decrease in the half-life of the "fast" component to 1.5 days. In addition, another one of the subjects had been on a calorie-restricted diet (~800 cal/day) for 6 months prior to the study and continued on the diet during the study. After 60 days on the study over 50% of the injected radioactivity still remained in the body, the estimated half-life of the "slow" phase was 92 days. As such, a program of daily ingestion of 800 mg of manganese per day ($MnCl_2$ solution) was instigated in an effort to "flush" out the remaining ^{54}Mn from the body. This resulted in a dramatic increase in the rate of excretion of the original ^{54}Mn with a new apparent half-life of 28 days. At day 85, another iv injection of ^{54}Mn was administered whilst maintaining the 800 mg manganese per day regimen. This time there was no apparent "slow" elimination phase, just a single "fast" phase with a calculated half-life of 5 days.

The clearance of ^{54}Mn (administered iv as $MnCl_2$ solution) from blood, plasma and tissues was measured from 3 different groups of volunteers: a control group (19 men and women between 20-30 years old), a group of healthy working miners (20 miners between 23 and 60 years old) and a group of 18 patients with chronic manganese poisoning (18 to 56 years old ex-miners) (Cotzias et al., 1968). Initial clearance was very rapid, with only about 1% of the initial radioactivity recorded remaining in the blood or plasma after 10 minutes. The healthy miners showed significantly slower clearance rates than the control group for this initial phase, 2.03±0.7 and 1.34±0.3 minutes respectively. The healthy miners also had higher concentrations of ^{55}Mn in their blood than the control Group, presumably from their occupational exposure. The healthy miners showed faster half-lives of ^{54}Mn from tissues analysed with the whole body and liver half-lives significantly different to the control group (15±2 and 13±3 days compared to 37.5±7.5 and 24.6±7 days respectively). The authors suggested this implied that the healthy miners had elevated tissue levels of ^{55}Mn.

A separate publication, (Mena et al., 1967), presented results taken from the same cohort of volunteers, miners and patients. The half-lives reported (total body turnover) were 35.5±8.4 days (control), 12.5±2.3 days (healthy miners) and 26.5±4.8 days for patients.

(Critchfield and Keen, 1992) investigated plasma from fasted adult male subjects that was labelled *in vitro* with $^{54}MnCl_2$ and then fractionated using several techniques. Molecular sieve chromatography showed that the major ^{54}Mn-containing peak had a very low molecular weight, although four other significant peaks, one of which corresponded to the mass of transferrin (Tf), were also observed. The ^{54}Mn content of the Tf peak increased with increasing incubation time *in vitro*, suggesting the oxidation of Mn^{+2} to Mn^{+3} before its association with Tf. This time-dependent effect was verified using affinity chromatography consisting of immobilized anti-Tf. However, electrophoretic analyses of plasma yielded equivocal results, indicating a limited value of this method for investigating plasma manganese localization. Pre-incubation of the plasma with Fe^{3+} resulted in decreased ^{54}Mn in the Tf area of the chromatogram further supporting the idea that Fe and Mn competitively bind to Tf at similar sites.

The correlation of blood and urine manganese concentrations to estimated manganese exposure was investigated in 141 male subjects working in a manganese oxide and salt producing plant (Roels et al., 1987a; Roels et al., 1987b). The average urine manganese concentration was ten times as high in the exposed workers compared to a control group. Following cessation to manganese exposure, the biological half-life of manganese in urine was estimated to be less than 30 hours.

Workers from manganese ore milling and dry-cell battery manufacturing plants were studied to access the extent of absorption and exposure to manganese dioxide (Gan et al., 1988). This was a relatively old study and lacked detailed methodology (Klimisch Code 4), however it contains very relevant human data. The highest manganese exposures had a mean manganese air level of 4.16 mg/m^3, with over half of the samples collected having values exceeding a TLV of 5 mg/m^3. Despite the dust being non-respirable, the authors reported good correlations (r=0.69 blood, r=0.77 urine) between the blood or urine manganese levels and the manganese-in-air on a group basis. The average blood manganese concentration for the exposed workers was 22.59 ug/L, which was significantly higher than control workers (13.04 ug/L). The average urine manganese concentration for the exposed workers was 5.97 ug/L, which was higher than control workers (1.73 ug/L). However, a poor correlation was found between the blood manganese and urinary manganese levels on an individual basis.

The investigation of potential nervous system effects of manganese workers employed in manganese-alloy producing plants showed an increased hand tremor related to manganese exposure (Bast-Pettersen et al., 2004). One hundred male manganese-alloy plant workers were age matched to referents from plants with similar working conditions to manganese-alloy plants. The arithmetic mean of urine manganese concentration was four times higher in the exposed workers compared to the referents (P<0.001). Overall, the mean blood manganese concentration was slightly higher in the exposed workers compared to the referents (10.4 vs. 9.2 ug/L, P 0.002). When the exposed subjects were stratified into low, medium or high blood manganese levels (<8.7 ug/L, 8.7-11.2 ug/L and >11.2 ug/L), then the high blood manganese subjects showed statistically (P<0.001) more tremor than the age matched referents on the Static Steadiness test. The geometric mean inhalable manganese in the workroom air was 301 ug/m^3, of which approximately 10% was found in the respirable aerosol fraction.

5.1.2 Animals

A very early study utilised the ^{52}Mn isotope and autoradiography to study the distribution and excretion of different doses of $^{52}MnCl_2$ after iv administration to adult male mice (Kato, 1963). This was a very old study (Klimisch Code 4), however it was reasonably well documented. The excretion after 7 days following a tracer amount of dose was approximately 60%, whereas over 90% of a higher dose of $^{52}MnCl_2$ was excreted over the same period.

The clearance of ^{54}Mn from the lower respiratory tract of dogs that had been exposed to an aerosol of $^{54}MnO_2$ (count median diameter of particle size = 0.07±0.01 - units not stated) was reported to be biphasic, with an effective clearance half-life of 34 days (Morrow et al., 1964). This again was a very old study (Klimisch Code 4), however it was reasonably well documented and provides information on dogs.

The importance of the faeces as the major route for excretion of manganese sulphate administered either intravenously or by stomach tube was demonstrated in rats with rectal ligation (Papavasiliou et al., 1966). This again was a very old study (Klimisch Code 4), however it provided results using a technique that would probably not now be permitable. Almost total retention of administered radioactivity was found, with almost no measurable radioactivity detected in the urine.

The whole-body elimination of a ^{54}Mn tracer following a single ip injection of $^{54}MnCl_2$ in the monkey was resolved into 2 exponential components with half-lives of 6 and 95 days (Dastur et al., 1971). This was an old study with Klimisch Code rating of 4, however it does provide data on monkeys.

The excretion (turnover) rate of a ^{54}Mn tracer administered ip ($^{54}MnCl_2$) to groups of mice was found to be directly related to the level of manganese in their diet over a wide range (Britton and Cotzias, 1966). As the study was very old it only had a limited amount of detail and was assigned a Klimisch Code of 4. The longer the mice had been on the controlled diet (restricted manganese content) the slower the excretion of the tracer. Addition of "manganous sulphate" to the diet immediately after administration of the tracer accelerated the excretion of the tracer. The authors proposed that variable excretion rather than variable absorption regulates the concentration of manganese in tissues.

The distribution and elimination of ^{54}Mn following a 1 hour nose-only inhalation of either a tracer dose, 3 µg Mn/m^3, or a high dose, 129 mg Mn/m^3, to male rats was followed for up to 121 hours post-dose (Wieczorek and Oberdorster, 1989). The size distribution of the manganese chloride particles in the aerosols administered had median aerodynamic diameters of 1.6 µm and 1.1 µm for the high and low doses respectively. The half-life of radioactivity from the lung was biphasic, with fast phases of 0.2 and 1.75 days, and slow phases of 10.5 and 12.7 days, for the high and low doses respectively. The relative uptake of the brain appeared to be independent of the inhaled concentration and did not exceed 1% of the lung deposition on day 0; maximum brain levels were observed on days 7 and 28 for the high and low doses respectively. The authors also noted that much higher levels of manganese in the large intestine after the high dose implied that an increase in manganese excretion by auxiliary GI routes to biliary excretion were in operation.

The effects of different levels of dietary manganese on the excretion of a ^{54}Mn tracer ($^{54}MnCl_2$) in rats showed that the biological half-life was significantly accelerated by increased dietary manganese (Lee and Johnson, 1988). In this extensive and well-designed study, the authors also reported that although an oral dose of the ^{54}Mn tracer was absorbed four times higher by fasted rats than in un-fasted rats, the excretion rate was unaffected. Within the same experiment, the biological half-life was relatively unaffected by the route of administration, whereby animals dosed ip with the tracer gave very similar biological half-lives to those orally dosed. The trend of a decreasing biological half-life with increasing levels of dietary manganese was also seen in rats fed either a 65% starch or 65% sucrose diet. The authors concluded that their results indicated that both absorption and excretion are important in maintaining manganese homeostasis in rats.

Table 5.1.1 A summary of the biological half-lives (days) (Lee and Johnson, 1988):

Dietary level of manganese (mg Mn/kg)	**Non-fasted**		**Fasted**		**65% Starch diet**	**65% Sucrose diet**
	Orally dosed	IP dosed	Orally dosed	IP dosed	Orally dosed	Orally dosed
Very low (0.4)					33.1	31.3
Low (2.8-4.1)	27.5-30.4	31.8-32.6	30.7	32.5	19.9	18.8
High (44-50)	10.3-12.9	10.9-12.5	12.7	12.8	11.4	8.8
Highest (82.4)	7.3	10.3				

In an additional study (Lee and Johnson, 1989) the authors confirmed the trend of a decreasing biological half-life with increasing levels of dietary manganese in rats fed either a soy protein-rich or casein-rich diets.

The excretion of radioactivity (as measured by whole-body retention) following the ip injection of a ^{54}Mn tracer ($^{54}MnCl_2$) to groups of mice was accelerated at increasing levels of dietary manganese (Sato et al., 1996). Dietary consumption of the top dose, 8000 mg Mn/kg diet (manganese was incorporated into the diets from manganese chloride solutions), was noted as being lower than the other dose groups. The elimination of radioactivity from the tissues that initially contained relatively high levels of radioactivity, the liver, kidneys, pancreas and spleen, was similar to the whole-body elimination and also showed the increase in excretion rate with increasing dietary manganese.

Table 5.1.2 Estimated biological half-lives of a ^{54}Mn tracer over 21 days from mice that were fed differing levels of dietary manganese after administration of the ^{54}Mn tracer (Sato et al., 1996).

Dietary manganese (mg Mn/kg diet)	**Initial biological half-life (days)**	**Terminal biological half-life (days)**
80 (control)	2.3±0.2	17.5±1.0
240	2.3±0.2	11.9±0.3
800	1.9±0.1	11.8±0.4
2400	1.3±0.1	9.5±1.4
8000	0.9±0.1	8.5±0.7

The toxicokinetics of manganese was investigated in male rats (n = 4-5 per group) following either a single iv or oral dose of $MnCl_2$ at 6.0 mg Mn/kg (Zheng et al., 2000). Plasma manganese concentrations returned to normal levels by 12 hours after dosing following both oral and iv administration. Upon iv administration of $MnCl_2$, manganese rapidly disappeared from blood with a terminal elimination $t_{1/2}$ of 1.83 h and CL_8 of 0.43 L/h/kg. However, the terminal elimination following oral administration was significantly ($p<0.01$) longer at 4.56 h. Taking into consideration the oral bioavailability, the volume of distribution for the oral group was also significantly greater ($p<0.05$), approximately 2.5-fold, than the iv group.

Differences in clearance rates of radioactivity were observed following dosing by intratracheal instillation of 55 µg of either soluble $^{54}MnCl_2$ or insoluble $^{54}Mn_3O_4$ to male Sprague-Dawley rats (Drown et al., 1986). The particle size of the insoluble manganese tetraoxide was determined by scanning electron microscopy to be less than 5µm in diameter, with 90% of the material having a diameter of 1 µm or less. Radioactivity from the $^{54}MnCl_2$ was cleared from the lungs at 4 times the rate of radioactivity from $^{54}Mn_3O_4$ during the first week after dosing. After this period, both the manganese tissue levels and clearance rates were generally similar following both dose forms of manganese.

The pulmonary clearance following a single intratracheal instillation to rats of manganese sulphate, manganese phosphate, or manganese tetraoxide was similar for each of the three compounds at dose levels up to a 0.16 µg Mn/kg (Vitarella et al., 2000a). All pulmonary clearance half-times of manganese were less than 0.5 day and all three compounds were essentially cleared from the lungs within 3 days following instillation.

Table 5.1.3 Elimination kinetics of manganese from the lungs of rats following a single intratracheal instillation of manganese sulphate, phosphate or tetraoxide (Vitarella et al., 2000a)

	Manganese Sulphate	**Manganese Tetraoxide**	**Manganese Phosphate**
Dose level	48 µg/rat	48 µg/rat	48 µg/rat
Particle size (mass median diameter)	4.9 µm	1.67 µm (66%) and 15.5 µm (34%)	4.9 µm
Concentration in lungs (T=0, µg Mn/g)	20.4±9.5	22.8±6.5	24.9±2.1
Concentration in lungs (T=3 days, µg Mn/g) – all results within control levels	0.32±0.05	0.32±0.05	0.32±0.05

The solubility of these three manganese salts in a simulated lung fluid containing proteins was 20-fold greater than in a simulated lung fluid without proteins. The solubility of manganese sulphate was also at least 10-fold greater than the other two manganese salts. The authors concluded that these data suggest that dissolution mechanisms only played a role in the pulmonary clearance of $MnSO_4$, while non-absorptive (e.g., mechanical transport) mechanisms predominate for the less soluble phosphate and oxide forms of manganese.

Table 5.1.4 Solubility of manganese salts in simulated lung fluids (Vitarella et al., 2000a)

	Solubility (µg Mn/g solution)		
	Manganese Sulphate	**Manganese Tetraoxide**	**Manganese Phosphate**
Gamble's - mimics extra-cellular epithelial lining fluid as found in alveoli (Moss, 1979)	2.1±0.1	0.13±0.002	0.15±0.002
Hatch's - modified lung lining fluid that contains proteins found in airway lining fluids (Hatch, 1992)	40.7±6.8	2.5±0.1	6.0±0.1

Adult male CD rats were exposed (inhalation) for 6 h/day for 7 days/week (14 exposures) to either manganese sulphate ($MnSO_4$), manganese tetraoxide (Mn_3O_4) or manganese phosphate in the mineral form hureaulite ($Mn_5(PO)_4)_2[(PO_3)(OH)]_2 \cdot 4H_2O$) at 3 different dose levels (Vitarella et al., 2000b; Dorman et al., 2001a). Immediately after the last exposure, the rats were iv dosed with a ^{54}Mn tracer and whole-body measurements of radioactivity determined for up to 16 weeks post-dose.

Table 5.1.5 Selected pharmacokinetic parameters of ^{54}Mn elimination of a tracer iv dose following repeated short-term exposure of rats to aerosols of either manganese sulphate, manganese tetraoxide or manganese phosphate (Vitarella et al., 2000b; Dorman et al., 2001a).

Variable	**Controls**	**3 mg Mn/m^3 (6 h/day for 14 days)**		
		Manganese Sulphate (MMAD 2.1 µm)	**Manganese Tetraoxide (MMAD 1.8 µm)**	**Manganese Phosphate (MMAD 1.6 µm)**
t½α (days) – initial elimination phase	3.8±0.32, 5.7±1.37, 4.0±1.1	2.4±0.24	2.9±0.28	3.3±1.0
t½β (days) – terminal elimination phase	31.8±3.3, 34.1±4.4, 31.6±5.2	27.1±1.4	32.6±4.5	32.0±5.2
Cl_b (Kbeq/day x10^{-3})	2.6±0.1, 2.3±0.1	4.1±0.4*	3.0±0.3*	-
Cl_b (Kbeq/day)	0.057±0.005	-	-	0.082±0.006*
AUC (Kbeq/day)	392±13.4, 444±31.1, 242±19.6	253±22.9*	344.2±33.3	159±11.6*

Key:
* – statistically significant increase over controls ($p<0.05$)
t½ - half-life
Cl - whole-body clearance
AUC - area under the curve calculated to infinity

No significant differences were seen in the elimination half-lives, either in the initial rapid phase or the second terminal phase, between the treated groups and controls. However, significant increases in clearance and significant decreases in AUC were found following this repeated short-term exposure at 3 mg Mn/m^3 exposure levels but not at the 0.03 mg Mn/m^3 compared to controls. The increased clearance rates from the high dose groups combined with the significantly elevated bile concentrations of non-radiolabelled manganese implied that enhanced biliary excretion of manganese was occurring at the highest dose levels. Similar conclusions regarding the increased rates of clearance and biliary excretion were reached in a study that aimed to investigate the influence of dietary manganese on the pharmacokinetics of inhaled manganese sulphate in male CD rats (Dorman et al., 2001b). Interestingly, the initial hypothesis of this study that brain and other target tissue manganese concentrations resulting from the inhalation of manganese sulphate would be influenced by dietary manganese concentrations, was not upheld.

In a further study in rats by the same workers, the influence of old age and gender on the pharmacokinetics of inhaled manganese sulfate (MMAD 1.85-2.03 µm) and manganese phosphate (hureaulite, MMAD 1.47 µm) was investigated (Dorman et al., 2004a). Young male and female rats exposed to $MnSO_4$ at 0.5 mg Mn/m^3 had increased ^{54}Mn clearance rates when compared with air-exposed controls, while senescent males did not develop higher ^{54}Mn clearance rates.

Following a single 90-min nose-only exposure of male rats to either a $^{54}MnHPO_4$ aerosol (0.39 mg Mn/m^3; MMAD 1.68 µm) or a $^{54}MnCl_2$ aerosol (0.54 mg Mn/m^3; MMAD 2.51 µm), the clearance of ^{54}Mn from the respiratory system was compared (Brenneman et al., 2000; Dorman et al., 2002). Although the lung showed similar clearance kinetics, the manganese clearance from the olfactory and respiratory epithelium appeared to be influenced by the particle solubility. Manganese clearance from the more soluble manganese chloride had shorter half-lives, particularly the terminal elimination, than manganese clearance from the less soluble manganese phosphate. These results are summarised in Table 5.1.6.

Table 5.1.6 ^{54}Mn Clearance from the respiratory system following a single 90-min nose-only exposure of male rats to either a $^{54}MnHPO_4$ aerosol or a $^{54}MnCl_2$ aerosol (Brenneman et al., 2000; Dorman et al., 2002).

Variable	**Lung**		**Olfactory Epithelium**		**Respiratory Epithelium**	
	$^{54}MnHPO_4$	$^{54}MnCl_2$	$^{54}MnHPO_4$	$^{54}MnCl_2$	$^{54}MnHPO_4$	$^{54}MnCl_2$
t½α (days) – initial phase elimination	0.28±0.03	0.27±0.09	0.45±0.15	0.30±0.11	0.54±0.08	0.40±0.02
t½β (days) – terminal phase elimination	3.1±0.5	2.5±0.8	11.4±1.5	8.3±1.2	19.0±4.8	10.9±1.4

A model was developed with rats to quantitate endogenous gut losses of manganese in which the parenterally administered isotope was distributed like fed isotope. A mathematical model of manganese metabolism in rats fed ^{54}Mn was developed using the SAAM and CONSAM computer programs. It was determined that the liver, not the pancreas, was the major source of endogenous gut losses of manganese. Young, growing rats fed 45 µg of Mn/g diet were calculated to absorb 8.2% of their manganese intake and then to lose 37% of the absorbed manganese through gut endogenous losses (Davis et al., 1993).

5.1.3 Biliary and pancreatic excretion

The importance of biliary excretion as the major route for excretion of manganese administered intravenously ($^{54}MnCl_2$) was demonstrated in rats with biliary ligation (Papavasiliou et al., 1966). This was a very old study (Klimisch Code 4), however it provided results using a technique that would probably not now be permitable. Biliary ligation diminished but did not abolish the excretion of radioactivity and the authors concluded that biliary excretion was just one of several gastrointestinal routes that excrete manganese. They also found that iv injection into the portal vein led to a considerably more rapid excretion of manganese than when administered through a peripheral vein. In biliary ligated animals more radioactivity was retained after iv injection into the portal vein than a peripheral vein.

An investigation into the rat bile components that manganese was bound to, concluded that Mn^{2+} cations were bound to substances of relatively low molecular weight, around 100,000 daltons (Tichy et al., 1973). This was an old study with limited methodology details and hence had a Klimisch Code rating of 4. The authors also noted that an additional fraction (not containing manganese) separated by column chromatography was seen in the bile from the treated rats but not in controls. They suggested that this fraction contained bile pigments loosed from their carriers by an unknown mechanism in the presence of Mn^{2+}. They also found that human bile when mixed *in vitro* with an aqueous solution of $MnCl_2$ gave the same fractionation pattern as the rat bile *in vivo*.

The excretion of radioactivity following a single iv dose of 3 mg Mn/kg of $^{54}MnCl_2$ was measured in rats for up to 5 days post-dose (Klaassen, 1974). Detailed methodology was absent in this publication, although it was cross-referenced and hence had a Klimisch Code rating of 4. Over 50% of the dose was excreted in the faeces within the first 24 hours and a further 17% in the next 24 hours, confirming that biliary excretion was the major elimination pathway for manganese. Less than 0.1% of dose was excreted in the urine over the 5-day period. Whilst the biliary excretion of manganese increased as the administered dose increased from 0.1 to 3 mg Mn/kg, there was no further increase in the biliary excretion rate (µg/min/kg) from the 3 to the 10 mg Mn/kg dose level. The authors also reported a species difference in the biliary excretion of manganese of 8.5, 1.5 and 0.5 µg/min/kg in rats, rabbits, and dogs respectively at a 3 mg Mn/kg iv dose level.

A concern that neonatal rats could accumulate manganese (particularly in the brain) due to an undeveloped excretory mechanism for manganese was addressed by a group of researchers (Ballatori et al., 1987). They concluded that suckling rats did have a well-developed excretory pathway for manganese and that the avid retention of tracer quantities of manganese by the neonate was most likely as a consequence of the scarcity of this essential trace metal in its diet. The whole-body elimination of manganese from suckling rats was dependent on the administered dose, whereby almost complete retention (90%) was seen in 8-17 day old rats from a tracer or 0.1 mg Mn/kg ip dose compared to elimination of 30-50% of dose in 4 days from a 1 or 10 mg Mn/kg dose. Interestingly, the biliary excretion of manganese, the primary elimination route of manganese, was only 30-60% lower in 14-day old rats compared to adults. Bile flow rates were reported as being 50-75% lower in the 14-day old rats. The authors reported that there was an apparent biliary transport maximum reached at a dose of 10 mg Mn/kg in both the 14-day old and adult rats, which agreed with data previously published for adult rats (Klaassen, 1974).

Dose dependent biliary and pancreatic excretion of divalent manganese serves to regulate the percentage of ingested manganese retained by the body and to limit increases in liver and other systemic tissue manganese concentrations (Aschner et al., 2005; Dorman et al., 2006b). Pancreatic excretion of manganese is generally considered to be a minor route of excretion (Davis et al., 1993), although after high doses of manganese auxiliary GI routes of excretion (other than bile), including pancreatic excretion become more important (Wieczorek and Oberdorster, 1989).

5.2 Summary and discussion of excretion data

The elimination of absorbed manganese is primarily through the bile and, as such, the main excretion route for manganese is ultimately in the faeces; however this route can also include unabsorbed dose following oral dosing, which is one of the complicating factors when trying to measure proportions of dose absorbed. Very little manganese is excreted in the urine or other potential routes of excretion such as milk or sweat.

The very low proportion of manganese dose excreted in the urine (<1%) means that analysing the urine from workers potentially exposed to manganese is not straightforward. Although the manganese concentration in the urine from groups of workers exposed to manganese was typically 5 to 10-fold higher than control groups, this correlation did not extend to measurements made on an individual basis or to their blood manganese levels (Roels et al., 1987a; Roels et al., 1987b; Gan et al., 1988). The biological half-life of manganese in urine is estimated to be less than 30 hours following cessation of exposure to manganese.

The exceedingly fast initial clearance of manganese from blood means that the measurement of blood manganese levels is not suitable for the estimation of very recent exposure but is more of a reflection of overall body burden.

The level of manganese in scalp hair has shown to be useful in determining a population's overall exposure to manganese (Stauber et al., 1987). However the analytical procedure and the interpretation of the data are fraught with problems because of the need to distinguish between endogenous manganese (from the body) and exogenous manganese from the environment. In which case this method is generally unsuitable in a workplace where the potential exposure is from airborne manganese.

When interpreting data on oral absorption of manganese or manganese elimination, the body's natural homeostatic control of manganese must be taken into account, particularly so if this is following oral absorption. Notwithstanding this a number of early studies focused on the elimination of manganese using ^{54}Mn as a tracer usually as a test meal. The typical whole-body terminal half-lives for manganese in healthy volunteers are around 30-40 days (Mena et al., 1969; Johnson et al., 1991; Finley et al., 1994; Finley et al., 2003). As with the absorption of manganese, factors such as the pre-body burden of manganese and dietary levels of manganese, considerably affect its rate of elimination. A very dramatic example of how the body's natural homeostatic regulation of manganese can both slow down and speed up the elimination of manganese was discussed in detail earlier in this section (Mahoney and Small, 1968).

Following the iv dosing of a ^{54}Mn tracer it was established that the elimination of manganese is at least biphasic with an initial fast elimination phase, typically around 3-4 days in healthy subjects (Mahoney and Small, 1968). As with the slower terminal elimination phase, this initial fast phase can be modulated by manganese body burden.

The elimination of absorbed manganese is dependent on bile production and flow into the small intestine. The efficiency of this process is delayed in the rat neonate following birth and may lead to delayed elimination of manganese following high doses of manganese. However, in the human neonate, efficient bile flow normally occurs within the first three days after birth, therefore the accumulation of manganese in human neonates may not be as much of a potential problem as with rat neonates. The manganese eliminated in the bile usually then undergoes enterohepatic recirculation. There is evidence from a study in rats that auxiliary GI routes of elimination to that of the bile are also in operation (Wieczorek and Oberdorster, 1989), however this was from an inhalation study and so this could have been due to clearance of manganese from the lung by mucocilliary elevator and subsequent direct excretion into the GI tract.

Table 5.2.1 Summary of the human whole-body elimination of manganese

Subject group	Sex	Terminal phase (days)	Reference	Klimisch Code
High manganese diet	F	14 (13-16)	(Finley et al., 2003)	2
Healthy miners	M	15±2	(Mena et al., 1969)	4
Anaemic patients	M+F	23±3	(Mena et al., 1969)	4
High manganese loading	NS	33	(Mahoney and Small, 1968)	2
Controls	M+F	37±7	(Mena et al., 1969)	4
Controls	M	48±21	(Finley et al., 1994)	2
Controls	F	34±16	(Finley et al., 1994)	2
Controls	M+F	36.5-41	(Mahoney and Small, 1968)	2
Manganism patients	M	34±5	(Mena et al., 1969)	4
Manganism patients	M	28±8	(Cotzias et al., 1968)	2
Low manganese diet	F	30 (28-33)	(Finley et al., 2003)	2
Calorie restricted	NS	92	(Mahoney and Small, 1968)	2

Key:
NS – Not specified

It is apparent that the body's natural homeostatic regulation will increase the rate of elimination of manganese if there is a high existing body burden and/or a continued high uptake of manganese.

Table 5.2.2 Examples of the elimination of manganese in animals

Species	Manganese Treatment Regimen	Elimination measurement	Initial elimination rate (days)	Terminal elimination rate (days)	Reference:	Klimisch Code
Mice	80 (control) mg/kg diet	Whole body following an ip injection of a ^{54}Mn tracer	2.3±0.2	17.5±1.0	(Sato et al., 1996)	2
	240 mg/kg diet		2.3±0.2	11.9±0.3		
	800 mg/kg diet		1.9±0.1	11.8±0.4		
	2400 mg/kg diet		1.3±0.1	9.5±1.4		
	8000 mg/kg diet		0.9±0.1	8.5±0.7		
Rats	~4 mg/kg diet	Whole body following an ip injection of a ^{54}Mn tracer		31.8-32.6	(Lee and Johnson, 1988)	2
	~45 mg/kg diet			10.9-12.5		
	~82 mg/kg diet			10.3		
Rats	Controls	Whole body following an iv injection of a $^{54}MnCl_2$ tracer	3.8-5.7	31-34	(Vitarella et al., 2000b; Dorman et al., 2001a)	2
	Short-term inhalation of manganese sulphate		2.4±0.24	27.1±1.4		
	Short-term inhalation of manganese tetraoxide		2.9±0.28	32.6±0.45		
	Short-term inhalation of manganese phosphate		3.3±1.0	32.0±5.2		
Dogs	Inhalation of $^{54}MnO_2$	Lower respiratory tract		34	(Morrow et al., 1964)	4
Monkeys	Controls	Whole body following an ip injection of a ^{54}Mn maleate tracer	6	95	(Dastur et al., 1971)	4
Monkeys	Subchronic inhalation of manganese sulphate	Olfactory bulb		4.9	(Dorman et al., 2006b)	2
		Globus pallidus, putamen, caudate		15.7-16.7		
		Olfactory cortex		19.4		
		Pituitary		23.6		
		Cerebellum		32.3		

6 PBPK MODELLING

The complex processes involved in how the body regulates manganese (Section 2.1.2) have shown that the toxicokinetics of manganese are non-linear (e.g. changes in rates of absorption or elimination depending on dose/body burden) and as such linear extrapolation either intra- or inter-species is not appropriate. Physiologically based pharmacokinetic modelling (PBPK) can be useful for predicting these non-linear behaviours as they are based on first principles instead of being strictly empirical. A PBPK modelling process can be well suited to calculating tissue doses of chemicals over a wide range of exposure conditions in a range of species (Andersen, 2003).

Whilst there is limited qualitative data showing that manganese can be delivered to the brain via the olfactory route, the relative contribution and rate of olfactory delivery compared to systemic delivery (via blood following pulmonary uptake) is more difficult to quantify. The results from a pharmacokinetic model (Leavens et al., 2007) developed to address these points using data from the nose-only exposure of rats to $^{54}MnCl_2$ (Brenneman et al., 2000) and $^{54}MnHPO_4$ (Dorman et al., 2002) indicated the following key points:

- The majority of ^{54}Mn tracer was transported from the blood to the striatum rather than via the olfactory route.
- The rate of elimination of the ^{54}Mn tracer from the olfactory mucosa following $^{54}MnCl_2$ exposure was 2-fold greater than following $^{54}MnHPO_4$, which was consistent with their respective solubilities.
- The rate constants for the transport of the ^{54}Mn tracer from the olfactory bulb to the olfactory tract and tubercle were the same for both forms of manganese (both forms of manganese were the Mn^{2+} form).

There were a number of limitations with the model, such as having to use tissue ^{54}Mn concentrations (liver, kidney and pancreas) instead of blood ^{54}Mn concentrations and assuming homogeneous compartments and first-order transport, which were acknowledged by the authors.

A further series of pharmacokinetic models using published rodent data were developed to evaluate:

1. The dose dependencies of manganese uptake and elimination (Teeguarden et al., 2007a).
2. The hepatic processing of manganese after ingestion and inhalation (Teeguarden et al., 2007b).
3. The physiological approaches accounting for background and tracer kinetics of manganese (Teeguarden et al., 2007c).
4. Factors that contribute to the brain accumulation of manganese during inhalation exposure (Nong et al., 2008).

The first of these publications (Teeguarden et al., 2007a) used a two-compartmental distribution model to investigate the increased elimination of a ^{54}Mn tracer in response to increases in dietary or inhaled manganese levels. The results of this work essentially provided relatively simple kinetic models to describe the body's natural homeostatic regulation of manganese. The following key indications were made:

- Increases in dietary or inhaled manganese levels led to an increase in elimination rate constants as opposed to changes between a central compartment and a deeper compartment.
- Reduced rate constants for absorption that occurred after chronic exposure were less evident after a single acute exposure.
- However, increased elimination rate constants were seen after acute exposure.
- An increase in elimination rate was seen following inhalation exposure only when inhalation became a significant contributor to the overall exposure.

The whole-body elimination measurement was used for the model, presumably as manganese blood concentrations provide limited kinetic data for analysis. Even the liver manganese levels change with time due to the different biological processes that exert control on liver manganese concentrations. The short-term processes (minutes/hours) are likely to differ from the long-term processes (hours/days). The authors noted that the kinetic processes for the uptake and elimination of orally

absorbed manganese are likely to be different following iv or inhalation administration whereby the manganese enters the systemic circulation without passing through the liver. The influence of the absorption process followed by direct delivery to the liver is commonly known as the first-pass effect and can considerably reduce the bioavailability (the amount reaching the systemic circulation) of a material compared to iv administration.

The second of these publications (Teeguarden et al., 2007b) specifically examined the hepatic processing of manganese following either ingestion or inhalation using essentially the same data sets as the previous publication. Revisions to the model included adding some physiologic constraints such as blood flows controlling the exchange of manganese between the systemic blood compartment and the liver. The authors reported that the model now provided good fits of the elimination kinetics of systemic manganese following combined oral and inhalation exposure to manganese tetraoxide and manganese sulphate. One of the main findings was that manganese leaving the systemic tissues was extracted much less efficiently than manganese arriving at the liver from the GI tract tissues. Manganese appears to be able to exist in blood in several forms and its oxidation state may be an important determinant of tissue toxicodynamics and subsequent neurotoxicity (Reaney et al., 2006). Uptake of dietary manganese appears to be in the divalent form, Mn^{2+}, where it is complexed with α2-macroglobulins and other plasma proteins and transported by the portal blood to the liver. The liver seems to be very efficient at processing these manganese complexes and eliminating the excess manganese in the bile. The uptake of manganese by routes other than the oral route, inhalation, for example, will bypass this first-pass effect. Manganese absorbed into the blood not following oral administration can be oxidised by ceruloplasmin and the resulting Mn^{3+} can bind to transferrin (Andersen et al., 1999). As such, Teeguarden et al., described blood manganese as containing two distinct pools: that which is highly extracted by liver (free Mn^{2+} and non-transferrin protein-complexed Mn^{2+}) and that which is much more poorly cleared by liver (e.g., transferrin-bound Mn^{3+}).

The model used in the third publication in this series (Teeguarden et al., 2007c) initially used data from a very old study (Furchner et al., 1966) which reported the tissue radioactivity following an ip dose of a ^{54}Mn tracer for up to 89 days post-dose to rats fed a normal diet (~45 ppm Mn). A very interesting outcome was that manganese did not fit the conventional PK modelling approach. Typically, a test chemical would be expected to be distributed into a central compartment which is in rapid equilibrium with the blood and also into a deep compartment where the movements into and out of are not rapid and have specific intercompartmental rate constants. Manganese, however, appeared to have deep compartments that were not specific to a single tissue, but instead represented components of every tissue whereby manganese was slowly taken up and released. This model was then used to try and predict the ^{54}Mn tracer kinetics after inhalation by comparison to data from a rat study which used a 1-hour nose-only exposure to $^{54}MnCl_2$ (Wieczorek and Oberdorster, 1989). This was not particularly successful, with the largest discrepancies seen in the liver. These results were not unexpected by the authors and as with the previous publications (Teeguarden et al., 2007a; Teeguarden et al., 2007b) the authors discussed how the uptake of manganese by inhalation will bypass the intestinal and hepatic control processes. It also appeared that uptake of manganese by the ip route had partial hepatic processing before entry into the systemic circulation and, as such, was closer to the oral uptake process than inhalation uptake.

The final publication in this series (Nong et al., 2008) focused on trying to simulate manganese brain (striatum) and liver concentrations in rats following inhalation exposure to manganese sulphate by comparison to data from 14 and 90-day repeated exposure studies (Dorman et al., 2001a; Dorman et al., 2001b; Dorman et al., 2004a). The first PBPK model (Model A) maintained tissue concentrations by simple partitioning with slow inter-compartmental transfer from free manganese in tissues to deeper tissue stores of manganese (Figure 6.2). This model appeared to be consistent with steady-state and tracer kinetics for dietary studies and low manganese inhalation levels, but was severely deficient (over-prediction of manganese tissue concentrations) for the higher repeated dose inhalation exposures. A revised model (Model B) was developed that included saturable binding in tissues (liver and brain) with equilibrium binding constants defined by slow association and dissociation constants.

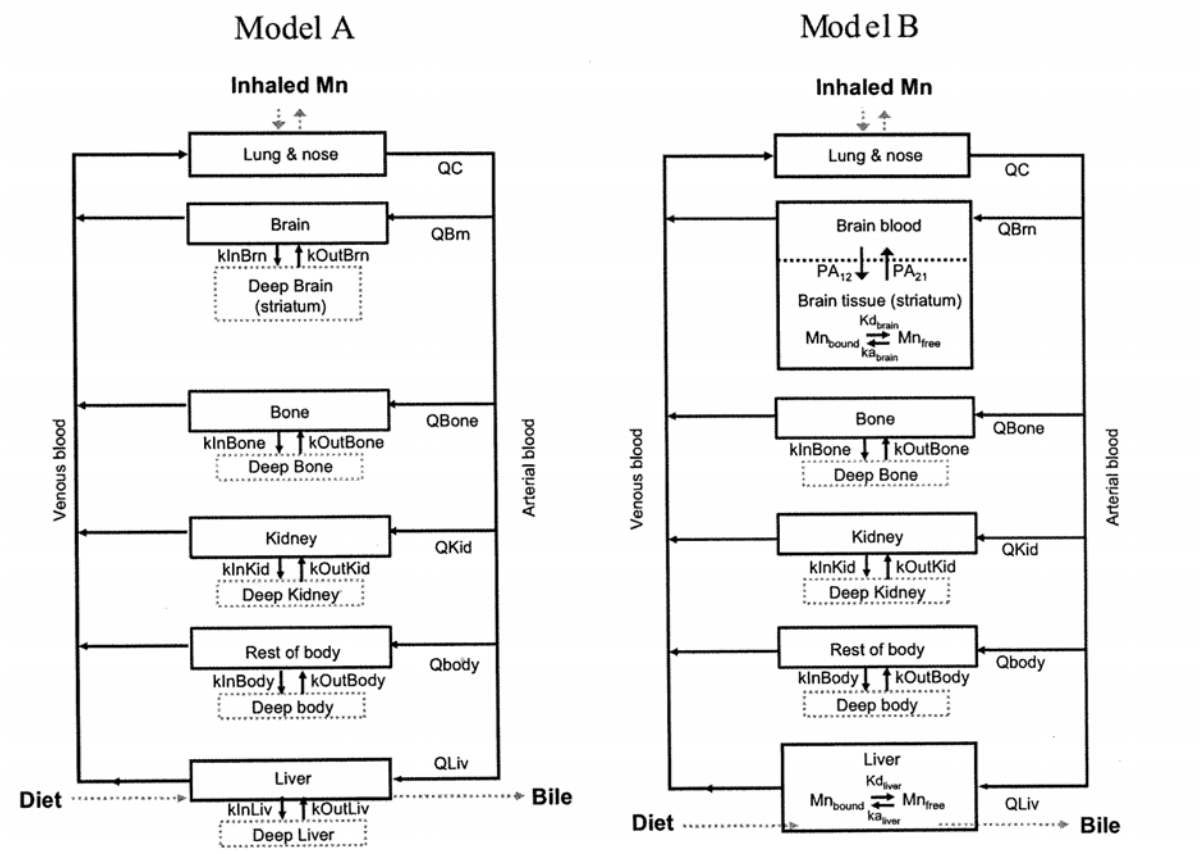


Figure 6.1 PBPK Model A described transfer from the well-mixed to the deep tissues occurred with rate constants (k_{in} and k_{out}). Model B had tissue binding kinetics using dissociation and association constants (*kd* and *ka*), and maximum concentration of binding capacity (B_{max}). Permeability diffusion constants (PA_{12} and PA_{21}) in Model B maintain manganese concentration gradient between striatal tissue and capillary blood (Nong et al., 2008).

These revisions meant that Model B was able to reflect the dose-dependent rise in the liver and striatum concentrations with increasing inhalation exposures, the accumulation of manganese in the striatum, as well as the subsequent decrease in manganese tissue levels after the end of the exposure period. The authors reported that the calculated bound and free manganese striatum levels were more sensitive to tissue binding when inhaled manganese concentrations were below 0.2 mg Mn/m^3. Unfortunately the authors did not speculate whether this could have been a trigger value for a change in manganese kinetics that could be correlated to neurological/toxicological observations. The model indicated that there were different rate constants for uptake and efflux of manganese to the brain, which correlates with manganese entering the brain via carrier-mediated transport and leaving the brain via diffusion only, a much slower process than carrier-mediated transport (Yokel and Crossgrove, 2004).

A follow-on publication scaled the model developed in rats to describe manganese tissue accumulation in nonhuman primates exposed to manganese sulphate by inhalation and also manganese in the diet (Nong et al., 2009). This model was further developed than the previous model and also described the dose-dependent processes seen with manganese, such as saturable binding tissue capacities, asymmetrical influx/efflux transport and increased rate of biliary excretion. The model showed that as inhaled concentrations of manganese in both the monkey and rat increased above 0.2 mg Mn/m^3 the intrinsic control mechanisms associated with biliary excretion could not keep up with increasing pulmonary uptake. The authors concluded that the PBPK models developed for laboratory animals are sufficiently well-characterised to warrant development of similar models for human exposures, and consideration of such models as cornerstones of future risk assessments.

It appears that during the development of this series of PBPK models for manganese, many of the confounding factors affecting the evaluation of the toxicokinetics of manganese discussed earlier (see Section 2.1.2) required significant revisions to the conventional PK modelling approach. As such it seems likely that other factors not yet specifically investigated by these models, such as soluble/insoluble forms of manganese, olfactory uptake, and potential target tissues (other brain regions, testes, bone) will also require considerable model development and development. Due to the complexity of the toxicokinetics of manganese, the development of a suitable PBPK model is a challenging area of research. However, the use of PBPK models to inform human health risk assessments is generally becoming more acceptable.

7 REFERENCES, ABSTRACTS AND EVALUATIONS

References, abstracts and evaluations

Abrams E, Lassiter JW, Miller WJ, Neathery MW, Gentry RP and Scarth RD (1976) Absorption as a factor in manganese homeostasis. *J Anim Sci* **42:**630-636.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. An old study but useful investigation into the effect of widely different dietary levels of manganese on 54Mn absorption from oral dosing.

Akoume MY, Perwaiz S, Yousef IM and Plaa GL (2003) Synergistic role of 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7alpha-hydroxylase in the pathogenesis of manganese-bilirubin-induced cholestasis in rats. *Toxicol Sci* **73:**378-385.

Abstract: Manganese (Mn) and bilirubin (BR) induce intrahepatic cholestasis when injected sequentially. It was suggested that accumulation of newly synthesized cholesterol in the canalicular membrane is an initial step in the development of cholestasis. To clarify the involvement of cholesterol in the pathogenesis of Mn-BR-induced cholestasis, we examined biliary secretion and liver subcellular distribution of lipids in the cholestatic conditions (Mn-BR combination). We also examined hepatic metabolism of cholesterol under cholestatic and noncholestatic (Mn or BR given alone) conditions. The Mn-BR combination reduced bile flow by 50%, and bile acid, phospholipids, and cholesterol output by 42, 75, and 90%, respectively. There was a dramatic impairment of cholate excretion but not chenodeoxycholate excretion. Phosphatidylcholine species secreted into bile were unchanged, and microsomal total phospholipid content was significantly increased. Although there was no changes in liver membrane phospholipid content, the cholesterol/phospholipid ratio was increased by more than 50% in the canalicular fraction. Despite the increased concentration of cholesterol in canalicular membrane the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, key enzyme in cholesterol synthesis, and cholesterol 7alpha-hydroxylase, key enzyme in cholesterol conversion to bile acids, were significantly reduced. However, the injection of Mn alone significantly increased both enzymes, while BR alone inhibited cholesterol 7alpha-hydroxylase by 62%, without affecting HMG-CoA reductase. In conclusion, the critical cholestatic events in Mn-Br-induced cholestasis appear to be mediated through the synergistic effects of Mn and BR acting on synthesis and degradation of cholesterol.

Evaluation: Klimisch Code 5. Non-pivotal.

Altstatt LB, Pollack S, Feldman MH, Reba RC and Crosby WH (1967) Liver manganese in hemochromatosis. *Proc Soc Exp Biol Med* **124:**353-355.

Evaluation: Klimisch Code 5. Non-pivotal.

Andersen ME (2003) Toxicokinetic modeling and its applications in chemical risk assessment. *Toxicol Lett* **138:**9-27.

Abstract: In recent years physiologically based pharmacokinetic (PBPK) modeling has found frequent application in risk assessments where PBPK models serve as important adjuncts to studies on modes of action of xenobiotics. In this regard, studies on mode of action provide insight into both the sites/mechanisms of action and the form of the xenobiotic associated with toxic responses. Validated PBPK models permit calculation of tissue doses of xenobiotics and metabolites for a variety of conditions, i.e. at low-doses, in different animal species, and in different members of a human population. In this manner, these PBPK models support the low-dose and interspecies extrapolations that are important components of current risk assessment methodologies. PBPK models are sometimes referred to as physiological toxicokinetic (PT) models to emphasize their application with compounds causing toxic responses. Pharmacokinetic (PK) modeling in general has a rich history. Data-based PK compartmental models were developed in the 1930's when only primitive tools were available for solving sets of differential equations. These models were expanded in the 1960's and 1970's to accommodate new observations on dose-dependent elimination and flow-limited metabolism. The application of clearance concepts brought many new insights about the disposition of drugs in the body. In the 1970's PBPK/PT models were developed to evaluate metabolism of volatile compounds of occupational importance, and, for the first time, dose-dependent processes in toxicology were included in PBPK models in order to assess the conditions under which saturation of metabolic and elimination processes lead to non-linear dose response relationships. In the 1980's insights from chemical engineers and occupational toxicology were combined to develop PBPK/PT models to support risk

assessment with methylene chloride and other solvents. The 1990's witnessed explosive growth in risk assessment applications of PBPK/PT models and in applying sensitivity and variability methods to evaluate model performance. Some of the compounds examined in detail include butadiene, styrene, glycol ethers, dioxins and organic esters/aids. This paper outlines the history of PBPK/PT modeling, emphasizes more recent applications of PBPK/TK models in health risk assessment, and discusses the risk assessment perspective provided by modern uses of these modeling approaches.

Evaluation: Klimisch Code 5. Review of toxicokinetics.

Andersen ME, Gearhart JM and Clewell HJ, 3rd (1999) Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. *Neurotoxicology* **20:**161-171.

Abstract: Manganese (Mn)-deficiency or Mn-excess can lead to adverse biological consequences. Central nervous system tissues, rich in dopaminergic neurons, are the targets whether the Mn gains entrance by inhalation, oral ingestion, or intravenous administration. Risk assessments with Mn need to ensure that brain concentrations in the globus pallidus and striatum stay within the range of normal. This paper first provides a critical review of the biological factors that determine the disposition of Mn in tissues within the body. Secondly, it outlines specific data needs for developing a physiologically based pharmacokinetic (PBPK) model for Mn to assist in conducting risk assessments for inhaled and ingested Mn. Uptake of dietary Mn appears to be controlled by several dose-dependent processes: biliary excretion, intestinal absorption, and intestinal elimination. Mn absorbed in the divalent form from the gut via the portal blood is complexed with plasma proteins that are efficiently removed by the liver. Absorption of Mn via inhalation, intratracheal instillation or intravenous infusions bypasses the control processes in the gastrointestinal tract. After absorption into the blood system by these alternate routes, Mn is apparently oxidized by ceruloplasmin and the trivalent Mn binds to the iron carrying protein, transferrin. Brain uptake of Mn occurs via transferrin receptors located in various brain regions. Transferrin-bound trivalent Mn is not as readily removed by the liver, as are protein complexes with divalent Mn. Thus, Mn delivered by these other dose routes would be available for uptake into tissues for a longer period of time than the orally administered Mn, leading to quantitative differences in tissue uptake for different dose routes. Several important data gaps impede organizing these various physiological factors into a multi-dose route PK model for Mn. They include knowledge of (1) oxidation rates of Mn in blood, (2) uptake rates of protein-bound forms of Mn by the liver, (3) neuronal transfer rates within the CNS, and (4) quantitative analyses of the control processes that regulate uptake of ingested Mn by the intestines and liver. These data gaps are the main obstacles to developing a risk assessment strategy for Mn that considers contributions of both inhalation and ingestion of this essential nutrient in determining brain Mn concentrations.

Evaluation: Klimisch Code 5. Pharmacokinetic Review

Aschner M (2006) The transport of manganese across the blood-brain barrier. *Neurotoxicology* **27:**311-314.

Abstract: The mammalian central nervous system (CNS) possesses a unique and specialized capillary adaptation, referred to as the blood-brain barrier (BBB). The BBB maintains an optimal neuronal microenvironment, regulating blood-tissue exchange of macromolecules and nutrients. The BBB is characterized by individual endothelial cells that are continuously linked by tight junctions, inhibiting the diffusion of macromolecules and solutes between adjacent endothelial cells. This review will focus on pertinent issues to BBB maintenance, and survey recent dogmas on the transport mechanisms for the essential metal, manganese, across this barrier. Specifically, putative carriers for manganese into and out of the brain will be discussed.

Evaluation: Klimisch Code 5. Review.

Aschner M and Aschner JL (1990) Manganese transport across the blood-brain barrier: relationship to iron homeostasis. *Brain Res Bull* **24:**857-860.

Abstract: The binding characteristics of manganese (Mn) to transferrin (Tf) were examined on G-75 Sephadex gel columns. When 54MnCl2 was combined with Tf and immediately fractionated on the Sephadex column, 49% of 54Mn was found to Tf. The fraction of 54Mn which was Tf-bound was dependent upon the incubation period, and increased in a time-dependent fashion. In vivo, 6 hr of intravenous administration of ferric-hydroxide dextran complex significantly inhibited (p less than 0.05) 54Mn brain uptake as compared to its uptake in iron-free dextran-treated rats. These results suggest that iron (Fe) homeostasis may play an important role in the regulation of Mn transport across the blood-brain barrier (BBB).

Evaluation: Klimisch Code 2. Although a brief communication, this is an important mechanistic study with results that suggest that iron homeostasis may play an important role in the regulation of manganese transport across the blood-brain barrier (BBB). Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Aschner M, Connor JR, Dorman DC, Malecki EA and Vrana KE (2002) Manganese in Health and Disease (From Transport to Neurotoxicity). *Handbook of Neurotoxicology* **1:**195-209.
Evaluation: Klimisch Code 5. Review.

Aschner M and Dorman DC (2006) Manganese: pharmacokinetics and molecular mechanisms of brain uptake. *Toxicol Rev* **25:**147-154.
Abstract: Manganese is an essential mineral that is found at low levels in virtually all diets. Manganese ingestion represents the principal route of human exposure, although inhalation also occurs, predominantly in occupational cohorts. Regardless of intake, animals generally maintain stable tissue manganese levels as a result of homeostatic mechanisms that tightly regulate the absorption and excretion of this metal. However, high-dose exposures are associated with increased tissue manganese levels, causing adverse neurological, reproductive and respiratory effects. In humans, manganese-induced neurotoxicity is associated with a motor dysfunction syndrome, commonly referred to as manganism or Parkinsonism, which is of paramount concern and is considered to be one of the most sensitive endpoints. This article focuses on the dosimetry of manganese with special focus on transport mechanisms of manganese into the CNS. It is not intended to be an exhaustive review of the manganese literature; rather it aims to provide a useful synopsis of contemporary studies from which the reader may progress to other research citations as desired. Specific emphasis is directed towards recent published literature on manganese transporters' systemic distribution of manganese upon inhalation exposure as well as the utility of magnetic resonance imaging in quantifying brain manganese distribution.
Evaluation: Klimisch Code 5. Review publication.

Aschner M, Erikson KM and Dorman DC (2005) Manganese dosimetry: species differences and implications for neurotoxicity. *Crit Rev Toxicol* **35:**1-32.
Abstract: Manganese (Mn) is an essential mineral that is found at low levels in food, water, and the air. Under certain high-dose exposure conditions, elevations in tissue manganese levels can occur. Excessive manganese accumulation can result in adverse neurological, reproductive, and respiratory effects in both laboratory animals and humans. In humans, manganese-induced neurotoxicity (manganism) is the overriding concern since affected individuals develop a motor dysfunction syndrome that is recognized as a form of parkinsonism. This review primarily focuses on the essentiality and toxicity of manganese and considers contemporary studies evaluating manganese dosimetry and its transport across the blood-brain barrier, and its distribution within the central nervous system (CNS). These studies have dramatically improved our understanding of the health risks posed by manganese by determining exposure conditions that lead to increased concentrations of this metal within the CNS and other target organs. Most individuals are exposed to manganese by the oral and inhalation routes of exposure; however, parenteral injection and other routes of exposure are important. Interactions between manganese and iron and other divalent elements occur and impact the toxicokinetics of manganese, especially following oral exposure. The oxidation state and solubility of manganese also influence the absorption, distribution, metabolism, and elimination of manganese. Manganese disposition is influenced by the route of exposure. Rodent inhalation studies have shown that manganese deposited within the nose can undergo direct transport to the brain along the olfactory nerve. Species differences in manganese toxicokinetics and response are recognized with nonhuman primates replicating CNS effects observed in humans while rodents do not. Potentially susceptible populations, such as fetuses, neonates, individuals with compromised hepatic function, individuals with suboptimal manganese or iron intake, and those with other medical states (e.g., pre-parkinsonian state, aging), may have altered manganese metabolism and could be at greater risk for manganese toxicity.
Evaluation: Klimisch Code 5. Review article.

Aschner M, Vrana KE and Zheng W (1999) Manganese uptake and distribution in the central nervous system (CNS). *Neurotoxicology* **20:**173-180.
Abstract: Information about the nature of manganese (Mn)-binding ligands in plasma and serum, and its transport mechanism across the blood-brain barrier (BBB) is sparse. Most studies to date have focused on distribution, excretion, and accumulation of intravenous and intraperitoneal solutions of soluble divalent salts of Mn. Mn is transported in the blood primarily in the divalent oxidation state (Mn2+) and crosses the BBB via specific carriers at a rate far slower than in other tissues. Mn transport across the BBB occurs both in the 2+ and 3+ oxidation state. Within the CNS, Mn accumulates primarily within astrocytes, presumably because the astrocyte-specific enzyme, glutamine synthetase (GS), represents an important regulatory target of Mn. Compared to Mn2+, Mn3+ has a slower elimination rate and therefore, may have a greater tendency to accumulate in tissues. Furthermore, in view of the dependence of Mn accumulation within the CNS on iron (Fe) homeostasis, the oxidation state of Mn may represent a key determinant in the differential distribution, accumulation and secretion profiles of

Mn, a fact that has received little attention in experimental biology toxicology. Accordingly, the distribution and membrane transport of Mn emphasizes the importance of: 1) the oxidation state of Mn, as it governs the affinity of Mn to endogenous ligands, and 2) the reaction of Mn3+ with transferrin, the plasma iron-carrying protein. This review will focus on transport kinetics of Mn across the BBB (both in the 2+ and 3+ oxidation state), the putative role of transferrin in the transport of Mn across the BBB, the transport of Mn by astrocytes, as well as the physiological significance of Mn to the function GS.

Evaluation: Klimisch Code 5. Review

Azaz A, Thomas A, Miller V, Ward I and Fell GS (1995) Manganese in long-term paediatric parenteral nutrition. *Arch Dis Child* **73:**89.

Evaluation: Klimisch Code 4. A letter to the editor and hence only a brief outline.

Bales CW, Freeland-Graves JH, Lin PH, Stone JM and Dougherty V (1987) *Plasma Uptake of Manganese - Influence of Dietary factors*. American Chemical Society, Washington DC.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Results appear to be lacking detailed statistical analysis.

Ballatori N, Miles E and Clarkson TW (1987) Homeostatic control of manganese excretion in the neonatal rat. *Am J Physiol* **252:**R842-847.

Abstract: Previous studies in neonatal and suckling animals showed that immature animals have a greatly diminished capacity to excrete manganese and therefore were considered to be unable to regulate tissue manganese concentrations. In contrast, the present studies indicate that suckling rats have the capacity to excrete excess manganese at rates nearly comparable to those of adults. Eight- to 10-day-old rats given a tracer dose of 54MnCl2 (essentially carrier free), either via gavage or by intraperitoneal injection showed little elimination of the 54Mn until the 18-19th day of life, when there was an abrupt increase in the rate of the metal's excretion. However, when manganese was given in doses of 1 and 10 mg/kg, the young animals excreted from 30-70% of the dose in only 4 days, at which time a new rate of excretion was achieved. This enhanced rate of excretion remained constant until the 18-19th day of life, when it was again accelerated. Biliary excretion of manganese, the primary route for the elimination of the metal, was only 30-60% lower in 14-day-old rats compared with adults at doses ranging from tracer to 10 mg 54Mn/kg. For both the 14-day-old and adult rats, an apparent biliary transport maximum was reached at a dose of 10 mg Mn/kg. These studies indicate that the excretory pathways for manganese are well developed in the neonatal rat. The avid retention of tracer quantities of manganese by the neonate may be a consequence of the scarcity of this essential trace metal in its diet.

Evaluation: Klimisch Code 2. Well designed study to address specific issues the results of which were then suitably discussed. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Baly DL, Lonnerdal B and Keen CL (1985) Effects of high doses of manganese on carbohydrate homeostasis. *Toxicol Lett* **25:**95-102.

Abstract: The effects of manganese (Mn) toxicity on carbohydrate homeostasis was examined in Sprague-Dawley and Osborne-Mendel rats. Mn injection was followed by increases in Mn concentration in both liver and pancreas. Concentrations of Mn in the pancreas increased more rapidly than in the liver. Plasma insulin levels decreased, plasma glucose levels increased, and a spike in glucagon concentration was observed following Mn injection. Increases in blood glucose in response to Mn injection were also observed in 24- and 48-h fasted rats, although the magnitude of the increase was less than that observed in fed rats. Both strains of rats appeared to respond similarly to Mn injection. The present studies demonstrate that acute Mn injection can affect glucose homeostasis. These effects may be mediated through altered endocrine pancreatic function.

Evaluation: Klimisch Code 5. Non-pivotal.

Banta RG and Markesbery WR (1977) Elevated manganese levels associated with dementia and extrapyramidal signs. *Neurology* **27:**213-216.

Abstract: In a patient with elevated manganese levels, dementia, and an extrapyramidal syndrome, brain biopsy showed numerous neuritic plaques and neurofibrillary tangles typical of Alzheimer's disease. Manganese was elevated beyond toxic levels in serum, hair, urine, feces, and brain. The possible relationship of elevated manganese levels, dementia, and the extrapyramidal syndrome warrants further studies of similar cases.

Evaluation: Klimisch Code 3. Although the publication records the distribution of manganese, there is no firm information on the method or route of administration nor the quantity of manganese administered. Several possibilities were suggested for the elevated manganese levels, such as abuse of vitamins and minerals or that the patient had worked for less than 2 months in a steel mill some 30 years earlier or that he had a genetic manganese metabolic dysfunction; however no firm link was found.

Bast-Pettersen R and Ellingsen DG (2005) The Klove-Matthews static steadiness test compared with the DPD TREMOR. Comparison of a fine motor control task with measures of tremor in smokers and manganese-exposed workers. *Neurotoxicology* **26:**331-342.

Abstract: The aim of this study was to compare two tests for tremor/fine motor control as regards their sensitivity in relation to effects on tremor of exposure to manganese and cigarette smoke. One hundred manganese-exposed workers were compared with 100 age-matched referents. The subjects were tested with the Klove-Matthews static steadiness test (hole tremormeter) and the DPD TREMOR (accelerometer). The manganese-exposed subjects showed increased postural tremor compared to the referents. The tremor had a larger frequency dispersion among the exposed subjects than among the referents as assessed by the TREMOR 7.0 test system, indicating that the tremor had a pattern where the power was burned at a wider spectre of frequencies among the exposed subjects than among the referents. The two tests differed in their ability to demonstrate tremor, depending of the type of exposure (manganese or cigarette smoke). The static steadiness test was better than the TREMOR at discriminating between manganese-exposed subjects and referents while the TREMOR was better at discriminating between smokers and non-smokers than the static steadiness test. The tests also differed in their ability to demonstrate tremor depending on the frequency of tremor. The Tremor Intensity I was higher for subjects with higher tremor frequency, while no such effect was found for the static steadiness test parameters. When studying the effects of exposures where the quality of the tremor is unknown, it is suggested to include at least two tremor tests, one based on acceleration and the other on displacement.

Evaluation: Klimisch Code 5. This publication uses data from another study that has already been reviewed.

Bast-Pettersen R, Ellingsen DG, Hetland SM and Thomassen Y (2004) Neuropsychological function in manganese alloy plant workers. *Int Arch Occup Environ Health* **77:**277-287.

Abstract: OBJECTIVES: The objective was to investigate potential nervous system effects of manganese (Mn) exposure in workers employed in manganese-alloy-producing plants. METHODS: One hundred male Mn alloy plant workers were compared with 100 age-matched referents. The subjects were examined with a comprehensive neuropsychological test battery. Exposure was assessed by measurement of Mn concentrations in the workroom air, blood and urine. RESULTS: The geometric mean (GM) concentration of inhalable Mn in workroom air was 301 microg/m(3). The GM concentration of Mn in whole blood (181 nmol/l vs 160 nmol/l) (P=0.002) and urine (0.9 nmol/mmol creatinine vs 0.4 nmol/mmol creatinine) (P<0.001) was higher among the exposed subjects than among the referents. The Mn-exposed subjects had increased postural tremor while conducting a visually guided tremor test (static steadiness test) compared with the referents (mean number of contacts 94 vs 59 (P= 0.001); duration of contacts (in seconds) 5.1 vs 3.5 (P=0.003)). The tremor had larger frequency dispersion, indicating that the tremor included a wider variety of frequencies, among the exposed subjects than among the referents, assessed by the "TREMOR" test system. Smoking habits (self-reported) influenced the tremor parameters significantly, the Mn-exposed smokers having more tremor than the non-smoking Mn-exposed subjects. No differences between the groups were found in tests for cognitive functions, reaction time or in symptom reporting. CONCLUSION: The Mn-exposed subjects had increased hand tremor compared with their referents. The tremor was related to exposure parameters. Smoking habits (self-reported) influenced the tremor parameters.

Evaluation: Klimisch Code 2. Very well documented and reported study. The main findings were on the neuropsychological function in manganese alloy workers although there was some data on manganese blood and urine levels as well as manganese levels in inhalable and respirable air. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Bast-Pettersen R, Skaug V, Ellingsen D and Thomassen Y (2000) Neurobehavioral performance in aluminum welders. *Am J Ind Med* **37:**184-192.

Abstract: METHODS: Twenty aluminum welders (mean age 33 years; range 21-52), who had been exposed to aluminum for an average of 8.1 years (range 2-21), were tested for tremor and reaction time and screened for neuropsychiatric symptoms in a cross-sectional study. The welders' median urinary aluminum concentration was 1.5 micromol/L (range 0. 7-4.8). Aluminum in air, measured inside the respiratory protection, was 0.9 mg/m(3) (range 0.6-3.8). The welders were compared with twenty construction workers matched for age. RESULTS: Welders reported more symptoms than referents did

(median 2 vs. 1; P=0.047). Although the welders as a group performed better than the referents on a tremor test, years of exposure, but not age, was predictive of poorer performance. The welders' reaction times were rapid by clinical standards (mean simple reaction time (SRT): 221 milliseconds; mean continuous performance test (CPT): 364 milliseconds). Although, as a group, they performed better than the referents, there was a statistically significant relation between longer reaction times and aluminum in air (air-Al). CONCLUSIONS: The relations between hand steadiness and years exposed, and between reaction time and air-Al, could indicate slight effects from exposure to aluminum. The possibility of selection of workers with high manual skills into welding work and a possible job-related training effect, might partly serve to explain the good performance among the welders.

Evaluation: Klimisch Code 5. Publication focuses on aluminum exposure.

Bertinchamps A and Cotzias GC (1958) Biliary excretion of Manganese. *Federation Proceedings* **17:**428.

Evaluation: Klimisch Code 4. No details only a short paragraph of description.

Bertinchamps AJ, Miller ST and Cotzias GC (1966) Interdependence of routes excreting manganese. *Am J Physiol* **211:**217-224.

Evaluation: Klimisch Code 3. Very old study, methodology dated and brief. A lot of discussion around the second "wave" of manganese in bile flow.

Bird ED, Anton AH and Bullock B (1984) The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. *Neurotoxicology* **5:**59-65.

Abstract: Manganese (Mn) may produce neurotoxicity in man through inhalation of Mn dust. Animals exposed to excessive Mn develop neurological abnormalities, and neuropathological lesions in the brain mainly in the globus pallidus with decreased concentrations of the neurotransmitter, dopamine (DA), in the brain. Monkeys exposed to Mn by inhalation did not produce any abnormal movements. After two years, the animals were sacrificed and certain brain areas were compared to controls. There were significant decreases in DA concentration in caudate and globus pallidus, and there was a 60-80% increase in Mn concentration in the basal ganglia of the brain. The DA system in the basal ganglia is vulnerable to the effects of Mn, but the amount of Mn inhaled and the period of exposure would appear to determine whether abnormal neurological signs develop.

Evaluation: Klimisch Code 3. The methodology lacks details necessary to assess the scientific integrity. The authors had no explanations for the absence of behavioural or neurological signs in the treated monkeys even though they recorded 60-80% increases in manganese concentrations in the brain. This publication was very unclear as to the composition of the administered dose, for example, the methods refer to manganese dust, the table titles manganese dioxide and within the tables MnO. The study design also appears to have been severely lacking in the basic blood manganese monitoring during the study, particularly as there was no other apparent mechanism in place to confirm the dose delivery. The manganese analysis that was performed on the brains took place a year after the study termination, no reason given. Overall, there appears to have been a lack of attention to the actual dose composition, the effectiveness of the administration and any measurement of whether or how much dose was actually absorbed.

Bleich S, Degner D, Sprung R, Riegel A, Poser W and Ruther E (1999) Chronic Manganism: Fourteen Years of Follow-up. *J Neuropsychiatry Clin Neurosci* **11:**117-.

Evaluation: Klimisch Code 4. Brief communication of a case report.

Bock NA, Paiva FF, Nascimento GC, Newman JD and Silva AC (2008) Cerebrospinal fluid to brain transport of manganese in a non-human primate revealed by MRI. *Brain Res* **1198:**160-170.

Abstract: Manganese overexposure in non-human primates and humans causes a neurodegenerative disorder called manganism thought to be related to an accumulation of the metal in the basal ganglia. Here, we assess changes in the concentration of manganese in regions of the brain of a non-human primate (the common marmoset, Callithrix jacchus) following four systemic injections of 30 mg/kg MnCl2 H2O in the tail vein using T1-weighted magnetic resonance imaging (MRI) and compare these to changes in the rat following the same exposure route and dose. The doses were spaced 48 h apart and we imaged the animals 48 h after the final dose. We find that marmosets have significantly larger T1-weighted image enhancements in regions of the brain compared to rats, notably in the basal ganglia and the visual cortex. To confirm this difference across species reflects actual differences in manganese concentrations and not variations in the MRI properties of manganese, we measured the longitudinal relaxivity of manganese (chi1) in the in vivo brain and found no significant species' difference. The high manganese uptake in the marmoset basal ganglia and visual cortex can be explained by CSF-brain transport from the large lateral ventricles and we confirm this route of uptake with time-course MRI during a tail-vein infusion of manganese. There is also high uptake in the substructures of the

hippocampus that are adjacent to the ventricles. The large manganese accumulation in these structures on overexposure may be common to all primates, including humans.

Evaluation: Klimisch Code 2. Very well documented, reported and discussed study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The dose level used for the marmoset caused liver damage in 50% of the animals and as such may have compromised the study conclusions.

Bolte S, Normandin L, Kennedy G and Zayed J (2004) Human exposure to respirable manganese in outdoor and indoor air in urban and rural areas. *J Toxicol Environ Health A* **67:**459-467.

Abstract: Methylcyclopentadienyl manganese tricarbonyl (MMT), is an additive in gasoline, and its combustion leads to the emission of Mn particles, which increase atmospheric metal concentrations. The objective of this study was to determine the level of outdoor and indoor respirable Mn (MnR) in Montreal, Canada, where MMT has been used since 1976. Ten women were involved in this study: five living in an urban area, near an expressway with high traffic density, and five residing in a rural area characterized by low traffic density. Outdoor and indoor air samples were collected each week (5 in total) during 3 consecutive days; blood samples were collected at the end of the air sampling period. The average concentration of outdoor MnR in the urban area was 0.025 microg/m3, which is significantly different from the average of 0.005 microg/m3 found in the rural area. The average indoor MnR concentration was also significantly different from the average MnR indoor concentrations within both areas. The mean blood Mn concentrations were not significantly different between the urban area (0.017 microg/m3) and the rural area (0.007 microg/m3). The average outdoor MnR concentrations within both areas. The mean blood Mn concentrations were not significantly different between the two groups. Data suggest that a high outdoor atmospheric MnR leads to a high indoor MnR, but not to an increase in blood Mn levels.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Bonilla E (1985) Chronic Manganese Poisoning and Striatal Adenylate Cyclase Activity. *Invest Clin* **26:**45-50.

Evaluation: Klimisch Code 5. Non-pivotal.

Bonilla E and Diez-Ewald M (1974) Effect of L-DOPA on brain concentration of dopamine and homovanillic acid in rats after chronic manganese chloride administration. *J Neurochem* **22:**297-299.

Evaluation: Klimisch Code 5. Non-pivotal.

Brain JD, Heilig E, Donaghey TC, Knutson MD, Wessling-Resnick M and Molina RM (2006) Effects of iron status on transpulmonary transport and tissue distribution of Mn and Fe. *Am J Respir Cell Mol Biol* **34:**330-337.

Abstract: Manganese transport into the blood can result from inhaling metal-containing particles. Intestinal manganese and iron absorption is mediated by divalent metal transporter 1 (DMT1) and is upregulated in iron deficiency. Since iron status alters absorption of Fe and Mn in the gut, we tested the hypothesis that iron status may alter pulmonary transport of these metals. DMT1 expression in the lungs was evaluated to explore its role in metal transport. The pharmacokinetics of intratracheally instilled 54Mn or 59Fe in repeatedly bled or iron oxide-exposed rats were compared with controls. Iron oxide exposure caused a reduction in pulmonary transport of 54Mn and 59Fe, and decreased uptake in other major organs. Low iron status from repeated bleeding also reduced pulmonary transport of iron but not of manganese. However, uptake of manganese in the brain and of iron in the spleen increased in bled rats. DMT1 transcripts were detected in airway epithelium, alveolar macrophages, and bronchial-associated lymphoid tissue in all rats. Focal increases were seen in particle-containing macrophages and adjacent epithelial cells, but no change was observed in bled rats. Although lung DMT1 expression did not correlate with iron status, differences in pharmacokinetics of instilled metals suggest that their potential toxicity can be modified by iron status.

Evaluation: Klimisch Code 2. Generally well documented, some details cross-referenced. Links with two other publications, Heilig 2005 and Thompson 2006, from the same group of workers. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Brenneman KA, Cattley RC, Ali SF and Dorman DC (1999) Manganese-induced developmental neurotoxicity in the CD rat: is oxidative damage a mechanism of action? *Neurotoxicology* **20:**477-487.

Abstract: Inhalation of high concentrations of manganese (Mn) is associated with an extrapyramidal motor disorder in humans. Oxidative damage, mediated by increased levels of Mn in dopaminergic brain regions and mitochondria, is a hypothesized mechanism of action for Mn-induced neuronal degeneration and loss. To test this proposed mechanism, developing CD rats, which may be at an

increased risk for Mn-induced neurotoxicity, were exposed orally to 0, 25, or 50 mg/kg/day of MnCl2 from postnatal day (PND) 1 to 49 Brain regional and mitochondrial Mn levels, brain regional reactive oxygen species (ROS) levels, and whole-brain nuclear and mitochondrial 8-OHdG levels were used to evaluate Mn-mediated oxidative damage. High-dose Mn exposure was associated with increased spontaneous motor activity on PND 21 and decreased body weights on PND 49. On PND 21, Mn concentrations were increased in brain regions and mitochondrial fractions in both low- and high-dose groups. ROS levels were elevated in cerebellum but not striatum. On PND 49, Mn concentrations in brain regions and mitochondrial fractions were increased only in the high-dose group. Mn exposure did not significantly alter 8-OHdG levels in either mitochondrial or nuclear DNA. Selective uptake of Mn by the striatum or mitochondrial fraction was not demonstrated at either time point. These data allow us to conclude that oral exposure to high levels of Mn in developing CD rats resulted in increased brain regional and mitochondrial Mn levels, increased motor activity, and decreased body weights but not in selective accumulation of Mn in the striatum or mitochondrial fraction of any brain region or elevations in striatal ROS or whole-brain 8-OHdG levels. These findings do not support the hypothesis that oxidative damage, as assessed by ROS and 8-OHdG levels, is a mechanism of action in Mn-induced developmental neurotoxicity in the CD rat.

Evaluation: Klimisch Code 5. Non-pivotal.

Brenneman KA, Wong BA, Buccellato MA, Costa ER, Gross EA and Dorman DC (2000) Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. *Toxicol Appl Pharmacol* **169:**238-248.

Abstract: Inhalation exposure of humans to high concentrations of manganese (Mn) is associated with elevated Mn levels in the basal ganglia and an extrapyramidal movement disorder. In the rat, direct olfactory transport of Mn from the nose to the brain has been demonstrated following intranasal instillation of (54)MnCl(2). However, the contribution this route makes to brain Mn delivery following inhalation is unknown and was the subject of our study. Male 8-week old CD rats underwent a single 90-min nose-only exposure to a (54)MnCl(2) aerosol (0.54 mg Mn/m(3); MMAD 2.51 microm). The left and right sides of the nose and brain, including the olfactory pathway and striatum, were sampled at 0, 1, 2, 4, and 8 days postexposure. Control rats were exposed to (54)MnCl(2) with both nostrils patent to evaluate the symmetry of Mn delivery. Another group of rats had the right nostril plugged to prevent nasal deposition of (54)MnCl(2) on the occluded side. Gamma spectrometry (n = 6 rats/group/time point) and autoradiography (n = 1 rat/group/time point) were used to compare the levels of (54)Mn found on the left and right sides of the nose and brain to determine the contribution of olfactory uptake to brain (54)Mn levels. Brain and nose samples from the side with the occluded nostril had negligible levels of (54)Mn activity, validating the nasal occlusion procedure. High levels of (54)Mn were observed in the olfactory bulb and tract/tubercle on the side or sides with an open nostril within 1-2 days following inhalation exposure. These results demonstrated, for the first time, that the olfactory route contributes the majority (up to >90%) of the (54)Mn found in the olfactory pathway, but not in the striatum, of the rat brain up to 8 days following a single inhalation exposure. These findings suggest that the olfactory route may make a significant contribution to brain Mn levels following inhalation exposure in the rat.

Evaluation: Klimisch Code 2. Although not conducted under GLP or claim to be following internationally accepted guidelines, the details of methodology and validation of techniques are very well documented. The results demonstrated that the olfactory route contributes the majority (up to >90%) of the (54)Mn found in the olfactory pathway, but not in the striatum of the rat brain up to 8 days following a single inhalation exposure. The results also contribute the key issue of the relevance of the rat model for human manganese neurotoxicity.

Britton AA and Cotzias GC (1966) Dependence of manganese turnover on intake. *Am J Physiol* **211:**203-206.

Evaluation: Klimisch Code 4. An old study with limited detailed methodology.

Burnett WT, Jr., Bigelow RR, Kimball AW and Sheppard CW (1952) Radio-manganese studies on the mouse, rat and pancreatic fistula dog. *Am J Physiol* **168:**620-625.

Evaluation: Klimisch Code 3. Very old study, very little experimental detail.

Cahill DF, Bercegeay MS, Haggerty RC, Gerding JE and Gray LE (1980) Age-related retention and distribution of ingested Mn3O4 in the rat. *Toxicol Appl Pharmacol* **53:**83-91.

Evaluation: Klimisch Code 3. Not very detailed methodology. Very interesting results, however some of the conclusions drawn in the discussion are unsound. The authors concluded that at the low dose of Mn3O4 the livers had nearly 50% of manganese whilst at the high dose this had significantly fallen: "the infant liver's capacity to sequester manganese had been exceeded and the excess had been released to other organs, in particular that the fraction in the brain had increased". This interpretation

is potentially flawed, as they hadn't taken into consideration the following 1. They were only measuring the radiolabelled manganese distribution which was at a tracer level and were not taking into consideration the total manganese burden. 2. The amount of available manganese to neonatal rats is restricted, as mothers' milk contains very low levels of manganese compared to solid diet. 3. How the homeostatic regulation would handle any additional manganese presented. 4. Why this change in distribution between the liver and brain had not been seen with the MnCl2 doses of which 13-fold more was retained at the higher dose? 5. The species of manganese presented in the Mn3O4. Whilst the publication gives details of the specific activity of the radiolabelled 54Mn and a detailed particle size distribution of the low dose Mn3O4, there was no further description of how the radiolabel was incorporated into the high dose. A radiolabled tracer needs to be completely homogenous with the non-labelled component, typically by mixing in a solution, otherwise it cannot be considered representative. In the case of an insoluble material, e.g. Mn3O4, the particle size distribution of both the radio and non-radiolabel material also need to be homogenous. Neither of these issues were addressed in the publication and, as such, interpretation of the high dose Mn3O4 results are compromised.

Casalino E, Sblano C, Landriscina V, Calzaretti G and Landriscina C (2004) Rat liver glutathione S-transferase activity stimulation following acute cadmium or manganese intoxication. *Toxicology* **200:**29-38.

Abstract: The effect of cadmium or manganese administration on rat liver glutathione S-transferase (GST) has been investigated. The activity of this enzyme in liver cytosol, where almost all the cellular activity is present, had increased by more than 36% 24 h after a single i.p. injection of CdCl(2) (2.5 mg kg(-1) b.w.) or MnCl(2) (2.0 mg kg(-1) b.w.). After shorter and longer time intervals, a lower enzyme activity stimulation was observed in both cases. When liver cytosol was incubated for 10 min with 75 microM CdCl(2) or 40 microM MnCl(2), no effect was observed on enzyme activity. The increase in GST following cadmium or manganese administration was blocked by prior administration of actinomycin D, indicative of a possible transcription-dependent response. The liver soluble GST from both control and metal-treated rats was not at all affected by Vitamin E, in the range of 20-300 microM. By contrast, hematin was seen to be a competitive inhibitor of this liver enzyme from both types of rats by using CDNB as substrate and the K(i) value was equal to 0.22 microM. The possibility that under the conditions used class alpha GST isoenzymes are affected by cadmium or manganese is discussed.

Evaluation: Klimisch Code 5. Non-pivotal.

Cawte J (1984) Emic accounts of a mystery illness: the Groote Eylandt syndrome. *Aust N Z J Psychiatry* **18:**179-187.

Abstract: The Aboriginal people of Groote Eylandt, in the Northern Territory of Australia, are suffering from an unusual disease complex having neurological, psychiatric and teratological features, which admits no ready explanation. The island people at various times blame it on the spirits, or accuse enemies, or take some responsibility upon themselves. In this paper, 'emic' accounts of the illness (those current among the members of the society) are described in order to compare them with 'etic' accounts of those who study the society from the outside. Since emic views regulate people's behaviours toward illness, it is suggested these views should complement and inform etic views of researchers and therapists. This principle might apply to all mysterious or poorly understood illness.

Evaluation: Klimisch Code 5. Not used directly for the assessment of the TK of manganese.

Cawte J and Florence M (1987) Environmental source of manganese on Groote Eylandt, northern Australia. *Lancet* **1:**1484.

Evaluation: Klimisch Code 5. Not used directly for the assessment of the TK of manganese.

Chan AW, Lai JC, Minski MJ, Lim L and Davison AN (1981) Manganese concentrations in rat organs:Effect after life-long manganese treatment. *Biochem Soc Trans* **9:**229.

Evaluation: Klimisch Code 4. Very brief publication lacking in detail.

Chan W, Raghib M, H. and Rennert O, M. (1987) *Absorption Studies of Manganese from Milk Diets in Suckling Rats*. American Chemical Society, Washington, D.C.

Evaluation: Klimisch Code 2. Reasonable detail in methodology. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Cikrt M (1972) Biliary excretion of 203 Hg, 64 Cu, 52 Mn, and 210 Pb in the rat. *Br J Ind Med* **29:**74-80.

Evaluation: Klimisch Code 3. Lacking in detailed methodology. 52Mn was used, which has a very short half-life, no details given, nor an overall mass balance on the results. Animals were starved for 24 hours before dosing, no comparison within the study design to non starved animals.

Cikrt M (1973) Enterohepatic circulation of 64Cu, 52Mn and 203Hg in rats. *Arch Toxikol* **31:**51-59.
Evaluation: Klimisch Code 3. Old study, methodology lacks detail. The rats were starved for 24 hours prior to manganese administration which is likely to have affected their homeostatic regulation of manganese and thus the proportion excreted in the bile. No overall mass balances reported.

Cikrt M and Vostal J (1969) Study of manganese resorption in vitro through intestinal wall. *Int Z Klin Pharmakol Ther Toxikol* **2:**280-285.
Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. A very old study, reasonable amount of detail, however methodology is dated.

Cotzias GC, Horiuchi K, Fuenzalida S and Mena I (1968) Chronic manganese poisoning. Clearance of tissue manganese concentrations with persistance of the neurological picture. *Neurology* **18:**376-382.
Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Consideration is also given to the age of the study and human relevance.

Critchfield JW and Keen CL (1992) Manganese + 2 exhibits dynamic binding to multiple ligands in human plasma. *Metabolism* **41:**1087-1092.
Abstract: Plasma from fasted adult male subjects was labeled in vitro with 54MnCl2 and then fractionated using several techniques. Molecular sieve chromatography showed that the major 54Mn-containing peak had a very low molecular weight (VLMW), although four other significant peaks, one of which corresponded to the mass of transferrin (Tf), were also observed. The 54Mn content of the Tf peak increased with increasing incubation time in vitro, suggesting the oxidation of Mn+2 to Mn+3 before its association with Tf. This time-dependent effect was verified using affinity chromatography consisting of immobilized anti-Tf. Electrophoretic analyses of plasma yielded equivocal results, indicating a limited value of this method for investigating plasma manganese localization. The above findings are discussed in the context of factors that influence the oxidation and metabolism of Mn2+ in human plasma.
Evaluation: Klimisch Code 2. There is detailed methodology and validation of the techniques used as well as discussion of the limitations of the results obtained. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Cross DJ, Flexman JA, Anzai Y, Maravilla KR and Minoshima S (2008) Age-related decrease in axonal transport measured by MR imaging in vivo. *Neuroimage* **39:**915-926.
Abstract: Axonal transport is a crucial process for neuronal homeostasis and cell functions. In vitro studies have indicated transport rates decrease with age. Disruption of axonal transport has been implicated in age-associated neurodegenerative disorders. We hypothesized that aged rats would show decreased transport in the brain, which could be measured using in vivo manganese-enhanced MR imaging (Mn-MRI) and parametric estimation. Serial T1-weighted images were obtained at pre- and post-administration of MnCl(2) in rats scanned longitudinally (n=4) and in a separate aged group (n=3). Subtraction analysis was performed for group-wise statistical comparison on a pixel-by-pixel basis. Change in intensity over time was plotted for the olfactory bulb and anterior and posterior olfactory tract. Bulk transport of material was estimated over an initial 72 h. Tracer kinetic estimation of time-intensity data, based on a mass transport model, used intensity change in the bulb as input function for subsequent changes in the tract. Time to the peak of Mn(2+) flow was estimated for both anterior and posterior tracts. Results indicated age-related decreases in axonal transport rate and bulk transport of material in the olfactory tract of living rat brains. Longitudinally scanned, mid-age group was decreased by 58% and the aged group by 71% of young rate (neuronal transport=4.07+/-1.24 mm/h, 1.72+/-0.89 mm/h, and 1.16+/-0.18 mm/h for young, mid-age, and aged, respectively). Neuronal transport rate decreases correlated with increased age. The use of kinetic analysis combined with dynamic manganese enhanced MR imaging provides a unique opportunity to study this important neuronal process.
Evaluation: Klimisch Code 5. This publication focuses on the use of a technique.

Cross DJ, Minoshima S, Anzai Y, Flexman JA, Keogh BP, Kim Y and Maravilla KR (2004) Statistical mapping of functional olfactory connections of the rat brain in vivo. *Neuroimage* **23:**1326-1335.

Abstract: The olfactory pathway is a unique route into the brain. To better characterize this system in vivo, rat olfactory functional connections were mapped using magnetic resonance (MR) imaging and manganese ion (Mn2+) as a transport-mediated tracer combined with newly developed statistical brain image analysis. Six rats underwent imaging on a 1.5-T MR scanner at pre-administration, and 6, 12, 24, 36, 48, and 72 h and 5.5, 7.5, 10.5, and 13.5 days post-administration of manganese chloride (MnCl2) into the right nasal cavity. Images were coregistered, pixel-intensity normalized, and stereotactically transformed to the Paxinos and Watson rat brain atlas, then averaged across subjects using automated image analysis software (NEUROSTAT). Images at each time point were compared to pre-administration using a one-sample t statistic on a pixel-by-pixel basis in 3-D and converted to Z statistic maps. Statistical mapping and group averaging improved signal to noise ratios and signal detection sensitivity. Significant transport of Mn2+ was observed in olfactory structures ipsilateral to site of Mn2+ administration including the bulb, lateral olfactory tract (lo) by 12 h and in the tubercle, piriform cortex, ventral pallidum, amygdala, and in smaller structures such as the anterior commissure after 24 h post-administration. MR imaging with group-wise statistical analysis clearly demonstrated bilateral transsynaptic Mn2+ transport to secondary and tertiary neurons of the olfactory system. The method permits in vivo investigations of functional neuronal connections within the brain.
Evaluation: Klimisch Code 5. This publication focuses on the use of a technique.

Crossgrove J and Zheng W (2004) Manganese toxicity upon overexposure. *NMR Biomed* **17:**544-553.
Abstract: Manganese (Mn) is a required element and a metabolic byproduct of the contrast agent mangafodipir trisodium (MnDPDP). The Mn released from MnDPDP is initially sequestered by the liver for first-pass elimination, which allows an enhanced contrast for diagnostic imaging. The administration of intravenous Mn impacts its homeostatic balance in the human body and can lead to toxicity. Human Mn deficiency has been reported in patients on parenteral nutrition and in micronutrient studies. Mn toxicity has been reported through occupational (e.g. welder) and dietary overexposure and is evidenced primarily in the central nervous system, although lung, cardiac, liver, reproductive and fetal toxicity have been noted. Mn neurotoxicity results from an accumulation of the metal in brain tissue and results in a progressive disorder of the extrapyramidal system which is similar to Parkinson's disease. In order for Mn to distribute from blood into brain tissue, it must cross either the blood-brain barrier (BBB) or the blood-cerebrospinal fluid barrier (BCB). Brain import, with no evidence of export, would lead to brain Mn accumulation and neurotoxicity. The mechanism for the neurodegenerative damage specific to select brain regions is not clearly understood. Disturbances in iron homeostasis and the valence state of Mn have been implicated as key factors in contributing to Mn toxicity. Chelation therapy with EDTA and supplementation with levodopa are the current treatment options, which are mildly and transiently efficacious. In conclusion, repeated administration of Mn, or compounds that readily release Mn, may increase the risk of Mn-induced toxicity.
Evaluation: Klimisch Code 5. Review publication.

Crossgrove JS, Allen DD, Bukaveckas BL, Rhineheimer SS and Yokel RA (2003) Manganese distribution across the blood-brain barrier. I. Evidence for carrier-mediated influx of manganese citrate as well as manganese and manganese transferrin. *Neurotoxicology* **24:**3-13.
Abstract: Manganese (Mn) is an essential element and a neurotoxicant. Regulation of Mn movement across the blood-brain barrier (BBB) contributes to whether the brain Mn concentration is functional or toxic. In plasma, Mn associates with water, small molecular weight ligands and proteins. Mn speciation may influence the kinetics of its movement through the BBB. In the present work, the brain influx rates of 54Mn2+, 54Mn citrate and 54Mn transferrin (54Mn Tf) were determined using the in situ brain perfusion technique. The influx rates were compared to their predicted diffusion rates, which were determined from their octanol/aqueous partitioning coefficients and molecular weights. The in situ brain perfusion fluid contained 54Mn2+, 54Mn citrate or 54Mn Tf and a vascular volume/extracellular space marker, 14C-sucrose, which did not appreciably cross the BBB during these short experiments (15-180 s). The influx transfer coefficient (Kin) was determined from four perfusion durations for each Mn species in nine brain regions and the lateral ventricular choroid plexus. The brain Kin was (5-13) x 10(-5), (3-51) x 10(-5), and (2-13) x 10(-5) ml/s/g for 54Mn2+, 54Mn citrate, and 54Mn Tf, respectively. Brain Kin values for any one of the three Mn species generally did not significantly differ among the nine brain regions and the choroid plexus. However, the brain Kin for Mn citrate was greater than Mn2+ and Mn Tf Kin values in a number of brain regions. When compared to calculated diffusion rates, brain Kin values suggest carrier-mediated brain influx of 54Mn2+, 54Mn citrate and 54Mn Tf. 55Mn citrate inhibited 54Mn citrate uptake, and 55Mn2+ inhibited 54Mn2+ uptake, supporting the conclusion of carrier-mediated brain Mn influx. The greater Kin values for Mn citrate than Mn2+ and its presence as a major non-protein-bound Mn species in blood plasma suggest Mn citrate may be a major Mn species entering the brain.

Evaluation: Klimisch Code 2. Well documented and discussed study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Crossgrove JS and Yokel RA (2004) Manganese distribution across the blood-brain barrier III. The divalent metal transporter-1 is not the major mechanism mediating brain manganese uptake. *Neurotoxicology* **25:**451-460.

Abstract: Manganese (Mn) is essential for and toxic to the brain. Brain Mn uptake utilizes both diffusion and transporter-mediated pathways. The divalent metal transporter-1 (DMT-1) has been suggested to mediate brain Mn uptake. The b/b Belgrade rat does not express significant amounts of functional DMT-1. In the present work, brain influx transfer coefficients of (54) Mn ion and (54) Mn transferrin (Mn Tf) were determined in b/b and +/b Belgrade and Wistar rats using the in situ brain perfusion technique. Brain Mn uptake was not significantly different among the three rat strains for either Mn species. We hypothesized that Mn may enter brain endothelial cells by a DMT-1-independent process but not be able to distribute across those cells into brain tissue due to the absence of DMT-1 activity. To test this hypothesis the brain capillary endothelial cells were isolated from b/b and +/b Belgrade rats and Wistar rats after in situ brain perfusion. Some animals received cerebrovascular washout after in situ brain perfusion to ascertain any affect of genotype on (54) Mn adsorption to the endothelial cell luminal surface. Less than 30% of the brain (54) Mn after (54) Mn ion or (54) Mn Tf perfusion remained associated with endothelial cells, suggesting the majority had distributed into brain extracellular fluid (ECF) and/or brain cells. Mn appears to distribute across the rat blood-brain barrier (BBB) into the brain by one or more carrier-mediated processes other than the DMT-1.

Evaluation: Klimisch Code 5. Review publication.

Crossgrove JS and Yokel RA (2005) Manganese distribution across the blood-brain barrier. IV. Evidence for brain influx through store-operated calcium channels. *Neurotoxicology* **26:**297-307.

Abstract: Manganese (Mn) is a required co-factor for many ubiquitous enzymes; however, chronic Mn overexposure can cause manganism, a parkinsonian-like syndrome. Previous studies showed Mn influx into brain is carrier-mediated, though the putative carrier(s) were not established. Studies conducted with cultured bovine brain microvascular endothelial cells (bBMECs), which comprise the blood-brain barrier, revealed (54)Mn (II) uptake positively correlated with pH, was temperature-dependent, and was sodium- and energy-independent. Brain (54)Mn uptake correlated inversely with calcium (Ca) concentration, but (45)Ca uptake was unaltered by high Mn concentration. Lanthanum (La), a non-selective inhibitor of several Ca channel types, as well as verapamil and amiloride, inhibitors of voltage-operated Ca channels, failed to inhibit Mn uptake into cells. Nickel (Ni), another non-selective inhibitor of several Ca channel types, inhibited Mn and Ca uptake into cells by 88 and 85%, respectively. Cyclopiazonic acid (CPA) and thapsigargin, which activate store-operated calcium channels (SOCCs), increased (54)Mn and (45)Ca uptake into cultured bBMECs. In situ brain perfusion studies were conducted in adult, male Sprague-Dawley rats to verify the cell culture results. Both nickel and verapamil produced a non-significant decrease in Mn and Ca influx. Lanthanum significantly increased Mn influx to 675 and 450% of control in parietal cortex and caudate, respectively, while producing no significant effect on Ca influx. Vanadate, which inhibits Ca-ATPase, inhibited Mn uptake into cultured blood-brain barrier cells, but not into perfused rat brain. Overall these results suggest that both Ca-dependent and Ca-independent mechanisms play a role in brain Mn influx. This work provides evidence that store-operated Ca channels, as well as another mechanism at the blood-brain barrier, likely play a role in carrier-mediated Mn influx into the brain.

Evaluation: Klimisch Code 5. Non-pivotal.

Dastur DK, Manghani DK and Raghavendran KV (1971) Distribution and fate of 54Mn in the monkey: studies of different parts of the central nervous system and other organs. *J Clin Invest* **50:**9-20.

Abstract: The fate and distribution of isotopic manganese administered as a single carrier-free dose of 200 muCi of maleate-(54)Mn to 12 rhesus monkeys was studied at different time periods from the 6th hr to the 278th day. Whole-body activity was measured, and all body organs and tissues and different parts of the central nervous system (CNS) were evaluated for specific activity, exponential analysis, and relative retention. Exponential analysis revealed a pattern of discharge with a fast and a slow component for the whole body and for many of the viscera. All parts of the CNS and, to a lesser degree, the thyroid and muscle showed an almost steady state of activity after the initial uptake. While the whole body and most organs and tissues appeared to discharge their radioactivity with the passage of time, first rapidly and then gradually, the CNS, endocrine glands, and muscle tissues showed persistent levels of specific activity. All components of the brain exhibited increasing relative retention, the lentiform nucleus and the cerebellum showing this more. It is suggested that the selective vulnerability of the brain in manganese miners might result from this inability on the part of the CNS

to discharge the (54)Mn with time. This investigation confirms and amplifies our earlier similar study on the rat.

Evaluation: Klimisch Code 4. A very old study with relatively brief details.

Dastur DK, Manghani DK, Raghavendran KV and Jeejeebhoy KN (1969) Distribution and fate of Mn54 in the rat, with special reference to the C.N.S. *Q J Exp Physiol Cogn Med Sci* **54:**322-331.

Evaluation: Klimisch Code 4. A very old study with brief details. The ip dose route was used which was not relevant for manganese.

Davidsson L, Cederblad A, Hagebo E, Lonnerdal B and Sandstrom B (1988) Intrinsic and extrinsic labeling for studies of manganese absorption in humans. *J Nutr* **118:**1517-1521.

Abstract: A dual-radioisotope method was used to simultaneously study whole-body manganese retention from a chicken liver based meal intrinsically labeled with 54Mn and extrinsically labeled with 52Mn. Manganese retention was monitored in a sensitive whole-body counter during approximately 30 d in six young adult women. Both radioisotopes were retained to a similar degree and excreted at identical rates. Retention at d 5 was 14.4 +/- 10.3 and 14.0 +/- 9.9% while retention at d 10 was 5.0 +/- 3.1 and 5.0 +/- 3.0% (X +/- SD) for 54Mn and 52Mn, respectively. From these results we conclude that the intrinsic and extrinsic Mn isotopes did form a common pool before absorption. The results can therefore be regarded as a direct validation of the use of extrinsic labeling for studies of Mn retention for estimating Mn absorption in man.

Evaluation: Klimisch Code 2 - very good detail about methodology and validation of techniques. Very relevant to human consumption of manganese from the diet and provides percentages of manganese retained on two occasions. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The variation between subjects was not explored. Retention data was not used to back extrapolate to obtain an initial absorption estimate, thus the earlier retention data is likely to be an overestimate of absorption compared to other studies.

Davidsson L, Cederblad A, Lonnerdal B and Sandstrom B (1989a) Manganese absorption from human milk, cow's milk, and infant formulas in humans. *Am J Dis Child* **143:**823-827.

Abstract: Manganese absorption from human milk, cow's milk, and infant formulas was studied in humans by using extrinsic labeling of the diets with manganese 54 or manganese 52 and whole-body retention measurements. The fractional manganese absorption from human milk (8.2% +/- 2.9%) was significantly different when compared with cow's milk (2.4% +/- 1.7%), soy formula (0.7% +/- 0.2%), and whey-preponderant cow's milk formula with 12 mg/L of iron (1.7% +/- 1.0%) and without iron fortification (2 mg/L of iron) (3.1% +/- 2.8%), while no significant difference was observed between a whey-preponderant cow's milk formula with 7 mg/L of iron (5.9% +/- 4.8%) and human milk. The total amount of absorbed manganese was significantly higher from the non--iron-fortified cow's milk formula (2 mg/L of iron) as compared with human milk, while no significant differences were observed for the other milks and formulas.

Evaluation: Klimisch Code 2 - well documented and designed study using paired observations, with subjects acting as their own control. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Davidsson L, Cederblad A, Lonnerdal B and Sandstrom B (1989b) Manganese retention in man: a method for estimating manganese absorption in man. *Am J Clin Nutr* **49:**170-179.

Abstract: Whole-body retention of 54Mn was studied in man by measurements in a sensitive whole-body counter after intake of an extrinsically labeled infant formula. Reproducible retention figures at day 10 were observed after repeated administrations to six subjects; 2.3 +/- 1.1, 3.3 +/- 3.1, and 2.4 +/- 1.4% (means +/- SD) for three separate occasions. Interindividual variation of manganese retention after intake of the same labeled diet was, however, shown to be substantial. Retention at day 10 was 2.9 +/- 1.8% (means +/- SD) and varied from 0.6 to 9.2% when measured in 14 healthy subjects. Large interindividual variations in rate of excretion were observed. The retention measurements for days 10-30, however, could be closely fitted to a single exponential function for each individual. The results regarding intraindividual and interindividual variation in Mn retention indicate that factors influencing Mn absorption can be identified only by repeated administrations using each subject as his/her own control.

Evaluation: Klimisch Code 2. Very well documented and thorough investigations. Proposed methodology for estimating absorption from retention data. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Davidsson L, Cederblad A, Lonnerdal B and Sandstrom B (1991) The effect of individual dietary components on manganese absorption in humans. *Am J Clin Nutr* **54:**1065-1070.

Abstract: Our knowledge of dietary factors affecting manganese absorption in man is very limited. In this study we used a recently developed radionuclide technique to measure manganese absorption in human adults. Using paired observations, we explored the effects of adding calcium and manganese to human milk on manganese absorption. Furthermore, the effects of adding phytate, phosphate, and ascorbic acid to infant formula as well as iron and magnesium to wheat bread were evaluated. Addition of calcium to human milk resulted in a significant decrease in manganese absorption whereas no significant differences in manganese absorption were observed as a result of the other test meals administered with and without the evaluated dietary component, respectively. Thus, manganese absorption was not significantly affected by most dietary factors evaluated in this study, except for the addition of calcium to human milk.

Evaluation: Klimisch Code 2 - well documented and designed study using paired observations, with subjects acting as their own control. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP.

Davidsson L, Lonnerdal B, Sandstrom B, Kunz C and Keen CL (1989c) Identification of transferrin as the major plasma carrier protein for manganese introduced orally or intravenously or after in vitro addition in the rat. *J Nutr* **119:**1461-1464.

Abstract: It is known that the metabolic handling of manganese (Mn) introduced via the diet or by intravenous injection is quite different. We hypothesized that this difference could be due in part to different proteins carrying Mn in plasma that could affect tissue uptake and retention. To test this idea, 54Mn was administered orally or intravenously to rats, and blood samples were taken by cardiac puncture at various time points postdosing. Plasma proteins were separated using fast protein liquid chromatography with a combination of anion exchange and gel filtration columns. Using these methods, independent of the route of 54Mn administration, transferrin was identified as the major Mn-binding protein in plasma. The identity was further confirmed by SDS-polyacrylamide gradient gel electrophoresis and Western blotting. These results conclusively show that 54Mn in plasma is carried by transferrin, regardless of route of administration and time postdosing.

Evaluation: Klimisch Code 2. Well documented study with key findings. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Davis CD and Greger JL (1992) Longitudinal changes of manganese-dependent superoxide dismutase and other indexes of manganese and iron status in women. *Am J Clin Nutr* **55:**747-752.

Abstract: The effect of dietary factors on manganese-dependent superoxide dismutase (MnSOD) activity in humans has not been studied. We longitudinally evaluated changes in MnSOD activity and other indices of manganese and iron status in 47 women during a 124-d supplementation study. Subjects received one of four treatments: placebo, 60 mg iron, 15 mg manganese, or both mineral supplements daily. Manganese supplementation resulted in significant increases in lymphocyte MnSOD activity and serum manganese concentrations from baseline values but no changes in urinary manganese excretion or in any indices of iron status. Oral contraceptive use and the stage of the menstrual cycle did not confound the use of lymphocyte MnSOD activity or serum manganese to monitor manganese status, but fat intake affected both indices. This work demonstrated that lymphocyte MnSOD activity can be used with serum manganese concentrations to monitor manganese exposure in humans.

Evaluation: Klimisch Code 5. Non-pivotal.

Davis CD, Malecki EA and Greger JL (1992a) Interactions among dietary manganese, heme iron, and nonheme iron in women. *Am J Clin Nutr* **56:**926-932.

Abstract: The relationship among dietary intake of heme iron, nonheme iron, and manganese on indexes of hematological and nutritional status in regard to manganese of 47 women consuming their typical diets was investigated. Increasing dietary iron intake, by consuming more nonheme iron in the diet, had questionable effects on hematological status (hematocrit values and ferritin and transferrin concentrations) and negative effects on nutritional status in regard to manganese (serum manganese, urine manganese, and lymphocyte manganese-dependent superoxide dismutase activity). In contrast, heme-iron intake was positively correlated with hematological status and had no consistent effect on nutritional status in regard to manganese. Differences in dietary manganese intake had no consistent effect on indices of manganese or iron status, possibly because foods that contain significant amounts of manganese (green vegetables, breads, and cereals) often contain significant amounts of nonheme iron. Thus, increasing dietary manganese intake by consuming these foods is apt to have limited impact on manganese status because of the interaction between nonheme iron and manganese.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. The study did not utilise 54Mn to assess retention and so comparative absorption data is not reported.

Davis CD, Wolf TL and Greger JL (1992b) Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. *J Nutr* **122:**1300-1308.

Abstract: The interactive effects of manganese and iron on true absorption and endogenous losses of manganese were investigated by feeding rats three levels of manganese (0.9, 48 or 188 micrograms Mn/g diet) and two levels of iron (19 or 276 micrograms Fe/g diet) for 7 wk. After 45 d, half of the rats were fed 54Mn and half were injected intraportally with 54Mn complexed to albumin. The relative distribution of 54Mn in tissues was generally similar for rats when 54Mn was administered in these two ways. Manganese-deficient animals retained more of the isotope, had both higher apparent and higher true absorption of manganese, had a greater proportion of 54Mn in their livers and had a lower proportion of 54Mn in their muscles compared with animals fed adequate or high levels of manganese. High iron intake inhibited manganese true absorption, reduced tissue manganese concentrations and inhibited heart manganese-dependent superoxide dismutase activity. However, the greatest effect of dietary iron was on mucosal cell manganese concentrations. Endogenous losses of manganese were approximately 8% of the amount of manganese actually absorbed regardless of intake. Thus, control of absorption in the gut seems to be the major way that manganese homeostasis is maintained. Furthermore, iron seems to be depressing manganese absorption by inhibiting manganese uptake into the mucosal cells.

Evaluation: Klimisch Code 2. Good level of methodology detail and use of statistical analysis. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The insoluble manganese carbonate was used as the source of manganese in the diet, however there was no discussion or justification of why this form of manganese was used. The radio-labelled tracer was the soluble manganese chloride.

Davis CD, Zech L and Greger JL (1993) Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses. *Proc Soc Exp Biol Med* **202:**103-108.

Abstract: Manganese homeostasis is believed to be maintained by excretion of excess absorbed manganese through the gut, but the extent of endogenous gut losses of manganese has not been quantitated. We developed a model with rats to quantitate endogenous gut losses of manganese in which the parenterally administered isotope was distributed like fed isotope. Intraportally injected 54Mn complexed to albumin distributed in tissues like the fed isotope, but carrier-free 54Mn injected intraperitoneally, intravenously, or intraportally, or 54Mn complexed to transferrin and injected intraportally did not. Thus, manganese appears to be complexed to albumin or an albumin-like protein when it leaves the intestine. A mathematical model of manganese metabolism in rats fed 54Mn was developed using the SAAM and CONSAM computer programs. It was determined that the liver, not the pancreas, was the major source of endogenous gut losses of manganese. Young, growing rats fed 45 micrograms of Mn/g diet were calculated to absorb 8.2% of their manganese intake and then to lose 37% of the absorbed manganese through gut endogenous losses.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. However, the authors did note that there had been problems with coprophagy which could compromise results.

Deimling MJ and Schnell RC (1984) Effect of manganese on the hepatic microsomal mixed function oxidase enzyme system in the rat. *Fundam Appl Toxicol* **4:**1009-1018.

Abstract: Experiments were conducted to examine the effect of manganese on the hepatic mixed function oxidase system in the rat. Acute treatment with manganese chloride (1-10 mg Mn/kg, ip) produced a significant prolongation of hexobarbital hypnosis in male rats on Days 2 and 3 following metal administration. The threshold dose of manganese to produce this alteration in response was 5 mg Mn/kg and the altered response returned to control values by Day 5. The prolonged hexobarbital hypnosis resulted from Mn inhibition of the hepatic microsomal mixed function oxidase system, the activity of which was assessed using aniline (23%), ethylmorphine (26%), and hexobarbital (27%) as substrates. Manganese treatment also produced significantly reduced levels of cytochrome P-450 (23%) and b5 (21%), but the substrate-induced spectral binding of all three substrates was not altered significantly by Mn when expressed as delta A per nanomole of cytochrome P-450. The activity of NADPH cytochrome c reductase was also significantly decreased (25%) by Mn treatment. Following the in vitro addition of Mn in concentrations ranging from 1 X 10(-6) to 1 X 10(-3) M Mn to microsomes derived from naive rats, there was no decrease in the metabolism of aniline or hexobarbital or cytochrome P-450 levels. Significant inhibition in ethylmorphine metabolism was observed with Mn concentrations of 1 X 10(-4) M and greater. These experiments indicate that acute

Mn treatment can alter drug response as the result of decreased hepatic biotransformation which occurs by an indirect mechanism.

Evaluation: Klimisch Code 5. Non-pivotal.

Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC and Wong BA (2002) Olfactory transport: a direct route of delivery of inhaled manganese phosphate to the rat brain. *J Toxicol Environ Health A* **65:**1493-1511.

Abstract: Experiments examining the dosimetry of inhaled manganese generally focus on pulmonary deposition and subsequent delivery of manganese in arterial blood to the brain. Growing evidence suggests that nasal deposition and transport along olfactory neurons represents another route by which inhaled manganese is delivered to certain regions of the rat brain. The purpose of this study was to evaluate the olfactory uptake and direct brain delivery of inhaled manganese phosphate ((54)MnHPO(4)). Male, 8-wk-old, CD rats with either both nostrils patent or the right nostril occluded underwent a single, 90-min, nose-only exposure to a (54)MnHPO(4) aerosol (0.39 mg (54)Mn/m(3); MMAD 1.68 microm, sigma(g) 1.42). The left and right sides of the nose, olfactory pathway, striatum, cerebellum, and rest of the brain were evaluated immediately after the end of the (54)MnHPO(4) exposure and at 1, 2, 4, 8, and 21 d postexposure with gamma spectrometry and autoradiography. Rats with two patent nostrils had equivalent (54)Mn concentrations on both sides of the nose, olfactory bulb, and striatum, while asymmetrical (54)Mn delivery occurred in rats with one occluded nostril. High levels of (54)Mn activity were observed in the olfactory bulb and tubercle on the same side (i.e., ipsilateral) to the open nostril within 1-2 d following (54)MnHPO(4) exposure, while brain and nose samples on the side ipsilateral to the nostril occlusion had negligible levels of (54)Mn activity. Our results demonstrate that the olfactory route contributes to (54)Mn delivery to the rat olfactory bulb and tubercle. However, this pathway does not significantly contribute to striatal (54)Mn concentrations following a single, short-term inhalation exposure to (54)MnHPO(4).

Evaluation: Klimisch Code 1. Well documented, designed and reported. Extensive discussion. No restrictions, the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, Arden James R, Struve MF and Wong BA (2005a) Maternal-fetal distribution of manganese in the rat following inhalation exposure to manganese sulfate. *Neurotoxicology* **26:**625-632.

Abstract: Studies examining the pharmacokinetics of manganese during pregnancy have largely focused on the oral route of exposure and have shown that the amount of manganese that crosses the rodent placenta is low. However, limited information exists regarding the distribution of manganese in fetal tissues following inhalation. The objective of this study was to determine manganese body burden in CD rats and fetuses following inhalation of a MnSO4 aerosol during pregnancy. Animals were evaluated following pre-breeding (2 weeks), mating (up to 14 days) and gestational (from gestation day (GD) 0 though 20) exposure to air or MnSO4 (0.05, 0.5, or 1 mg Mn/m(3)) for 6h/day, 7 days/week. The following maternal samples were collected for manganese analysis: whole blood, lung, pancreas, liver, brain, femur, and placenta. Fetal tissues were examined on GD 20 and included whole blood, lung, liver, brain, and skull cap. Maternal lung manganese concentrations were increased following exposure to MnSO4 at >or=0.05 mg Mn/m(3). Maternal brain and placenta manganese concentrations were increased following exposure of pregnant rats to MnSO4 at >or=0.5 mg Mn/m(3). Increased fetal liver manganese concentrations were observed following in utero exposure to MnSO4 at >or=0.5 mg Mn/m(3). Manganese concentrations within all other fetal tissues were not different from air-exposed controls. The results of this study demonstrate that the placenta partially sequesters inhaled manganese, thereby limiting exposure to the fetus.

Evaluation: Klimisch Code 2. Well designed and documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, James RA, Struve MF and Wong BA (2005b) Tissue manganese concentrations in lactating rats and their offspring following combined in utero and lactation exposure to inhaled manganese sulfate. *Toxicol Sci* **84:**12-21.

Abstract: There is little information regarding the tissue distribution of manganese in neonates following inhalation. This study determined tissue manganese concentrations in lactating CD rats and their offspring following manganese sulfate (MnSO4) aerosol inhalation. Except for the period of parturition, dams and their offspring were exposed to air or MnSO4 (0.05, 0.5, or 1 mg Mn/m3) for 6 h/day, 7 days/week starting 28 days prior to breeding through postnatal day (PND) 18. Despite increased manganese concentrations in several maternal tissues, MnSO4 inhalation exposure did not affect body weight gain, terminal (PND 18) body weight, or organ weights in the dams. Exposure to

MnSO4 at 1 mg Mn/m3 resulted in decreased pup body weights on PND 19 and decreased brain weights in some PND 14 to PND 45 pups. Exposure to MnSO4 at > or =0.05 mg Mn/m3 was associated with increased stomach content, blood, liver, and skull cap manganese concentrations in PND 1 pups, increased brain, lung, and femur manganese concentrations in PND 14 pups, and elevated olfactory bulb, cerebellum, and striatum manganese concentrations in PND 19 pups. When compared to controls, MnSO4 exposure to > or =0.5 mg Mn/m3 increased liver and blood manganese concentrations in PND 14 pups and increased liver, pancreas, and femur manganese concentrations in PND 19 pups. Manganese concentrations returned to control values in all offspring tissues by PND 45 +/- 1. Our data demonstrate that neonatal tissue manganese concentrations observed following MnSO4 inhalation are dependent on the MnSO4 exposure concentration and the age of the animal.

Evaluation: Klimisch Code 2. Well designed and documented. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP.

Dorman DC, McManus BE, Marshall MW, James RA and Struve MF (2004a) Old age and gender influence the pharmacokinetics of inhaled manganese sulfate and manganese phosphate in rats. *Toxicol Appl Pharmacol* **197:**113-124.

Abstract: In this study, we examined whether gender or age influences the pharmacokinetics of manganese sulfate (MnSO(4)) or manganese phosphate (as the mineral form hureaulite). Young male and female rats and aged male rats (16 months old) were exposed 6 h day(-1) for 5 days week(-1) to air, MnSO(4) (at 0.01, 0.1, or 0.5 mg Mn m(-3)), or hureaulite (0.1 mg Mn m(-3)). Tissue manganese concentrations were determined in all groups at the end of the 90-day exposure and 45 days later. Tissue manganese concentrations were also determined in young male rats following 32 exposure days and 91 days after the 90-day exposure. Intravenous (54)Mn tracer studies were also performed in all groups immediately after the 90-day inhalation to assess whole-body manganese clearance rates. Gender and age did not affect manganese delivery to the striatum, a known target site for neurotoxicity in humans, but did influence manganese concentrations in other tissues. End-of-exposure olfactory bulb, lung, and blood manganese concentrations were higher in young male rats than in female or aged male rats and may reflect a portal-of-entry effect. Old male rats had higher testis but lower pancreas manganese concentrations when compared with young males. Young male and female rats exposed to MnSO(4) at 0.5 mg Mn m(-3) had increased (54)Mn clearance rates when compared with air-exposed controls, while senescent males did not develop higher (54)Mn clearance rates. Data from this study should prove useful in developing dosimetry models for manganese that consider age or gender as potential sensitivity factors.

Evaluation: Klimisch Code 2. Well designed and documented study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Dorman DC, McManus BE, Parkinson CU, Manuel CA, McElveen AM and Everitt JI (2004b) Nasal toxicity of manganese sulfate and manganese phosphate in young male rats following subchronic (13-week) inhalation exposure. *Inhal Toxicol* **16:**481-488.

Abstract: Growing evidence suggests that nasal deposition and transport along the olfactory nerve represents a route by which inhaled manganese and certain other metals are delivered to the rodent brain. The toxicological significance of olfactory transport of manganese remains poorly defined. In rats, repeated intranasal instillation of manganese chloride results in injury to the olfactory epithelium and neurotoxicity as evidenced by increased glial fibrillary acidic protein (GFAP) concentrations in olfactory bulb astrocytes. The purpose of the present study was to further characterize the nasal toxicity of manganese sulfate (MnSO(4)) and manganese phosphate (as hureaulite) in young adult male rats following subchronic (90-day) exposure to air, MnSO(4) (0.01, 0.1, and 0.5 mg Mn/m(3)), or hureaulite (0.1 mg Mn/m(3)). Nasal pathology, brain GFAP levels, and brain manganese concentrations were assessed immediately following the end of the 90-day exposure and 45 days thereafter. Elevated end-of-exposure olfactory bulb, striatum, and cerebellum manganese concentrations were observed following MnSO(4) exposure to > or = 0.01, > or = 0.1, and 0.5 mg Mn/m(3), respectively. Exposure to MnSO(4) or hureaulite did not affect olfactory bulb, cerebellar, or striatal GFAP concentrations. Exposure to MnSO(4) (0.5 mg Mn/m(3)) was also associated with reversible inflammation within the nasal respiratory epithelium, while the olfactory epithelium was unaffected by manganese inhalation. These results confirm that high-dose manganese inhalation can result in nasal toxicity (irritation) and increased delivery of manganese to the brain; however, we could not confirm that manganese inhalation would result in altered brain GFAP concentrations.

Evaluation: Klimisch Code 2. Well documented, designed and reported. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Focuses on nasal toxicity more than toxicokinetics.

Dorman DC, Struve MF, Clewell HJ, 3rd and Andersen ME (2006a) Application of pharmacokinetic data to the risk assessment of inhaled manganese. *Neurotoxicology* **27:**752-764.

Abstract: There is increased interest within the scientific community concerning the neurotoxicity of manganese owing in part to the use of methylcyclopentadienyl manganese tricarbonyl (MMT) as a gasoline fuel additive and an enhanced awareness that this essential metal may play a role in hepatic encephalopathy and other neurologic diseases. Neurotoxicity generally arises over a prolonged period of time and results when manganese intake exceeds its elimination leading to increases in brain manganese concentration. Neurotoxicity can occur following high dose oral, inhalation, or parenteral exposure or when hepatobiliary clearance of this metal is impaired. Studies completed during the past several years have substantially improved our understanding of the health risks posed by inhaled manganese by determining exposure conditions that lead to increased concentrations of manganese within the central nervous system and other target organs. Many of these studies focused on phosphates, sulfates, and oxides of manganese since these are formed and emitted following MMT combustion by an automobile. These studies have evaluated the role of direct nose-to-brain transport of inhaled manganese and have examined differences in manganese toxicokinetics in potentially sensitive subpopulations (e.g., fetuses, neonates, individuals with compromised hepatic function or sub-optimal manganese intake, and the aged). This manuscript reviews the U.S. Environmental Protection Agency's current risk assessment for inhaled manganese, summarizes these contemporary pharmacokinetic studies, and considers how these data could inform future risk assessments of this metal following inhalation.

Evaluation: Klimisch Code 5. Review article.

Dorman DC, Struve MF, James RA, Marshall MW, Parkinson CU and Wong BA (2001a) Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. *Toxicol Appl Pharmacol* **170:**79-87.

Abstract: Dissolution rate can influence the pulmonary clearance of a metal and thus affect its delivery to the brain and other organs. The goal of this study was to determine the exposure-response relationship for the relatively soluble sulfate (MnSO(4)) and insoluble tetroxide (Mn(3)O(4)) forms of inhaled manganese in adult male CD rats. Rats were exposed 6 h/day for 7 days/week (14 exposures) to either MnSO(4) or Mn(3)O(4) at 0, 0.03, 0.3, or 3 mg Mn/m(3). End-of-exposure olfactory bulb, striatum, cerebellum, bile, lung, liver, femur, serum, and testes (n = 6 rats/concentration/chemical) manganese concentrations and whole-body (54)Mn elimination were then determined. Increased whole-body (54)Mn clearance rates were observed in animals from the high-dose (3 mg Mn/m(3)) MnSO(4) and Mn(3)O(4) exposure groups. Elevated manganese concentrations in the lung were observed following MnSO(4) and Mn(3)O(4) exposure to > or=0.3 mg Mn/m(3). Increased olfactory bulb and femur manganese concentrations were also observed following MnSO(4) exposure at > or=0.3 mg Mn/m(3). Elevated striatal, testes, liver, and bile manganese concentrations were observed following exposure to MnSO(4) at 3 mg Mn/m(3). Elevated olfactory bulb, striatal, femur, and bile manganese concentrations were observed following exposure to Mn(3)O(4) at 3 mg Mn/m(3). Animals exposed to MnSO(4) (3 mg Mn/m(3)) had lower lung and higher olfactory bulb and striatal manganese concentrations compared with levels achieved following similar Mn(3)O(4) exposures. Our results suggest that inhalation exposure to soluble forms of manganese results in higher brain manganese concentrations than those achieved following exposure to an insoluble form of manganese.

Evaluation: Klimisch Code 2. Very good detail in methodology, study design and clear discussion. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Dorman DC, Struve MF, James RA, McManus BE, Marshall MW and Wong BA (2001b) Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. *Toxicol Sci* **60:**242-251.

Abstract: Concerns exist as to whether individuals with relative manganese deficiency or excess may be at increased risk for manganese toxicity following inhalation exposure. The objective of this study was to determine whether manganese body burden influences the pharmacokinetics of inhaled manganese sulfate (MnSO(4)). Postnatal day (PND) 10 rats were placed on either a low (2 ppm), sufficient (10 ppm), or high (100 ppm) manganese diet. The feeding of the 2 ppm manganese diet was associated with a number of effects, including reduced body weight gain, decreased liver manganese concentrations, and reduced whole-body manganese clearance rates. Beginning on PND 77 +/- 2, male littermates were exposed 6 h/day for 14 consecutive days to 0, 0.092, or 0.92 mg MnSO(4)/m(3). End-of-exposure tissue manganese concentrations and whole-body (54)Mn elimination rates were determined. Male rats exposed to 0.092 mg MnSO(4)/m(3) had elevated lung manganese concentrations when compared to air-exposed male rats. Male rats exposed to 0.92 mg MnSO(4)/m(3)

developed increased striatal, lung, and bile manganese concentrations when compared to air-exposed male rats. There were no significant interactions between the concentration of inhaled MnSO(4) and dietary manganese level on tissue manganese concentrations. Rats exposed to 0.92 mg MnSO(4)/m(3) also had increased (54)Mn clearance rates and shorter initial phase elimination half-lives when compared with air-exposed control rats. These results suggest that, marginally manganese-deficient animals exposed to high levels of inhaled manganese compensate by increasing biliary manganese excretion. Therefore, they do not appear to be at increased risk for elevated brain manganese concentrations.

Evaluation: Klimisch Code 2. Well documented and reported. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The initial hypothesis that brain and other target tissue manganese concentrations resulting from the inhalation of manganese sulphate would be influenced by dietary manganese concentrations was not upheld.

Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA and Wong BA (2006b) Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. *Toxicol Sci* **92:**201-210.

Abstract: High-dose human exposure to manganese results in manganese accumulation in the basal ganglia and dopaminergic neuropathology. Occupational manganese neurotoxicity is most frequently linked with manganese oxide inhalation; however, exposure to other forms of manganese may lead to higher body burdens. The objective of this study was to determine tissue manganese concentrations in rhesus monkeys following subchronic (6 h/day, 5 days/week) manganese sulfate (MnSO(4)) inhalation. A group of monkeys were exposed to either air or MnSO(4) (0.06, 0.3, or 1.5 mg Mn/m(3)) for 65 exposure days before tissue analysis. Additional monkeys were exposed to MnSO(4) at 1.5 mg Mn/m(3) for 15 or 33 exposure days and evaluated immediately thereafter or for 65 exposure days followed by a 45- or 90-day delay before evaluation. Tissue manganese concentrations depended upon the aerosol concentration, exposure duration, and tissue. Monkeys exposed to MnSO(4) at > or = 0.06 mg Mn/m(3) for 65 exposure days or to MnSO(4) at 1.5 mg Mn/m(3) for > or = 15 exposure days developed increased manganese concentrations in the olfactory epithelium, olfactory bulb, olfactory cortex, globus pallidus, putamen, and cerebellum. The olfactory epithelium, olfactory bulb, globus pallidus, caudate, putamen, pituitary gland, and bile developed the greatest relative increase in manganese concentration following MnSO(4) exposure. Tissue manganese concentrations returned to levels observed in the air-exposed animals by 90 days after the end of the subchronic MnSO(4) exposure. These results provide an improved understanding of MnSO(4) exposure conditions that lead to increased concentrations of manganese within the nonhuman primate brain and other tissues.

Evaluation: Klimisch Code 2. Well designed, documented and reported. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Dorman DC, Struve MF and Wong BA (2001c) Pharmacokinetic Factors That Influence Manganese Delivery to the Brain. *CIIT Activities* **21:**1-11.

Evaluation: Klimisch Code 5. Review

Dorman DC, Struve MF, Wong BA, Dye JA and Robertson ID (2006c) Correlation of brain magnetic resonance imaging changes with pallidal manganese concentrations in rhesus monkeys following subchronic manganese inhalation. *Toxicol Sci* **92:**219-227.

Abstract: High-dose manganese exposure is associated with parkinsonism. Because manganese is paramagnetic, its relative distribution within the brain can be examined using magnetic resonance imaging (MRI). Herein, we present the first comprehensive study to use MRI, pallidal index (PI), and T(1) relaxation rate (R1) in concert with chemical analysis to establish a direct association between MRI changes and pallidal manganese concentration in rhesus monkeys following subchronic inhalation of manganese sulfate (MnSO(4)). Monkeys exposed to MnSO(4) at > or = 0.06 mg Mn/m(3) developed increased manganese concentrations in the globus pallidus, putamen, olfactory epithelium, olfactory bulb, and cerebellum. Manganese concentrations within the olfactory system of the MnSO(4)-exposed monkeys demonstrated a decreasing rostral-caudal concentration gradient, a finding consistent with olfactory transport of inhaled manganese. Marked MRI signal hyperintensities were seen within the olfactory bulb and the globus pallidus; however, comparable changes could not be discerned in the intervening tissue. The R1 and PI were correlated with the pallidal manganese concentration. However, increases in white matter manganese concentrations in MnSO(4)-exposed monkeys confounded the PI measurement and may lead to underestimation of pallidal manganese accumulation. Our results indicate that the R1 can be used to estimate regional brain manganese concentrations and may be a reliable biomarker of occupational manganese exposure. To our

knowledge, this study is the first to provide evidence of direct olfactory transport of an inhaled metal in a nonhuman primate. Pallidal delivery of manganese, however, likely arises primarily from systemic delivery and not directly from olfactory transport.

Evaluation: Klimisch Code 2. Well documented and reported study. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP.

Dorman DC and Wong BA (2006) Neurotoxicity of inhaled manganese: a reanalysis of human exposure arising from showering. *Med Hypotheses* **66:**199-200.

Evaluation: Klimisch Code 5. Rebuttal Letter to Elsner and Spangler, 2005.

Dorner K, Dziadzka S, Hohn A, Sievers E, Oldigs HD, Schulz-Lell G and Schaub J (1989) Longitudinal manganese and copper balances in young infants and preterm infants fed on breast-milk and adapted cow's milk formulas. *Br J Nutr* **61:**559-572.

Abstract: 1. Mn and Cu intake and retention in twenty full-term infants and six preterm infants were studied on the basis of 72 h balances. The age of the infants was 2-16 weeks and the gestational age of the preterm infants (triplets) 34 and 36 weeks. Three nutrition schemes were pursued: breast-fed, formula-fed with unsupplemented adapted formula and formula-fed with trace element supplementation. 2. The mean Mn concentration of all breast-milk samples (n 2339) was 6.2 micrograms/l. The two formulas had similar Mn concentrations (77 and 99 micrograms/l) but had different Fe, Cu (121 and 619 micrograms/l), Zn and I contents. The mean Cu concentration in mother's milk was 833 micrograms/l. 3. The following mean daily Mn intakes and retentions (micrograms/kg) respectively were measured: breast-fed full-term 1.06 (SD 0.43) and 0.43 (SD 0.65), formula-fed full-term 14.2 (SD 3.1) and 2.8 (SD 4.8), formula-fed preterm 15.0 (SD 2.2) and 0.06 (SD 5.87). The results for Cu were 114.5 (SD 22.3) and 88.0 (SD 46.5) micrograms/kg in breast-fed, 19.8 (SD 4.2) and 4.6 (-11.5-9.6) in the unsupplemented formula-fed and 106.4 (SD 18.9) and 55.5 (SD 20.3) in the supplemented formula full-term infant group. No significant influence of the trace element contents of the formulas on the relative retention of Mn or Cu was found. 4. Young preterm infants, and to some degree young full-term infants, often had negative Mn balances caused by a high faecal excretion. The formulas with a Mn concentration below 100 micrograms/l gave a sufficient supply of Mn. Preterm infants fed on the unsupplemented formula had a marginal Cu supply and their first balances were negative (-3.8 (SD 1.8) micrograms/kg). 5. In accordance with the estimated safe and adequate daily dietary intakes (recommended dietary allowances), formula-fed infants receive much more Mn than breast-fed infants and their absolute retention is higher. 6. Cu from breast-milk had a significantly better biological availability than that from cow's milk formula. If retentions similar to those in breast-fed infants are intended, we conclude, therefore, that cow's milk formula should be fortified with Cu up to a level of at least 600 micrograms/l.

Evaluation: Klimisch Code 5. Not particularly relevant.

Drown DB, Oberg SG and Sharma RP (1986) Pulmonary clearance of soluble and insoluble forms of manganese. *J Toxicol Environ Health* **17:**201-212.

Abstract: Manganese is an essential metal of toxicologic concern primarily because of exposure via inhalation. Environmental forms of Mn exist mainly as insoluble oxides, yet much of the research information available relates to the soluble salts. In the present study, adult male Sprague-Dawley rats were intratracheally instilled with either soluble MnCl2 or insoluble Mn3O4 labeled with 54Mn. Lungs and other major organs were sampled over a span of 3 mo after dosing with the respective chemicals, which were equivalent to 8 mu Ci and 1 mumol of manganese in 0.2 ml of buffer. There was rapid clearance of Mn from the lungs in the case of both chemicals; the chloride cleared at an initial rate of nearly four times that of the oxide. Despite this early difference, the amount of 54Mn remaining in the lungs after 2 wk was similar for both compounds. The level of 54Mn in the liver, kidney, spleen, and testes peaked at the 3-d sampling point in the case of the oxide, whereas the chloride peaked in these organs within 4 h. At 1 wk after administration, however, the 54Mn activity was comparable for both compounds in most organs sampled. Mn uptake in the brain was also more rapid with the chloride form, but both compounds remained at high levels for several weeks.

Evaluation: Klimisch Code 2. Methodology describes preparation of both the soluble and insoluble radiolabel. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Methodology relative brief in some instances such as manganese levels in diet not stated. Otherwise the study appears well conducted and reported and contains key results.

Ejima A, Imamura T, Nakamura S, Saito H, Matsumoto K and Momono S (1992) Manganese intoxication during total parenteral nutrition. *Lancet* **339:**426.

Evaluation: Klimisch Code 4. Very little detail given as this was a letter to the Lancet. However, the author does link a known daily and cumulative dose of manganese with toxic symptoms which regressed when manganese supplementation had ceased.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J and Oberdorster G (2006) Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect* **114:**1172-1178.

Abstract: BACKGROUND: Studies in monkeys with intranasally instilled gold ultrafine particles (UFPs; <100 nm) and in rats with inhaled carbon UFPs suggested that solid UFPs deposited in the nose travel along the olfactory nerve to the olfactory bulb. METHODS: To determine if olfactory translocation occurs for other solid metal UFPs and assess potential health effects, we exposed groups of rats to manganese (Mn) oxide UFPs (30 nm; approximately 500 microg/m(superscript)3(/superscript)) with either both nostrils patent or the right nostril occluded. We analyzed Mn in lung, liver, olfactory bulb, and other brain regions, and we performed gene and protein analyses. RESULTS: After 12 days of exposure with both nostrils patent, Mn concentrations in the olfactory bulb increased 3.5-fold, whereas lung Mn concentrations doubled; there were also increases in striatum, frontal cortex, and cerebellum. Lung lavage analysis showed no indications of lung inflammation, whereas increases in olfactory bulb tumor necrosis factor-alpha mRNA (approximately 8-fold) and protein (approximately 30-fold) were found after 11 days of exposure and, to a lesser degree, in other brain regions with increased Mn levels. Macrophage inflammatory protein-2, glial fibrillary acidic protein, and neuronal cell adhesion molecule mRNA were also increased in olfactory bulb. With the right nostril occluded for a 2-day exposure, Mn accumulated only in the left olfactory bulb. Solubilization of the Mn oxide UFPs was <1.5% per day. CONCLUSIONS: We conclude that the olfactory neuronal pathway is efficient for translocating inhaled Mn oxide as solid UFPs to the central nervous system and that this can result in inflammatory changes. We suggest that despite differences between human and rodent olfactory systems, this pathway is relevant in humans.

Evaluation: Klimisch Code 2. Well designed documented and reported. Manganese oxide UFP should be of relevance to arc-welding. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Ellingsen D, Bast-Pettersen R, Hetland S and Thomassen Y (2000) Health survey of workers exposed to manganese in smelting plants - a cross-sectional study. *National Institute of Occupational Health Norway* **1**.

Abstract: In 1995, the Norwegian labour inspectorate proposed to lower the OEL for Mn from 2.5 mg/m^3 (dust) and 1.0 mg/m^3 (fume) to a general level of 0.2 mg/m^3. The authors have therefore investigated whether Mn exposure in the Norwegian ferromanganese industry had any impact on the worker's health. Mn is produced in Norway at the following locations: Porsgrunn, Sauda, and Øye. The employees of the respective plants took part in this investigation.

Evaluation: Klimisch Code 5. The publication is in Norwegian, however the data appears to be have been reported in a later publication in English, which has been reviewed elsewhere in this report.

Ellingsen DG, Haug E, Ulvik RJ and Thomassen Y (2003a) Iron status in manganese alloy production workers. *J Appl Toxicol* **23:**239-247.

Abstract: The aim of this study was to investigate markers of iron status in production workers with current and long-term exposure to manganese (Mn) alloys. A total of 100 Mn-exposed male workers were compared with 100 male controls matched for age in a cross-sectional study. The geometric mean urinary Mn concentration in the exposed workers was 0.9 nmol mmol(-1) creatinine (range = 0.1-126.3), compared with 0.4 nmol mmol(-1) creatinine (range = 0.1-13.1) in the controls. The index group had been exposed to Mn for 20 years on average (range = 2.1-41.0). The geometric mean concentration of soluble transferrin receptor was lower in the exposed subjects than in the controls (2.2 vs 2.6 mg l(-1); $P < 0.001$) and the concentration was negatively associated with current exposure to "soluble" Mn in the inhalable aerosol fraction and with current smoking habits. An association was found between the concentration of serum soluble transferrin receptor and the concentration of Mn in whole blood (Pearson's $r = 0.48$; $P < 0.001$) in the controls. The results suggest that Mn-exposed workers have higher intracellular iron concentration in the erythrocyte precursors than the controls, resulting in a down-regulation of transferrin receptors on the surface of these cells. The concentrations of Mn in the blood of occupationally non-exposed individuals appear to be influenced by iron status, even at physiological iron levels.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. A well reported and discussed study.

Ellingsen DG, Hetland SM and Thomassen Y (2003b) Manganese air exposure assessment and biological monitoring in the manganese alloy production industry. *J Environ Monit* **5:**84-90.

Abstract: One hundred workers carried personal air sampling equipment during three days to assess exposure to inhalable and respirable Mn. A novel four-step chemical fractionation procedure developed for the speciation of Mn in workroom aerosols was applied for selected aerosol filters. Blood and urine samples were analysed for Mn. The geometric mean (GM) concentrations of inhalable (n = 265) and respirable (n = 167) Mn determined in all filters were 254 microg m(-3) and 28 microg m(-3) respectively. Only 10.6% (95% CI 8.9-12.5) respirable Mn was found in the inhalable fraction when inhalable and respirable samples collected in parallel were considered (n = 153 pairs). There was a high correlation (Pearson's r = 0.70; p < 0.001) between respirable and inhalable Mn. The largest amounts of Mn in the inhalable aerosol fraction were found as Mn0 and Mn2+ (47.4%), whereas 28% was practically "insoluble". The associations between B-Mn and aerosol concentrations of Mn were weak, but an association was found between U-Mn and respirable Mn; Pearson's r being 0.38 between "soluble" respirable Mn and U-Mn. No significant association was found between the "insoluble" components (probably SiMn) and Mn in biological samples.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. A well reported and discussed study.

Elsner RJ and Spangler JG (2005) Neurotoxicity of inhaled manganese: public health danger in the shower? *Med Hypotheses* **65:**607-616.

Abstract: CONTEXT: Manganese (Mn) is an essential trace element but is neurotoxic at high doses. Showering with Mn-laden water has never been evaluated as a central nervous system (CNS) delivery vector for Mn, even though intranasally administered Mn in laboratory animals circumvents the blood-brain barrier and passes directly into the brain via olfactory pathways. OBJECTIVE: To review the literature on Mn and attempt to quantify potential human CNS exposure to manganese from showering. DATA SOURCES: We systematically searched Medline 11/9/02 and again on 3/9/04. The following search terms were used: manganese, water, drinking water, shower, showering, bath, bathing and inhalation, then combined with "water or drinking water or showering or shower or bathing or inhalation." STUDY SELECTION: Animal experimental investigations, human epidemiological studies, and consensus and governmental reports were utilized. DATA EXTRACTION: Data were extracted by both authors and extrapolations to humans were calculated by one of us (JGS) controlling for age, length of exposure and known respiratory differences between rats and humans. DATA SYNTHESIS: During a decade of showering in Mn-contaminated water, models for children and adults show higher doses of aerosolized Mn (3-fold and 112-fold greater, respectively) than doses reported to cause Mn brain deposition in rats. CONCLUSIONS: Long-term shower exposure to Mn-laden water may pose a significant risk for CNS neurotoxicity via olfactory uptake in up to 8.7 million Americans. If our results are confirmed, regulatory agencies must rethink existing Mn drinking water standards.

Evaluation: Klimisch Code 3. The authors reviewed the literature and used the extracted data to extrapolate the perceived human risk. The authors concluded that long-term shower exposure to manganese-laden water may pose a risk for CNS neurotoxicity via olfactory uptake in up to 8.7 million Americans. However, there were several flaws in their assumptions and calculations which were discussed in a further publication by the authors of one of the original papers whose data had been used by Elsner and Spangler to support their hypothesis.

EN481 ECfS (1993) Workplace atmospheres - Size fraction definitions for measurement of airborne particles, in: *EN 481*.

Evaluation: Klimisch Code 5. European Standard 481.

Eriksson H, Gillberg PG, Aquilonius SM, Hedstrom KG and Heilbronn E (1992) Receptor alterations in manganese intoxicated monkeys. *Arch Toxicol* **66:**359-364.

Abstract: The density of four different receptors and one marker of dopamine uptake sites were analyzed in monkey brains after manganese exposure (0.1 g manganese per month during 26 months, a dose comparable to that workers might inhale in dusty environments) by means of quantitative receptor autoradiography. The binding of 3H-mazindol to the dopamine uptake sites was reduced by 75% in both the head of the caudate nucleus and putamen, while it remained unchanged in the other regions analyzed. The binding of the D1 receptor ligand 3H-SCH 23,390 was reduced about 45% in the same areas as mazindol binding, while the density of D2 receptors was unaffected. The muscarinic acetylcholine receptors as well as GABAA receptors remained also unchanged in all brain areas analyzed after manganese exposure. Thus the dopaminergic neurons must be considered to be vulnerable to manganese concentrations attainable in the work environment. Our results also indicate

that postsynaptic structures containing D1 receptors are sensitive while cells containing D2 receptors are either spared or compensated for by up-regulation of the number of receptors on remaining sites.
Evaluation: Klimisch Code 5. Non-pivotal.

Eriksson H, Lenngren S and Heilbronn E (1987a) Effect of long-term administration of manganese on biogenic amine levels in discrete striatal regions of rat brain. *Arch Toxicol* **59:**426-431.
Abstract: The effect of long-term manganese exposure of rats on biogenic amine levels in striatal brain regions is described. Four groups of male Sprague-Dawley rats received manganese as MnCl2 continuously in the drinking water for 60, 100, 165 and 265 days, respectively. Discrete regions within the caudate-putamen were punched out. Dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid were determined by high performance liquid chromatography with electrochemical detection. Rats exposed for 60 and 165 days showed significantly increased levels of dopamine and 3,4-dihydroxyphenylacetic acid in discrete regions of the dorsal caudate-putamen. The affected regions were possibly not identical in the two age groups but they were adjacently situated. These alterations were not found in rats exposed for 100 or 265 days.
Evaluation: Klimisch Code 5. Non-pivotal.

Eriksson H, Magiste K, Plantin LO, Fonnum F, Hedstrom KG, Theodorsson-Norheim E, Kristensson K, Stalberg E and Heilbronn E (1987b) Effects of manganese oxide on monkeys as revealed by a combined neurochemical, histological and neurophysiological evaluation. *Arch Toxicol* **61:**46-52.
Abstract: Four monkeys were exposed to a total of 8 g each of manganese as oxide by repetitive subcutaneous injections during 5 months, after which they were left for 1 week to 6 months before they were sacrificed. All animals developed hyperactive behaviour after about 2 months. About 5 months after the start of the exposure the animals became hypoactive with an unsteady gait, and subsequently an action tremor appeared in some of the animals. The animals lost power in both upper and lower limbs and the movements of the hands and feet were very clumsy. The serum content of manganese rose 10-40 times during the exposure time and the content in brain was generally increased more than 10 times, with the highest content found in globus pallidus and putamen. The observed neurochemical effects were also largest in globus pallidus and putamen. In these regions there was a considerable depletion of dopamine and 3,4-dihydroxyphenylacetic acid, while the homovanillic acid content remained almost unchanged. A severe neuronal cell loss was observed in globus pallidus but not in other regions. This is in accordance with results from the most recent neuropathological study of a human suffering from chronic manganese poisoning [Yamada et al. (1986) Acta Neuropathol 70: 273-278] where globus pallidus was devoid of neuronal cells while the content of pigmented cells in substantia nigra was normal. Our data suggest a reduction in number of dopaminergic nerve terminals, as the activity of the dopamine synthesizing enzyme DOPA-decarboxylase was also lowered.
Evaluation: Klimisch Code 5. Non-pivotal.

Fechter LD (1999) Distribution of manganese in development. *Neurotoxicology* **20:**197-201.
Abstract: Elimination of manganese is closely related to uptake in the normal adult and is believed to play a critical role in maintaining manganese homeostasis in the face of changing manganese intake. Data from immature rats, mice and cats have suggested that elimination of manganese undergoes a period of maturation with adult patterns of excretion developing at about the time of weaning. In addition, the uptake of manganese from the intestine appears to be more efficient in young animals than in adults. These two sets of findings raise the possibility that exposure to elevated manganese levels during the perinatal period might yield excessive concentrations of this metal in the developing organism. Such an outcome might lead to manganese accumulations in organ systems where subsequent mobilization might be difficult and might produce permanent toxic injury. This review evaluates the patterns of manganese uptake and distribution following prenatal and pre-weaning exposure using a variety of model systems. The data demonstrate that manganese does cross the placenta and enter fetal tissue although the extent of material crossing the placenta appears to be limited. The issue of neonatal manganese elimination following tracer and toxic exposure levels to manganese is addressed. The data show that that the neonatal rodent is significantly more effective in eliminating manganese than previously believed based upon tracer studies. Finally, data are presented on regional brain manganese distribution. These data highlight the lack of agreement on whether manganese is concentrated in specific brain areas.
Evaluation: Klimisch Code 5. Review - not very comprehensive.

Fechter LD, Johnson DL and Lynch RA (2002) The relationship of particle size to olfactory nerve uptake of a non-soluble form of manganese into brain. *Neurotoxicology* **23:**177-183.
Abstract: The essential element, manganese, can produce chronic neuromotor impairment related to basal ganglia (BG) damage when it is presented in excessive quantities. The uptake and elimination patterns

of manganese following ingestion have been well studied and, under normal conditions, excretion appears to keep manganese levels under tight control. Less is known about inhalation exposure, but it has been proposed that the lung might serve as a long-term reservoir for manganese transport into blood. Recent data suggest that a third route of exposure, transport by the olfactory nerve directly to the brain, might have importance in toxicology since such a route would bypass liver uptake and biliary excretion of manganese. In this study, we sought to determine how particle size and the use of a poorly soluble form of manganese might influence net systemic absorption of manganese dust and the potential role of the olfactory nerve in transport of manganese dioxide. Rats were exposed in nose-only exposure chambers to manganese dioxide (MnO2) aerosols of 1.3 and 18 microm mass median aerodynamic diameter (MMAD). The concentration of aerosols was kept constant at 3 mg/m3 as Mn. Following 15 days of exposure (five times per week for 3 weeks), rats were euthanized and tissues harvested for manganese determination carried out by graphite furnace atomic absorption spectroscopy. Small-particle MnO2 exposure resulted in an elevation in olfactory bulb manganese concentration, presumably through uptake by the olfactory nerve, but the effect was highly variable. While small increases in cortical and neostriatal manganese levels were also observed in these rats, they did not reach statistical significance. By contrast, there was no evidence of olfactory nerve MnO2 uptake in rats receiving the large-particle exposure.

Evaluation: Klimisch Code 3. Whilst small-particle MnO2 exposure resulted in an elevation in olfactory bulb manganese concentration, the effect was highly variable. Other results did not reach statistical significance and there was no evidence of olfactory nerve MnO2 uptake in rats receiving the large-particle exposure. As such, the data from this study are not considered to significantly contribute to the toxicokinetic review of manganese.

Fell JM, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ and Milla PJ (1996) Manganese toxicity in children receiving long-term parenteral nutrition. *Lancet* **347:**1218-1221.

Abstract: BACKGROUND: In patients receiving long-term parenteral nutrition (PN), cholestatic disease and nervous system disorders have been associated with high blood concentrations of manganese. In such patients, the normal homoeostatic mechanisms of the liver and gut are bypassed and the requirement for this trace element is not known; nor has it been certain whether hypermanganesaemia causes the cholestasis or vice versa. We explored the direction of effect by serial tests of liver function after withdrawal of manganese supplements from children receiving long-term PN. We also examined the relation between blood manganese concentrations and brain lesions, as indicated by clinical examination and magnetic resonance imaging (MRI). METHODS: From a combined group of 57 children receiving PN we identified 11 with the combination of hypermanganesaemia and cholestasis; one also had a movement disorder. Manganese supplements were reduced in the first three and withdrawn in the remainder. MRI was done in two of these children. We also looked at manganese concentrations and MRI scans in six children who had received PN for more than 2 years without developing liver disease. FINDINGS: In the hypermanganesaemia/cholestasis group, four of the 11 patients died. In the seven survivors baseline whole-blood manganese was 615-1840 nmol/L, and after 4 months it had declined by a median of 643 nmol/L ($p < 0.01$). Over the same interval total bilirubin declined by a median of 70 mumol/L ($p < 0.05$). Two of these children had movement disorders, one of whom survived to have an MRI scan; this showed, with T1 weighted images, bilateral symmetrically increased signal intensity in the globus pallidus and subthalamic nuclei. Such changes were also seen in five other children--one from the hypermanganesaemia/cholestasis group and four of six in the long-term PN group without liver disease (in all of whom blood manganese was above normal). INTERPRETATION: The cholestasis complicating PN is multifactorial, but these results add to the evidence that manganese contributes. In view of the additional hazard of basal ganglia damage from high manganese levels in children receiving long-term PN, we recommend a low dose regimen of not more than 0.018 mumol/kg per 24 h together with regular examination of the nervous system.

Evaluation: Klimisch Code 2. Well documented and discussion of results. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Finkelstein Y, Zhang N, Fitsanakis VA, Avison MJ, Gore JC and Aschner M (2008) Differential deposition of manganese in the rat brain following subchronic exposure to manganese: a T1-weighted magnetic resonance imaging study. *Isr Med Assoc J* **10:**793-798.

Abstract: BACKGROUND: Manganism is a central nervous system disorder caused by toxic exposure to manganese. Manganism has been related to occupational exposures, liver diseases, prolonged parenteral nutrition, and abuse of illicit drugs. Initially manifested by a reversible neuropsychiatric syndrome (locura manganica), the main symptoms and signs of manganism are emotional lability, compulsive behavior and visual hallucinations. Locura manganica is followed by an irreversible extrapyramidal syndrome, the onset of which occurs years after chronic exposure. OBJECTIVES: To

characterize the regional distribution of Mn in the rat brain after subchronic exposure to Mn. This animal model holds special clinical relevance, reflecting the earlier clinical stages of manganism before chronic exposure to Mn exerts its irreversible effects. METHODS: Sprague-Dawley rats were intravenously injected with MnCl2 weekly, for a total of 14 weeks - approximately 1/10 of the lifetime of the rat. T1-weighted magnetic resonance imaging was used to detect the distribution of Mn deposition in brain tissues, as evidenced by areas of T1-weighted hyperintense signals. RESULTS: A consistent region-specific pattern of T1-weighted hyperintensities was observed in the brains of Mn-treated rats. Cortical hyperintensities were prominent in the hippocampus and dentate gyrus. Hyperintensities were also observed in the olfactory bulbs, pituitary gland, optic nerves and chiasma, pons, midbrain tegmentum, habenula, lentiform and caudate nuclei, thalamus, chorioid plexus and cerebellar hemispheres. CONCLUSIONS: Prominent Mn depositions, evidenced by T1-weighted hyperintensities in the hippocampus after subacute exposure to Mn, are compatible with the clinical picture of manganism during its early stages, and may explain its pathophysiology.

Evaluation: Klimisch Code 2. Study well discussed and documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Finley JW (1999) Manganese absorption and retention by young women is associated with serum ferritin concentration. *Am J Clin Nutr* **70:**37-43.

Abstract: BACKGROUND: The interaction between iron and manganese in the gut is well characterized but iron status has not been shown to affect manganese absorption. OBJECTIVE: The objective of this study was to determine whether iron status as determined by serum ferritin concentrations affects manganese absorption, retention, balance, and status. DESIGN: The subjects were healthy young women; 11 had serum ferritin concentrations >50 microg/L and 15 had serum ferritin concentrations <15 microg/L. In a crossover design, subjects consumed diets that supplied either 0.7 or 9.5 mg Mn/d for 60 d. Manganese absorption and retention were assessed during the last 30 d of each dietary period by using an oral dose of 54Mn; balance was assessed simultaneously. RESULTS: Dietary manganese did not affect manganese status, but high serum ferritin depressed arginase activity. The interaction of ferritin status and dietary manganese affected 54Mn absorption and biological half-life. Absorption was greatest in subjects with low ferritin concentrations when they were consuming the low-manganese diet, and was least in subjects with high ferritin concentrations. Biological half-life was longest when subjects with high ferritin concentrations consumed the low-manganese diet, and was shortest in all subjects consuming the high-manganese diet. Manganese balance was only affected by the amount of manganese in the diet. CONCLUSIONS: These results show that iron status, as measured by serum ferritin concentration, is strongly associated with the amount of manganese absorbed from a meal by young women. When greater amounts of manganese are absorbed, the body may compensate by excreting manganese more quickly.

Evaluation: Klimisch Code 2. Well documented and particularly thorough analysis and discussion of the data. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Finley JW, Caton JS, Zhou Z and Davison KL (1997) A surgical model for determination of true absorption and biliary excretion of manganese in conscious swine fed commercial diets. *J Nutr* **127:**2334-2341.

Abstract: Some trace elements, such as Mn, Cu and Zn, are absorbed and quickly resecreted into the gut through the bile. When this occurs, the unabsorbed nutrient and the absorbed and resecreted nutrient may mix in the gut, preventing quantitative calculation of either. We have developed a surgical model that prevents this complication. Pigs (20-40 kg) were fitted with cannulas in the bile duct, lumen of the duodenum, portal vein, ileocolic vein and jugular vein. After recovery for 6-8 d, pigs were given an oral dose of 9.25 mBq of 54Mn. The flow rate of blood past the portal vein was determined by infusion of P-amino hippuric acid into the ileocolic vein. Absorption was quantified by multiplying the concentration of 54Mn in the portal blood by the flow rate. Biliary excretion was determined by quantitative collection of bile, and previously collected bile was reinfused into the gut lumen. Urine and feces were also quantitatively collected. A postoperative time of 6-8 d was sufficient for pigs to recover from the effects of surgery and anesthesia, as assessed by several measures of metabolic function and food and water intake. True absorption was calculated to be 0.5%. 54Mn in the urine and bile began to increase after 4 d. When the pigs were killed after 12 d, only 0.5% of the 54Mn remained in the carcass. Results of this study show that pigs surgically modified by the described procedure can recover fully and can serve as a model to study intestinal absorption and biliary excretion of nutrients. Furthermore, initial studies using 54Mn showed that the model is applicable to studying Mn metabolism and suggest the need for a more detailed study of Mn absorption and biliary excretion.

Evaluation: Klimisch Code 3. Although the technique is well documented, there is no evidence that this has been successfully used in pigs and that the biliary excretion was functioning correctly in the surgically modified animals, e.g. using a model test compound. There is too little detail on the radiolabelled manganese dosed (which salt, whether it was as a solution, capsule etc.). Overall, the scientific reliability is questionable.

Finley JW, Johnson PE and Johnson LK (1994) Sex affects manganese absorption and retention by humans from a diet adequate in manganese. *Am J Clin Nutr* **60:**949-955.

Abstract: Men (n = 20) and women (n = 20) consuming a diet adequate in manganese were fed 0.037 mBq 54Mn in a test meal. Subjects were counted in a whole-body counter for 70 d to determine whole-body retention of 54Mn. Data from days 10 to 20 and from days 19 to 70 were analyzed by linear regression to calculate absorption and biological half-life. Men absorbed significantly less 54Mn than women, but the 54Mn absorbed had a longer half-life in men. Estimates of absorption were higher, and estimates of half-life were lower, when data from days 10 to 20 were used compared with days 19 to 70. There was a significant association between manganese absorption and plasma ferritin concentrations and between manganese absorption and biological half-life. We conclude that men and women differ in manganese metabolism and that such differences may be related to iron status. We also conclude that regression estimates of absorption determined by using whole-body retention curves depend on the portion of the data used.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. The interpretation of the study may have been confounded by iron deficiency in 11 out of 20 female subjects, while only 1 out of 20 males had iron deficiency. As such, the sex differences reported may have been significantly influenced by the iron-deficient subjects which were predominately female. Details regarding the source of the radioisotope were missing.

Finley JW, Penland JG, Pettit RE and Davis CD (2003) Dietary manganese intake and type of lipid do not affect clinical or neuropsychological measures in healthy young women. *J Nutr* **133:**2849-2856.

Abstract: Because manganese (Mn) is potentially toxic, and because dietary fat type may affect Mn absorption, the objectives of the current study were to determine whether diets containing very low or very high amounts of Mn and enriched in either saturated or unsaturated fats affected measures of neuropsychological and basic metabolic function. Healthy young women were fed for 8 wk each, in a crossover design, diets that provided 0.8 or 20 mg of Mn/d. One half of the subjects received 15% of energy as cocoa butter, and one half received 15% of energy as corn oil. A meal containing (54)Mn was fed after 4 wk, and subjects underwent whole-body counting for the next 21 d. Blood draws and neuropsychological tests were administered at regular intervals during the dietary periods. When subjects consumed the diets low in Mn, compared with the high Mn diets, they absorbed a significantly higher percentage of (54)Mn, but had a significantly longer biological half-life of the absorbed (54)Mn. Manganese intake did not affect any neurological measures and only minimally affected psychologic variables. These data show that efficient mechanisms operate to maintain Mn homeostasis over the range of intakes that may be encountered in a mixed Western diet. Thus, dietary intakes of Mn from 0.8 to 20 mg for 8 wk likely do not result in Mn deficiency or toxicity signs in healthy adults.

Evaluation: Klimisch Code 2. Well documented and reported study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Fitsanakis VA, Zhang N, Anderson JG, Erikson KM, Avison MJ, Gore JC and Aschner M (2008) Measuring brain manganese and iron accumulation in rats following 14 weeks of low-dose manganese treatment using atomic absorption spectroscopy and magnetic resonance imaging. *Toxicol Sci* **103:**116-124.

Abstract: Chronic exposure to manganese (Mn) may lead to a movement disorder due to preferential Mn accumulation in the globus pallidus and other basal ganglia nuclei. Iron (Fe) deficiency also results in increased brain Mn levels, as well as dysregulation of other trace metals. The relationship between Mn and Fe transport has been attributed to the fact that both metals can be transported via the same molecular mechanisms. It is not known, however, whether brain Mn distribution patterns due to increased Mn exposure vs. Fe deficiency are the same, or whether Fe supplementation would reverse or inhibit Mn deposition. To address these questions, we utilized four distinct experimental populations. Three separate groups of male Sprague-Dawley rats on different diets (control diet [MnT], Fe deficient [FeD], or Fe supplemented [FeS]) were given weekly intravenous Mn injections (3 mg Mn/kg body mass) for 14 weeks, whereas control (CN) rats were fed the control diet and received sterile saline injections. At the conclusion of the study, both blood and brain Mn and Fe levels were determined by atomic absorption spectroscopy and magnetic resonance imaging. The data indicate that

changes in dietary Fe levels (either increased or decreased) result in regionally specific increases in brain Mn levels compared with CN or MnT animals. Furthermore, there was no difference in either Fe or Mn accumulation between FeS or FeD animals. These data suggest that dietary Fe manipulation, whether increased or decreased, may contribute to brain Mn deposition in populations vulnerable to increased Mn exposure.

Evaluation: Klimisch Code 2. Study well discussed and documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

FNB/IOM (2001) Manganese. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc., in: *Food and Nutrition Board. Institute of Medicine*, pp 394-419, National Academy Press.

Evaluation: Klimisch Code 5. Reference to manganese dietary intake.

Freeland-Graves JH, Behmardi F, Bales CW, Dougherty V, Lin PH, Crosby JB and Trickett PC (1988) Metabolic balance of manganese in young men consuming diets containing five levels of dietary manganese. *J Nutr* **118:**764-773.

Abstract: Five healthy men, ages 19-20, were fed a diet for 105 d to measure manganese balance during consumption of conventional foods. The study was divided into five periods of 21, 21, 38, 11 and 14 d, in which the daily dietary intakes of manganese (Mn) were 2.89, 2.06, 1.21, 3.79 and 2.65 mg, respectively. During the last 7 d of each dietary period, subjects resided in a metabolic unit and fasting blood samples were drawn on two mornings. Feces and urine were collected during the last 6 d and integumental losses were collected during the last 60 h of each period. The mean Mn balances for periods 1-5 were -0.083, -0.018, -0.088, +0.657 and +0.136 mg/d, respectively. Corresponding apparent retentions were -2.90, -0.88, -7.40, +17.33 and +5.12%. The mean sum of endogenous and exogenous losses when intake was theoretically zero was calculated to be 392 micrograms/d. When these total losses were combined with the mean positive retention, the theoretical mean dietary level of Mn required for positive balance for these male subjects was 3.5 mg/d or 50 micrograms/kg.

Evaluation: Klimisch Code 5. Non-pivotal.

Freeland-Graves JH and Lin PH (1991) Plasma uptake of manganese as affected by oral loads of manganese, calcium, milk, phosphorus, copper, and zinc. *J Am Coll Nutr* **10:**38-43.

Abstract: Six adult subjects were administered a series of manganese (Mn) tolerance tests to investigate the influence of various minerals on Mn plasma uptake. Oral loads given to all six subjects included 40 mg manganese alone, or with 800 mg calcium (Ca) as either calcium carbonate (CaCO3) or 545 ml 2% milk. Four of the subjects also received loads of 800 mg phosphorus (P), 2 mg copper (Cu), and 50 mg zinc (Zn) with the 40 mg Mn. Baseline Mn tolerance tests for all subjects produced a rapid increase in plasma Mn, followed by return to baseline. The addition of Ca as either CaCO3 or 2% milk to the oral Mn essentially blocked the plasma uptake of Mn. No significant differences were found between the source of Ca in its inhibitory effect. Plasma Ca uptake was lower when Mn was simultaneously administered, but the results were not significantly different. Ionized levels of plasma Ca did not change significantly. The addition of Cu to the Mn load decreased the area under the curve for plasma Mn by about half, but it was not significantly different in the four subjects. In contrast, the addition of Zn to the Mn produced a significant increase in plasma Mn. Phosphorus has no influence on plasma uptake of Mn. These results indicate that the plasma uptake of Mn is greatly reduced by concomitant ingestion of Ca but may be increased by an oral load of Zn.

Evaluation: Klimisch Code 4. Too brief methodology, for example the sex of the subjects is not stated.

Furchner JE, Richmond CR and Drake GA (1966) Comparative metabolism of radionuclides in mammals. 3. retention of manganese-54 in the mouse, rat, monkey and dog. *Health Phys* **12:**1415-1423.

Abstract: Radiomanganese (54Mn) was administered to mice and rats by the oral, intravenous and intraperitoneal routes and to dogs and monkeys by the oral and intravenous routes. Whole-body-counting techniques, utilizing 4pi liquid scintillation counters, were used. to determine whole-body-retention parameters. Retention functions consisting of the sum of three or four exponential expressions were adequate descriptions of retention until the body burden was less than 1 per cent of the administered dose. The longest effective half times after intravenous injections were 119, 146, 99 and 68 days for mice, rats, monkeys and dogs, respectively. Tissue distribution studies in rats showed that, for most tissues, concentration as a function of time roughly paralleled whole-body retention. Both bone and brain were found to have a slower rate of loss than other tissues. Using a parabolic relation between body weights and the integrals of the retention functions, it was estimated that 6 x 10-3 [mu]c/ml was the maximum permissible concentration in water when the total body is the critical

organ. This value is in good agreement with the current ICRP value of 8 x 10-3. However, the value calculated for the lower large intestine (1 x 10-3 [mu]c/ml) must remain the MPCw for 54Mn

Evaluation: Klimisch Code 4. Very old study with very little methodology. However, the data from this study was used for the pharmacokinetic modeling used by Teeguarden et al., 2007c.

Furst A (1978) Tumorigenic effect of an organomanganese compound on F344 rats and Swiss albino mice. *J Natl Cancer Inst* **60:**1171-1173.

Abstract: Trioctanoin suspensions of manganese dioxide and manganese powder were injected im into inbred F344 rats and Swiss albino mice. The manganese powder was also administered orally to the rats. No difference in tumor incidence was noted between treated and control animals. In contrast, manganese (manganous) acetylacetonate administered im to rats produced a statistically significant number of fibrosarcomas at the sites of injection.

Evaluation: Klimisch Code 5. Non-pivotal.

Gan SL, Tan KT and Kwok SF (1988) Biological threshold limit values for manganese dust exposure. *Singapore Med J* **29:**105-109.

Evaluation: Klimisch Code 4. Methodology lacking, no data on timings of blood and urine sampling for example. However, this is an important study as it was using real human occupational exposures.

Garcia-Aranda JA, Wapnir RA and Lifshitz F (1983) In vivo intestinal absorption of manganese in the rat. *J Nutr* **113:**2601-2607.

Abstract: The mechanisms of intestinal absorption of Mn in rats and the effects of low-molecular-weight ligands in this process were investigated using an in vivo perfusion system. Segments of either jejunum or ileum were perfused with isotonic solutions containing 0.0125 to 0.1 mM MnSO4 X 7H2O, in the presence or absence of double its concentration of either L-histidine (His) or citrate (Cit). In all cases the absorption of Mn declined with time; for example, in the absence of ligand Mn absorption fell from (means +/- SEM) 16.0 +/- 2.2 at 30 minutes to 2.3 +/- 4.1 pmol/(minute X cm) after 90 minutes of perfusion. Comparable declines occurred both in the jejunum and in the ileum in the presence of His or Cit. The initial absorption rates of Mn, obtained by extrapolation, were higher in the jejunum when His or Cit were present than when no ligands were included in the perfusate [means +/- SD, with His, 66.4 +/- 11.9; with Cit, 79.5 +/- 6.2; none = 17.8 +/- 3.3 pmol/(minute X cm)]. In the ileum, optimum absorption with His was observed between pH 7 and 8. The kinetics of in vivo Mn ileal absorption in the presence of His yielded a Kt of 0.056 mM and an estimated Vmax of 158 pmol/(minute X cm). The coefficient of diffusion was calculated to be 1.5 X 10(-3) cm2/minute. These data are compatible with a high affinity, low capacity, active transport mechanism for Mn in the rat intestine and suggest a limited role for small-molecular-weight ligands associated with both diffusional or active translocation processes.

Evaluation: Klimisch Code 3. Limitations in the methodology used, for example no measurements were taken until 30 minutes after the dose solution had been added. This meant no steady-state absorption was seen and extensive extrapolation was used. As such, the dynamics of manganese transport at the start of the experiment were not directly measured. The transport accross the intestinal wall was not considered.

Gianutsos G, Morrow GR and Morris JB (1997) Accumulation of manganese in rat brain following intranasal administration. *Fundam Appl Toxicol* **37:**102-105.

Abstract: Manganese chloride (50-800 micrograms) was injected unilaterally into the right nostril of rats and its accumulation in the central nervous system (CNS) was monitored. Brain manganese levels were elevated in a dose-dependent, time-dependent, and tissue-dependent manner. Elevated levels of manganese were detected in the right olfactory bulb and olfactory tubercle within 12 hr after instillation and remained elevated for at least 3 days. As little as 100 micrograms of manganese chloride was sufficient to increase brain manganese levels. No changes were detected on the left side of the brain. The manganese content of the striatum, the target site for manganese neurotoxicity, was unchanged following acute administration, but was elevated when two injections were made 1 week apart. These results suggest that air-borne manganese can be retrogradely transported along olfactory neurons to the CNS and can reach deeper brain structures under appropriate exposure conditions.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Methodology was lacking, for example type of diet and level of manganese in diet. Study did not address whether the dosing technique could have damaged the nasal lining and hence compromise the results.

Gianutsos G, Seltzer MD, Saymeh R, Wu ML and Michel RG (1985) Brain manganese accumulation following systemic administration of different forms. *Arch Toxicol* **57:**272-275.

Abstract: The content and retention of manganese in the blood and brain of mice exposed to different forms of the metal was compared. Mice received an acute sc injection of manganese as the chloride or oxide (Mn3O4) or as the organic MMT. A single injection markedly elevated brain manganese concentrations within 1 day and elevated levels were maintained for at least 21 days. Repeated injections led to further increases in both brain and blood, although the levels in the brain appeared to persist at consistently high levels for longer periods. The chloride form produced higher brain levels than either of the other two forms. These results appear to suggest that the slowly developing neurotoxicity in response to manganese exposure may be due to a persistent retention of manganese by the brain.

Evaluation: Klimisch Code 3. The study uses subcutaneous injections of 3 different forms of manganese in 3 different dose vehicles due to the limitations of solubility of the manganese forms - as such it is difficult to relate the data obtained to real-life exposure. It is not particularly well documented, the dose levels are quoted as meq, which makes it difficult to compare to other data.

Gibbons RA, Dixon SN, Hallis K, Russell AM, Sansom BF and Symonds HW (1976) Manganese metabolism in cows and goats. *Biochim Biophys Acta* **444:**1-10.

Abstract: When $^{54}MnCl_2$ was incubated with fresh bovine or caprine serum for 20 h and the serum subjected to electrophoresis at pH 9.5, the ^{54}Mn bound to transferrin and alpha2-macroglobulin in proportions which varied with the temperature of incubation and the temperature of electrophoresis. Between 0 and 37 degrees C, the higher the temperature of incubation the larger the proportion bound to transferrin and the lower the proportion bound to alpha2-macroglobulin. The temperature at which electrophoresis was performed had little effect on the proportion of ^{54}Mn bound to transferrin, but increasing temperature reduced the proportion of ^{54}Mn bound to alpha2-macroglobulin. Mn^{2+} did not bind to purified transferrin in vitro in the absence of an oxidising agent. In the presence of permanganate, Mn^{3+} was formed and chelated by transferrin at physiological pH. In fresh serum this oxidation step may be performed by ceruloplasmin or molecular oxygen. Mn^{2+} was bound reversibly to alpha2-macroglobulin but this protein played no part in the oxidation of divalent manganese and had no effect on the protein binding of trivalent manganese. Manganese in the divalent state, either free as Mn^{2+} or bound to alpha2-macroglobulin, is removed from blood plasma very efficiently by the liver. However, the manganic-transferrin complex normally found in circulation is not rapidly removed from plasma. The liver can remove large amounts of excess manganous manganese which it presumably excretes; the small essential fraction of the manganese absorbed is oxidised to the trivalent state and bound to transferrin.

Evaluation: Klimisch Code 2. Adequate methodology description considering the age of the study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC and Lindquist S (2009) Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* **41:**308-315.

Abstract: Parkinson's disease (PD), dementia with Lewy bodies and multiple system atrophy, collectively referred to as synucleinopathies, are associated with a diverse group of genetic and environmental susceptibilities. The best studied of these is PD. alpha-Synuclein (alpha-syn) has a key role in the pathogenesis of both familial and sporadic PD, but evidence linking it to other predisposition factors is limited. Here we report a strong genetic interaction between alpha-syn and the yeast ortholog of the PD-linked gene ATP13A2 (also known as PARK9). Dopaminergic neuron loss caused by alpha-syn overexpression in animal and neuronal PD models is rescued by coexpression of PARK9. Further, knockdown of the ATP13A2 ortholog in Caenorhabditis elegans enhances alpha-syn misfolding. These data provide a direct functional connection between alpha-syn and another PD susceptibility locus. Manganese exposure is an environmental risk factor linked to PD and PD-like syndromes. We discovered that yeast PARK9 helps to protect cells from manganese toxicity, revealing a connection between PD genetics (alpha-syn and PARK9) and an environmental risk factor (PARK9 and manganese). Finally, we show that additional genes from our yeast screen, with diverse functions, are potent modifiers of alpha-syn-induced neuron loss in animals, establishing a diverse, highly conserved interaction network for alpha-syn.

Evaluation: Klimisch Code 5. Mechanistic study.

Greenberg DM and Campbell WW (1940) Studies in Mineral Metabolism with the Aid of Induced Radioactive Isotopes: IV-Manganese. *Proc Natl Acad Sci U S A* **26:**448-452.

Evaluation: Klimisch Code 3. Very old study with outdated techniques.

Greenberg DM, Copp DH and Cuthbertson EM (1943) Studies in Mineral Metabolism with the aid of Artificial Radioactive Isotopes. VII. The Distribution and Excretion, particularly by the way of the Bile, of Iron, Cobalt, and Manganese. *J. Biol. Chem.* **147:**749-756.
Evaluation: Klimisch Code 3. Very old study with outdated techniques.

Greger JL and Snedeker SM (1980) Effect of dietary protein and phosphorus levels on the utilization of zinc, copper and manganese by adult males. *J Nutr* **110:**2243-2253.
Abstract: Zinc, copper and manganese utilization were examined in eight adult males fed a low protein-moderate phosphorus diet (LPrMP), a low protein-high phosphorus diet (LPrHP), a high protein-moderate phosphorus diet (HPrMP) and a high protein-high phosphorus diet (HPrHP) during a 51-day balance study. The low and high protein diets contained 8.1 g and 24.1 g nitrogen daily, respectively. The moderate and high levels of phosphorus were 1,010 and 2,525 mg phosphorus daily. Subjects lost less zinc in the feces when fed the HPrMP diet than when fed the other three diets. The level of dietary protein and phosphorus all statistically affected fecal zinc excretion. Urinary zinc excretion was significantly greater when subjects consumed the high protein diets rather than the low protein diets. Apparent retention of zinc was greatest when subjects were fed the HPrMP diet rather than the other three diets. This effect was statistically attributable to the interaction between dietary protein and phosphorus. Serum zinc levels were significantly elevated when subjects consumed the high protein rather than the low protein diets. Serum zinc levels were correlated to urinary zinc excretion ($r = 0.788$, $P < 0.001$), apparent retention of zinc ($r = 0.385$, $P < 0.05$) and serum copper levels ($r = 0.395$, $P < 0.05$). Apparent absorption and retention of copper were significantly greater when subjects were fed the high protein rather than the low protein diets. They dietary treatments did not affect the urinary excretion of copper, serum copper levels or the apparent absorption and retention of manganese by these subjects.
Evaluation: Klimisch Code 5. Non-pivotal.

Gruden N (1984) The Influence of Iron on Manganese Metabolism in the First Three Weeks of Rat's Life. *Nutrition Reports International* **30:**553-557.
Evaluation: Klimisch Code 3. Very brief methodology.

Guilarte TR, McGlothan JL, Degaonkar M, Chen MK, Barker PB, Syversen T and Schneider JS (2006) Evidence for cortical dysfunction and widespread manganese accumulation in the nonhuman primate brain following chronic manganese exposure: a 1H-MRS and MRI study. *Toxicol Sci* **94:**351-358.
Abstract: Exposure to high levels of manganese (Mn) is known to produce a complex neurological syndrome with psychiatric disturbances, cognitive impairment, and parkinsonian features. However, the neurobiological basis of chronic low-level Mn exposure is not well defined. We now provide evidence that exposure to levels of Mn that results in blood Mn concentrations in the upper range of environmental and occupational exposures and in certain medical conditions produces widespread Mn accumulation in the nonhuman primate brain as visualized by T1-weighted magnetic resonance imaging. Analysis of regional brain Mn distribution using a "pallidal index equivalent" indicates that this approach is not sensitive to changing levels of brain Mn measured in postmortem tissue. Evaluation of longitudinal 1H-magnetic resonance spectroscopy data revealed a significant decrease ($p = 0.028$) in the N-acetylaspartate (NAA)/creatine (Cr) ratio in the parietal cortex and a near significant decrease ($p = 0.055$) in frontal white matter (WM) at the end of the Mn exposure period relative to baseline. Choline/Cr or myo-Inositol/Cr ratios did not change at any time during Mn exposure. This indicates that the changes in the NAA/Cr ratio in the parietal cortex are not due to changes in Cr but in NAA levels. In summary, these findings suggest that during chronic Mn exposure a significant amount of the metal accumulates not only in the basal ganglia but also in WM and in cortical structures where it is likely to produce toxic effects. This is supported by a significantly decreased, in the parietal cortex, NAA/Cr ratio suggestive of ongoing neuronal degeneration or dysfunction.
Evaluation: Klimisch Code 2. Reasonably well documented methods and results. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Gupta SK, Murthy RC and Chandra SV (1980) Neuromelanin in manganese-exposed primates. *Toxicol Lett* **6:**17-20.
Abstract: Monkeys developed muscular weakness and rigidity of the lower limbs after 18 months exposure to manganese. These are neurological signs typical of chronic manganese intoxication. Marked neuronal degeneration with depigmentation was noticed in the region of substantia nigra. Significance of depigmentation in relation to the depletion in the contents of brain dopamine in chronic manganese intoxication has been discussed.
Evaluation: Klimisch Code 5. Non-pivotal. No actual quantitation of manganese recorded.

Gwiazda R, Lucchini R and Smith D (2007) Adequacy and consistency of animal studies to evaluate the neurotoxicity of chronic low-level manganese exposure in humans. *J Toxicol Environ Health A* **70:**594-605.

Abstract: The adequacy of existing animal studies to understand the effects of chronic low-level manganese exposures in humans is unclear. Here, a collection of subchronic to chronic rodent and nonhuman primate studies was evaluated to determine whether there is a consistent dose-response relationship among studies, whether there is a progression of effects with increasing dose, and whether these studies are adequate for evaluating the neurotoxicity of chronic low-level manganese exposures in humans. Neurochemical and behavioral effects were compared along the axis of estimated internal cumulative manganese dose, independent of the route of exposure. In rodents, motor effects emerged at cumulative doses below those where occupationally exposed humans start to show motor deficits. The main neurochemical effects in rodents were an increase in striatal gamma-aminobutyric acid (GABA) concentration throughout the internal cumulative dose range of 18 to 5300 mg Mn/kg but a variable effect on striatal dopamine concentration emerging at internal cumulative doses above approximately 200 mg Mn/kg. Monkey studies showed motor deficits and effects on the globus pallidus at relatively low doses and consistent harmful effects on both the globus pallidus and the caudate and putamen at higher doses (> 260 mg Mn/kg). Internal cumulative manganese doses of animal studies extend more than two orders of magnitude (< 1 to 5300 mg Mn/kg) above the doses at which occupationally exposed humans show neurological dysfunction (10-15 mg Mn/kg). Since the animal data indicate that manganese neurotoxicity may be different at low compared to elevated exposures, most existing animal model studies might be of limited relevance for the risk assessment of chronic low-level manganese exposure to humans.

Evaluation: Klimisch Code 5. A review of existing studies.

Hanlon DP, Gale TF and Ferm VH (1975) Permeability of the syrian hamster placenta to manganous ions during early embryogenesis. *J Reprod Fertil* **44:**109-112.

Evaluation: Klimisch Code 3. Brief methodology. Publication describes distribution of Mn2+.

Hatch GE (1992) *Comparative Biochemistry of Airway Lining Fluid*. Boca Raton, Florida.

Evaluation: Klimisch Code 5. (Reference for simulated lung fluid).

Heilig E, Molina R, Donaghey T, Brain JD and Wessling-Resnick M (2005) Pharmacokinetics of pulmonary manganese absorption: evidence for increased susceptibility to manganese loading in iron-deficient rats. *Am J Physiol Lung Cell Mol Physiol* **288:**L887-893.

Abstract: High levels of airborne manganese can be neurotoxic, yet little is known about absorption of this metal via the lungs. Intestinal manganese uptake is upregulated by iron deficiency and is thought to be mediated by divalent metal transporter 1 (DMT1), an iron-regulated factor known to play a role in dietary iron absorption. To better characterize metal absorption from the lungs to the blood and test whether iron deficiency may modify this process, the pharmacokinetics of pulmonary manganese and iron absorption by control and iron-deficient rats were compared. Levels of DMT1 expression in the lungs were determined to explore potential changes induced by iron deficiency that might alter metal absorption. The pharmacokinetic curves for intratracheally instilled 54Mn and 59Fe were significantly different, suggesting that pulmonary uptake of the two metals involves different mechanisms. Intratracheally instilled iron-deficient rats had significantly higher blood 54Mn levels, whereas blood 59Fe levels were significantly reduced compared with controls. The same trend was observed when radioisotopes were delivered by intravenous injection, indicating that iron-deficient rats have altered blood clearance of manganese. In situ analysis revealed the presence of DMT1 transcripts in airway epithelium; however, mRNA levels did not change in iron deficiency. Although lung DMT1 levels and metal absorption did not appear to be influenced by iron deficiency, the differences in blood clearance of instilled manganese identified by this study support the idea that iron status can influence the potential toxicity of this metal.

Evaluation: Klimisch Code 2. Generally well documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Recovery data quoted, which although low for one group didn't appear to affect the overall results.

Heilig EA, Thompson KJ, Molina RM, Ivanov AR, Brain JD and Wessling-Resnick M (2006) Manganese and iron transport across pulmonary epithelium. *Am J Physiol Lung Cell Mol Physiol* **290:**L1247-1259.

Abstract: Pathways mediating pulmonary metal uptake remain unknown. Because absorption of iron and manganese could involve similar mechanisms, transferrin (Tf) and transferrin receptor (TfR) expression in rat lungs was examined. Tf mRNA was detected in bronchial epithelium, type II alveolar cells, macrophages, and bronchus-associated lymphoid tissue (BALT). Tf protein levels in lung and

bronchoalveolar lavage fluid did not change in iron deficiency despite increased plasma levels, suggesting that lung Tf concentrations are regulated by local synthesis in a manner independent of body iron status. Iron oxide exposure upregulated Tf mRNA in bronchial and alveolar epithelium, macrophages, and BALT, but protein was not significantly increased. In contrast, TfR mRNA and protein were both upregulated by iron deficiency. To examine potential interactions with lung Tf, rats were intratracheally instilled with (54)Mn or (59)Fe. Unlike (59)Fe, interactions between (54)Mn and Tf in lung fluid were not detected. Absorption of intratracheally instilled (54)Mn from the lungs to the blood was unimpaired in Belgrade rats homozygous for the functionally defective G185R allele of divalent metal transporter-1, indicating that this transporter is also not involved in pulmonary manganese absorption. Pharmacological studies of (54)Mn uptake by A549 cells suggest that metal uptake by type II alveolar epithelial cells is associated with activities of both L-type Ca(2+) channels and TRPM7, a member of the transient receptor potential melastatin subfamily. These results demonstrate that iron and manganese are absorbed by the pulmonary epithelium through different pathways and reveal the potential role for nonselective calcium channels in lung metal clearance.

Evaluation: Klimisch Code 5. Non-pivotal.

Henriksson J, Tallkvist J and Tjalve H (1999) Transport of manganese via the olfactory pathway in rats: dosage dependency of the uptake and subcellular distribution of the metal in the olfactory epithelium and the brain. *Toxicol Appl Pharmacol* **156:**119-128.

Abstract: The dosage dependency of the uptake of Mn from the olfactory epithelium via olfactory neurons into the brain was studied after intranasal administration of the metal in rats. The results indicate that the Mn transport is saturable both regarding the uptake into the olfactory epithelium and the transfer to the olfactory bulb. Further, our data indicate that Mn moves relatively freely from the olfactory bulb to the olfactory cortex at an amount dependent on the level of influx into the bulb. The transport to the rest of the brain was related to the amounts in the olfactory bulb and the olfactory cortex, but the relative proportion reaching this area increased with increasing doses. Cell fractionations showed that the Mn was present both in the cytosol and in association with various cell constituents. Gel filtrations of the cytosol on a Superdex 30 column showed that about 20% of the Mn in the brain and about 3% in the olfactory epithelium was eluted together with high-molecular-weight materials (MW > 10,000), whereas the rest was eluted in the total volume and may represent unbound metal. It is likely that the metal has been loosely associated with protein(s) or other constituents at the application to the column, but that this association is too loose to be retained during the passage through the column. Our results show that the olfactory neurons provide a pathway with a considerable capacity to transport Mn into the brain. We propose that the neurotoxicity of inhaled Mn is related to an uptake via this route.

Evaluation: Klimisch Code 2. Well documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The authors concluded that their results showed that the olfactory neurons provided a pathway with a considerable capacity to transport manganese into the brain and that the neurotoxicity of inhaled manganese is related to an uptake via this route. This statement failed to take into account the difference in the relative size of the olfactory bulb in rats and humans and presumably assumed that the same pathway with the same capacity was present in humans, although unfortunately this was not discussed. Similarly it fails to address the suitability of the rat as a model for manganese neurotoxicity in humans.

Hernandez EH, Discalzi G, Dassi P, Jarre L and Pira E (2003) Manganese Intoxication: The Cause of an Inexplicable Epileptic Syndrome in a 3 Year Old Child. *Neurotoxicology* **24:**633-639.

Abstract: Excess manganese (Mn) can cause several neurotoxic effects, however only a few studies have reported epileptic syndromes related to manganese intoxication. We describe an epileptic syndrome due to manganese intoxication in a 3 year old male child. His blood manganese was elevated, but no other abnormal values or toxic substances were found in blood or urine. The electroencephalogram (EEG) showed a picture of progressive encephalopathy, while brain magnetic resonance was normal. The patient's conditions rapidly worsened to epileptic status despite the use of antiepileptic drugs. Chelating treatment with $CaNa_2EDTA$ was initiated to remove excess manganese and promptly succeeded in reverting epileptic symptoms. Concurrently, manganese blood levels and electroencephalogram progressively normalized. Thereafter it has been possible to discontinue antiepileptic treatment, and the patient remains in excellent conditions without any treatment.

Evaluation: Klimisch Code 4. Not very detailed methodology as it is a case report.

Hietanen E, Kilpio J and Savolainen H (1981) Neurochemical and biotransformational enzyme responses to manganese exposure in rats. *Arch Environ Contam Toxicol* **10:**339-345.

Abstract: Adult male rats were exposed to 0.5% manganese as MnCl2 in their drinking water for 1, 4, or 6 weeks. Manganese content was measured in brain, liver, kidney, and intestine. Peak manganese

concentrations were found in all tissues after one week exposure. Hepatic aryl hydrocarbon hydroxylase, ethoxycoumarin O-deethylase, and epoxide hydrase activities increased after one week manganese exposure, while intestinal and renal activities decreased. The activities returned nearly to the control level at six weeks of exposure. The UDPglucuronosyltransferase activity increased in the liver, kidney, and intestinal mucosa after one week exposure, decreasing thereafter nearly to the control level. In the brain, most significant changes were found after six weeks exposure when the succinate dehydrogenase activity decreased. The results suggest an adaptation to manganese absorption during continuous exposure. The biotransformation enzymes respond first to manganese exposure followed by neurochemical changes in the central nervous system.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Relatively brief methodology as some detail is in cross-references. However, publication does seem well designed and discussed.

Hobbesland A, Kjuus H and Thelle DS (1999) Study of cancer incidence among 6363 male workers in four Norwegian ferromanganese and silicomanganese producing plants. *Occup Environ Med* **56:**618-624.

Abstract: OBJECTIVES: Little has been known about the risk of cancer associated with occupational exposure to manganese. The objective of this study was therefore to examine the associations between duration of specific work and cancer incidence among employees in four Norwegian ferromanganese and silicomanganese producing plants. METHODS: Among men first employed in 1933-91 and with at least 6 months in these plants, the incident cases of cancer during 1953-91 were obtained from The Cancer Registry of Norway. The numbers of various cancers were compared with expected figures calculated from age and calendar time specific rates for Norwegian men during the same period. Internal comparisons of rates were performed with Poisson regression analysis. The final cohort comprised 6363 men. RESULTS: A total of 607 cases of cancer were observed against 596 cases expected (standardised incidence ratio (SIR) 1.02). Internal comparisons of rates showed a positive trend between the rate of all cancers and duration of furnace work. A slightly weaker trend was also found for duration of blue collar non-furnace work when lags of 25 or 30 years were applied in the analyses. However, several results indicated that the incidence of all cancers among the non-furnace workers decreased during the period of active employment. CONCLUSIONS: Furnace and non-furnace workers may have exposures that increase the incidence of several cancers. The low incidence of cancer among non-furnace workers during the period of ongoing exposure cannot be explained. As this study cannot identify any causal factors, the role of exposure to manganese remains unclear.

Evaluation: Klimisch Code 5. Non-pivotal.

Holzgraefe M, Poser W, Kijewski H and Beuche W (1986) Chronic enteral poisoning caused by potassium permanganate: a case report. *J Toxicol Clin Toxicol* **24:**235-244.

Abstract: To our knowledge, this is the first case report of a multiple, low dosage ingestion of manganese. A 66-year-old male patient is presented, who ingested 125 ml of a 8% solution of potassium permanganate (10 g) within 4 weeks. As early as 2 weeks after the beginning of poisoning, psychological alterations were noted. Neurological examination revealed disturbances of many subsystems of the CNS. Visually evoked potentials showed prolongation of the P2-latency, not reported in earlier publications. Levels for manganese were elevated in peripheral blood as well as in hair samples. Treatment with calcium trisodium pentetate decreased serum levels and increased urine excretion of manganese. Nine months after poisoning, the first signs of progressive Parkinson disease became evident. The time-course of neurological symptoms seems to depend on a critical dose of manganese.

Evaluation: Klimisch Code 5. Not evaluated.

Hudson NJ, Evans AT, Yeung CK and Hewitt PJ (2001) Effect of process parameters upon the dopamine and lipid peroxidation activity of selected MIG welding fumes as a marker of potential neurotoxicity. *Ann Occup Hyg* **45:**187-192.

Abstract: There is growing concern over the neurotoxic effects of chronic occupational exposure to metal fume produced by welding. Elevated iron and manganese levels in the brain have been linked to an increase in lipid peroxidation, dopamine depletion and predisposition to the development of a Parkinson's type condition in advanced cases. Chemical and toxicological analysis of selected welding fumes, generated by model processes, were used in order to evaluate their potential to release solutes that promote oxidation of dopamine and peroxidation of brain lipids in cell free assays. This study compared the effect of shield gas, electrode type and voltage/currect upon the dopamine and brain lipid peroxidation potential of selected welding fume, obtained from metal inert gas (MIG) welding systems. Overall, fume extracts were found to enhance dopamine oxidation and inhibit lipid peroxidation. Significant differences were also found in the oxidising potential of fume generated under differing process

conditions; it may therefore be possible to determine the potential neurotoxicity of fumes using this system.
Evaluation: Klimisch Code 5. Mechanism of neurotoxicity.

Hughes BP and Cotzias GC (1960) Manganese Metabolism and Adrenocortical Activity. *Federation Proceedings* **19:**249.
Evaluation: Klimisch Code 5. Non-pivotal

Hughes ER and Cotzias GC (1961) Adrenocorticosteroid hormones and manganese metabolism. *Am J Physiol* **201:**1061-1064.
Evaluation: Klimisch Code 5. Non-pivotal.

Hughes ER, Miller ST and Cotzias GC (1966) Tissue concentrations of manganese and adrenal function. *Am J Physiol* **211:**207-210.
Evaluation: Klimisch Code 5. Non-pivotal.

Hurley LS, Keen CL and Baly DL (1984) Manganese deficiency and toxicity: effects on carbohydrate metabolism in the rat. *Neurotoxicology* **5:**97-104.
Abstract: Although manganese deficiency and manganese toxicity both have pathological consequences, the underlying biochemical lesions have not been well defined. Manganese is involved in carbohydrate metabolism; either deficiency or excess results in abnormal carbohydrate metabolism. Clinical studies have shown that patients with chronic manganism have hypoglycemia following a glucose load. One report has been published of a diabetic patient who responded to oral manganese with a consistent drop in blood glucose. Rats fed a manganese deficient diet respond to an oral glucose load with a diabetic type of glucose tolerance curve. Insulin release from the pancreas of manganese deficient animals in response to a glucose stimulus is lower than controls. Reduced insulin output occurs in both the first phase (release of stored hormone) and second phase (release of stored and newly synthesized hormone) of insulin output. Thus dietary manganese deficiency can result in abnormal insulin production producing impaired carbohydrate metabolism. Manganese toxicity also affects carbohydrate metabolism. Rats given intraperitoneal injections of high levels of manganese show a rapid hyperglycemia and hypoinsulinemia, followed by a reactionary hypoglycemia. The changes in blood glucose and blood insulin levels correlated with changes in liver and pancreatic manganese concentrations, suggesting that some of the effects of manganese on carbohydrate metabolism may be due to a direct effect on insulin release and gluconeogenesis.
Evaluation: Klimisch Code 5. Non-pivotal.

Hussain S, Lipe GW, Slikker W and Ali SF (1997) The Effects of Chronic Exposure of Manganese on Antioxidant Enzymes in Different Regions of Rat Brain. *Neuroscience Research Communications* **21:**135-144.
Evaluation: Klimisch Code 5. Non-pivotal.

Inoue T, Kimura A, Aoki K, Tohma M and Kato H (1997) Developmental pattern of 3-oxo-delta 4 bile acids in neonatal bile acid metabolism. *Arch Dis Child Fetal Neonatal Ed* **77:**F52-56.
Abstract: AIMS: To investigate whether a fetal pathway of bile acid synthesis persists in neonates and infants. METHODS: 3-oxo-delta 4 bile acids were determined qualitatively and quantitatively in the urine, meconium, and faeces of healthy neonates and infants, using gas chromatography-mass spectrometry. RESULTS: The mean percentage of 3-oxo-delta 4 bile acids in total bile acids in urine at birth was significantly higher than that at 3 or 7 days, and at 1 or 3 months of age. The concentration of this component in meconium was significantly higher than that in faeces at 7 days and at 1 or 3 months of age. CONCLUSIONS: The presence of large amounts of urinary 3-oxo-delta 4 bile acids may indicate immaturity in the activity of hepatic 3-oxo-delta 4-steroid 5 beta-reductase in the first week of postnatal life. Large amounts of this component in meconium may be due to the ingestion of amniotic fluid by the fetus during pregnancy.
Evaluation: Klimisch Code 5 - Cross reference for human neonatal bile

Ji F, Luo XG, Lu L, Liu B and Yu SX (2006) Effect of manganese source on manganese absorption by the intestine of broilers. *Poult Sci* **85:**1947-1952.
Abstract: Two experiments were conducted to investigate the effect of Mn source on Mn absorption by the intestine of broilers. In Experiment 1, the effect of Mn source, including MnSO(4), 2 Mn-amino acid chelates (Mn-Gly and Mn-Met) synthesized in our laboratory, 3 Mn-amino acid complexes with different complex strengths (Mn-Met E, Mn-AA A, and Mn-AA B), and 2 mixtures of MnSO(4) with Gly or Met, on Mn absorption was assessed with ligated loops of different small intestinal segments of

broilers. In Experiment 2, the absorption of Mn from MnSO(4), Mn-AA A, and Mn-AA B was compared with intact broilers fed ad libitum. The criterion used for comparison was the Mn content of hepatic portal vein plasma. The absorption of Mn was higher ($P < 0.0002$) by ligated ileal loops than by duodenal and jejunal ones. Met supplementation increased ($P < 0.03$) the absorption of Mn as MnSO(4). The absorption of Mn as Mn-AA A and Mn-AA B with moderate and strong complex strengths, respectively, were higher ($P < 0.05$) than those of Mn as MnSO(4) and Mn-Met E with weak complex strength. On d 7 and 9 of Experiment 2, the Mn content of portal vein plasma was higher ($P < 0.03$) for Mn-AA B with strong complex strength than for MnSO(4). On d 9, Mn content in plasma was higher ($P < 0.01$) for Mn-AA B with strong complex strength than for Mn-AA A with a moderate one. The results from this study confirm that the ileum was the main site of Mn absorption for broilers, and Met was more effective in facilitating Mn absorption than Gly as a ligand. Organic Mn was more efficiently absorbed than inorganic Mn (MnSO(4)); the absorption of organic Mn with moderate and strong complex strengths was greater than that of the organic Mn, which was weak, and the absorption of organic Mn with strong complex strength was greater than that of the organic Mn with a moderate strength.

Evaluation: Klimisch Code 2. Well documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Johnson PE, Lykken GI and Korynta ED (1991) Absorption and biological half-life in humans of intrinsic and extrinsic 54Mn tracers from foods of plant origin. *J Nutr* **121:**711-717.

Abstract: Absorption and biological half-life of 54Mn were measured in adult men and women fed foods labeled intrinsically or extrinsically with 54Mn. Each subject consumed a series of three test meals consisting of a food labeled intrinsically, a food labeled extrinsically or MnCl2 (control) served in random order. The foods tested were lettuce, spinach, wheat and sunflower seeds. Lettuce meals and their controls contained 9.65 mumol Mn; other meals contained 22.50 mumol Mn. In addition to the test food or MnCl2, each meal consisted of vegetable oil (5 g), salt (NaCl, 0.15 g) and crackers (10 g), which provided 0.55 mumol Mn. There were no differences in percentage of Mn absorption or biological half-life of 54Mn for any of the intrinsically/extrinsically labeled food pairs. Absorption of 54Mn from MnCl2 (8.90%) was greater than from lettuce (5.20%), spinach (3.81%), wheat (2.16%) or sunflower seeds (1.71%), but the biological half-life did not vary with the source of Mn. Absorption of 54Mn from lettuce was significantly (P less than 0.05) greater than from wheat or sunflower seeds. Although the Mn dose in the test meal was less for lettuce than for the other foods, there was no difference in Mn absorption from MnCl2 between the subjects fed lettuce and subjects fed other foods. There was no correlation of either 54Mn absorption or biological half-life with whole blood or plasma Mn.

Evaluation: Klimisch Code 2 - very well documented. Study confirmed extrinsic use of labelled manganese in foods from plant origin. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Kato M (1963) Distribution and Excretion of Radiomanganese Administered to the Mouse. *Q J Exp Physiol Cogn Med Sci* **48:**355-369.

Evaluation: Klimisch Code 4. A very old study. However, the level of documentation is good considering its age.

Kaur G, Hasan SK and Srivastava RC (1980) The distribution of manganese-54 in fetal, young and adult rats. *Toxicol Lett* **5:**423-426.

Abstract: The distribution of 54Mn in various organs of pregnant rats and of their 19-day-old fetuses and in non-pregnant female rats of various ages has been studied 18 h after an i.v. injection of 54MnCl2. The results indicate that early neonates are more susceptible to manganese (Mn) than the growing rats. The localization of 54Mn in liver and brain of the embryo was highly significant.

Evaluation: Klimisch Code 4. Very brief methodology.

Kawamura R, Ikuta H, Fukuzumi S, Yamada M and Tsubaki S (1941) Intoxication by Manganese in Well Water. *Kitasato Archives of Expeimental Medicine* **18:**145-169.

Evaluation: Klimisch Code 4. Descriptions and investigations seem relatively adequate considering the age of the publication.

Keen CL, Baly DL and Lonnerdal B (1984) Metabolic Effects of High Doses of Manganese in Rats. *Biological Trace Element Research* **6:**309-315.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Methodology lacking in detail. For example, no control group is described and this study did not use radiolabel. From the figures of concentration vs time it is assumed that there was 0 hour tissue concentrations which acted as controls, although this is not stated. However since there are 3 dose levels the effects of the higher doses compared to the low dose can clearly be seen.

Keen CL, Bell JG and Lonnerdal B (1986) The effect of age on manganese uptake and retention from milk and infant formulas in rats. *J Nutr* **116:**395-402.

Abstract: Manganese nutrition of the neonate is poorly understood, due in part to a paucity of information on the amount and availability of manganese in infant foods. We have developed a suckling pup model to assess the uptake of manganese from fluid diets by using extrinsic labeling. Human milk, cow milk and infant formulas were fed by intubation to fasted rat pups and adults. Rats were killed after varying time periods, and tissues were removed and counted. A period of 6 h was found to be adequate to allow for stomach emptying while limiting tissue redistribution; 24 h was found to reflect pup manganese retention. From human milk, manganese retention was highest (greater than or equal to 80%) in pups less than or equal to 15 days of age; in older pups average retention decreased to 40%. Using d 14 pups to assess relative Mn uptake from diets, wholebody Mn uptake was highest from cow milk (approximately 89%); uptake from human and cow milk formula was similar (approximately 80%) whereas it was lower from soy formula (approximately 60%). These findings suggest that bioavailability of Mn from infant diets is very high during the suckling period. Since most formulas contain considerably more manganese than is found in human milk, Mn deficiency may be less of a concern than possible toxicity from formulas.

Evaluation: Klimisch Code 4. Methodology lacking detail.

Keen CL and Zidenberg-Cherr S (1994) *Manganese Toxicity in Humans and Experimental Animals, in "Manganese in Health and Disease".* CRC Press, Boca Raton.

Evaluation: Klimisch Code 5. Non-pivotal.

Kimbell JS (2006) Nasal dosimetry of inhaled gases and particles: where do inhaled agents go in the nose? *Toxicol Pathol* **34:**270-273.

Abstract: The anatomical structure of the nasal passages differs significantly among species, affecting airflow and the transport of inhaled gases and particles throughout the respiratory tract. Since direct measurement of local nasal dose is often difficult, 3-dimensional, anatomically accurate, computational models of the rat, monkey, and human nasal passages were developed to estimate regional transport and dosimetry of inhaled material. The computational models predicted that during resting breathing, a larger portion of inspired air passed through olfactory-lined regions in the rat than in the monkey or human. The models also predicted that maximum wall mass flux (mass per surface area per time) of inhaled formaldehyde in the nonsquamous epithelium was highest in monkeys (anterior middle turbinate) and similar in rats and humans (dorsal medial meatus in the rat and mid-septum in the human, near the squamous/nonsquamous epithelial boundary in both species). For particles that are 5 microm in aerodynamic diameter, preliminary simulations at minute volume flow rates predicted nasal deposition efficiencies of 92%, 11% and 25% in the rat, monkey, and human, respectively, with more vestibular deposition in the rat than in the monkey or human. Estimates such as these can be used to test hypotheses about mechanisms of toxicity and supply species-specific information for risk assessment, thus reducing uncertainty in extrapolating animal data to humans.

Evaluation: Klimisch Code 5 - Computer Simulation.

Kimura M, Yagi N and Itokawa Y (1978) Effect of subacute manganese feeding on serotonin metabolism in the rat. *J Environ Pathol Toxicol* **2:**455-461.

Abstract: To clarify the effect of subacute manganese feeding on serotonin and mineral metabolism, Wistar rats were separated into two groups and fed two different diets, one a normal diet and the another a manganese-supplemented diet. After three weeks on these dietary regimens, the rats on the manganese-supplemented diet manifested the following abnormalities: blood pressure was decreased; brain serotonin was decreased; L-aromatic amino acid decarboxylase activity in brain was decreased. Manganese levels in heart, lung, and kidney increased, whereas sodium, potassium, magnesium and calcium levels in the brainstem decreased.

Evaluation: Klimisch Code 5. Non-pivotal.

Kitagawa Y and Wada O (1990) [Pharmacokinetics of trace elements by noncompartmental analysis in rats (Part 1): Significance of the pharmacokinetic parameters]. *Nippon Eiseigaku Zasshi* **44:**1097-1106.

Abstract: Six-week-old male Wistar rats were administrated the radioisotopes of five trace elements (iron, zinc, copper, manganese and iodine) intravenously in order to elucidate the significance of their pharmacokinetics by noncompartmental analysis. The mean residence time (MRT) and variance of residence time (VRT) increased in the order Mn, Cu, I, Fe and Zn. Neither MRT nor VRT indicated any statistical significance between I and Fe. These results suggested that Mn and Cu were voided rapidly from the plasma, whereas Zn persisted in the plasma for the longest time among these elements. Though I and Fe showed quite different plasma disappearance curves, both were considered to diminish at almost the same speed. The volume of distribution at steady state (Vdss) increased in the order Cu (32 ml), Fe (62 ml), I (149 ml), Mn (185 ml) and Zn (1012 ml). The distribution coefficient (Kd) of these elements increased in the same order as Vdss did. For Cu and Fe, Vdss was intermediate between the plasma volume and total body fluid volume of the rat, while, for the others, Vdss was greater than the total body fluid volume. In particular, Vdss of Zn was the greatest among these elements. Hence, the present study suggested that the plasma concentrations of Cu and Fe may reflect their body contents fairy well, though those of I, Fe and Zn can hardly do so. Vdss and Kd are, therefore, considered to be useful as supplementary diagnostic indices to understand the plasma concentrations of trace elements. Systemic clearance (CLs) increased in the order Fe (0.02 ml/min), Zn (0.07 ml/min), I (0.21 ml/min), Cu (0.37 ml/min) and Mn (4.61 ml/min). The CLs of Mn was similar to the hepatic plasma flow rate of the rat in size, indicating that the Cls of Mn may be one of the greatest among trace elements. It appeared, therefore, that when administered intravenously, Mn may be transferred from the plasma to the tissues more easily than the other elements.

Evaluation: Klimisch Code 4. Not possible to evaluate as publication is in Japanese.

Klaassen CD (1974) Biliary excretion of manganese in rats, rabbits, and dogs. *Toxicol Appl Pharmacol* **29:**458-468.

Evaluation: Klimisch Code 4. Detailed methodology absent in publication, although it is referenced.

Klimisch HJ, Andreae M and Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol* **25:**1-5.

Evaluation: Klimisch Code 5. Reference publication for Klimisch Codes.

Kobayashi H, Uchida M, Sato I, Suzuki T, Hossain MM and Suzuki K (2003) Neurotoxicity and Brain Regional Distribution of Manganese in Mice. *Journal of Toxicology* **22:**679-689.

Evaluation: Klimisch Code 4. Relatively brief details of methodology.

Kobayashi K, Kuroda J, Shibata N, Hasegawa T, Seko Y, Satoh M, Tohyama C, Takano H, Imura N, Sakabe K, Fujishiro H and Himeno S (2007) Induction of metallothionein by manganese is completely dependent on interleukin-6 production. *J Pharmacol Exp Ther* **320:**721-727.

Abstract: Metallothionein (MT) is a cysteine-rich protein that binds to and is inducible by heavy metals such as cadmium and zinc. However, the precise mechanism of MT induction by other metals remains unclear. In the present study, we investigated the mechanism of MT induction by manganese, focusing on the involvement of cytokine production. Administration of MnCl(2) to mice resulted in the induction of MT dose-dependently in the liver with little accumulation of manganese. Speciation analysis of metals in the liver cytosol showed that the major metal bound to the induced MT was zinc. Administration of MnCl(2) caused an increase in mRNA levels of interleukin-6 (IL-6) in the liver as well as an increase in serum levels of IL-6 but not those of other inflammatory cytokines. Subsequently, serum levels of serum amyloid A (SAA), an acute-phase protein induced by IL-6, increased with a peak at 24 h. However, no increase in serum alanine aminotransferase activity was observed, suggesting that manganese enhanced the production of IL-6 and SAA without causing liver injury. In response to IL-6, the expression of a zinc transporter, ZIP14, was enhanced in the liver, possibly contributing to the synthesis of hepatic zinc-MT. In IL-6-null mice, the induction of hepatic MT by treatment with MnCl(2) was completely suppressed to the control level. These results suggest that manganese is a unique metal that induces the synthesis of hepatic MT completely depending on the production of IL-6 without accompanying liver injury.

Evaluation: Klimisch Code 5. Non-pivotal.

Kojima S, Hirai M, Kiyozumi M, Sasawa Y, Nakagawa M and Shin-o T (1983) Studies on poisonous metals. X. Metabolic fate of manganese after oral administration of excessive manganese chloride in rats. *Chem Pharm Bull (Tokyo)* **31:**2459-2465.

Evaluation: Klimisch Code 3. Very brief description of methods. Radio-labelled manganese was not used, yet a lot of comparisons were made based upon percentage of dose, no details on how this was calculated, or the overall recoveries using this methodology. Data lacks comprehensive statistical analysis (statistical significance) to confirm observations and conclusions. The study lacked any data on whether the

percentage absorption was affected by the increases in dose, which could have an impact on the distribution and excretion data.

Komaki H, Maisawa S, Sugai K, Kobayashi Y and Hashimoto T (1999) Tremor and seizures associated with chronic manganese intoxication. *Brain Dev* **21:**122-124.

Abstract: Tremor and seizures developed in a 2-year-old girl receiving total parenteral nutrition. T1-weighted images on MRI revealed areas of hyperintensity in the basal ganglia, brainstem and cerebellum. Blood manganese was elevated. The symptoms and MRI abnormalities disappeared after withdrawal of manganese administration. The recommendation of daily parenteral manganese intake was discussed.

Evaluation: Klimisch Code 4. A case report.

Komura J and Sakamoto M (1993) Subcellular and gel chromatographic distribution of manganese in the mouse brain: relation to the chemical form of chronically-ingested manganese. *Toxicol Lett* **66:**287-294.

Abstract: The subcellular distribution of manganese and the binding characteristics of manganese to protein in the mouse brain were examined on G-75 Sephadex gel columns. Four manganese compounds were included at 2 g/kg in each food eaten by ddY mice for 12 months. The cerebral cortex manganese concentrations in the virtually insoluble manganese compounds were significantly higher than those in the control group. The brain striatal subcellular distribution and gel chromatographic profiles of manganese were similar among the divalent manganese compounds. On the contrary, the behaviour of MnO2 was little different from the divalent manganese compounds. There was more manganese associated with fast-migrating ligands in the striatal cytosol of the manganese-exposed group than in the control groups.

Evaluation: Klimisch Code 3. Limited methodology description. Although the animals were exposed to different sources of manganese in their diet for 12 months, there was no calculation of an estimated dosage received (mg Mn/kg/day) or results of manganese in blood to be able to compare uptake of manganese. Since the diets were comparing soluble and insoluble forms of manganese, differences in absorption should have been considered. The study design meant that for a potential key finding, the subcellular distribution of manganese in the corpus striatum, there was only n=2 and thus statistical significance could not be tested.

Kondakis XG, Makris N, Leotsinidis M, Prinou M and Papapetropoulos T (1989) Possible health effects of high manganese concentration in drinking water. *Arch Environ Health* **44:**175-178.

Abstract: Three areas in the same region of northwest Peloponnesos, Greece, that had varying concentrations of manganese (Mn) in drinking water were selected for study. The Mn concentrations in areas A, B, and C were 3.6-14.6 micrograms/l, 81.6-252.6 micrograms/l, and 1 800-2 300 micrograms/l, respectively. A random sample (62 in area A, 49 in area B, and 77 in area C) of males and females who were at least 50 y of age were submitted to a thorough neurological examination and their whole-blood Mn and hair Mn concentrations were determined. Although all areas were similar with respect to social and dietary characteristics, significant differences were observed for prevalence of chronic manganese poisoning (CMnP) symptoms and hair Mn concentration. The means (both sexes) of neurological scores were 2.7, 3.9, and 5.2, respectively, for areas A, B, and C (Kruskal-Wallis, chi 2 = 6.44, 2 df, p less than .05 for males; chi 2 = 7.8, 2 df, p less than .05 for females). Hair Mn concentrations were also significantly different, the means for which were 3.51, 4.49, and 10.99 micrograms/g dry weight, respectively (both sexes [p less than .001 for each sex separately]). These results indicate that progressive increases of Mn concentration in drinking water are associated with progressively higher prevalences of neurological signs of CMnP and Mn concentration in hair of older persons.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Adequate description of methodology, however no detail on actual consumption levels of manganese from combined water and food. As such, it is impossible to actually estimate total oral consumption of manganese and thus correlate with study findings. However, results did show a correlation between hair manganese concentrations and levels of manganese in drinking water.

Koshida Y, Kato M and Hara T (1963) Autoradiographic Observations of Manganese in Adult and Embryo Mice. *Q J Exp Physiol Cogn Med Sci* **48:**370-378.

Evaluation: Klimisch Code 3. Very old study. Out-dated technique (52Mn).

Koshida Y, Kato M and Hara T (1965) Distribution of Radiomanganese in Embryonic Tissues of the Mouse. *Annotationes Zoologicae Japonenses* **38:**1-7.

Evaluation: Klimisch Code 3. Very old study with limited detail. Outdated techniques (52Mn).

Kostial K, Blanusa M, Maljkovic T, Kello D, Rabar I and Stara JF (1989) Effect of a metal mixture in diet on the toxicokinetics and toxicity of cadmium, mercury and manganese in rats. *Toxicol Ind Health* **5:**685-698.

Abstract: The purpose of this work was to determine whether a metal mixture added to diet influences the toxicokinetics and toxicity of some elements. The metal mixture (MM) used in these experiments was ash (slag) from a coal gasification plant. The effect of this mixture (5 percent in diet) on the toxicokinetic and on the acute or subchronic toxicity of Cd, Hg, Mn was determined in rats of different ages. Rats were exposed for five weeks in toxicokinetic and six weeks in acute toxicity experiments. Sucklings were exposed through their mothers, which received the MM in diet over the pregnancy and lactation period. In toxicokinetic studies, half of the animals additionally received Cd, Hg or Mn (100, 50 or 2000 ppm, respectively) in drinking water. In subchronic experiments, six-week-old albino rats of both sexes were given MM in the diet and Cd, Hg or Mn in drinking water for 16 weeks. In toxicokinetic studies, 115mCd, 203Hg or 54Mn were administered orally or intraperitoneally to mothers and pups. Exposure to MM had no effect on the absorption, retention and organ distribution of these elements determined six days after radioisotope administration. In acute toxicity studies, exposure to MM in diet had no effect on LD50 values obtained eight days after oral administration of Cd, Hg or Mn to rats of different ages. In subchronic experiments, the effect of individual metals (Cd, Hg or Mn) was similar in animals with and without simultaneous exposure to the metal mixture (only a slight potentiation of a few health-effect parameters of cadmium was noticed in some animals). It is concluded that oral exposure to MM in the diet had almost no effect on the toxicokinetics and toxicity of Cd, Hg and Mn. This could be explained by the low level or low bioavailability of elements from MM, by the metal-metal interaction within the mixture or by the choice of health effect indicators determined. These results are presented as one of the potential approaches for studying the health effect of a metal mixture as occurring in the environment.

Evaluation: Klimisch Code 5. Non-pivotal.

Kostial K, Kello D, Jugo S, Rabar I and Maljkovic T (1978a) Influence of age on metal metabolism and toxicity. *Environ Health Perspect* **25:**81-86.

Abstract: The metabolism and toxicity of lead, cadmium, mercury, and manganese in the postnatal period was studied in rats. Absorption, whole body retention, and organ distribution of 203Pb, 115mCd, 203Hg, and 54Mn were determined after oral and parenteral administration of these radioisotopes. The acute oral toxicity (LD50) was determined after a single application of metal chlorides. The results obtained in sucklings show a very high intestinal absorption of all metals which is partly attributed to milk diet; a higher whole body retention, higher blood levels and a much higher accumulation in the brain; and a higher oral toxicity. These results indicate age specific differences in the pharmacokinetics of metals in sucklings. It seems reasonable to consider the early neonatal age as a critical period for metal accumulation and therefore for metal toxicity. The results are interpreted on the basis of current concepts of developmental physiology and pharmacology and suggestions for future research trends are made.

Evaluation: Klimisch Code 4. Lacking in detail.

Kostial K, Kello D, Rabar I, Maljkovic T and Blanusa M (1978b) Influence of ash from coal gasification on the pharmacokinetics and toxicity of cadmium, manganese and mercury in suckling and adult rats. *Arh Hig Rada* **30:**319-326.

Evaluation: Klimisch Code 5. Non-pivotal.

Lai CP, Minski MJ, Chan AW, Lim L and Davison AN (1981) Brain regional manganese distribution after chronic manganese treatment. *Biochem Soc Trans* **9:**228.

Evaluation: Klimisch Code 4. Very brief publication lacking in detail.

Lai JC, Chan AW, Leung TK, Minski MJ and Lim L (1992) Neurochemical changes in rats chronically treated with a high concentration of manganese chloride. *Neurochem Res* **17:**841-847.

Abstract: Several neurochemical parameters were studied in brain regions of rats chronically treated with a high concentration of manganese chloride (20 mg MnCl2.4H2O per ml. of drinking water) throughout development until adulthood. Large increases in Mn accumulation were found in all brain regions (hypothalamus, +530%; striatum, +479%; other regions, +152 to +250%) of Mn-treated adult rats. In these animals, Ca levels were decreased (-20 to -46%) in cerebellum, hypothalamus, and cerebral cortex but were increased (+186%) in midbrain. Mg levels were decreased (-12 to -32%) in pons and medulla, midbrain, and cerebellum. Fe levels were increased (+95%) in striatum but were decreased (-28%) in cerebral cortex. Cu levels were increased (+43 to +100%) in pons and medulla and striatum but Zn levels were decreased (-30%) in pons and medulla. Na levels were increased (+22%) in striatum but those of K and Cl remained unchanged. Type A monoamine oxidase activities were decreased (-13

to -16%) in midbrain, striatum, and cerebral cortex, but type B monoamine oxidase activities decreased (-13%) only in hypothalamus. Acetylcholinesterase activities were increased (+20 to +22%) in striatum and cerebellum. The results are consistent with our hypothesis that chronic manganese encephalopathy not only affects brain metabolism of Mn but also that of other metals.

Evaluation: Klimisch Code 4. Detailed methodology was not included in publication but further references were supplied.

Laitung JK and Mercer DM (1983) Manganese absorption through a burn. *Burns Incl Therm Inj* **10:**145-146.

Abstract: A case of manganese absorption occurring in association with a burn is presented. We maintain that acute manganese toxicity was a likely cause of liver dysfunction in our patient.

Evaluation: Klimisch Code 5. Not relevant for the TK of manganese.

Leavens TL, Rao D, Andersen ME and Dorman DC (2007) Evaluating transport of manganese from olfactory mucosa to striatum by pharmacokinetic modeling. *Toxicol Sci* **97:**265-278.

Abstract: Increased brain manganese (Mn) following inhalation can result from direct transport via olfactory neurons and blood delivery. Human health risk assessments for Mn should consider the relative importance of these pathways. The objective of this study was to develop a pharmacokinetic model describing the olfactory transport and blood delivery of Mn in rats following acute MnCl(2) or MnHPO(4) inhalation. Model compartments included the olfactory mucosa (OM), olfactory bulb, olfactory tract and tubercle, and striatum. Intercompartmental transport of Mn was described as ipsilateral, anterograde movement to deeper brain regions. Each compartment contained free and bound Mn and included blood influx and efflux. First-order rate constants were used to describe transport. Model parameters were estimated by comparing the model with published experimental data in rats exposed by inhalation to (54)MnCl(2) or (54)MnHPO(4) with both nostrils patent or one nostril occluded. The model-derived elimination rate constant from the OM was higher for the chloride salt (0.022 per hour) compared with the phosphate salt (0.011 per hour), consistent with their relative solubilities. Rate constants for Mn transport among the other compartments were similar for both Mn forms. Our results indicate that direct olfactory transport provided the majority of Mn tracer in the olfactory regions during the 21 days following exposure to (54)MnHPO(4) and 8 days following exposure to (54)MnCl(2). Only a small fraction of Mn tracer from the tract and tubercle was predicted to be delivered to the striatum, 3 and 0.1% following (54)MnHPO(4) or (54)MnCl(2) exposure, respectively.

Evaluation: Klimisch Code 5. Pharmacokinetic Model

Lee DY and Johnson PE (1988) Factors affecting absorption and excretion of 54Mn in rats. *J Nutr* **118:**1509-1516.

Abstract: The effects of different levels of dietary Mn and different models of 54Mn administration and the effect of sucrose and starch on absorption and excretion of the isotope were studied. Rats were fed diets containing between 1.3 and 82.4 mg Mn/kg for 7 or 14 d and administered 54Mn by gavage or a test meal containing 5 or 20 micrograms Mn. Additional rats for each dietary treatment received 54Mn by intramuscular or intraperitoneal injection. Amount of Mn in the oral dose did not affect 54Mn absorption, but increasing dietary Mn reduced Mn absorption and enhanced 54Mn excretion. Absorption of 54Mn by fasted, gavaged rats was four times higher than in unfasted gavaged rats or in fasted rats fed test meals. Orally administered 54Mn had a shorter biological half-life than injected 54Mn and tissue distribution of 54Mn differed in rats given 54Mn by different routes. Rats fed between 1.4 and 2.8 mg Mn/kg diet grew as well as or better than those fed amounts similar to the recommended level (50 mg/kg). Sucrose-fed rats absorbed more than 54Mn than starch-fed rats. Rats fed sucrose excreted injected 54Mn faster than rats fed starch. Concentrations of liver Mn in sucrose-fed rats were higher than in starch-fed rats. Our results indicate that both absorption and excretion are important in maintaining Mn homeostasis in rats.

Evaluation: Klimisch Code 2. Well documented, good experimental study design and use of statistics to formulate conclusions. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The form of manganese in the diet was not stated.

Lee DY and Johnson PE (1989) 54Mn absorption and excretion in rats fed soy protein and casein diets. *Proc Soc Exp Biol Med* **190:**211-216.

Abstract: Rats were fed diets containing either soy protein or casein and different levels of manganese, methionine, phytic acid, or arginine for 7 days and then fed test meals labeled with 2 microCi of 54Mn after an overnight fast. Retention of 54Mn in each rat was measured every other day for 21 days using a whole-body counter. Liver manganese was higher (P less than 0.0001) in soy protein-fed rats (8.8 micrograms/g) than in casein-fed rats (5.2 micrograms/g); manganese superoxide dismutase activity

also was higher in soy protein-fed rats than in casein-fed rats (P less than 0.01). There was a significant interaction between manganese and protein which affected manganese absorption and biologic half-life of 54Mn. In a second experiment, rats fed soy protein-test meals retained more 54Mn (P less than 0.001) than casein-fed rats. Liver manganese (8.3 micrograms/g) in the soy protein group was also higher than that (5.7 micrograms/g) in the casein group (P less than 0.0001), but manganese superoxide dismutase activity was unaffected by protein. Supplementation with methionine increased 54Mn retention from both soy and casein diets (P less than 0.06); activity of manganese superoxide dismutase increased (P less than 0.05) but liver manganese did not change. The addition of arginine to casein diets had little effect on manganese bioavailability. Phytic acid affected neither manganese absorption nor biologic half-life in two experiments, but it depressed liver manganese in one experiment. These results suggest that neither arginine nor phytic acid was the component in soy protein which made manganese more available from soy protein diets than casein diets.

Evaluation: Klimisch Code 2. Well documented, good experimental study design and use of statistics to formulate conclusions. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Lewis J, Bench G, Myers O, Tinner B, Staines W, Barr E, Divine KK, Barrington W and Karlsson J (2005) Trigeminal uptake and clearance of inhaled manganese chloride in rats and mice. *Neurotoxicology* **26:**113-123.

Abstract: Inhaled manganese (Mn) can enter the olfactory bulbs via the olfactory epithelium, and can then be further transported trans-synaptically to deeper brain structures. In addition to olfactory neurons, the nasal cavity is innervated by the maxillary division of the trigeminal nerve that projects to the spinal trigeminal nucleus. Direct uptake and transport of inhaled metal particles in the trigeminal system has not been investigated previously. We studied the uptake, deposition, and clearance of soluble Mn in the trigeminal system following nose-only inhalation of environmentally relevant concentrations. Rats and mice were exposed for 10-days (6 h/day, 5 days/week) to air or MnCl2 aerosols containing 2.3 +/- 1.3 mg/m3 Mn with mass median aerodynamic diameter (MMAD) of 3.1 +/- 1.4 microm for rats and 2.0 +/- 0.09 mg/m3 Mn MnCl2 with MMAD of 1.98 +/- 0.12 microm for mice. Mn concentrations in the trigeminal ganglia and spinal trigeminal nucleus were measured 2 h (0-day), 7-, 14-, or 30-days post-exposure using proton induced X-ray emission (PIXE). Manganese-exposed rats and mice showed statistically elevated levels of Mn in trigeminal ganglia 0-, 7- and 14-days after the 10-days exposure period when compared to control animals. The Mn concentration gradually decreased over time with a clearance rate (t1/2) of 7-8-days. Rats and mice were similar in both average accumulated Mn levels in trigeminal ganglia and in rates of clearance. We also found a small but significant elevation of Mn in the spinal trigeminal nucleus of mice 7-days post-exposure and in rats 0- and 7-days post-exposure. Our data demonstrate that the trigeminal nerve can serve as a pathway for entry of inhaled Mn to the brain in rodents following nose-only exposure and raise the question of whether entry of toxicants via this pathway may contribute to development of neurodegenerative diseases.

Evaluation: Klimisch Code 3. Data claims to show the direct trigeminal uptake of manganese following nose-only inhalation in mice and rats. The data failed to show that the manganese deposition was not the results of systemic circulation and refers to unpublished data. This data is needed to uphold the claims made. The study also failed to investigate single nasal occlusion, the results of which could have helped uphold the claims made.

Mahoney JP and Small WJ (1968) Studies on manganese. 3. The biological half-life of radiomanganese in man and factors which affect this half-life. *J Clin Invest* **47:**643-653.

Abstract: The biological half-life of manganese and some factors influencing it have been studied in man. The disappearance of manganese from the body in normal subjects is described by a curve having two exponential components. An average of 70% of the injected material was eliminated by the "slow" pathway. The half-time characterizing this component showed a small variation in normal subjects and had an average value of 39 days. The half-time for the "fast" component also showed a small variation and had an average value of 4 days.In a normal subject presumed to have a low manganese intake due to a voluntary low caloric intake, the percentage eliminated by the slow pathway increased to 84% and the half-time characterizing the pathway increased to 90 days. The half-time of the "fast" component was the same as for the normal group. 2 months after initiation of the study in this subject, a large "flushing" dose of manganese markedly increased the elimination rate which was described by a single exponential curve. A mildly iron-deficient subject showed a marked decrease in the percentage of manganese eliminated by the "slow" pathway accompanied by a less dramatic decrease in the half-time characterizing this pathway. Oral iron therapy, which corrected the mild anemia, caused a decrease in the elimination rate and the altered curve was described by a single exponential component. Preloading two subjects with manganese resulted in a great decrease in the fraction eliminated by the "slow" pathway with less effect on the half-time. The subject with the largest preloading dose showed no

"slow" component at all. Observations on the red cells of some of these subjects showed that a small but definite fraction was incorporated into the erythrocytes.In the mildly iron-deficient subject, our observations suggest an interrelationship between manganese and iron metabolism.

Evaluation: Klimisch Code 2. Although a very old study, the methodology is well described. The results are very interesting particularly after a large "flushing" dose of manganese. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Malik JK and Srivastava AK (1987) Studies on the interaction between manganese and fenitrothion in rats. *Toxicol Lett* **36:**221-226.

Abstract: The effect of manganese pretreatment on acute toxicity of fenitrothion (FTH) was investigated in male rats by assessing the degree of enzymatic alterations. Oral administration of FTH (260 mg/kg) markedly inactivated cholinesterase (ChE) and carboxylesterase and elevated the activities of acid phosphatase, alanine aminotransferase and aspartate aminotransferase in different tissues 3 h after dosing. Pretreatment of rats with manganese (10 mg/kg, i.p.) 3 days prior to FTH application (260 mg/kg, p.o.) significantly enhanced these enzymatic changes. The results indicate that inhibition of esterases and elevation in other enzymes induced by manganese are likely to contribute to the increased enzymatic alterations observed following combined treatment.

Evaluation: Klimisch Code 5. Non-pivotal.

Matrone G, Hartman RH and Clawson AJ (1959) Studies of a manganese-iron antagonism in the nutrition of rabbits and baby pigs. *J Nutr* **67:**309-317.

Evaluation: Klimisch Code 5. Non-pivotal.

Maynard LS and Cotzias GC (1955) The partition of manganese among organs and intracellular organelles of the rat. *J Biol Chem* **214:**489-495.

Evaluation: Klimisch Code 5. Non-pivotal.

McLeod BE and Robinson MF (1972) Metabolic balance of manganese in young women. *Br J Nutr* **27:**221-227.

Evaluation: Klimisch Code 4. An old study with a limited amount of detailed methodolgy.

McMillan G (2005) Is electric arc welding linked to manganism or Parkinson's disease? *Toxicol Rev* **24:**237-257.

Abstract: Manganese and its inorganic compounds are widely used in many industries and have been accepted as occupational neurotoxins that have caused a distinct and disabling clinical entity, manganism, in several types of work, notably where exposure is by way of dust. There is inconclusive and inconsistent evidence that, in these occupations, subclinical neurological effects, detectable only by neurobehavioural studies, may be caused by low doses. This has prompted a re-evaluation of occupational exposure limits. Some countries, including the UK, already demand much higher levels of protection against exposure than 5 years ago. Welding is the most common source of occupational exposure as manganese is an essential component of steel and so its compounds are inevitable components of fume emitted from steel welding processes. There it is found in respirable particles, often as complex oxides (spinels), sometimes within a core protected by a silicon oxide shell - as distinct from the much simpler form of particle formed by disintegration in processes such as mining and ore milling where manganism has been diagnosed convincingly. Millions of workers are at risk of exposure to manganese-containing compounds in fumes from electric arc welding of steel. In recent years it has been asserted that neurological and neurobehavioural disorders may develop consequent to exposure to steel welding fumes and that employment as a welder is associated with the unusually early onset of Parkinson's disease. Causal relationships have been postulated. Welders have been recorded as having been exposed to high levels of manganese-containing fume, especially where they have worked in confined, unventilated spaces, although this appears from limited data to be the exception rather than the rule. Even then the dose received is generally less than in mining or ore crushing. When care is taken to exclude exposures from hardfacing and burning and cutting arc processes, where manganese may form a high percentage of the fume, manganese compounds usually form a relatively low percentage of the composition of welding fume particles, <2.0%, much outweighed by iron. Although these manganese-compound-containing welding fume particles are insoluble in water, the manganese compounds in particles that are retained in the alveoli may be absorbed, at least in part. Manganese concentrations in biological material samples in some exposed groups reflect this relative to unexposed workers. Some of the transfer systems for absorption and transport, including across the blood-brain barrier, are used in competition with iron which is present in abundance in welding fume. This may reduce absorption of manganese in welders and thus reduce

the opportunity for sufficient doses to cause neurotoxicological consequences. Scrutiny of the literature covering the last 40 years has revealed only five cases that meet sufficient criteria for manganism to just cross the diagnostic threshold, and even then they carry a degree of doubt with them. This low incidence alone gives notice that welders have not been and are not at high risk of clinically apparent damage from exposure to manganese. If this needs to be further emphasised, there is the fact that the literature contains no confirmed cases of manganism in welders. Assertions of abnormal results in neurobehavioural studies of welders have raised the possibility of there being a subclinical form of manganism with loss of fine motor control as one of its features. While observations of such changes in workers in other industries have caused regulators in some countries to apply more stringent controls of exposure, as yet the results lack convincing consistency and there is no indication of any dose-effect relationship. If welding fume can have these motor effects it would be a heavy and perhaps career-ending blow to those affected. It would not be prudent to dismiss the warnings sounded by the results of studies of welders, no matter how flawed these investigations are, but wiser and better to act with vigour to reduce exposure and monitor the effectiveness of this additional protection whilst conducting high quality research to allow sound conclusions to be drawn as to whether there actually is a subclinical disorder. Idiopathic Parkinson's disease is a common disorder affecting 1-2% of those in the general population aged >65 years. It has been suggested, on flawed and contested evidence, not that welding causes the disease but rather that employment as a welder carries with it the risk of developing this disease at a younger age than if that trade had not been followed. Manganese in welding fume has been nominated as the neurotoxin. This may be biologically feasible if manganese destroys insufficient receptor cells to produce clinical manganism but sufficient to enhance the effects of a reduced supply of dopamine to give the manifestations of already developing idiopathic Parkinson's disease earlier in the course of destruction of the substantia nigra than if all receptors were intact.

Evaluation: Klimisch Code 5. Review - not evaluated.

Mena I, Horiuchi K, Burke K and Cotzias GC (1969) Chronic manganese poisoning. Individual susceptibility and absorption of iron. *Neurology* **19:**1000-1006.

Evaluation: Klimisch Code 4. A very old study and as such the detail is lacking. However, the study does appear to be well designed and reported and contains very relevant human information on the toxicokinetics of manganese.

Mena I, horiuchi K and Lopez G (1974) Factors Enhancing Entrance of Manganese into the Brain: Iron Deficiency and Age. *J Nucl Med* **15:**516.

Evaluation: Klimisch Code 3. A very brief report in the proceedings of an annual meeting.

Mena I, Marin O, Fuenzalida S and Cotzias GC (1967) Chronic manganese poisoning. Clinical picture and manganese turnover. *Neurology* **17:**128-136.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Consideration is also given to the age of the study and human relevance.

Mertz W (1974) The newer essential trace elements, chromium, tin, nickel, vanadium and silicon. *Proc Nutr Soc* **33:**307-313.

Evaluation: Klimisch Code 5. Non-pivotal.

Michalke B, Halbach S and Nischwitz V (2007) Speciation and toxicological relevance of manganese in humans. *J Environ Monit* **9:**650-656.

Abstract: Although manganese is an essential trace element, concerns are rising about the Mn exposure of humans being related to neurotoxic effects. This review summarizes several aspects of this topic to provide updated information on Mn related investigations, including chemical speciation of Mn-compounds. The paper starts with some chemical aspects of Mn and its compounds, enlightening oxidation states in general and in biological matrices. This is followed by considerations on natural sources of human exposure, on occupational sources and on anthropogenically caused environmental sources, for example from the use of methylcyclopentadienyl manganese tricarbonyl (MMT). Next, the paper deals with Mn levels in the human organism, showing normal Mn concentrations in various tissues or body fluids, and continues with the toxicology of Mn, i.e. absorption, distribution and excretion. Of specific concern is the transfer of Mn to the brain which is the relevant neurotoxic target. In this context, parallels and differences between primary and Mn-dependent Parkinsonism are discussed, concluding with a risk assessment and a consideration of susceptible groups. The main part of this review focuses on recent investigations on Mn speciation. Analytical problems and their solutions are also described for correct identification of relevant Mn-compounds in matrices of human

origin. Finally, future needs are discussed, such as further investigations on those Mn-species which may overcome neural barrier control, on disease-modulated barrier control, on susceptibility to certain Mn-species, and on the interaction of Mn with Fe-homeostasis in the brain.

Evaluation: Klimisch Code 5. Review.

Miller ST, Cotzias GC and Evert HA (1975) Control of tissue manganese: initial absence and sudden emergence of excretion in the neonatal mouse. *Am J Physiol* **229:**1080-1084.

Abstract: All adult animals and humans tested up to this time have controlled their tissue manganese concentrations by controlling primarily the rate of the metal's excretion. In sharp contrast, neonatal mice did not excrete manganese for the first 17-18 days of life, although absorption of the natural 55Mn as well as distribution, tissue accumulation, and mitochondrial accumulation of the radioactive 54Mn were vigorous. This suggested as initially avid accumulation of this essential micronutrient, supplied in scarce traces in mouse milk (54 ng/ml) by mothers consuming very much higher dietary concentrations (55,000 ng/g). The tissue accumulation was demonstrated analytically and was particularly impressive in the brain, which can be susceptible to both manganese poisoning and deficiency.

Evaluation: Klimisch Code 3. A reasonable amount of method detail for an old study, however the conclusions were later to be proved doubtful.

Mirowitz SA and Westrich TJ (1992) Basal ganglial signal intensity alterations: reversal after discontinuation of parenteral manganese administration. *Radiology* **185:**535-536.

Abstract: The authors describe magnetic resonance (MR) imaging findings in a patient receiving long-term total parenteral nutrition (TPN) therapy in whom parenteral manganese administration was experimentally discontinued. MR imaging performed while the patient was receiving standard TPN solution demonstrated marked hyperintensity of the globi pallidi on T1-weighted images. Following cessation of parenteral manganese administration for 1 year, repeat MR imaging demonstrated regression of the abnormal signal intensity.

Evaluation: Klimisch Code 4. A short paper lacking in some detail, for example there was no mention of blood manganese levels.

Missy P, Lanhers MC, Lisiane C, Joyeux M and Burnel D (2000) Effects of Subchronic Exposure to Manganese Chloride on Tissue Distribution of Three Essential Elements in Rats. *International Journal of Toxicology* **19:**313-321.

Abstract: A subchronic treatment of manganese chloride (MnCl2) was administered to rats by intraperitoneal (IP) route (6 mgMn/kg of body weight/day) or oral (PO) route (75 mg Mn/kg of body weight/day) for 4 weeks. After a 2-week interval, different tissues plus the blood were sampled. An increase of manganese (Mn) concentrations was observed in most of the tissues, particularly in the nervous system (brain and spinal cord) and in femur, with the exception of liver, adrenal glands, and esophagus by IP treatment and liver, jejunum, ileum, and adipose tissue by PO treatment. Tissue accumulation of Mn was greater by IP treatment. During each of the two treatments, urinary and fecal excretion of Mn increased. The presence of Mn observed in whole blood, bone marrow, and spleen after IP treatment could be explained by the existence of competition between iron (Fe) and Mn that may appear, notably, as a disturbance in the functioning of the respiratory chain in the cells (incomplete O2 reduction and formation of free radicals and oxygenated compounds), leading to cellular degeneration. In these experimental conditions, no obvious competition could be observed with zinc (Zn) and copper (Cu). Despite a large accumulation of Mn in the bones, no disturbance of the phosphorus-calcium metabolism was observed.

Evaluation: Klimisch Code 2. Description of methodology seems adequate. Excellent agreement between the manganese levels in the different control groups. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Moore W, Jr., Hall L, Crocker W, Adams J and Stara JF (1974) Metabolic aspects of methylcyclopentadienyl manganese tricarbonyl in rats. *Environ Res* **8:**171-177.

Evaluation: Klimisch Code 5. Non-pivotal, outside scope of TK review.

Morganti JB, Lown BA, Stineman CH, D'Agostino RB and Massaro EJ (1985) Uptake, distribution and behavioral effects of inhalation exposure to manganese (MnO2) in the adult mouse. *Neurotoxicology* **6:**1-15.

Abstract: Adult male mice were exposed either to sublethal levels of MnO2 dust or filtered air (control group) 7 hours/day, 5 days/week for 16 to 32 weeks. Following a 16 week initial exposure period, randomly selected samples (8 animals) from both the control and Mn-exposed groups were observed for

behavioral performance (ambulations and rearings in the open-field, "hole-in-board" explorations, rotarod) and learning (passive avoidance) and tissue Mn levels were determined via atomic absorption spectrometry. Exposure continued for the remaining animals and the sampling procedure was repeated biweekly for an additional 8 time points. At week 32, Mn exposure was terminated. However, biweekly testing of the remaining animals continued for an additional 3 time points. Mn-exposed animals had significantly higher blood, liver, kidney, lung, cerebrum, cerebellum plus brainstem, and testis Mn levels than control animals. With the exception of the liver, these levels declined with increasing exposure time. No histopathologic effects attributable to Mn-exposure were observed. However, significant overall effects on growth and behavior were obtained. Specifically, Mn-exposed subjects weighed more, executed more rearings in the open-field, and tended to exhibit longer latencies to enter the open-field. When the post-exposure data were analyzed separately, no significant effects were obtained. While no general relationship was obtained between tissue Mn levels and behavior, selected behavioral measures did correlate with tissue Mn levels. Animals exposed via feeding to comparable Mn levels across the same length of exposure employed in the inhalation study did not demonstrate any significant behavioral alterations.

Evaluation: Klimisch Code 3. After 8 hours inhalation exposure to MnO2 dust the mean amount of manganese in the GI tract of mice was 5.127 mg. This value was then used to calculate the level of manganese to incorporate into diet (1 mg Mn/g diet) in order to provide comparable levels of manganese exposure to a second group of mice for comparative purposes (behaviour etc.). This calculation appears flawed, as it totally rules out any possibility of uptake from the lungs directly or even if mucocilliary transport of inhaled particles took place whether these would have the same bioavailability as dietary manganese.

Morrow PE, Gibb FR and Johnson L (1964) Clearance of Insoluble Dust from the Lower Respiratory Tract. *Health Phys* **10:**543-555.

Evaluation: Klimisch code 4. Very old study, however does provide estimated clearance data of 54Mn from the lower respiratory tract of dogs following 54MnO2 aerosol exposure.

Moss OR (1979) Simulants of lung interstitial fluid. *Health Phys* **36:**447-448.

Evaluation: Klimisch Code 5. (Reference for simulated lung fluid).

Murphy VA, Wadhwani KC, Smith QR and Rapoport SI (1991) Saturable transport of manganese(II) across the rat blood-brain barrier. *J Neurochem* **57:**948-954.

Abstract: Unanesthetized adult male rats were infused intravenously with solutions containing 54Mn (II) and one of six concentrations of stable Mn(II). The infusion was timed to produce a near constant [Mn] in plasma for up to 20 min. Plasma was collected serially and on termination of the experiment, samples of CSF, eight brain regions, and choroid plexus (CP) were obtained. Influx of Mn (JMn) was calculated from uptake of 54Mn into tissues and CSF at two different times. Plasma [Mn] was varied 1,000-fold (0.076-78 nmol/ml). Over this plasma concentration range, JMn increased 123 times into CP, 18-120 times into brain, and 706 times into CSF. CP and brain JMn values fit saturation kinetics with Km (nmol/ml) equal to 15 for CP and 0.7-2.1 for brain, and Vmax (10(-2) nmol.g-1.s-1) of 27 for CP and 0.025-0.054 for brain. Brain JMn except at cerebral cortex had a nonsaturable component. CSF JMn varied linearly with plasma [Mn]. These findings suggest that Mn transport into brain and CP is saturable, but transport into CSF is nonsaturable.

Murthy RC, Srivastava RS, Gupta SK and Chandra SV (1980) Manganese induced testicular changes in monkeys. *Exp Pathol (Jena)* **18:**240-244.

Abstract: Oral administration of manganese chloride (25 mg/kg b. w. daily) to monkeys for a period of 18 months produced congestion and marked increase in weight of testis. Histopathologic examination revealed interstitial oedema and degeneration of seminiferous tubules. Activities of succinic dehydrogenase, glucose-6-phosphate dehydrogenase and acid phosphatase were significantly inhibited whereas NADH-diaphorase and alkaline phosphatase activities showed only slight inhibition in seminiferous tubules of treated monkeys. It was concluded that chronic exposure to manganese does not produce sever degenerative changes in the testis earlier than metal induced encephalopathy in primates.

Evaluation: Klimisch Code 5. Non-pivotal.

Newland MC, Ceckler TL, Kordower JH and Weiss B (1989) Visualizing manganese in the primate basal ganglia with magnetic resonance imaging. *Exp Neurol* **106:**251-258.

Abstract: The paramagnetism of manganese was exploited to obtain proton nuclear magnetic resonance (MR) images of manganese-rich tissue in the central nervous system in vivo. One Macaca fascicularis monkey inhaled MnCl2 aerosol prior to imaging. A second M. fascicularis and two Cebus apellas were

administered MnCl2 in various doses intravenously. The monkeys' brains were imaged before and after manganese administration in coronal and horizontal planes that included the basal ganglia and substantia nigra. A T1-weighted pulse sequence exploited manganese's reduction of spin-lattice relaxation times and clearly distinguished several separate and specific regions after manganese administration: the caudate nucleus, the lenticular nuclei, the substantia nigra, a region corresponding to subthalamic nucleus and ventromedial hypothalamus, and the pituitary gland. The kinetics of manganese accumulation were important in determining the imaged intensity of these regions but the route of parenteral administration was not. Spin-lattice relaxation times showed that T1 was shortened at lower doses of manganese and remained shortened longer in the globus pallidus and pituitary gland while little effect appeared in gray and white matter. T1 effects in caudate and putamen effects were intermediate. These data suggest selective affinity for manganese in globus pallidus and pituitary.

Evaluation: Klimisch Code 2. Reasonably well documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Reference to blood manganese concentrations are made to support a point in the text, but the results are not fully detailed. Only one animal was exposed to manganese by inhalation.

Newland MC, Cox C, Hamada R, Oberdorster G and Weiss B (1987) The clearance of manganese chloride in the primate. *Fundam Appl Toxicol* **9:**314-328.

Abstract: Two macaque monkeys inhaled trace amounts of 54MnCl2 for 30 min. Subsequently the chest, head, and fecal radioactivity were monitored for over a year. The chest data curve required a sum of three exponential terms, with half-times ranging from 0.2 to 187 days, to attain a satisfactory fit. Head levels peaked 40 days after acute inhalation exposure and remained elevated for over a year. The excretion of manganese through the feces was best described by a sum of two exponentials. One had a half-time of less than 1 day and the second had a half-time of 50 to 60 days. A third macaque received a 6-week continuous exposure to 54Mn through a subcutaneous osmotic pump. With this route, manganese clearance from the head occurred at a faster rate than after acute inhalation exposure. Fecal elimination following continuous subcutaneous exposure resembled that following acute inhalation. Kinetic analyses suggested that the long half-times of manganese in the head following inhalation reflected both slow disappearance from the head and replenishment from other depots.

Evaluation: Klimisch Code 3. Comparisons of elimination kinetics were made between one monkey that had received 200 mg of manganese though a subcutaneous osmotic pump, to two monkeys that received a trace (~0.02 ug) dose of manganese from a single 30-minute inhalation. The exceptionally large differences in dose levels meant that the comparisons made and conclusions drawn between the dose routes was unsound. In addition, due to the methodology used there was no differentiation between total head measurement and activity in the brain or skull. In particular, the phrase taken from the discussion " The observation of very long biological half-times for the elimination of brain manganese chloride following inhalation of manganese chloride raises the possibility that long-term exposure to even low levels of manganese will cause significant accumulation in the brain" cannot be substantiated from this study.

Nishiyama K, Suzuki Y, Fujii N, Yano H, Ohnishi K and Miyai T (1977) Biochemical changes and manganese distribution in monkeys exposed to manganese dioxide dust. *Tokushima J Exp Med* **24:**137-145.

Evaluation: Klimisch Code 2. Relatively good detailed methodology for an old study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Limited group sizes (2 or 3) meant no statistical evaluation. No estimates of absorption or elimination of dose performed.

Nong A, Taylor MD, Clewell HJ, 3rd, Dorman DC and Andersen ME (2009) Manganese tissue dosimetry in rats and monkeys: accounting for dietary and inhaled Mn with physiologically based pharmacokinetic modeling. *Toxicol Sci* **108:**22-34.

Abstract: Manganese (Mn) is an essential nutrient required for normal tissue growth and function. Following exposures to high concentrations of inhaled Mn, there is preferential accumulation of Mn in certain brain regions such as the striatum and globus pallidus. The goal of this research was to complete a physiologically based pharmacokinetic (PBPK) model for Mn in rats and scale the model to describe Mn tissue accumulation in nonhuman primates exposed to Mn by inhalation and diet. The model structure includes saturable tissue binding with association and dissociation rate constants, asymmetric tissue permeation flux rate constants to specific tissues, and inducible biliary excretion. The rat PBPK model described tissue time-course studies for various dietary Mn intakes and accounted for inhalation studies of both 14-day and 90-day duration. In monkeys, model parameters were first calibrated using steady-state tissue Mn concentrations from rhesus monkeys fed a diet containing 133 ppm Mn. The model was then applied to simulate 65 exposure days of weekly (6 h/day; 5 days/week) inhalation

exposures to soluble MnSO(4) at 0.03 to 1.5 mg Mn/m(3). Sensitivity analysis showed that Mn tissue concentrations in the models have dose-dependencies in (1) biliary excretion of free Mn from liver, (2) saturable tissue binding in all tissues, and (3) differential influx/efflux rates for tissues that preferentially accumulate Mn. This multispecies PBPK model is consistent with the available experimental kinetic data, indicating preferential increases in some brain regions with exposures above 0.2 mg/m(3) and fairly rapid return to steady-state levels (within several weeks rather than months) after cessation of exposure. PBPK models that account for preferential Mn tissue accumulation from both oral and inhalation exposures will be essential to support tissue dosimetry-based human risk assessments for Mn.

Evaluation: Klimisch Code 5. Pharmacokinetic Model.

Nong A, Teeguarden JG, Clewell HJ, 3rd, Dorman DC and Andersen ME (2008) Pharmacokinetic modeling of manganese in the rat IV: Assessing factors that contribute to brain accumulation during inhalation exposure. *J Toxicol Environ Health A* **71:**413-426.

Abstract: A recently published physiologically based pharmacokinetic (PBPK) model successfully accounted for steady-state tissue manganese (Mn) concentration seen with normal dietary intakes and for biphasic, whole-body time-course profiles observed with tracer (54Mn) dosing. In this present study, PBPK modeling was used to evaluate Mn kinetics and brain concentrations in rats exposed to Mn both in their diet and by inhalation. Three published studies were used: (1) rats fed on diets ranging from 2 to 100 ppm, (2) rats on 125 ppm in diet and exposed via inhalation at 0.0 to 3.00 mg Mn/m3 each day for 14 d, and (3) rats to 0.1 or 0.5 mg Mn/m3 for 6 h/d, 5 d/wk over a 90-d period. The original model structure with well-mixed and "deep" compartments for each tissue could not describe rapid increases in tissue concentrations and rapid declines seen in high concentration inhalation studies. A second structure was developed that included (1) saturable, high-affinity binding of Mn in all tissues and (2) asymmetric diffusion from blood into brain (i.e., transport into and out of specific brain regions such as the striatum was described with different diffusion constants). This second model was consistent with liver and striatum experimental data. Preferential increases in some brain regions were predicted for exposures above 0.2 mg/m3 and had a rapid (i.e., 1 or 2 wk) return to steady-state levels. Multi-dose-route PBPK models for Mn based on this alternative model structure can be readily scaled to evaluate tissue Mn kinetics in other species and for human populations. Once validated across test animals, these PBPK models will be useful in tissue-dose based risk assessment with manganese.

Evaluation: Klimisch Code 5. Pharmacokinetic Model

Normandin L, Ann Beaupre L, Salehi F, St -Pierre A, Kennedy G, Mergler D, Butterworth RF, Philippe S and Zayed J (2004) Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. *Neurotoxicology* **25:**433-441.

Abstract: The central nervous system is an important target for manganese (Mn) intoxication in humans; it may cause neurological symptoms similar to Parkinson's disease. Manganese compounds emitted from the tailpipe of vehicles using methylcyclopentadienyl manganese tricarbonyl (MMT) are primarily Mn phosphate, Mn sulfate, and Mn phosphate/sulfate mixture. The purpose of this study is to compare the patterns of Mn distribution in various brain regions (olfactory bulb, frontal parietal cortex, globus pallidus, striatum and cerebellum) and other tissues (lung, liver, kidney, testis) and the neurobehavioral damage following inhalation exposure of rats to three Mn species. Rats (n=15 rats per Mn species) were exposed 6 h per day, 5 days per week for 13 consecutive weeks to metallic Mn, Mn phosphate or Mn phosphate/sulfate mixture at about 3000 microgm(-3) and compared to controls. At the end of the exposure period, spontaneous motor activity was measured for 36 h using a computerized autotrack system. Mn in tissues was determined by instrumental neutron activation analysis (INAA). The Mn concentrations in the brain were significantly higher in rats exposed to Mn phosphate and Mn phosphate/sulfate mixture than in control rats or rats exposed to metallic Mn. Exposure to Mn phosphate/sulfate mixture caused a decrease in the total ambulatory count related to locomotor activity. Our results confirm that Mn species and solubility have an influence on the brain distribution of Mn in rats.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. The inclusion of metallic manganese was apparently included in order to establish the neurotoxicity of the pure element. This seems an odd choice for a number of reasons, the relevance to human exposure seems very unlikely, manganese oxide(s) would have been a better choice. Since the delivery method was via inhalation, the results for the metallic manganese in the lungs was less than 2-fold greater than the control animals, yet the lung manganese levels from the manganese salts were 30 to 50-fold greater than controls. No discussion of this observation was presented. The highest increases in manganese brain tissue levels from the manganese salts was seen in the olfactory bulb, yet the olfactory bulb following the metallic manganese exposure was not measured with no explanation!

Norwegian-Labour-Inspectorate (2007) Determination of occupational exposure limits for manganese and inorganic manganese compounds.

Abstract: Based on existing human studies, there are 2 critical effects justifying new OEL for Mn and its inorganic compounds: increased hand tremor and increased prolactin concentration

Evaluation: Klimisch Code 4. A review of existing data, only the summary and conclusions provided.

Onoda K, Hasegawa A, Sunouchi M, Tanaka S, Tanaka A, Omori Y and Urakubo G (1977) Studies on the Fate of Poisonous Metals in Experimental Animals (VII). *Journal of Food Hygiene Society* **19:**208-215.

Evaluation: Klimisch Code 4. Relatively brief methodology, lacks use of statistical analysis.

Orimo S and Ozawa E (2001) Short-term administration of an essential trace elements preparation (Elemenmic) causes a high whole blood manganese concentration and manganese deposition in basal ganglia. *Intern Med* **40:**1162-1163.

Evaluation: Klimisch Code 4. A short communication.

Panic B (1967) In vitro binding of manganese to serum transferrin in cattle. *Acta Vet Scand* **8:**228-233.

Evaluation: Klimisch Code 5. Non-pivotal.

Papavasiliou PS, Miller ST and Cotzias GC (1966) Role of liver in regulating distribution and excretion of manganese. *Am J Physiol* **211:**211-216.

Evaluation: Klimisch Code 4. A very old study with some interesting techniques!

Park JD, Chung YH, Kim CY, Ha CS, Yang SO, Khang HS, Yu IK, Cheong HK, Lee JS, Song CW, Kwon IH, Han JH, Sung JH, Heo JD, Choi BS, Im R, Jeong J and Yu IJ (2007a) Comparison of high MRI T1 signals with manganese concentration in brains of cynomolgus monkeys after 8 months of stainless steel welding-fume exposure. *Inhal Toxicol* **19:**965-971.

Abstract: Several pharmacokinetic studies on inhalation exposure to manganese (Mn) have already demonstrated that Mn readily accumulates in the olfactory and brain regions. However, a shortening of the magnetic resonance imaging (MRI) T1 relaxation time or high T1 signal intensity in specific sites of the brain, including the globus pallidus and subcortical frontal white matter, as indicative of tissue manganese accumulation has not yet been clearly established for certain durations of known doses of welding-fume exposure in experimental animals. Accordingly, to investigate the movement of manganese after welding-fume exposure, six cynomolgus monkeys were acclimated and assigned to three dose groups: unexposed, low dose (31 mg/m(3) total suspended particulate [TSP], 0.9 mg/m(3) of Mn), and high dose (62 mg/m(3) TSP, 1.95 mg/m(3) of Mn) of total suspended particulate. The primates were exposed to manual metal arc stainless steel (MMA-SS) welding fumes for 2 h per day in an inhalation chamber system equipped with an automatic fume generator. Magnetic resonance imaging (MRI) studies were conducted before the initiation of exposure and thereafter every month. The tissue Mn concentrations were then measured after a plateau was reached regarding the shortening of the MRI T1 relaxation time. A dose-dependent increase in the Mn concentration was found in the lungs, while noticeable increases in the Mn concentrations were found in certain tissues, such as the liver, kidneys, and testes. Slight increases in the Mn concentrations were found in the caudate, putamen, frontal lobe, and substantia nigra, while a dose-dependent noticeable increase was only found in the globus pallidus. Therefore, the present results indicated that a shortening of the MRI T1 relaxation time corresponded well with the Mn concentration in the globus pallidus after prolonged welding-fume exposure.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The treatment related results from the low dose group, tissue manganese levels,were generally greater than the high dose group, possibly because the two dose levels were too close together.

Park JD, Kim KY, Kim DW, Choi SJ, Choi BS, Chung YH, Han JH, Sung JH, Kwon IH, Mun JH and Yu IJ (2007b) Tissue distribution of manganese in iron-sufficient or iron-deficient rats after stainless steel welding-fume exposure. *Inhal Toxicol* **19:**563-572.

Abstract: Welders can be exposed to high levels of manganese through welding fumes. Although it has already been suggested that excessive manganese exposure causes neurotoxicity, called manganism, the pathway of manganese transport to the brain with welding-fume exposure remains unclear. Iron is an essential metal that maintains a homeostasis in the body. The divalent metal transporter 1 (DMT1) transports iron and other divalent metals, such as manganese, and the depletion of iron is known to upregulate DMT1 expression. Accordingly, this study investigated the tissue distribution of manganese in iron-sufficient and iron-deficient rats after welding-fume exposure. The feeding of an iron-deficient

diet for 4 wk produced a depletion of body iron, such as decreased iron levels in the serum and tissues, and upregulated the DMT1 expression in the rat duodenum. The iron-sufficient and iron-deficient rats were then exposed to welding fumes generated from manual metal arc stainless steel at a concentration of 63.5 +/- 2.3 mg/m3 for 2 h per day over a 30-day period. Animals were sacrificed on days 1, 15, and 30. The level of body iron in the iron-deficient rats was restored to the control level after the welding-fume exposure. However, the tissue distributions of manganese after the welding-fume exposure showed similar patterns in both the iron-sufficient and iron-deficient groups. The concentration of manganese increased in the lungs and liver on days 15 and 30, and increased in the olfactory bulb on day 30. Slight and heterogeneous increases of manganese were observed in different brain regions. Consequently, these findings suggest that the presence of Fe in the inhaled welding fumes may not have a significant effect on the uptake of Mn into the brain. Thus, the condition of iron deficiency did not seem to have any apparent effect on the transport of Mn into the brain after the inhalation of welding fumes.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The publication was adequately documented and reported. The single dose level of welding-fume exposure used in the study was selected to reproduce an environment similar to human industrial exposure. As higher dose levels had not been used, the results of manganese tissue distribution were not particularly significant or informing to the toxicokinetics of manganese.

Ponzoni S, Gaziri LC, Britto LR, Barreto WJ and Blum D (2002) Clearance of manganese from the rat substantia nigra following intra-nigral microinjections. *Neurosci Lett* **328:**170-174.

Abstract: Chronic exposure to manganese (Mn) positively correlates with the occurrence of Parkinsonism but little is known about mechanisms of its neurotoxicity. In the present study, we determined the clearance of Mn from rat substantia nigra after its nigral injection and correlated it with the establishment of apomorphine-induced rotational behaviour and loss of striatal tyrosine hydroxylase (TH) immunoreactivity. Our results suggest that Mn is slowly cleared from the substantia nigra, following a first-order kinetics with a t(1/2) of 3 days. Appearance of apomorphine-induced rotational behaviour and loss of TH immunoreactivity within the striatum follows metal clearance were both detected 24 hours after intra-nigral Mn microinjection and maximal 72 hours after injection. The present data suggest that the cellular mechanisms induced by Mn and leading to dopaminergic cell death, occurred shortly after its injection and that the metal concentration needs to reach a threshold value to induce neurotoxic effects. This would indicate that nigral damages are a direct consequence of Mn accumulation.

Evaluation: Klimisch Code 5. Non-pivotal.

Quintanar L (2008) Manganese Neurotoxicity: A Bioinorganic Chemist's Perspective. *Inorganica Chimica Acta* **361:**875-884.

Abstract: Manganese is an essential metal for life, yet chronic exposure to this metal can cause a neurodegenerative disease named manganism, with symptoms that resemble Parkinson's disease. Mn accumulates in the striatum and damages this brain structure that controls motor function; however, the molecular mechanisms underlying this neurodegenerative disease are poorly understood. In this short review, a summary of the current knowledge on the mechanisms involved in Mn neurotoxicity is given, with a special emphasis on the features that suggest specific protein-manganese interactions. The mechanisms of Mn uptake into the brain are discussed, displaying its similarities to Fe metabolism. Cellular trafficking of Mn is also reviewed, pointing out at its connection to Ca homeostasis, and its relevance for understanding Mn-induced neuronal death. The main purpose of this review is to provide a glimpse of an unexplored bioinorganic facet of a Mn-induced neurodegenerative disease.

Evaluation: Klimisch Code 5. Review.

Rabin O, Hegedus L, Bourre JM and Smith QR (1993) Rapid brain uptake of manganese(II) across the blood-brain barrier. *J Neurochem* **61:**509-517.

Abstract: 54Mn2+ uptake into brain and choroid plexus from the circulation was studied using the in situ rat brain perfusion technique. Initial uptake from blood was linear with time (30 s to 6 min) and extrapolated to zero with an average transfer coefficient of approximately 6 x 10(-5) ml/s/g for brain and approximately 7 x 10(-3) ml/s/g for choroid plexus. Influx from physiologic saline was three- to fourfold more rapid and exceeded that predicted for passive diffusion by more than one order of magnitude. The lower uptake rate from blood could be explained by plasma protein binding as the free fraction of 54Mn2+ in rat plasma was < or = 30%. Purified albumin, transferrin, and alpha 2-macroglobulin were each found to bind 54Mn2+ significantly and to restrict brain 54Mn2+ influx. The results demonstrate that 54Mn2+ is readily taken up into the CNS, most likely as the free ion, and that

transport is critically affected by plasma protein binding. The results support the hypothesis that Mn2+ transport across the blood-brain barrier is facilitated by either an active or a passive mechanism.

Evaluation: Klimisch Code 2. Reasonable level of detailed methodology. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Reaney SH, Bench G and Smith DR (2006) Brain accumulation and toxicity of Mn(II) and Mn(III) exposures. *Toxicol Sci* **93:**114-124.

Abstract: Concern over the neurotoxic effects of chronic moderate exposures to manganese has arisen due to increased awareness of occupational exposures and to the use of methylcyclopentadienyl manganese tricarbonyl, a manganese-containing gasoline antiknock additive. Little data exist on how the oxidation state of manganese exposure affects toxicity. The objective of this study was to better understand how the oxidation state of manganese exposure affects accumulation and subsequent toxicity of manganese. This study utilized a rat model of manganese neurotoxicity to investigate how ip exposure to Mn(II)-chloride or Mn(III)-pyrophosphate at total cumulative doses of 0, 30, or 90 mg Mn/kg body weight affected the brain region distribution and neurotoxicity of manganese. Results indicate that Mn(III) exposures produced significantly higher blood manganese levels than equimolar exposures to Mn(II). Brain manganese concentrations increased in a dose-dependent manner, with Mn(III) exposures producing significantly higher (> 25%) levels than exposures to Mn(II) but with no measurable differences in the accumulation of manganese across different brain regions. Gamma amino butyric acid concentrations were increased in the globus pallidus (GP) with manganese exposure. Dopamine (DA) levels were altered in the GP, with the highest Mn(II) and Mn(III) exposures producing significantly different DA levels. In addition, transferrin receptor and H-ferritin protein expression increased in the GP with manganese exposure. These data substantiate the heightened susceptibility of the GP to manganese, and they indicate that the oxidation state of manganese exposure may be an important determinant of tissue toxicodynamics and subsequent neurotoxicity.

Evaluation: Klimisch Code 2. Well documented, design and discussed study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. It would have been interesting to see the results of additional tissues analysed for manganese concentration.

Rehnberg GL, Hein JF, Carter SD and Laskey JW (1980) Chronic manganese oxide administration to preweanling rats: manganese accumulation and distribution. *J Toxicol Environ Health* **6:**217-226.

Abstract: Mn accumulation was evaluated in selected tissues of preweanling rats dosed daily with particulate Mn3O4. Significant findings include a high rate of Mn accumulation in the preweanling rat; a Mn dose-related acceleration of postpartum liver iron depletion; a Mn dose-related depression in red blood cells, hematocrit, hemoglobin, body weight, and survival by 21 d postpartum; and a Mn distribution in tissues with liver greater than brain greater than or equal to kidney greater than testes at 18-21 d of age.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Fairly brief methodology. The top 2 dose levels caused increased mortality and bodyweight loss. The lower dose level did not produce statistically significant increases in manganese tissue concentrations. Data from this study does not correlate well with a follow-up study by the same workers published in 1981.

Rehnberg GL, Hein JF, Carter SD, Linko RS and Laskey JW (1981) Chronic ingestion of Mn3O4 by young rats: tissue accumulation, distribution, and depletion. *J Toxicol Environ Health* **7:**263-272.

Abstract: Mn accumulation, distribution, and disappearance were evaluated in selected tissues of preweanling rats dosed daily with particulate Mn3O4 for 12 or 27 d postpartum. Significant findings include a high rate of Mn absorption and localization in tissues, especially the cerebrum, hypothalamus, and pituitary. In these tissues, the return of Mn concentrations to control levels was much slower when Mn dosing was continued beyond 18-20 d postpartum. Data from this study does not correlate well with the previous study by the same workers published in 1980.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Fairly brief methodology.

Rehnberg GL, Hein JF, Carter SD, Linko RS and Laskey JW (1982) Chronic ingestion of Mn3O4 by rats: tissue accumulation and distribution of manganese in two generations. *J Toxicol Environ Health* **9:**175-188.

Abstract: Sprague-Dawley rats were chronically exposed to particulate Mn3O4 through two generations. At specific ages, observations were made of growth, tissue content, and distribution of Mn and Fe as

affected by chronic exposure to Mn through an Fe-sufficient diet and an Fe-deficient diet. Chronic dietary Mn3O4 resulted in dose-related increases in Mn accumulation, and a concomitant Fe deficiency promoted Mn accumulation. In general, the addition of substantial amounts of Mn to either diet depressed tissue Fe levels.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Fairly brief methodology.

Reynolds AP, Kiely E and Meadows N (1994) Manganese in long term paediatric parenteral nutrition. *Arch Dis Child* **71:**527-528.

Abstract: The current practice of providing manganese supplementation to neonates on long term parenteral nutrition is leading to a high incidence of hypermanganesaemia. Magnetic resonance imaging (MRI) studies in adults on long term manganese parenteral nutrition have shown changes in TI weighted MRI images and similar findings in a neonate receiving trace element supplementation are reported here. Whole blood manganese concentration in the infant was 1740 nmol/l (or 8.3 times upper reference limit). In all neonates on long term parenteral nutrition with evidence of cholestatic liver disease so far investigated, the whole blood manganese concentrations were > 360 nmol/l (reference range 73-210). Manganese supplementation to patients on long term parenteral nutrition requires reappraisal, particularly in those who develop cholestatic liver disease associated with parenteral nutrition.

Evaluation: Klimisch Code 4. A brief report on a case history.

Roels H, Lauwerys R, Buchet JP, Genet P, Sarhan MJ, Hanotiau I, de Fays M, Bernard A and Stanescu D (1987a) Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices. *Am J Ind Med* **11:**307-327.

Abstract: A cross-sectional epidemiological study was carried out among 141 male subjects exposed to inorganic manganese (Mn) in a Mn oxide and salt producing plant (mean age 34.3 years; duration of exposure, mean 7.1 years, range 1-19 years). The results were compared with those of a matched control group of 104 subjects. The intensity of Mn exposure was moderate as reflected by the airborne Mn levels and the concentrations of Mn in blood (Mn-B) and in urine (Mn-U). A significantly higher prevalence of cough in cold season, dyspnea during exercise, and recent episodes of acute bronchitis was found in the Mn group. Lung ventilatory parameters (forced vital capacity, FVC; forced expiratory volume in one second, FEV1; peak expiratory flow rate, PEFR) were only mildly altered in the Mn group (smokers) and the intensity and the prevalence of these changes were not related to Mn-B, Mn-U, or duration of exposure. There was no synergistic effect between Mn exposure and smoking on the spirometric parameters. Except for a few nonspecific symptoms (fatigue, tinnitus, trembling of fingers, increased irritability), the prevalence of the other subjective complaints did not differ significantly between the control and Mn groups. Psychomotor tests were more sensitive than the standardized neurological examination for the early detection of adverse effects of Mn on the central nervous system (CNS). Significant alterations were found in simple reaction time (visual), audioverbal short-term memory capacity, and hand tremor (eye-hand coordination, hand steadiness). A slight increase in the number of circulating neutrophils and in the values of several serum parameters (ie, calcium, ceruloplasmin, copper, and ferritin) was also found in the Mn group. There were no clear-cut dose-response relationships between Mn-U or duration of Mn exposure and the prevalence of abnormal CNS or biological findings. The prevalences of disturbances in hand tremor and that of increased levels of serum calcium were related to Mn-B. The response to the eye-hand coordination test suggests the existence of a Mn-B threshold at about 1 microgram Mn/100 ml of whole blood. This study demonstrates that a time-weighted average exposure to airborne Mn dust (total dust) of about 1 mg/m3 for less than 20 years may present preclinical signs of intoxication.

Evaluation: Klimisch Code 2. Well documented and discussed. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Roels H, Lauwerys R, Genet P, Sarhan MJ, de Fays M, Hanotiau I and Buchet JP (1987b) Relationship between external and internal parameters of exposure to manganese in workers from a manganese oxide and salt producing plant. *Am J Ind Med* **11:**297-305.

Abstract: In a plant producing manganese (Mn) oxides and salts, 11 different workplaces were identified. The current exposure to airborne Mn (total dust, personal sampling, n = 80) varied from 0.07 to 8.61 mg/m3. The geometric mean and median values amounted approximately to 1 mg/m3 and the 95th percentile was 3.30 mg/m3. The concentration of Mn in blood (Mn-B) in a group of 141 Mn-exposed male workers ranged from 0.10-3.59 micrograms/100 ml compared to 0.04-1.31 micrograms/100 ml in a group of 104 control subjects. The ranges of the concentrations of Mn in urine (Mn-U) were 0.06-140.6 and 0.01-5.04 micrograms/g creatinine for the exposed and control groups, respectively. The

average level of Mn-B in the Mn group was more than twice as high as in the control group (arithmetic mean, 1.36 vs 0.57 microgram/100 ml) and that of Mn-U was ten times higher in the Mn group (geometric mean, 1.56 vs 0.15 microgram/g creatinine). The Mn-B level did not change significantly after 8 h of Mn exposure, whereas the Mn-U level dropped rapidly when exposure ceased (half-life less than 30 h). On an individual basis, neither Mn-B nor Mn-U correlated with the current levels of Mn-air or duration of Mn exposure. There was also no relationship between Mn-B and Mn-U. On a group basis, there was no correlation between the mean Mn-B levels and the current levels of Mn-air at each workplace; however, a slight but significant correlation ($r = 0.62$, $p < 0.05$) was found between the geometric mean of Mn-U of each subgroup ($n = 11$) and the current level of Mn-air at their corresponding workplaces. On a group basis ($n = 6$), Mn-U did not correlate with the estimation of past integrated exposure of the workers, while group means of Mn-B significantly correlated with past integrated exposure. These results indicate that the individual evaluation of the Mn exposure intensity remains difficult on the basis of Mn-B and Mn-U. On a group basis however, Mn-U appears to reflect very recent exposure, while Mn-B is to some extent a reflection of the body burden of Mn.

Evaluation: Klimisch Code 2. Appears to be well designed, described and discussed. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP and Lison D (1997) Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. *Arch Toxicol* **71:**223-230.

Abstract: The absorption and cerebral distribution of manganese (Mn) have been studied with respect to the route of administration and the chemical form of the Mn compound. Different groups of adult male rats received either MnCl2, 4H2O or MnO2 once a week for 4 weeks at a dose of 24.3 mg Mn/kg body wt. (b.w.) by oral gavage (g.) or 1.22 mg Mn/kg b.w. by intraperitoneal injection (i.p.) or intratracheal instillation (i.t.). Control rats were treated with 0.9% saline. Four days after the last administration the rats were killed and the concentration of Mn measured in blood, hepatic and cerebral tissues (cortex, cerebellum, and striatum). The liver Mn concentration was not affected by the treatments whatever the chemical form or the route of administration of the Mn compound. Administration of MnCl2 by g., i.p., and i.t. routes produced equivalent steady-state blood Mn concentrations (about 1000 ng Mn/100 ml), representing increases of 68, 59, and 68% compared with controls, respectively. Mn concentrations were significantly increased in the cortex but to a lesser extent (g., 22%; i.p., 36%; i.t., 48%) and were higher in the cerebellum after i.p. and i.t. administrations than after oral gavage. Rats treated i.t. with MnCl2 showed an elective increase of the striatal Mn concentration (205%). In contrast, MnO2 given orally did not significantly increase blood and cerebral tissue Mn concentrations; the low bioavailability is most likely due to the lack of intestinal resorption. Administration of MnO2 i.p. and i.t., however, led to significant increases of Mn concentrations in blood and cerebral tissues. These increments were not significantly different from those measured after MnCl2 administration, except for striatal Mn after i.t. which was markedly less (48%) after MnO2 administration. A comparison of the blood Mn kinetics immediately after g. and i.t. treatment with MnCl2 or MnO2 indicated that the higher elevation of blood Mn concentration (> 2000 ng Mn/100 ml) after i.t. administration of MnCl2 could account for the elective uptake of Mn in the striatum observed in repeated dosing experiments. It is concluded that the modulation of Mn distribution in brain regions according to the route of administration and the chemical form of the Mn compound may be explained on the basis of different blood Mn kinetics and regional anatomic specificities of the striatal region.

Evaluation: Klimisch Code 2. A very well designed and documented study. A clear hypothesis is tested and the results thoroughly analysed and discussed. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Rose C, Butterworth RF, Zayed J, Normandin L, Todd K, Michalak A, Spahr L, Huet PM and Pomier-Layrargues G (1999) Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction. *Gastroenterology* **117:**640-644.

Abstract: BACKGROUND & AIMS: Manganese (Mn) deposition could be responsible for the T(1)-weighted magnetic resonance signal hyperintensities observed in cirrhotic patients. These experiments were designed to assess the regional specificity of the Mn increases as well as their relationship to portal-systemic shunting or hepatobiliary dysfunction. METHODS: Mn concentrations were measured in (1) brain samples from basal ganglia structures (pallidum, putamen, caudate nucleus) and cerebral cortical structures (frontal, occipital cortex) obtained at autopsy from 12 cirrhotic patients who died in hepatic coma and from 12 matched controls; and from (2) brain samples (caudate/putamen, globus pallidus, frontal cortex) from groups ($n = 8$) of rats either with end-to-side portacaval anastomosis, with biliary cirrhosis, or with fulminant hepatic failure as well as from sham-operated and normal rats. RESULTS:

Mn content was significantly increased in frontal cortex (by 38%), occipital cortex (by 55%), pallidum (by 186%), putamen (by 66%), and caudate (by 54%) of cirrhotic patients compared with controls. Brain Mn content did not correlate with patient age, etiology of cirrhosis, or history of chronic hepatic encephalopathy. In cirrhotic and portacaval-shunted rats, Mn content was increased in pallidum (by 27% and 57%, respectively) and in caudate/putamen (by 57% and 67%, respectively) compared with control groups. Mn concentration in pallidum was significantly higher in portacaval-shunted rats than in cirrhotic rats. No significant changes in brain Mn concentrations were observed in rats with acute liver failure. CONCLUSIONS: These findings suggest that brain Mn deposition results both from portal-systemic shunting and from liver dysfunction.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The publication was adequately well described and reported.

Roth JA (2006) Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biol Res* **39:**45-57.

Abstract: This review attempts to summarize and clarify our basic knowledge as to the various factors that potentially influence the risks imposed from chronic exposure to high atmospheric levels of manganese (Mn). The studies describe the interrelationship of the different systems in the body that regulate Mn homeostasis by characterizing specific, biological components involved in its systemic and cellular uptake and its elimination from the body. A syndrome known as manganism occurs when individuals are exposed chronically to high levels of Mn, consisting of reduced response speed, intellectual deficits, mood changes, and compulsive behaviors in the initial stages of the disorder to more prominent and irreversible extrapyramidal dysfunction resembling Parkinson's disease upon protracted exposure. Mn intoxication is most often associated with occupations in which abnormally high atmospheric concentrations prevail, such as in welding and mining. There are three potentially important routes by which Mn in inspired air can gain access the body to: 1) direct uptake into the CNS via uptake into the olfactory or trigeminal presynaptic nerve endings located in the nasal mucosa and the subsequent retrograde axonal transport directly into the CNS; 2) transport across the pulmonary epithelial lining and its subsequent deposition into lymph or blood; and/or 3) mucocilliary elevator clearance from the lung and the subsequent ingestion of the metal in the gastrointestinal tract. Each of these processes and their overall contribution to the uptake of Mn in the body is discussed in this review as well as a description of the various mechanisms that have been proposed for the transport of Mn across the bloodbrain barrier which include both a transferrin-dependent and a transferrin-independent process that may involve store-operated Ca channels.

Evaluation: Klimisch Code 5. Review article.

Salehi F, Krewski D, Mergler D, Normandin L, Kennedy G, Philippe S and Zayed J (2003) Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. *Toxicol Appl Pharmacol* **191:**264-271.

Abstract: Methylcyclopentadienyl manganese tricarbonyl (MMT) is an organic manganese (Mn) compound added to unleaded gasoline in Canada. The primary combustion products of MMT are Mn phosphate, Mn sulfate, and a Mn phosphate/Mn sulfate mixture. Concerns have been raised that the combustion products of MMT containing Mn could be neurotoxic, even at low levels of exposure. The objective of this study is to investigate exposure-response relationships for bioaccumulation and locomotor effects following subchronic inhalation exposure to a mixture of manganese phosphates/sulfate mixture. A control group and three groups of 30 male Sprague-Dawley rats were exposed in inhalation chambers for a period of 13 weeks, 5 days per week, 6 h a day. Exposure concentrations were 3000, 300, and 30 microg/m(3). At the end of the exposure period, locomotor activity and resting time tests were conducted for 36 h using a computerized autotrack system. Rats were then euthanized by exsanguination and Mn concentrations in different tissues (liver, lung, testis, and kidney) and blood and brain (caudate putamen, globus pallidus, olfactory bulb, frontal cortex, and cerebellum) were determined by neutron activation analysis. Increased manganese concentrations were observed in blood, kidney, lung, testis, and in all brain sections in the highest exposure group. Mn in the lung and in the olfactory bulb were dose dependent. Our data indicate that the olfactory bulb accumulated more Mn than other brain regions following inhalation exposure. Locomotor activity was increased at 3000 microg/m(3), but no difference was observed in resting time among the exposed groups. At the end of the experiment, rats exposed to 300 and 3000 microg/m(3) exhibited significantly decreased body weight in comparison with the control group. Biochemical profiles also revealed some significant differences in certain parameters, specifically alkaline phospatase, urea, and chlorate.

Evaluation: Klimisch Code 5. This appears to be the same study data as used in the Normandin 2004 publication (High dose of manganese phosphate/sulphate mixture). There are a few minor differences

that appear to be due to the number of animals per group and this publication also reports lower dose group data.

Sanchez DJ, Gomez M, Domingo JL, Llobet JM and Corbella J (1995) Relative efficacy of chelating agents on excretion and tissue distribution of manganese in mice. *J Appl Toxicol* **15:**285-288.

Abstract: The effect of repeated parenteral administration of a number of structurally diverse chelating agents on the excretion and tissue distribution of manganese was assessed in mice following 4 weeks of manganese exposure. Males Swiss mice received s.c. injections of manganese(II) chloride tetrahydrate (8.9 mg Mn kg-1 body wt.) for 4 weeks (5 days per week). After the end of this exposure period, cyclohexanediaminetetraacetic acid (CDTA), ethyleneglycol-bis-(beta-aminoethylether)-N,N-tetraacetic acid (EGTA), N-(2-hydroxyethyl)ethylenediamine triacetic acid (HEDTA), isonicotinyl hydrazine (INH), L-dopa, sodium 4.5-dihydroxy-1.3-benzenedisulphonate (Tiron), p-aminosalicylic acid (PAS) or 0.9% saline (control group) were given i.p. for five consecutive days. The doses of the chelators were approximately equal to one-eighth of their respective LD50 values. Urine and faeces were daily collected for 5 days. Twenty-four hours after the final chelator injection, mice were killed and manganese concentrations were determined in various tissues. Although CDTA, EGTA and HEDTA significantly enhanced the elimination of manganese into urine, none of the chelators increased faecal excretion. Tissue concentrations of manganese were significantly reduced only by CDTA. According to these results, among the compounds tested only CDTA would mobilize effectively manganese in manganese-loaded mice.

Evaluation: Klimisch Code 5. Non-pivotal.

Sandstrom B (1992) Dose dependence of zinc and manganese absorption in man. *Proc Nutr Soc* **51:**211-218.

Evaluation: Klimisch Code 5. Brief review on existing data for manganese absorption.

Sandstrom B, Davidsson L, Bosaeus I, Eriksson R and Alpsten M (1990) Selenium status and absorption of zinc (65Zn), selenium (75Se) and manganese (54Mn) in patients with short bowel syndrome. *Eur J Clin Nutr* **44:**697-703.

Abstract: Selenium level and activity of glutathione peroxidase in plasma were studied in seven patients with extensive short bowel resection due to Crohn's disease, before and during 27-54 weeks of intake of a vitamin and trace element supplement containing 50 micrograms of selenium as sodium selenite. Initial levels of selenium were normal in all except one of the patients. The supplementation had no or minor effects on plasma selenium levels and glutathione peroxidase activity. The absorption of zinc, manganese and selenium was measured with a radionuclide technique before and/or after the supplementation period in five of the patients. The absorption of zinc and manganese was similar to that observed earlier in healthy subjects, while the absorption of selenium was significantly lower. The results indicate that a higher selenium intake or a different form of selenium is needed in these patients to compensate for the impaired bowel function.

Evaluation: Klimisch Code 5. Non-pivotal.

Sandstrom B, Davidsson L, Cederblad A, Eriksson R and Lonnerdal B (1986) Manganese absorption and metabolism in man. *Acta Pharmacol Toxicol (Copenh)* **59 Suppl 7:**60-62.

Evaluation: Klimisch Code 4. Very brief description of methodology.

Sandstrom B, Davidsson L, Eriksson R, Alpsten M and Bogentoft C (1987) Retention of selenium (75Se), Zinc (65Zn) and manganese (54Mn) in humans after intake of a labelled vitamin and mineral supplement. *J Trace Elem Electrolytes Health Dis* **1:**33-38.

Abstract: The whole body retention of 75Se, 65Zn, and 54Mn after intake of a labelled vitamin and mineral supplement was followed in 12 healthy volunteers. The supplement had a vitamin and mineral content according to recommended dietary allowances or the so-called "safe and adequate levels" for trace elements, including 15 mg of zinc as zinc citrate, 50 micrograms of selenium as sodium selenite and 2.5 mg of manganese as manganese sulphate. The supplement was taken either in the fasting state or together with a light meal. Retention day 14 was 48 +/- 6%, 33 +/- 6% and 5 +/- 2% (mean +/- SD) for selenium, zinc and manganese, respectively, when the supplement was taken fasting and 45 +/- 3%, 8 +/- 1% and 1.0 +/- 0.2% when it was taken with food. During day 1-14, 27%, 1% and less than 0.01% of the administered selenium, zinc and manganese radionuclides, respectively, were excreted in the urine. Based on the rate of turnover of the radionuclides and the urinary losses of 75Se, the absorption of selenium, zinc and manganese from the supplement was estimated to be 89 +/- 5%, 38 +/- 7%, 9 +/- 3% (mean +/- SD) in the fasting state and 87 +/- 4%, 10 +/- 2% and 2 +/- 1% with food. These results indicate that when a supplement is taken with food the minerals are absorbed and metabolized in the same way as are native minerals in food. When the supplement is taken in the fasting state, the absorption of zinc and manganese can be substantially higher.

Evaluation: Klimisch Code 2. Reasonably well documented considering age of study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The study design would have been better if each subject had been used as own control (cross-over style).

Sato I, Matsusaka N, Kobayashi H and Nishimura Y (1996) Effects of dietary manganese contents on 54Mn metabolism in mice. *J Radiat Res (Tokyo)* **37:**125-132.

Abstract: Several parameters of 54Mn metabolism were noted in mice maintained on diets with manganese contents of 80 to 8000 mg/kg. Excretion of 54Mn was promoted as the dietary manganese contents increased. Clearance of 54Mn from the liver, kidneys, pancreas, and spleen was markedly accelerated by feeding mice a high-manganese diet, but clearance from the muscles, femurs, and brain was relatively insensitive to the dietary manganese. Manganese concentrations in the tissues were regulated homeostatically upto the dietary manganese content of 2400 mg/kg, but marked accumulations of manganese occurred when mice were given 8000 mg/kg diet. No toxic symptoms were found up to the 2400 mg/kg diet, but consumption of the 8000 mg/kg diet was less than for other diets. These results suggest that an oral intake of excess manganese is effective for promoting the excretion of 54Mn from a body contaminated with this isotope.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. However, relatively brief methodology detail and study could have benefited from statistical significance evaluation.

Schafer DF, Stephenson DV, Barak AJ and Sorrell MF (1974) Effects of ethanol on the transport of manganese by small intestine of the rat. *J Nutr* **104:**101-104.

Evaluation: Klimisch Code 3. An old study, methodology not very detailed. Study design very limited.

Scheuhammer AM and Cherian MG (1981) The influence of manganese on the distribution of essential trace elements. I. Regional distribution of Mn, Na, K, Mg, Zn, Fe, and Cu in rat brain after chronic Mn exposure. *Toxicol Appl Pharmacol* **61:**227-233.

Evaluation: Klimisch Code 4. Very brief methodology.

Schlesinger RB (1996) *Deposition and clearance of inhaled particles. In Concepts in inhalation toxicology*. Taylor & Francis, Philadelphia.

Evaluation: Klimisch Code 5.

Schwartz R, Apgar BJ and Wien EM (1986) Apparent absorption and retention of Ca, Cu, Mg, Mn, and Zn from a diet containing bran. *Am J Clin Nutr* **43:**444-455.

Abstract: To establish conditions for comparisons of mineral bioavailability from plant sources, seven male subjects consumed a constant diet containing bran fiber and phytate. Absorption and retention of Ca, Cu, Mg, Mn, and Zn were measured for 7-day periods through wk 2-7. Intakes of Mg, Mn, and Zn significantly exceeded the RDA; Ca and Cu intakes were only slightly in excess of RDA. All mineral retentions fluctuated from week to week but only Mg and Mn showed a consistent positive trend over time. Phytate excretions showed characteristic individual patterns, but did not appear to change with time. In contrast to previous observations fecal recovery of polyethyleneglycol (PEG) (MW = 4000) was consistently lower than recovery of simultaneously ingested Cr. Only five of the seven subjects returned close to 100% of Cr within 7 days. It was concluded that at least 4 wk were needed for adaptation in investigations involving more than one mineral when the experimental diet is adequate in the nutrients under investigation, that measurements of responses to treatment required 2-3 wk each, and that successive isotopically labeled test meals may overlap if they are spaced at 7-day intervals.

Evaluation: Klimisch Code 3. The method of estimating % absorption of manganese appears to be from an overall mass balance of the intake and recovery in faeces of manganese. For the first 2-week period this was negative, -2.0±4.9%, and the second 2-week period, positive 7.6±6.3%. Although no variation was quoted for the analysis of manganese, other metals in the study were approximately ±10%. Therefore this estimate of absorption of manganese is more likely to be measuring the method recovery of manganese rather than manganese absorption and therefore is not considered reliable.

Shukla GS and Chandra SV (1981) Manganese toxicity: lipid peroxidation in rat brain. *Acta Pharmacol Toxicol (Copenh)* **48:**95-100.

Abstract: Albino rats were given intraperitoneally manganese chloride (Mn2+, 4mg/kg) daily for a period of 30 days. Manganese significantly inhibited the lipid peroxidation potential of treated rat brain without altering the contents of iron and ascorbic acid, the two prooxidant factors. In vitro lipid peroxidation studies in the fresh and heated brain homogenates showed almost a non-enzymatic mechanism of

inhibition by this metal ion. 30 micrometers Mn2+ concentrations completely inhibited the formation of malonaldehyde (MDA) at 3 hours of incubation. Iron was found to reverse, to some extent, the effect of manganese on in vitro lipid peroxide formation in the mitochondrial fraction of brain and at concentrations of 5 micrometers Fe2+ the amount of MDA formed is comparable to that observed with 1 micro meter Fe2+ in the mitochondrial fraction without manganese. These observations suggest that the central nervous system toxicity of manganese may not be associated with accelerated in vivo lipid peroxidation. However, the mechanism of iron induced reversal on in vitro inhibition of lipid peroxidation by manganese is not understood, at present.

Evaluation: Klimisch Code 5. Non-pivotal.

Shukla GS and Chandra SV (1982) Effects of manganese on carbohydrate metabolism and mitochondrial enzymes in rats. *Acta Pharmacol Toxicol (Copenh)* **51:**209-216.

Abstract: The effect of daily intraperitoneal administration of Mn2+(4 mg/kg) was investigated on the metabolism of carbohydrates and certain enzymes involved in the oxidation of glucose in the rat liver and blood at the intervals of 30, 60 and 90 days after exposure. Mn2+ had no effect on the contents of blood reducing sugars and proteins, however the levels of pyruvic and lactic acids were reduced at 60 and 90 days after the metal treatment. The contents of liver glycogen and proteins remained unaffected while pyruvic acid content was decreased in Mn2+ treated rat liver throughout the experimental period. The activities of glycogen phosphorylase and lactate dehydrogenase decreased while that of phosphoglucoisomerase and glucose-6-phosphatase increased in the post mitochondrial supernatant at 60 and 90 days of Mn2+ exposure. The levels of hexokinase decreased and FDP-aldolase and fructose-1, 6-diphosphatase increased throughout the experimental period. The magnitude of alteration was found to be greater with the increase in the duration of Mn2+ treatment. Several of the mitochondrial enzymes in the liver were inhibited in the manganese exposed rats which may be responsible to inhibit the rate of dehydrogenation of Kreb cycle's intermediates along with the linked respiratory chain and eventually oxidation in the rat liver.

Evaluation: Klimisch Code 5. Non-pivotal.

Shukla GS and Chandra SV (1987) Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. *Arch Environ Contam Toxicol* **16:**303-310.

Evaluation: Klimisch Code 3. Very brief methodology, for example the time of sacrifice after the last dose was not detailed. Manganese chloride was only administered by repeated ip dosing whereas lead acetate was administered through drinking water.

Sierra P, Chakrabarti S, Tounkara R, Loranger S, Kennedy G and Zayed J (1998) Bioaccumulation of manganese and its toxicity in feral pigeons (Columba livia) exposed to manganese oxide dust (Mn3O4). *Environ Res* **79:**94-101.

Abstract: Manganese tetroxide (Mn3O4) is a product from the combustion of methylcyclopentadienyl manganese tricarbonyl. Exposure to high levels of manganese can lead to serious health effects especially to the central nervous and respiratory systems. Very few studies on the effects of long-term low level exposure to Mn3O4 have been reported. The present study was therefore conducted to examine the bioaccumulation and toxicity of manganese in various organs of feral pigeons (Columba livia) when exposed to low levels of Mn3O4 via inhalation and hence to find any possible relationship between these two parameters. A total of 22 pigeons was exposed to 239 micrograms/m3 of manganese for 7 h/day, 5 days/week for 5, 9, and 13 consecutive weeks. Manganese concentrations in various tissues, e.g., brain (mesencephalon), lung, liver, intestine, pancreas, kidney, muscle, bone, and whole blood, were measured by neutron activation analysis. Various biochemical parameters in blood, e.g., hematocrit, total proteins, glucose, uric acid, alanine aminotransferase, total iron, blood urea nitrogen and triglycerides, were also measured. Manganese concentrations in brain, lung, and bone were significantly higher in Mn3O4-exposed pigeons (0.59, 0.58, and 3.02 micrograms wet tissue, respectively) than in the control group (0.46, 0.19, 1.74 micrograms/g wet tissue, respectively). However, except for total proteins such exposure did not produce any changes in various biochemical parameters which were within the normal values. Thus these results have shown that, despite significant bioaccumulation of manganese in some tissues, no significant toxic effects could be seen.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. Adequate description of methodology and results. Only a single dose level used at much lower than occupational exposure limits.

Singh J, Kaw JL and Zaidi SH (1977) Early biochemical response of pulmonary tissue to manganese dioxide. *Toxicology* **8:**177-184.

Abstract: The biochemical response of pulmonary tissue to MnO2 dust burden of 30 days duration was studied in rats. The activities of enzymes from isolated fractions of rat lungs were not significantly altered, even though manganese content was increased significantly in tissues remote from lungs, indicating translocation of the dust from its intrapulmonary location.

Evaluation: Klimisch Code 3. Very brief methodology. Even though the text stated that the lungs from treated rats retained as high as 12-fold the normal lung content of manganese, the table of results showed: control lungs 33.2±5.2 manganese/g and treated lungs 30.3±5.1 manganese/g which contradicts the text. As such, the reliability of the data is compromised.

Singh PP and Junnarkar AY (1991) Behavioural and toxic profile of some essential trace metal salts in mice and rats. *Indian Journal of Pharmacology* **23:**153-159.

Evaluation: Klimisch Code 5. Non-pivotal.

Slikker W, Jr., Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Woodrow Setzer R, Swenberg JA and Wallace K (2004) Dose-dependent transitions in mechanisms of toxicity. *Toxicol Appl Pharmacol* **201:**203-225.

Abstract: Scientists and decision makers from all sectors agree that risk assessments should be based on the best available science. Several years ago, the Health and Environmental Sciences Institute (HESI), a global branch of the International Life Sciences Institute (ILSI), identified the need for better scientific understanding of dose-dependent transitions in mechanisms of toxicity as one avenue by which the best and latest science can be integrated into the decision making process. In July 2001, the HESI Project Committee on Dose-Dependent Transitions in Mechanisms of Toxicity established a group of academic, government, and industry scientists to engage in active technical discourse on the issue of dose-dependent transitions in mechanisms of toxicity. Over the next 18 months, case studies were examined. These case studies included acetaminophen, butadiene, ethylene glycol, formaldehyde, manganese, methylene chloride, the peroxisome proliferator-activated receptor, progesterone/hydroxyflutamide, propylene oxide, vinyl acetate, vinyl chloride, vinylidene chloride, and zinc (Slikker, W., Jr., Andersen, M.E., Bogdanffy, M.S., Bus, J.S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrer, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Setzer, R.W., Swenberg, J.A., Wallace, K., 2004. Dose-dependent transitions in mechanisms of toxicity: case studies. Toxicol. Appl. Pharmacol. 201(3), 226-294 (this issue)). The HESI Project Committee sponsored two technical workshops in 2003. The first of these workshops took place on February 12-13, 2003, and was co-sponsored by the Agency for Toxic Substances and Disease Registry, the American Chemistry Council, the National Institute of Environmental Health Sciences, the Society of Toxicology, and the U.S. Environmental Protection Agency. Additional support was provided by Health Canada. Invited experts from government, academia, and industry provided scientific perspectives and recommendations at the workshop. The purpose of the workshop was to examine approaches to dose-response analysis, learn from the case study examples, and gather feedback from invited participants on the impact of dose-dependent transitions on the risk assessment process. The second forum consisted of a workshop in March 2003 at the Society of Toxicology Annual Meeting in Salt Lake City, UT. This paper addresses the issues discussed at both workshops, and presents the consensus conclusions drawn by expert participants.

Evaluation: Klimisch Code 5. Non-pivotal.

Smialowicz RJ, Luebke RW, Rogers RR, Riddle MM and Rowe DG (1985) Manganese chloride enhances natural cell-mediated immune effector cell function: effects on macrophages. *Immunopharmacology* **9:**1-11.

Abstract: A single intramuscular injection of MnC12 in mice caused an increase in macrophage functional activity. Spleen cell antibody-dependent cell-mediated cytotoxicity against both chicken erythrocytes and P815 tumor cell targets was enhanced 24 h following a single injection of MnC12. Enhanced antibody-dependent cell-mediated cytotoxicity activity following MnC12 treatment was not associated with a change in spleen cellularities compared with saline-injected mice. Resident peritoneal macrophages from mice injected intramuscularly with MnC12 displayed enhanced phagocytic activity for chicken erythrocytes in the presence or absence of opsonizing antibody. Enhanced cytolytic activity against P815 mastocytoma target cells and enhanced cytostatic activity against MBL-2 lymphoma target cells was also observed for nonelicited resident peritoneal macrophages from mice injected intramuscularly with MnC12. There were no differences in the cellularity or relative number of adherent cells obtained from the peritoneal cavity of saline or MnC12-injected mice. These enhanced macrophage functions were associated with the induction of increased interferon levels in mice injected with MnC12.

Evaluation: Klimisch Code 5. Non-pivotal.

Smialowicz RJ, Riddle MM, Rogers RR, Luebke RW and Burleson GR (1988) Enhancement of natural killer cell activity and interferon production by manganese in young mice. *Immunopharmacol Immunotoxicol* **10:**93-107.

Abstract: The effect that MnCl2 has on murine splenic natural killer (NK) cell activity was investigated in infant (10 days old), pre-weanling (18 days old) and weanling (24 days old) C57BL/6J mice. A single intraperitoneal injection of 10, 20 or 40 micrograms MnCl2/g body weight caused a significant enhancement in NK activity, as determined by the in vitro 51Cr release assay. Comparable enhancement of NK activity was observed for age-matched mice injected intraperitoneally with polyinosinic polycytidylic acid (Poly I:C). Both MnCl2 and Poly I:C caused elevations in serum interferon levels. Time-course studies revealed that interferon levels returned to normal within 48 hours following injection with either MnCl2 or Poly I:C; however enhanced NK activity persisted for up to 48 hours in Poly I:C-injected mice and 72 hours in MnCl2-injected mice. The administration of rabbit anti-asialo GMl to MnCl2-injected mice completely abrogated the enhanced NK activity. In addition, the injection of rabbit anti-mouse interferon alpha, beta but not gamma completely abrogated the enhanced NK activity. In addition, the injection of rabbit anti-mouse interferon alpha, beta but not gamma completely abrogated the enhancement of NK activity by MnCl2 and to a lesser extent the enhancement of NK activity by Poly I:C. These results indicate that despite low levels of NK activity in pre-weanling mice, MnCl2 is capable of enhancing this activity by 8-9 fold. Furthermore, Mn-enhanced NK activity in these young mice appears to be mediated by the production of interferon alpha, beta.

Evaluation: Klimisch Code 5. Non-pivotal.

Smialowicz RJ, Rogers RR, Riddle MM, Luebke RW, Fogelson LD and Rowe DG (1987) Effects of manganese, calcium, magnesium, and zinc on nickel-induced suppression of murine natural killer cell activity. *J Toxicol Environ Health* **20:**67-80.

Abstract: The effects that divalent metals have on nickel-induced suppression of natural killer (NK) cell activity were studied in mice. Male CBA/J mice were given a single intramuscular injection of metal salt on a body weight basis. The metal doses used were the following: nickel chloride, 4.5-36 micrograms/g; manganese chloride, 20-80 micrograms/g. Twenty-four hours after metal injection, splenic NK cell activity was assessed using a 51Cr-release assay. Ni significantly (p less than 0.01) suppressed NK activity, while Mn significantly (p less than 0.01) enhanced NK activity. No alteration in NK activity was observed in mice injected with Mg, Ca, or Zn. Since these divalent metals have been shown to have antagonistic effects on Ni-induced carcinogenicity and toxicity, they were used in combination with Ni to determine if such antagonisms exist for NK cell activity. The injection of Ni and Mn in combination at a single site resulted in the enhancement of NK activity, although this enhancement was at a level below that observed following the injection of Mn alone. Injection of Mg, Zn, or Ca in combination with Ni did not affect NK activity compared to saline controls. In contrast, the injection of Ni in one thigh followed immediately by Mn, Mg, Ca, or Zn into the other thigh resulted in significant suppression of NK activity for all metals compared with saline controls. An interesting finding was that the injection of Ni followed immediately by Mn into the opposite thigh resulted in even greater reductions in NK activity than Ni alone. Suppression of NK activity by Ni and Mn injected at separate sites was not seen when Mn injection preceded Ni injection by 1 h. These data indicate that both the divalent metal and the timing of its injection relative to Ni injection are critical for altering Ni-induced suppression of NK cell activity.

Evaluation: Klimisch Code 5. Non-pivotal.

Smialowicz RJ, Rogers RR, Riddle MM, Luebke RW, Rowe DG and Garner RJ (1984) Manganese chloride enhances murine cell-mediated cytotoxicity: effects on natural killer cells. *J Immunopharmacol* **6:**1-23.

Abstract: Natural killer (NK) cell activity of mice given a single injection of manganese chloride (MnCl2) was significantly enhanced as measured in a 4-hr in vitro 51Cr release assay. Enhanced activity persisted for several days after injection. This cytotoxic activity was associated with nonadherent spleen cells and was completely eliminated by injecting MnCl2-treated mice with anti-asialo GM1 serum. Manganese-enhanced natural cytotoxicity was observed in several mouse strains with differing NK cell reactivity (CBA/J, C57BL/6, A/J, C3H/HeJ, and C57BL/6 beige mice) and with several tumor target cells with differing sensitivity to NK cytolysis (YAC-1, RBL-5, EL-4, and P815). The growth of B16-F10 melanoma lung tumors was inhibited in mice injected with MnCl2 one day before tumor challenge. Manganese chloride enhancement of NK cell activity appeared to be mediated by interferon (IFN). Low levels of IFN were detected in the serum of mice as early as 4 hr after MnCl2 injection. Rabbit anti-mouse IFN alpha, beta but not anti-mouse IFN gamma completely eliminated the MnCl2-enhanced NK cell activity in the spleens of mice. The observed enhancement of NK cell activity by MnCl2 is similar to that reported for more complex molecules that act by inducing IFN production.

Evaluation: Klimisch Code 5. Non-pivotal.

Spencer H, Asmussen CR, Holtzman RB and Kramer L (1979) Metabolic balances of cadmium, copper, manganese, and zinc in man. *Am J Clin Nutr* **32:**1867-1875.

Abstract: Balance studies of cadmium, copper, manganese, and zine were carried out under constant dietary conditions in eight adult males during two calcium intake levels of 200 and 800 mg/day and in an additional single case during a calcium intake of 1500 mg/day. The dietary content and the excretions of these elements in urine and stool were determined. The mean dietary content of cadmium was 32.9 micrograms/day, of copper 1020 micrograms/day, of manganese 2130 micrograms/day, and of zinc 12.4 mg/day. The ratio of the fecal/urinary cadmium excretion was approximately 1.5 and the main pathway of excretion of the other three elements was via the intestine, while the urinary excretions were very low. The different trace element balances were either slightly negative or in equilibrium, except that the zinc balances was positive in 50% of the cases. All balances should be considered maximal values, as the losses in sweat were not determined. The calcium intake level had little effect on the excretion and retention of these trace elements.

Evaluation: Klimisch Code 5. Non-pivotal.

St-Pierre A, Normandin L, Carrier G, Kennedy G, Butterworth R and Zayed J (2001) Bioaccumulation and locomotor effect of manganese dust in rats. *Inhal Toxicol* **13:**623-632.

Abstract: The primary goal of this study is to determine the effects of Mn exposure via inhalation. The bioaccumulation of Mn in different organs and tissues, the alteration of biochemical parameters, and the locomotor activity were assessed. A group of 26 male Sprague-Dawley rats (E) were exposed to 3750 microg/m(3) of Mn dust for 6 h/day, 5 days/wk for 13 consecutive weeks and compared to a control group of 12 rats (C) exposed to 4 microg/m(3). After exposure, neurological evaluation was carried out for 36 h (a night-day-night cycle) using a computerized autotrack system. Rats were then sacrificed by exsanguination, and Mn content in organs and tissues was determined by neutron activation analysis. Mn concentrations in lung, putamen, and cerebellum were significantly higher in E than in C (0.30 vs. 0.17, 0.89 vs. 0.44, 0.63 vs. 0.48 ppm; $p < .01$), as well as in the kidney, frontal cortex, and globus pallidus (1.15 vs. 0.96, 0.84 vs. 0.47, 1.28 vs. 0.55 ppm; $p < .05$). Potassium concentration was significantly lower in E than in C (5.11 vs. 5.79 mmol/L; $p < .05$), as was alkaline phosphatase (106.9 vs. 129.6 U/L; $p < .01$). Locomotor activity indicated higher distance covered in the first 12-h period for E (45 383 vs. 36 098 cm; $p < .05$) and lower resting time in the last 12-h period for E (36 326 vs. 37 393 s; $p < .05$). This study is the first of several ongoing studies in our laboratory that address health concerns associated with inhalation exposure to different Mn species and to different levels of exposure.

Evaluation: Klimisch Code 5. This appears to be the same study data as used in the Normandin 2004 publication (manganese metal data). There are a few minor differences that appear to be due to the number of animals per group. As such this publication was not considered to uniquely and unequivocally contain data that could inform the toxicokinetic assessment of manganese.

Stauber JL, Florence TM and Webster WS (1987) The use of scalp hair to monitor manganese in aborigines from Groote Eylandt. *Neurotoxicology* **8:**431-435.

Evaluation: Klimisch Code 4. Methodology was not very comprehensive. Highlighted the importance of distinguishing between endogenous and exogenous sources of manganese in hair when interpreting data.

Sung JH, Kim CY, Yang SO, Khang HS, Cheong HK, Lee JS, Song CW, Park JD, Han JH, Chung YH, Choi BS, Kwon IH, Cho MH and Yu IJ (2007) Changes in blood manganese concentration and MRI t1 relaxation time during 180 days of stainless steel welding-fume exposure in cynomolgus monkeys. *Inhal Toxicol* **19:**47-55.

Abstract: Welders are at risk of being exposed to high concentrations of welding fumes and developing pneumoconiosis or other welding-fume exposure-related diseases. Among such diseases, manganism resulting from welding-fume exposure remains a controversial issue, as although the movement of manganese into specific brain regions has been established, the similar movement of manganese presented with other metals, such as welding fumes, has not been clearly demonstrated as being similar to that of manganese alone. Meanwhile, the competition between Mn and iron for iron transporters, such as transferrin and DMT-1, to the brain has also been implicated in the welding-fume exposure. Thus, the increased signal intensities in the basal ganglia, including the globus pallidus and subcortical frontal white matter, based on T1-weighted magnetic resonances in welders, require further examination as regards the correspondence with an increased manganese concentration. Accordingly, to investigate the movement of manganese after welding-fume exposure, 6 cynomolgus monkeys were acclimated for 1 mo and assigned to 3 dose groups: unexposed, low dose of (total suspended

particulate [TSP] 31 mg/m3, 0.9 mg/m3 of Mn), and high dose of total suspended particulate (62 mg/m3 TSP, 1.95 mg/m3 of Mn). The primates were exposed to manual metal-arc stainless steel (MMA-SS) welding fumes for 2 h/day in an inhalation chamber system equipped with an automatic fume generator for 6 mo. Magnetic resonance imaging (MRI) studies of the basal ganglia were conducted before the initiation of exposure and thereafter every month. During the exposure, the blood chemistry was monitored every 2 wk and the concentrations of metal components in the blood were measured every 2 wk and compared with ambient manganese concentrations. The manganese concentrations in the blood did not show any significant increase until after 2 mo of exposure, and then reached a plateau after 90 days of exposure, showing that an exposure period of at least 60 days was required to build up the blood Mn concentration. Furthermore, as the blood Mn concentration continued to build, a continued decrease in the MRI T1 relaxation time in the basal ganglia was also detected. These data suggested that prolonged inhalation of welding fumes induces a high MRI T1 signal intensity with an elevation of the blood manganese level. The presence of a certain amount of iron or other metals, such as Cr and Ni, in the inhaled welding fumes via inhalation was not found to have a significant effect on the uptake of Mn into the brain or the induction of a high MRI T1 signal intensity.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. There is a numerical mistake in Table 2 (the value 21.19% is incorrect). The treatment related results from the low dose group, both blood manganese levels and MRI T1 relaxation times appear to higher (blood Mn) and shorter (MRI T1) than the high dose group, possibly because the two dose levels were too close together.

Tapin D, Kennedy G, Lambert J and Zayed J (2006) Bioaccumulation and locomotor effects of manganese sulfate in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. *Toxicol Appl Pharmacol* **211:**166-174.

Abstract: Methylcyclopentadienyl manganese tricarbonyl (MMT) is an organic compound that was introduced as an anti-knock additive to replace lead in unleaded fuel. The combustion of MMT results in the emission of fine Mn particulates mainly in the form of manganese sulfate and manganese phosphate. The objective of this study is to determine the effects of subchronic exposure to Mn sulfate in different tissues, on locomotor activity, on neuropathology, and on blood serum biochemical parameters. A control group and three groups of 30 male Sprague-Dawley rats were exposed 6-h/day, 5 days/week for 13 consecutive weeks at 30, 300, or 3,000 microg/m(3) Mn sulfate. Locomotor activity was measured during 36 h using an Auto-Track System. Blood and the following tissues were collected and analyzed for manganese content by neutron activation analysis: olfactory bulb, globus pallidus, caudate/putamen, cerebellum, frontal cortex, liver, lung, testis, and kidney. Neuronal cell counts were obtained for the caudate/putamen and the globus pallidus and clinical biochemistry was assessed. Manganese concentrations were increased in blood, kidney, lung, and testis and in all brain regions in the 3,000 microg/m(3) exposure group. Significant differences were also noted in the 300 microg/m(3) exposure group. Neuronal cell counts for the globus pallidus were significantly different between the two highest exposed groups and the controls. Locomotor activity for all exposure concentrations and resting time for the middle and highest concentrations for the two night resting periods were significantly increased. Total ambulatory count was decreased significantly for all exposure concentrations. Biochemical profiles also presented significant differences. No body weight loss was observed between all groups. These results suggest that neurotoxicity could occur at low exposure levels of Mn sulfate, one of the main combustion products of MMT.

Evaluation: Klimisch Code 2. Generally well documented. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. The tissue manganese levels were determined 42 hours after the end of exposure which means that it is not possible to directly compare this data to other studies. The manganese levels in the blood, both control and treated rats, was over 10-fold less than seen serum/plasma from rats in other similar studies. No plausible explanation was given for this.

Taylor PA and Price JD (1982) Acute manganese intoxication and pancreatitis in a patient treated with a contaminated dialysate. *Can Med Assoc J* **126:**503-505.

Evaluation: Klimisch Code 2. A good level of detail for a case report. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Teeguarden JG, Dorman DC, Covington TR, Clewell HJ, 3rd and Andersen ME (2007a) Pharmacokinetic modeling of manganese. I. Dose dependencies of uptake and elimination. *J Toxicol Environ Health A* **70:**1493-1504.

Abstract: Homeostatic mechanisms controlling uptake, storage, and elimination of dietary manganese (Mn) afford protection against fluctuations in tissue manganese (Mn) levels. Homeostatic control of inhaled Mn is less well understood, but important in assessing likely risks of Mn inhalation. Two compartmental kinetic models were used to characterize the influence of Mn exposure level and route (oral, inhalation) on uptake, elimination, and transport of Mn. The models were fitted to or used to interpret data from five whole-body Mn elimination studies: one dietary Mn balance study, two biliary elimination studies, and one acute and one chronic. As dietary Mn concentrations increased from low sufficiency (1.5 ppm) to sufficiency (20 ppm), control of Mn uptake shifts from the intestine (principally) to more proportional control by both intestinal tissues and liver. Using a two-compartment distribution model, the increased elimination of 54Mn tracer doses in response to increases in dietary Mn (rats and mice) or inhaled Mn (rats) resulted from elevation in Mn elimination rate constants rather than changes in intercompartmental transfer rate constants between a central compartment and deep compartment. The pharmacokinetic (PK) analysis also indicated differential control of absorption in single gavage oral dose studies versus continuous high oral doses in the feed. The gavage study indicated increased elimination rate constants, and the chronic study showed reduced rate constants for absorption. These dose dependencies in uptake and elimination are necessary inputs for comprehensive PK models guiding human health risk assessments with Mn.

Evaluation: Klimisch Code 5. Pharmacokinetic Model

Teeguarden JG, Dorman DC, Nong A, Covington TR, Clewell HJ, 3rd and Andersen ME (2007b) Pharmacokinetic modeling of manganese. II. Hepatic processing after ingestion and inhalation. *J Toxicol Environ Health A* **70:**1505-1514.

Abstract: Current concerns regarding inhalation exposure to Mn, a component from oxidation of the gasoline antiknock agent MMT, have stimulated interest in developing kinetic tools for describing the inhalation and combined inhalation/oral route kinetics of Mn. Kinetic approaches were integrated kinetic for (1) bulk tissue Mn kinetics and (2) hepato-intestinal control of oral-route Mn uptake into a integrated model structure connecting systemic and oral Mn. Linkages were developed between the hepato-intestinal and systemic tissues in order to evaluate differences in hepatic processing of orally absorbed Mn and systemic Mn. The integrated, unified model described the uptake, net absorption, and elimination of ingested Mn and the elimination kinetics of i.v. administered (systemic) Mn by treating Mn arriving at the liver from systemic versus portal blood differently. Hepatic extraction of orally absorbed Mn in rats predicted through simulation of the oral uptake data was 19, 54, and 78% at dietary exposures of 1.5, 11.2, and 100 ppm, respectively. In contrast, hepatic extraction of systemic Mn predicted through simulation of elimination kinetics i.v. tracer Mn was much less, 0.004, 0.005, or 0.009% at dietary levels of 2, 10, and 100 ppm, respectively. These differences in hepatic processing of blood Mn derived from different dose routes need to be accounted for in more complete PK models for Mn that are intended to support human health risk assessments.

Evaluation: Klimisch Code 5. Pharmacokinetic Model.

Teeguarden JG, Gearhart J, Clewell HJ, 3rd, Covington TR, Nong A and Andersen ME (2007c) Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics. *J Toxicol Environ Health A* **70:**1515-1526.

Abstract: Manganese (Mn), an essential metal nutrient, produces neurotoxicity in workers exposed chronically to high concentrations of Mn-containing dusts. Our long-term goal was to develop a physiologically based pharmacokinetic (PBPK) model to support health risk assessments for Mn. A PK model that accounts for Mn-tracer kinetics and steady-state tissue Mn in rats on normal diets (about 45 ppm Mn) is described. The focus on normal dietary intakes avoids inclusion of dose-dependent processes that maintain Mn homeostasis at higher dose rates. Data used for model development were obtained from published literature. The model represents six tissues: brain, respiratory tract, liver, kidneys, bone, and muscle. Each of these has a shallow tissue pool in rapid equilibration with blood and a deep tissue store, connected to the shallow pool by transfer rate constants. Intraperitoneal (i.p.) tracer Mn is absorbed into systemic blood and equilibrated with the shallow and deep pools of tissue Mn. The model was calibrated to match steady-state tissue concentrations and radiotracer kinetics following an i.p. dose of 54Mn. Successful simulations showed uptake of 0.8% of dietary Mn, and estimated tissue partition coefficients and transfer rate constants in the tissues. Inhalation tracer 54Mn studies could only be adequately modeled by assuming that deposited Mn was absorbed into deep tissue stores in the lung before becoming available to move via blood to other tissues. In summary, this present effort provides the basic structure of a multiroute PBPK model for Mn that should now be easily extended to include homeostatic control and inhalation exposures in order to support risk assessment calculations for Mn.

Evaluation: Klimisch Code 5. Pharmacokinetic Model

Thomassen Y, Ellingsen DG, Hetland S and Sand G (2001) Chemical speciation and sequential extraction of Mn in workroom aerosols: analytical methodology and results from a field study in Mn alloy plants. *J Environ Monit* **3:**555-559.

Abstract: Workers in the Mn alloy producing industry are exposed to aerosols containing a variety of Mn compounds (MnO, MnO,, Mn2O3, Mn3O4, FeMn and SiMn). This paper reports a novel four-step chemical speciation/ fractionation procedure developed for characterisation of workroom aerosols collected in Mn alloy producing plants. The following components of the aerosol have been quantified: "water soluble" Mn dissolved in 0.01 M ammonium acetate: Mn0 and Mn2+ dissolved in 25% acetic acid; Mn3+ and Mn4+ dissolved in 0.5%) hydroxylamine hydrochloride in 25%) acetic acid; and "insoluble" Mn digested in aqua regia and hydrofluoric acid. Dissolution of pure Mn compounds with well-defined stoichiometries were essentially complete in the respective leaching steps with detectable amounts of < 1% in others. Recoveries of a mixed quality control sample were also acceptable in the range 92-97% for the different oxidation states. The levels measured in the inhalable and respirable fractions in three Mn alloy producing plants were approximately 300 and 35 microg m(-3) of total Mn, respectively. The most obvious feature of the speciation results is that none of the work areas is characterised by a single Mn contaminant. The predominant oxidation states in the inhalable aerosol fraction are Mn0 and Mn2+ independent of job functions/departments. The occurrence of insoluble Mn compounds in both the inhalable and respirable aerosol fractions is significantly higher during production of SiMn.

Evaluation: Klimisch Code 2. Restrictions - no claims that study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. The publication has a very good level of detail in the methodology.

Thompson K, Molina R, Donaghey T, Brain JD and Wessling-Resnick M (2006) The influence of high iron diet on rat lung manganese absorption. *Toxicol Appl Pharmacol* **210:**17-23.

Abstract: Individuals chronically exposed to manganese are at high risk for neurotoxic effects of this metal. A primary route of exposure is through respiration, although little is known about pulmonary uptake of metals or factors that modify this process. High dietary iron levels inversely affect intestinal uptake of manganese, and a major goal of this study was to determine if dietary iron loading could increase lung non-heme iron levels and alter manganese absorption. Rats were fed a high iron (1% carbonyl iron) or control diet for 4 weeks. Lung non-heme iron levels increased approximately 2-fold in rats fed the high iron diet. To determine if iron-loading affected manganese uptake, 54Mn was administered by intratracheal (it) instillation or intravenous (iv) injection for pharmacokinetic studies. 54Mn absorption from the lungs to the blood was lower in it-instilled rats fed the 1% carbonyl iron diet. Pharmacokinetics of iv-injected 54Mn revealed that the isotope was cleared more rapidly from the blood of iron-loaded rats. In situ analysis of divalent metal transporter-1 (DMT1) expression in lung detected mRNA in airway epithelium and bronchus-associated lymphatic tissue (BALT). Staining of the latter was significantly reduced in rats fed the high iron diet. In situ analysis of transferrin receptor (TfR) mRNA showed staining in BALT alone. These data demonstrate that manganese absorption from the lungs to the blood can be modified by iron status and the route of administration.

Evaluation: Klimisch Code 2. Generally well documented. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP.

Thompson K, Molina RM, Donaghey T, Schwob JE, Brain JD and Wessling-Resnick M (2007) Olfactory uptake of manganese requires DMT1 and is enhanced by anemia. *FASEB J* **21:**223-230.

Abstract: Manganese, an essential nutrient, can also elicit toxicity in the central nervous system (CNS). The route of exposure strongly influences the potential neurotoxicity of manganese-containing compounds. Recent studies suggest that inhaled manganese can enter the rat brain through the olfactory system, but little is known about the molecular factors involved. Divalent metal transporter-1 (DMT1) is the major transporter responsible for intestinal iron absorption and its expression is regulated by body iron status. To examine the potential role of this transporter in uptake of inhaled manganese, we studied the Belgrade rat, since these animals display significant defects in both iron and manganese metabolism due to a glycine-to-arginine substitution (G185R) in their DMT1 gene product. Absorption of intranasally instilled 54Mn was significantly reduced in Belgrade rats and was enhanced in iron-deficient rats compared to iron-sufficient controls. Immunohistochemical experiments revealed that DMT1 was localized to both the lumen microvilli and end feet of the sustentacular cells of the olfactory epithelium. Importantly, we found that DMT1 protein levels were increased in anemic rats. The apparent function of DMT1 in olfactory manganese absorption suggests that the neurotoxicity of the metal can be modified by iron status due to the iron-responsive regulation of the transporter.

Evaluation: Klimisch Code 2. Generally well documented, although relatively brief methodology. Restrictions - no claims that the study had been conducted and reported according to internationally accepted

guidelines or in compliance with the principles of GLP. Potentially contentious issues in discussion where it suggests that as the percentage of air flow that reaches their olfactory mucosa is comparable between rats and humans despite the differences in size, shape, and breathing mode, that potential absorption of airborne metals by rats and humans would be similar.

Thomson AB, Olatunbosun D and Valverg LS (1971) Interrelation of intestinal transport system for manganese and iron. *J Lab Clin Med* **78:**642-655.

Evaluation: Klimisch Code 2. Good level of detail for an old publication and uses both human and rat data. Relevant route of administration (oral) and well designed study. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Tichy M, Cikrt M and Havrdova J (1973) Manganese binding in rat bile. *Arch Toxikol* **30:**227-236.

Evaluation: Klimisch Code 4. An old study with limited methodology details.

Tipton IH, Stewart PL and Dickson J (1969) Patterns of elemental excretion in long term balance studies. *Health Phys* **16:**455-462.

Evaluation: Klimisch Code 5. Non-pivotal.

Tjalve H, Henriksson J, Tallkvist J, Larsson BS and Lindquist NG (1996) Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. *Pharmacol Toxicol* **79:**347-356.

Abstract: In the olfactory epithelium the primary olfactory neurones are in contact with the environment and via the axonal projections they are also connected to the olfactory bulbs of the brain. Therefore, the primary olfactory neurones provide a pathway by which foreign materials may gain access to the brain. In the present study we used autoradiography and gamma spectrometry to show that intranasal instillation of manganese (54Mn2+) in rats results in initial uptake of the metal in the olfactory bulbs. The metal was then seen to migrate via secondary and tertiary olfactory pathways and via further connections into most parts of the brain and also to the spinal cord. Intranasal instillation of cadmium (109Cd2+) resulted in uptake of the metal in the anterior parts of the olfactory bulbs but not in other areas of the brain. This indicates that this metal is unable to pass the synapses between the primary and secondary olfactory neurones in the bulbs. Intraperitoneal administration of 54Mn2+ or 109Cd2+ showed low uptake of the metals in the olfactory bulbs, an uptake not different from the rest of the brain. Manganese is a neurotoxic metal which in man can induce an extrapyramidal motor system dysfunction associated with occupational inhalation of manganese-containing dusts or fumes. We propose that the neurotoxicity of inhaled manganese is related to an uptake of the metal into the brain via the olfactory pathways. In this way manganese can circumvent the blood-brain barrier and gain direct access to the central nervous system.

Evaluation: Klimisch Code 2. Generally well documented. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Tjalve H, Mejare C and Borg-Neczak K (1995) Uptake and transport of manganese in primary and secondary olfactory neurones in pike. *Pharmacol Toxicol* **77:**23-31.

Abstract: gamma-spectrometry and autoradiography were used to examine the axoplasmic flow of manganese in the olfactory nerves and to study the uptake of the metal in the brain after application of 54Mn2+ in the olfactory chambers of pikes. The results show that the 54Mn2+ is taken up in the olfactory receptor cells and is transported at a constant rate along the primary olfactory neurones into the brain. The maximal velocity for the transported 54Mn2+ was 2.90 +/- 0.21 mm/hr (mean +/- S.E.) at 10 degrees, which was the temperature used in the experiments. The 54Mn2+ accumulated in the entire olfactory bulbs, although most marked in central and caudal parts. The metal was also seen to migrate into large areas of the telencephalon, apparently mainly via the secondary olfactory axons present in the medial olfactory tract. A transfer along fibres of the medial olfactory tract probably also explains the labelling which was seen in the diencephalon down to the hypothalamus. The results also showed that there is a pathway connecting the two olfactory bulbs of the pike and that this can carry the metal. Our data further showed a marked accumulation of 54Mn2+ in the meningeal epithelium and in the contents of the meningeal sacs surrounding the olfactory bulbs. It appears from our study that manganese has the ability to pass the synaptic junctions between the primary and the secondary olfactory neurones in the olfactory bulbs and to migrate along secondary olfactory pathways into the telencephalon and the diencephalon.

Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Methodology relatively brief - uses references to other publications, otherwise well documented.

Van Assche F, VanTilborg W and Waeterschoot H (1997) Environmental risk assessment for essential elements, Case study: Zinc, in: *National Environmental Health Forum Monographs, metal Series No. 2*, pp 33-47, Adelaide, Australia.

Evaluation: Klimisch Code 5. Reference for homeostatic regulation.

Vitarella D, Moss O and Dorman DC (2000a) Pulmonary clearance of manganese phosphate, manganese sulfate, and manganese tetraoxide by CD rats following intratracheal instillation. *Inhal Toxicol* **12:**941-957.

Abstract: Manganese (Mn) is ubiquitous in ambient air due to both industrial and crustal sources. It is also a component of the octane-enhancing fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT). The combustion of MMT by the automobile engine results in the formation of Mn particulates including phosphate, sulfate, and oxide forms. The objectives of this study were to determine the contribution of particle dissolution on pulmonary clearance rates of Mn sulfate (MnSO(4)), Mn phosphate, and Mn tetraoxide (Mn(3)O(4)) in CD rats following an intratracheal instillation exposure. In addition, brain (striatal) Mn concentrations were evaluated following exposure. Adult CD rats were intratracheally instilled with 0, 0.04, 0.08, or 0.16 microg Mn/g of either MnSO(4), Mn phosphate, or Mn(3)O(4). Rats were euthanized at 0, 1, 3, or 14 days after instillation. Lung and striatal Mn concentrations were measured by neutron activation analysis. Pulmonary clearance following single intratracheal instillation of MnSO(4), Mn phosphate, or Mn(3)O(4) was similar for each of the three compounds at each of the three doses used. All pulmonary clearance half-times were less than 0.5 day. At the concentrations used, striatal Mn levels were unaffected, and lung pathology was unremarkable. The dissolution rate constant of the Mn particles was determined in vitro using lung simulant fluids. The solubility of the Mn compounds was in general 20 to 40 times greater in Hatch artificial lung lining fluid than in Gamble lung simulant fluid. The dissolution rate constant of the water-soluble MnSO(4) particles in Hatch artificial lung fluid containing protein was 7.5 x 10(-4) g (Mn)/cm(2)/day, which was 54 times that of relatively water-insoluble Mn phosphate and 3600 times that of Mn(3)O(4). The dissolution rate constants for these compounds were sevenfold slower in Gamble lung fluid simulant. For both solutions, the time for half the material to go into solution differed only by factors of 1/83 to 1/17 to 1 for MnSO(4), Mn phosphate, and Mn(3)O(4), respectively, consistent with measured differences in size distribution, specific surface, and dissolution rate constant. These data suggest that dissolution mechanisms only played a role in the pulmonary clearance of MnSO(4), while nonabsorptive (e.g., mechanical transport) mechanisms predominate for the less soluble phosphate and oxide forms of Mn.

Evaluation: Klimisch Code 2. Well documented and extensive discussion. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP

Vitarella D, Wong BA, Moss OR and Dorman DC (2000b) Pharmacokinetics of inhaled manganese phosphate in male Sprague-Dawley rats following subacute (14-day) exposure. *Toxicol Appl Pharmacol* **163:**279-285.

Abstract: Methylcyclopentadienyl manganese tricarbonyl (MMT) is used as a gasoline octane enhancer. Manganese phosphate is the primary respirable (PM(2.5)) MMT-combustion product emitted from the automobile tailpipe. The goal of this study was to determine the exposure-response relationship for inhaled manganese phosphate in adult male CD rats. Rats were exposed 6-h/day for either 5 days/week (10 exposures) or 7 days/week (14 exposures) to manganese phosphate at 0, 0.03, 0.3, or 3 mg Mn/m(3) (MMAD congruent with 1.5 micrometer). The following tissues collected at the end of the 2-week exposure: plasma, erythrocytes, olfactory bulb, striatum, cerebellum, lung, liver, femur, and skeletal muscle (n = 6 rats/exposure group) were analyzed for manganese content by neutron activation analysis. Intravenous (54)MnCl(2) tracer studies were also conducted following the 14th exposure (n = 6 rats/concentration), and whole-body gamma spectrometry was performed immediately after injection and at 1, 2, 4, 8, 12, and 16 weeks after (54)MnCl(2) administration. Increased manganese concentrations were observed in olfactory bulb, lung, femur, and skeletal muscle following exposure to 3 mg Mn/m(3) (10 or 14 exposures). Increased manganese concentrations were also observed in olfactory bulb, striatum, and lung following exposure to 0.3 mg Mn/m(3) (14 exposures only). Red blood cell and plasma manganese concentrations were increased only in rats exposed to 3 mg Mn/m(3) (10 exposures). Rats exposed to 3 mg Mn/m(3) also had an increased whole-body manganese clearance rate when compared to air-exposed control animals. Our results suggest that the rat olfactory bulb may accumulate more manganese than other brain regions following inhalation exposure.

Evaluation: Klimisch Code 2. Very good detail in methodology, study design and clear discussion. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP.

Wassermann D and Wassermann M (1977) The ultrastructure of the liver cell in subacute manganese administration. *Environ Res* **14:**379-390.
Evaluation: Klimisch Code 5. Non-pivotal.

Wedler FC (1993) Biological significance of manganese in mammalian systems. *Prog Med Chem* **30:**89-133.
Evaluation: Klimisch Code 5. Background information on manganese.

Weigand E, Kirchgessner M and Helbig U (1986) True Absorption and Endogenous Fecal Excretion of Manganese in Relation to its Dietary Supply in Growing Rats. *Biological Trace Element Research* **10:**265-279.
Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. Many details of the methodology were cross references.

Wieczorek H and Oberdorster G (1989) Kinetics of inhaled 54MnCl2 aerosols: influence of inhaled concentration. *Pol J Occup Med* **2:**248-260.
Abstract: Lung clearance, tissue distribution and elimination of manganese was studied in male Long-Evans rats. Animals were exposed for 1 hr by nose only to 54MnCl2 in concentrations of 54MnCl2: 129 mg Mn/m3 and 2.93 micrograms Mn/m3. Activity of 54Mn in lung, brain, liver, kidney, stomach, large and small intestine, blood, urine and feces was determined on days 0, 1, 2, 7, 14, 28, 60 and 121. Inhaled 54MnCl2 was cleared from the lung of rates biexponentially; at the high concentration, the fast and the slow phases had half-times, of 0.2 and 10.5 days, respectively. At the low concentration, the rapid and the slow phases had half times of 1.8 and 12.7 days, respectively. Relative uptake into the brain was independent of inhaled concentration and did not exceed 1 percent of lung deposition on day 0. After the high concentration, liver and kidney Mn levels peaked immediately at the end of exposure and decreased rapidly during the first two days. After the low exposure, liver and kidney accumulation was maximal on day 2 and then organ levels decreased like those of the high exposure group. Relative Mn content in the GI tract was similar after high and low exposure, except for the large intestine where much higher levels were measured in the early phase after inhalation of the high concentration. These data show that concentration of inhaled Mn has a significant influence on its organ distribution and elimination rates.
Evaluation: Klimisch Code 2. A concise discussion. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP. However, there is limited detailed methodology.

Witholt R, Gwiazda RH and Smith DR (2000) The neurobehavioral effects of subchronic manganese exposure in the presence and absence of pre-parkinsonism. *Neurotoxicol Teratol* **22:**851-861.
Abstract: Recent studies have implicated chronic elevated exposures to environmental agents, such as metals (e.g., manganese, Mn) and pesticides, as contributors to neurological disease. In particular, there is a concern that sensitive subpopulations such as the aged may be at increased risk for the onset of neurologic disorders because elevated exposures to Mn is associated with increased incidence of parkinsonism. Here, we utilized a rat model of pre-parkinsonism to investigate the effects of Mn exposure on neurotoxicity and the exacerbation of parkinsonism. A pre-parkinsonism state was induced using a unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA), followed 4 weeks later by Mn exposure (4.8 mg Mn/kgx3 intraperitoneal injections/week) for 5 weeks. Female Sprague-Dawley rats (n=44) were divided among the following treatments: (A) control, saline/vehicle; (B) Mn only; (C) 6-OHDA only; and (D) 6-OHDA+Mn. Brain Mn levels were measured by ICP-MS. Neurobehavioral function was assessed following Mn exposure using a functional observational battery (FOB) consisting of 10 neurobehavioral tests. Unilateral 6-OHDA lesions produced significant ipsilateral vs. contralateral striatal dopamine depletions (60-70%), but no measurable impairment of neurobehavioral function, thereby substantiating this pre-parkinsonism (i.e., subthreshold) model. In contrast, Mn exposure resulted in significant impairment of neurobehavioral function for eight of the 10 FOB tests. No effects of Mn exposure on striatal dopamine depletion were detected, despite the 3.4-fold increase in brain Mn levels over controls. Notably, Mn exposure in the presence of a pre-parkinsonism state significantly exacerbated the neurobehavioral impairment in the reactivity to handling (P<.049) and hopping contralateral rear limb (P<.033) FOB tests. While the persistence and Mn dose-response relationship of these neurobehavioral effects were not evaluated here, these results

nonetheless suggest that chronic Mn exposure may increase the risk of neurobehavioral impairment in subpopulations that are in a pre-parkinsonism state.
Evaluation: Klimisch Code 5. Mechanistic study.

Witzleben CL (1969) Manganese-induced cholestasis: concurrent observations on bile flow rate and hepatic ultrastructure. *Am J Pathol* **57:**617-625.
Evaluation: Klimisch Code 5. Non-pivotal.

Witzleben CL, Pitlick P, Bergmeyer J and Benoit R (1968) Acute manganese overload. A new experimental model of intrahepatic cholestasis. *Am J Pathol* **53:**409-422.
Evaluation: Klimisch Code 5. Non-pivotal.

World_Health_Organization (2004) Manganese in drinking-water. Background document for development of WHO guidelines for drinking-water quality.. *WHO/SDS/WSH/03.04/104* **http://www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf**.
Evaluation: Klimisch Code 5. Review, not evaluated.

Yamada M, Ohno S, Okayasu I, Okeda R, Hatakeyama S, Watanabe H, Ushio K and Tsukagoshi H (1986) Chronic manganese poisoning: a neuropathological study with determination of manganese distribution in the brain. *Acta Neuropathol* **70:**273-278.
Abstract: An autopsy case of a 52-year-old man suffering from chronic manganese poisoning (CMP) is reported with determination of the manganese distribution in the brain. The patient had been working in a manganese ore crushing plant since 1965. In 1967 he began to complain of difficulties in walking and diminished libido. Later, he developed various neuropsychiatric symptoms including euphoria, emotional incontinence, masked face, monotonous speech, "cock-walk", increased muscle tone, weakness of upper and lower extremities, tremor of the eye lids, and exaggeration of knee jerks. The major neuropathological change was degeneration of the basal ganglia, in which the pallidum was severely affected. The pallidum disclosed a loss and degeneration of nerve cells, which was especially marked in the medial segment, a prominent decrease of myelinated fibers, and moderate astrocytic proliferation. The substantia nigra was intact. Distribution of manganese in the brain of the present case of CMP was determined using flameless atomic absorption spectrometry and compared with control cases and also a case of Parkinson's disease (PD). There was no significant difference between the control cases and the case of PD in average concentration of manganese and its distribution in the brain. The present case of CMP showed no elevation in average concentration of manganese in the brain. However, there were some changes in its distribution. Thus, the continuance of neurological disorders in CMP is not linked to an elevated manganese concentration itself in the brain. CMP appears to be different from PD in neuropathology and manganese behavior in brain.
Evaluation: Klimisch Code 2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. However, the manganese distribution in the brain determinations were carried out 5 years after the end of exposure and 4 years after chelating treatment which effected a marked excretion of manganese. Hence the concentration of manganese remaining in the brain at autopsy were not elevated, although there may have been changes in distribution. Also the control samples selected were from 4 other control cases and one of a Parkinson's disease sufferer, however, none of these would have undergone chelation with EDTA and, as such, direct comparisons should be treated with caution.

Yamaguchi M, Inamoto K and Suketa Y (1986) Effect of essential trace metals on bone metabolism in weanling rats: comparison with zinc and other metals' actions. *Res Exp Med (Berl)* **186:**337-342.
Abstract: The effect of essential trace metals on bone metabolism was investigated in the femoral diaphysis of weanling rats. Oral administration of zinc (1.53-306 mumol/100 g body weight) for 3 days produced significant increases in alkaline phosphatase activity and DNA content. These biochemical indices were also increased by oral administration of chromium (III), cobalt, copper, manganese, and nickel with the dose of 1.53 mumol/100 g. With the dose of 15.3 mumol/100 g of above all metals, except zinc, the enzyme activity was significantly decreased in comparison with control, while DNA content was not decreased significantly. Moreover, the effect of zinc on alkaline phosphatase activity and DNA content was not enhanced by simultaneous administration of other metals (1.53 mumol/100 g). The present study indicates that, of the essential trace metals, zinc can effectively stimulate the bone growth and calcification with comparatively higher dose levels. This suggests a nutritional significance of zinc on bone growth.
Evaluation: Klimisch Code 5. Non-pivotal.

Yokel RA and Crossgrove JS (2004) Manganese toxicokinetics at the blood-brain barrier. *Res Rep Health Eff Inst***:**7-58; discussion 59-73.

Abstract: Increased manganese (Mn) use in manufacturing and in gasoline has raised concern about Mn-induced parkinsonism. Previous research indicated carrier-mediated brain entry but did not assess brain efflux. Using in situ rat brain perfusion, we studied influx across the blood-brain barrier (BBB*) of three predominant plasma Mn species available to enter the brain: Mn2+, Mn citrate, and Mn transferrin. Our results suggested transporter-mediated uptake of these species. The uptake rate was greatest for Mn citrate. Our results using the brain efflux index method suggested that diffusion mediates distribution from rat brain to blood. To characterize the carriers mediating brain Mn uptake, we used rat erythrocytes, an immortalized murine BBB cell line (b.End5), primary bovine brain endothelial cells (bBMECs), and Sprague Dawley and Belgrade rats. Studies with bBMECs and b.End5 cells suggested concentrative brain Mn2+ and Mn citrate uptake, respectively, consistent with carrier-mediated uptake. Mn2+ uptake positively correlated with pH, suggesting mediation by an electromotive force. Mn2+ uptake was not inhibited by iron or the absence of divalent metal transporter 1 (DMT-1) expression, suggesting an iron-transporter-independent mechanism. Mn2+ uptake inversely correlated with calcium and was affected by calcium channel modulators, suggesting a role for calcium channels. Rat erythrocyte results suggested monocarboxylate transporter 1 (MCT1) and anion exchange transporters do not mediate Mn citrate brain uptake. Considering carrier-mediated brain influx (but not efflux), repeated excessive Mn exposure should produce brain accumulation. Further work is necessary to identify the specific transporter or transporters mediating Mn distribution across the BBB.

Evaluation: Klimisch Code 5. Review publication.

Yokel RA, Crossgrove JS and Bukaveckas BL (2003) Manganese distribution across the blood-brain barrier. II. Manganese efflux from the brain does not appear to be carrier mediated. *Neurotoxicology* **24:**15-22.

Abstract: There is concern about manganese (Mn) neurotoxicity. Mn can enter the brain by carrier-mediated influx. There have been no previous reports of investigation of Mn efflux from the brain. We used an established method that determines the rate of efflux out of the brain across the blood-brain barrier (BBB) from the product of the brain distribution volume (Vbrain) and the apparent elimination rate constant (Kel). Vbrain is determined as 54Mn uptake into rat parietal brain slices versus time. Kel is determined from the percentage of 54Mn remaining in the brain at various times after its discrete injection into the parietal cortex, compared to a reference compound which is expected to very slowly diffuse out of the brain. The Mn ion, Mn citrate and Mn transferrin (Mn Tf) were studied. 14C-sucrose and 14C-dextran were used as reference compounds. The volume of distribution of the Mn species in brain slices was approximately 3-5 ml/g, indicating concentrative uptake. Mn, as the Mn ion or Mn citrate, was injected into the brain with sucrose or dextran to determine Kel. Based on the rapid exchange rate of Mn with ligands and on thermodynamic calculations, injection of Mn ion or Mn citrate into the brain would be expected to result in rapid formation of the same Mn species, predominantly the Mn ion, Mn citrates and Mn phosphate, in brain extracellular fluid. After injection into the brain Mn did not efflux from the brain more rapidly than sucrose or dextran, which diffuse across the BBB. Brain capillary diffusion of the Mn ion and Mn citrate would be expected to be slower than sucrose or dextran. The rate of Mn efflux from the brain is consistent with diffusion.

Evaluation: Klimisch Code 2. Well documented and discussed. Restrictions - no claims that the study had been conducted and reported according to internationally accepted guidelines or in compliance with the principles of GLP.

Yokel RA, Lasley SM and Dorman DC (2006) The speciation of metals in mammals influences their toxicokinetics and toxicodynamics and therefore human health risk assessment. *J Toxicol Environ Health B Crit Rev* **9:**63-85.

Abstract: Chemical form (i.e., species) can influence metal toxicokinetics and toxicodynamics and should be considered to improve human health risk assessment. Factors that influence metal speciation (and examples) include: (1) carrier-mediated processes for specific metal species (arsenic, chromium, lead and manganese), (2) valence state (arsenic, chromium, manganese and mercury), (3) particle size (lead and manganese), (4) the nature of metal binding ligands (aluminum, arsenic, chromium, lead, and manganese), (5) whether the metal is an organic versus inorganic species (arsenic, lead, and mercury), and (6) biotransformation of metal species (aluminum, arsenic, chromium, lead, manganese and mercury). The influence of speciation on metal toxicokinetics and toxicodynamics in mammals, and therefore the adverse effects of metals, is reviewed to illustrate how the physicochemical characteristics of metals and their handling in the body (toxicokinetics) can influence toxicity (toxicodynamics). Generalizing from mercury, arsenic, lead, aluminum, chromium, and manganese, it is clear that metal speciation influences mammalian toxicity. Methods used in aquatic toxicology to predict the interaction among metal speciation, uptake, and toxicity are evaluated. A classification

system is presented to show that the chemical nature of the metal can predict metal ion toxicokinetics and toxicodynamics. Essential metals, such as iron, are considered. These metals produce low oral toxicity under most exposure conditions but become toxic when biological processes that utilize or transport them are overwhelmed, or bypassed. Risk assessments for essential and nonessential metals should consider toxicokinetic and toxicodynamic factors in setting exposure standards. Because speciation can influence a metal's fate and toxicity, different exposure standards should be established for different metal species. Many examples are provided which consider metal essentiality and toxicity and that illustrate how consideration of metal speciation can improve the risk assessment process. More examples are available at a website established as a repository for summaries of the literature on how the speciation of metals affects their toxicokinetics.

Evaluation: Klimisch Code 5. Review.

Yoshikawa H (1974) Tolerance to Acute Metal Toxicity in Mice Having Received a Daily Injection of its Low Dose. *Industrial Health* **12:**175-177.

Evaluation: Klimisch Code 5. Non-pivotal.

Yu IJ, Park JD, Park ES, Song KS, Han KT, Han JH, Chung YH, Choi BS, Chung KH and Cho MH (2003) Manganese distribution in brains of Sprague-Dawley rats after 60 days of stainless steel welding-fume exposure. *Neurotoxicology* **24:**777-785.

Abstract: Welders working in a confined space, as in the shipbuilding industry, are at risk of being exposed to high concentrations of welding fumes and developing pneumoconiosis or other welding-fume exposure related diseases. Among such diseases, manganism resulting from welding-fume exposure remains a controversial issue, as the movement of manganese into specific brain regions has not yet been clearly established. Accordingly, to investigate the distribution of manganese in the brain after welding-fume exposure, male Sprague-Dawley rats were exposed to welding fumes generated from manual metal arc-stainless steel (MMA-SS) at concentrations of 63.6 +/- 4.1 mg/m(3) (low dose, containing 1.6 mg/m(3) Mn) and 107.1 +/- 6.3 mg/m(3) (high dose, containing 3.5 mg/m(3) Mn) total suspended particulate (TSP) for 2 h per day in an inhalation chamber over a 60-day period. Blood, brain, lung, and liver samples were collected after 2 h, 15, 30, and 60 days of exposure and the tissues analyzed for their manganese concentrations using an atomic absorption spectrophotometer. Although dose- and time-dependent increases in the manganese concentrations were found in the lungs and livers of the rats exposed for 60 days, only slight manganese increases were observed in the blood during this period. Major statistically significant increases in the brain manganese concentrations were detected in the cerebellum after 15 days of exposure and up until 60 days. Slight increases in the manganese concentrations were also found in the substantia nigra, basal ganglia (caudate nucleus, putamen, and globus pallidus), temporal cortex, and frontal cortex, thereby indicating that the pharmacokinetics and distribution of the manganese inhaled from the welding fumes were different from those resulting from manganese-only exposure.

Evaluation: Klimisch Code 3. Poor study design: for example less than 2-fold between high and low dose, the high dose was not high enough. Only a 2 hour exposure.

Zaprianov ZK, Tsaley DL, Gheorghieva RB and Kaloyanova FP (1985) New Toxicokinetic Exposure Tests Based on Atomic Absorption Analysis of Toenails. I. Manganese. *Heavy Met. Environ. 5th Int. Conf.* **1 and 2:**95-97.

Evaluation: Klimisch Code 4. Brief details.

Zhang S, Fu J and Zhou Z (2005) Changes in the brain mitochondrial proteome of male Sprague-Dawley rats treated with manganese chloride. *Toxicol Appl Pharmacol* **202:**13-17.

Abstract: To probe the mitochondrial involvement in Mn intoxicity, aliquots of brain mitochondria samples from control and treated (30 mg/kg manganese chloride, ip) male Sprague-Dawley rats were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and searched for protein abundance changes induced by Mn exposure. The electrophoretic separation resolved over 300 distinct spots as visualized by colloidal Coomassie blue (CCB), of which three spots were induced and three spots were inhibited after Mn exposure in all the five brain mitochondria preparations. Analysis by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) indicated that these spots are calcium-transporting ATPase type 2C (ATP-dependent Ca(2+) pump PMR1); 60-kDa heat shock protein; Mitochondrial transmembrane GTPase FZO1B; ATP-binding cassette, sub-family b; Long-chain-fatty-acid-CoA ligase; ATP Synthase Beta Chain; and Succinate dehydrogenase flavoprotein subunit. The changes of the mitochondrial ATP synthase beta-subunit and Succinate dehydrogenase flavoprotein subunit indicate an effected level of mitochondrial ATP content and/or ATP-producing capacity. This result provides suggestion that respiratory chain complexes were implicated in the mitochondrial dysfunction induced by Mn intoxicity. And the changes of 60-kDa heat shock protein

and ATP-dependent Ca(2+) pump PMR1 expression indicate that the Ca homeostasis and stress effect were involved in Mn intoxicity.

Evaluation: Klimisch Code 5. Non-pivotal.

Zhang S, Zhou Z and Fu J (2003) Effect of manganese chloride exposure on liver and brain mitochondria function in rats. *Environ Res* **93:**149-157.

Abstract: Manganese (Mn) is an essential trace element found in many enzymes. As is the case for many essential trace elements, excessive Mn is toxic. Individuals suffering from manganese toxicity exhibit several symptoms, which are similar to those frequently observed in cases of Parkinson's disease. In this investigation, we studied the effect of manganese chloride (7.5, 15.0, and 30.0 mg/kg body weight) on mitochondrial function and attempted to ascertain the mechanism of manganese-induced mitochondrial dysfunction. The production of reactive oxygen species in mitochondria of rat liver and brain was assayed using 2',7'-dichlorofluorescin diacetate, and the activities of respiratory chain enzymes were examined spectrophotometrically. Monoamine oxidase (MAO) activity was assayed by measuring reduction of benzylamine. Manganese and calcium content in mitochondria were determined by atomic absorption spectrophotometry. These results indicate that manganese chloride (MnCl2) can decrease MAO activity and inhibit the respiratory chain. Manganese can accumulate in mitochondria and inhibit efflux of calcium. There is a significant inverse correlation between the amount of superoxide radicals and the specific activities of the mitochondria enzymes. Mitochondrial function was significantly affected in both males and females.

Evaluation: Klimisch Code 5. Non-pivotal.

Zheng W, Kim H and Zhao Q (2000) Comparative toxicokinetics of manganese chloride and methylcyclopentadienyl manganese tricarbonyl (MMT) in Sprague-Dawley rats. *Toxicol Sci* **54:**295-301.

Abstract: The toxicokinetics of manganese (Mn) was investigated in male and female rats either following a single intravenous (iv) or oral dose of MnCl2 (6.0 mg Mn/kg), or following a single oral dose of methylcyclopentadienyl manganese tricarbonyl (MMT) (20 mg MMT/kg or 5.6 mg Mn/kg). The plasma concentrations of manganese were quantified by atomic absorption spectrophotometry (AAS). Upon iv administration of MnCl2, manganese rapidly disappeared from blood with a terminal elimination t1/2 of 1.83 h and CL8 of 0.43 L/h/kg. The plasma concentration-time profiles of manganese could be described by C = 41.9e(-424t) + 2.1e(-0.44t). Following oral administration of MnCl2, manganese rapidly entered the systemic circulation (Tmax = 0.25 h). The absolute oral bioavailability was about 13%. Oral dose of MMT resulted in a delayed Tmax(7.6 h), elevated Cmax (0.93 microg/ml), and prolonged terminal t1/2 (55.1 h). The rats receiving MMT had an apparent clearance (CL/F = 0.09 L/h x kg) about 37-fold less than did those who were dosed with MnCl2. Accordingly, the area under the plasma concentration-time curves (AUC) of manganese in MMT-treated rats was about 37-fold greater than that in MnCl2-treated rats. A gender-dependent difference in toxicokinetic profiles of plasma manganese was also observed. Female rats displayed a greater AUC than that of male rats. Although the apparent volume of distribution of manganese was similar in both sexes, the apparent clearance in males was about twice that observed in females. The results indicated that after oral administration, the MMT-derived manganese displayed higher and more prolonged plasma concentration-time profiles than MnCl2-derived manganese. Thus, MMT-derived manganese appeared likely to accumulate in the body following repeated exposure.

Evaluation: Klimisch Code 2 for MnCl2. Restrictions - no claims that the study had been conducted and reported according to international accepted guidelines or in compliance with the principles of GLP. It appears to be a good study design with sufficient plasma time-points to enable TK calculations. However, an assessment of Klimisch Code 3 for MMT is assigned as slow absorption of MMT could have been due to dose vehicle and also oral exposure to MMT is not its expected exposure route. The materials and methods only give details of male rats, yet results are quoted for female rats for MMT.