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Speciation of Manganese in Drinking Water

WRc Ref: UC9780
March 2014

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Report No.:	UC9780
Date:	March 2014
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Project No.:	15749-0
Client:	Drinking Water Inspectorate
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Manganese (Mn) is a transition element which can exist in a number of oxidation states. The main states in water are Mn(II), which is soluble and bioavailable, and Mn(IV), which is essentially insoluble. The World Health Organization (WHO) has established a health based value of 400 µg/l, based on an upper tolerable intake, while the standard set in the UK is 50 µg/l, based on avoidance of water discolouration and deposition in mains, rather than human health.

Although Mn is an essential mineral, neurotoxicity by inhalation has been widely described, particularly in workers and miners, where exposure is relatively high. Recently, there has been concern over a number of studies that have suggested that exposure to Mn in drinking water may have neurological adverse effects in terms of intellectual and cognitive development. A recent Canadian study indicated these effects below the WHO health based values but above the UK standard.

With these concerns in mind, this project reviewed the recent data on the potential neurotoxicity of Mn relating to oral intake via drinking water, both in experimental animals and humans, including the bioavailability of different states of Mn. The literature on the behaviour of Mn in water and its possible removal was also reviewed.

Four seasonal monitoring surveys were conducted on final drinking water at up to 20 sites in England and Wales that had been identified as being at potential risk of high Mn concentrations. 18 of these were public supplies and a further two were private supplies. The water samples were analysed for the presence of total Mn and soluble Mn. The soluble Mn was reported as the Mn(II) concentration and Mn(IV) concentration was calculated as the difference between total and soluble Mn.

Results

In summary, the review of the literature on studies that have been conducted in experimental animals, suggests that there is a biologically plausible hypothesis for an adverse effect on neurological development of Mn taken in orally via drinking water. There is an accumulation of Mn in those same areas of the brain which accompanies neurotoxicity, caused by inhalation of Mn, together with some behavioural and locomotor effects

The human epidemiological studies, particularly on children, are suggestive of an effect on intellectual and cognitive development. However, the types of studies conducted are not the most appropriate for measuring the longer-term effects such as those which may occur after

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accumulation of Mn in the brain. There are also problems in the accurate estimation of exposure via drinking water, and the detection of Mn in the body (through the measurement of blood or hair). Therefore, these experimental animal and human studies do not, at present, provide conclusive evidence that exposure to Mn in drinking water causes adverse neurological effects in humans; however, additional studies are currently in progress that may yield further information. It should be clearly stated that this is not a review of all the toxicological data on Mn but only considers the recent studies on possible neurological effects (mainly in children) of Mn in drinking water together with neurological studies in experimental animals. It does not consider toxicological effects on other target organs or the regulatory studies on Mn exposure by the oral route.

In the recent Canadian studies, populations were exposed to borehole drinking water with variable, but with some groups, naturally high levels of Mn, mainly in the bioavailable Mn(II) form (95%). In the other epidemiological studies in Bangladesh and China where drinking water Mn was measured, the concentrations were very high, with the cut-off for high and low exposure in several studies being 400-500 µg/l and concentrations up to 6000 µg/l detected. However, the Mn speciation in these waters was unknown. A monitoring survey was conducted in England and Wales to ascertain whether these final drinking waters were similar to those in the Quebec region of Canada. The four seasonal sampling exercises indicated that public water supplies had low levels of Mn (the great majority below 5 µg/l), and levels of Mn(II) were on average approximately 50% of total Mn. Therefore, the Quebec studies do not represent the typical situation in the public supplies of England and Wales, as the concentrations of Mn are low with a smaller proportion in the bioavailable Mn(II) form. The maximum level of Mn in public supplies (11.04 µg/l) represents a small proportion of an adequate dietary intake. The exception to these findings in England and Wales was in two private borehole supplies, which had high concentrations of Mn nearly all in the Mn(II) form, i.e. similar to the water in the Quebec boreholes. The British Geological Survey indicates that these boreholes are in a geological area which may have high deposits of Mn, although the final drinking water from a nearby public supply does not have an increased concentration of Mn.

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1. General Introduction

Manganese (Mn) is a transition metal which can exist in a range of oxidation states, +2, +3, +4, +6 and +7. The divalent form, Mn(II), predominates in most waters at pH 4-7. If the pH and redox potential of the water are increased (by the addition of lime and chlorine) then Mn(II) may be oxidised to Mn(IV). The complex series of oxidation and adsorption reactions which occur in aerobic water, means that manganese dioxide (MnO_2) is eventually formed. At times MnO_2 may be present as a colloid, which essentially behaves as a solution in terms of transport. MnO_2 is essentially a biologically unavailable, insoluble form of manganese.

The UK Committee on Toxicity (COT) Expert Group on Vitamins and Minerals has concluded that total manganese intakes of 12.2 mg/day (general population) and 8.7 mg/day (older people) would be unlikely to result in adverse effects (EVM, 2003).This Expert Group also state that for the general population a supplemental intake of up to 4 mg/day (approximately 70 µg/kg bw/day for a 60 kg adult) in addition to the diet would be unlikely to produce adverse effects (EVM 2003). These values are similar to the tolerable upper intake level currently set by the US Institute of Medicine (IOM) of 11 mg/day, which was used by the World Health Organization (WHO) to establish their health-based value of 0.4 mg/l (400 µg/l) in drinking water (WHO, 2011). The standard set in the UK is 0.05 mg/l (50 µg/l) based on water discolouration and deposition in mains rather than human health.

There has been some concern about the dietary data underlying the WHO guideline and also a number of studies, including those from Bangladesh and Greece, which have suggested that exposure to manganese in drinking water may have neurological effects in terms of learning and behaviour, although other studies have not demonstrated this relationship. A recent study from Canada (Bouchard *et al*., 2007, 2011) has suggested significant effects on IQ in children drinking water with manganese concentrations of median value of 216 µg/l (below the WHO guideline but above the UK standard) with a smaller, non-significant effect at 34 µg/l (below the UK standard). These changes in IQ in children are somewhat similar to those seen with lead.

The bioavailability of manganese in water is dependent on the species present and this may be an important factor, as the area used in the Bouchard Canadian study was chosen for high levels of manganese present in drinking water, and mainly sourced from boreholes. Therefore the levels and species may not be representative of UK drinking water. The published papers on this study did not state the species of Mn present, but further information has been supplied by the authors (Bouchard, personal communication) together with a subsequent paper by Carriere *et al.* (2011). The gathering of this information precluded the need to collect samples from Canada for analysis in the UK, which may have also led to subsequent transport and stability difficulties.

There is also a need to assess whether the neurological effects observed in recent studies on oral exposure via drinking water are biologically plausible in terms of the known toxicity of manganese.

With these current concerns, Defra and the Drinking Water Inspectorate commissioned the study, the results of which are contained in this report, with the following aims:

- Critically appraise the recent data on manganese toxicity focussing on studies relating to intake via drinking water, in particular, studies relating to intellectual and behavioural effects on children and scrutinising any available information on the oxidation state of the manganese that may cause these effects;

- Interpret these studies in the context of previous knowledge of manganese toxicity and other factors that influence intellectual and behavioural development;

- Review any studies on bioavailability of different oxidation states of manganese;

- Review studies on the fate of manganese in water treatment and supply, including the stability and solubility of different oxidation states of manganese;

- Summarise any studies that have investigated the oxidation state of manganese present in drinking water;

- Conduct a monitoring programme to determine the oxidation states of manganese present in samples of water from a small selection of public and private supplies in England and Wales and possibly some comparative sample from Canada - collect and analyse any samples in accordance with best practice including AQC; and

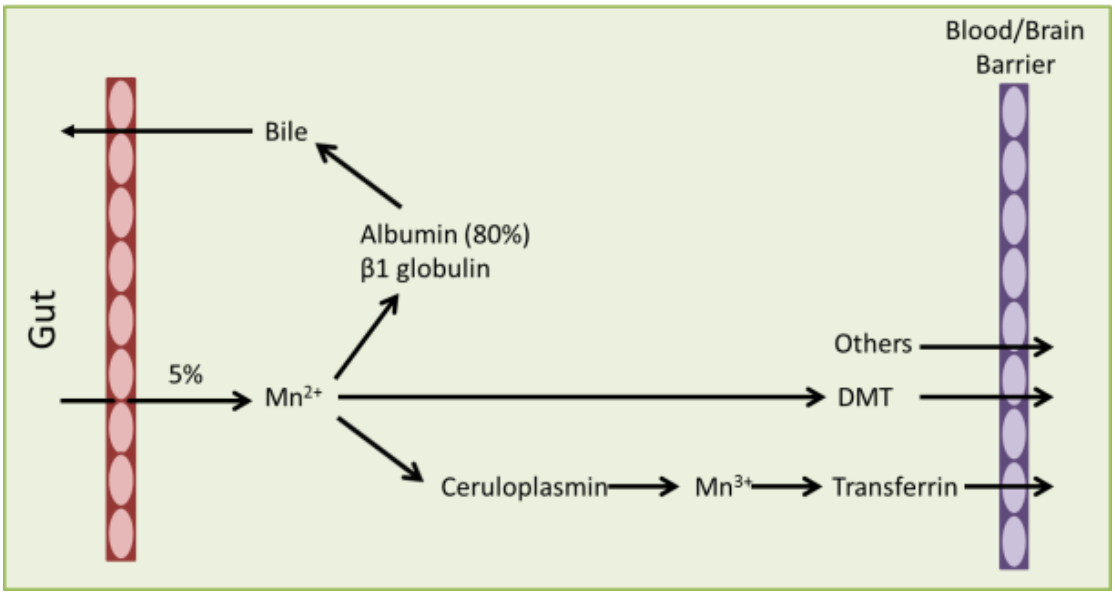
- Report the findings of the study and advise on any possible future areas of research.

2. Toxicokinetics and the Blood-Brain Barrier

2.1 Toxicokinetics of Manganese

A model for the oral absorption and distribution of manganese and its passage across the blood-brain barrier (BBB) to the presumed site in the brain of any effects on neurodevelopment is shown in Figure 2.1.

Figure 2.1 Model for the oral absorption of manganese and its passage through the blood-brain barrier



Although Mn can exist in up to 11 oxidation states, Mn(II) and Mn(III) are the most relevant for biological systems. This indicates that different states of Mn can be formed in the body, Mn(II) and Mn(IV) are the most common forms in the environment and taken up by the body, while Mn(II) is the form that is absorbed. Apart from occupational exposure to Mn via inhalation which has been well described, ingestion is the main route of exposure, with 3-5% of ingested Mn being absorbed by the gut in adults. However, absorption of Mn is higher in children, with up to 40% absorption by ingestion and, as a result, children are likely to be more susceptible to Mn toxicity than adults (Neal and Guilarte, 2012). Mn homeostasis is maintained by absorption and biliary excretion, the bile being the main route of Mn excretion. A fraction of Mn may be reabsorbed through the gut establishing an enterohepatic loop (reviewed by Neal and Guilarte, 2012). Infants, and in particular, neonates are likely to show further susceptible to Mn toxicity owing to their reduced capacity for biliary excretion, compared to adults (Neal and Guilarte, 2012).

The absorption of Mn from the gut is inversely related to the intake of Mn in rats and humans. The data indicate that liver and gastrointestinal tract tissue act in concert to increase absorption during low dietary levels of Mn and reduce absorption when dietary levels are high. Gastrointestinal absorption of Mn is also inversely related to the intake of iron, and so is higher in iron-deficient and anaemic subjects (reviewed in IEH, 2004).

The oxidation state of Mn appears to be a key determinant of its distribution, accumulation and excretion. While Mn(II) is rapidly cleared from the blood and efficiently excreted in the bile, Mn(III) is transported across membranes and has a slower elimination rate. So the exchange and equilibrium of states from Mn(II) (which is absorbed) to Mn(III) is an important factor in the distribution of Mn in the body and hence, any neurotoxic effect. Mn(II) may be oxidised to Mn(III) by ceruloplasmin, a protein which also facilitates the oxidation of Fe(II) to Fe(III) (Neal and Guilarte, 2012).

The majority (80%) of Mn in the blood is bound to albumin, globulin or transferrin with a small proportion as Mn-citrate. Mn then crosses the BBB via facilitated diffusion or across cell membranes by transporter mechanisms such as Divalent Metal Transporter (DMT1), Zrt-like/Irt-like 8 (ZIP 8), and transferrin-mediated mechanisms (reviewed by Neal and Guilarte, 2012), although the evidence for a role for DMT1 is more controversial (Yokel and Crossgrove, 2004; Yokel, 2006). Evidence for the role for transferrin (Tf) is stronger with Mn(II) tightly binding to form a Mn-Tf complex.

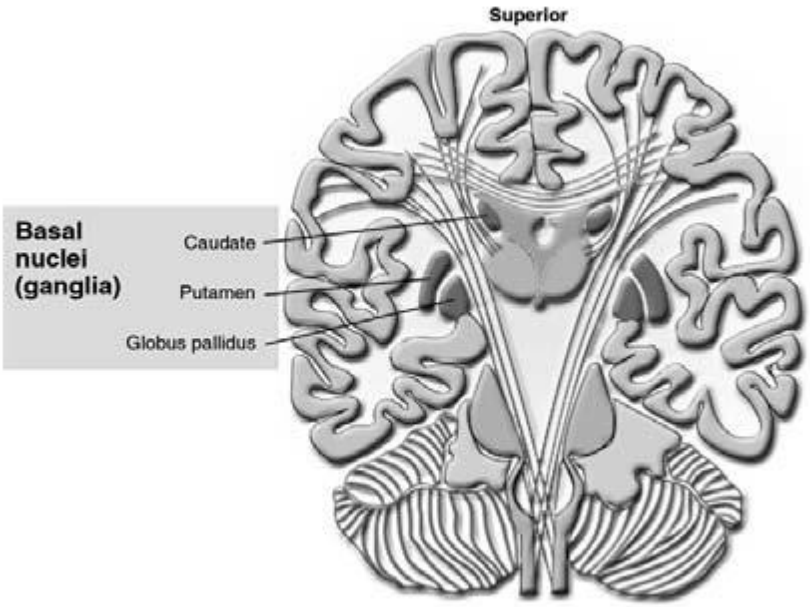
Once it has crossed the BBB, Mn can accumulate in a number of different types of brain cells, such as neurons, astrocytes and oligodendrocytes. There is evidence that, while active transport mediates passage into the brain, slow diffusion mediates the efflux of Mn from the brain. This suggests that repeated excessive Mn exposure might result in accumulation in the brain over time and its retention (Yokel, 2006; Neal and Guilarte, 2012). This has been shown in animals and in humans where brain Mn has been observed to increase from infancy to adulthood, with the highest concentrations being seen in the basal ganglia, the site of Mn-induced neurotoxicity (Yokel, 2006).

3. Oral Neurotoxicity Studies

3.1 Suggested Mechanisms of Manganese Neurotoxicity

The severe neurotoxic effects of inhalable manganese due to high occupational exposure have been well-described and the signs and symptoms, some of which may resemble Parkinson's Disease and include psychiatric effects, have become known as 'manganism'. Because of this resemblance, much of the work has concentrated on the areas of the brain concerned with movement, principally the organs of the basal ganglia (see Figure 3.1), the globus pallidus, putamen and caudate nucleus, the substantia nigra, and the dopaminergic system. These areas have been shown to be associated with Parkinson's Disease. The studies on these systems have included a range of exposure methods and time intervals, making interpretation and conclusions difficult.

Figure 3.1 Outline of the brain showing sites of Manganese accumulation and toxicity (taken from IEH, 2004)

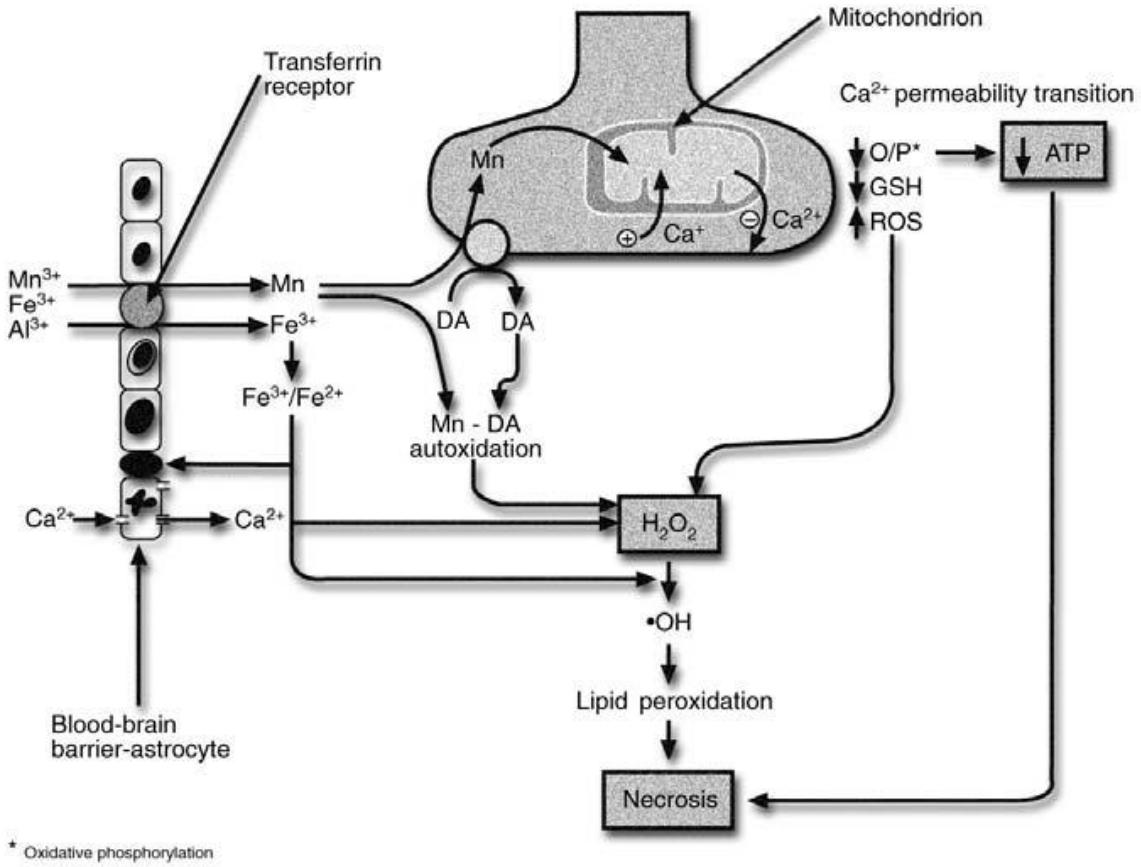


There is selective uptake of Mn within the globus pallidus and the substantia nigra, accumulating in neurons, astrocytes and oligodendrocytes. This uptake appears to be driven by Mn (mainly Mn(III)) bound to transferrin as these areas of the brain are rich in transferrin receptors. There appears to be no single metabolic dysfunction that would account for Mn neurotoxicity and some of the suggested mechanisms are shown in Figure 3.2 (IEH, 2004). Intracellular Mn is transported into the mitochondria of the cell through a calcium one-way uniporter where it accumulates (as there is no export process) and disrupts ATP synthesis

(Neal and Guilarte, 2013). This leads to decreased ATP levels and increased oxidative stress. This may lead to the formation of reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$), leading to free radical damage, lipid peroxidation and the auto-oxidation of the neurotransmitter, dopamine (DA). These damaging mechanisms may lead to cytotoxicity and dendritic degeneration and necrosis.

This brief outline of the potential neurotoxic mechanisms of Mn including the effects on dopamine and the accumulation in the areas of the brain near to the substantia nigra, highlights the similarities to the damage seen in Parkinson's Disease. However, it is less easy to attach these mechanisms to effects of Mn on behaviour and cognitive abilities particularly in children during development. However, there is evidence in animals that deficiencies in dopamine neurotransmission during early development may result in lasting behavioural and cognitive deficits (Neal and Guilarte, 2013).

Figure 3.2 Suggested mechanisms for manganese neurotoxicity (taken from IEH, 2004)



3.2 Review of Oral Neurotoxicological Studies

The purpose of this review is to assess studies on oral intake of manganese in experimental animals and humans and evaluate the evidence for neurotoxicological effects on development and behaviour in children. The brief review above on toxicokinetics suggests that although absorption from the gastrointestinal tract is poor in adults (3-5%), it is much greater in infants and children (40%), as bile is the main pathway for Mn excretion, and this is not fully developed in children. Therefore, there is evidence that Mn may be more readily absorbed and accumulated in children after oral exposure.

In this section, studies on the neurotoxicological effects of Mn after oral exposure in experimental animals and humans will be reviewed and assessed.

3.3 Literature Review Strategy

Literature searches were conducted using Science Direct, PubMed and Toxnet. The terms used for searching were as follows:

Manganese, Mn, MnCl2, oral, neurotoxicity, gavage, drinking water, Manganese chloride, Manganese II chloride, Manganese II nitrate, Manganese II oxide, Manganese II sulphate, Manganese III oxide, Manganese III sulphate, Manganese IV oxide, Manganese IV

All abstracts located were read and the ones that related to the objective were included in this review. Further reviews on manganese, such as the web report produced by the MRC Institute for Environment and Health entitled “Occupational exposure limits: Criteria document for manganese and inorganic manganese compounds” (IEH., 2004), was also used to identify relevant studies. When available the relevant papers were reviewed. In some cases where the full paper was not available, details were taken from the abstract and these are identified in the review below. The quality of the identified experimental animal studies was assessed using the Klimisch code as outlined in Table 3.1.

Table 3.1 Klimisch Classification System

Klimisch Code	Description
1	**Reliable without restrictions**: Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	**Reliable with restrictions**: Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	**Not reliable**: Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	**Not assignable**: Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

The studies described here are research-based and not conducted to regulatory standards. As such, they are mostly considered to be Klimisch rating 2 as they are published in peer-reviewed papers. However, they do not have details of experimental procedures as would be found in regulatory studies; for example, dose calculations are not given, although it is likely that the concentration of Mn would be calculated from the intake of water which would be supplied *ad libitum*. These studies also tended to use high doses in order to obtain and study a potentially adverse response, rather than in a regulatory study where the aim is to derive no and lowest effect levels. Some studies have also been added for completion, but are unreliable for risk assessment as high dose research studies. They do, however, study neurochemical effects, which are relevant to the possible neurological toxicity of Mn.

3.4 Oral neurotoxicity studies in Experimental animals

3.4.1 Ávila, D.S., Gubert, P., Fachinetto, R., Wagner, C., Aschner, M., Rocha, J.B., Soares, F.A. (2008) Involvement of striatal lipid peroxidation and inhibition of calcium influx into brain slices in neurobehavioral alterations in a rat model of short-term oral exposure to manganese. NeuroToxicology 20: 1062-1068

Study Design

- Adult Wistar rats (15/dose) were exposed to manganese chloride ($MnCl_2$•$4H_2O$) (technical purity: ~99%) at either 10 or 25 mg/ml (approximately 0.95 and 2.375 mg/kg bw/day, respectively) in their drinking water for 30 days.

- Bodyweights were monitored on days 0, 15 and 30 of treatment.

- Behavioural evaluations were performed on day 0 and at the end of the treatment period and included: open field (spontaneous ambulation and exploratory activity recorded over 6 minutes) and orofacial dyskinesia (tongue protusions, vacuous chewing movements recorded over 12 minutes).

- Biochemical parameters measured were: hepatic, renal striatal and hippocampal δ-aminolevulinate dehydratase (δ-ALA-D) activity, production of thiobarbituric acid reactive substances, protein carbonylation assay, calcium influx (measured with radiolabelled calcium $^{45}Ca^{2+}$) and protein content determination.

Valency of $MnCl_2$: II

Study Findings

Rats administered both doses of $MnCl_2$ displayed a decrease in body weight gain and increased locomotor activity which, at least in part, was linked to increased lipid peroxidation, inhibition of δ-ALA-D and decreased $^{45}Ca^{2+}$ influx into striatal slices. No extrapyramidal effects were noticed or orofacial dyskinesia. The authors suggested that striatal damage was indicative of early stages of manganese-induced damage. The kidney and liver were unaffected by the short term exposure to $MnCl_2$. A Lowest Observed Adverse Effect Level (LOAEL) of 10 mg/ml (approximately 2.375 mg/kg bw/day) was identified from this study.

Klimisch Rating: 2

3.4.2 Torrente, M., Colomina, M.T., Domingo, J.L. (2005) Behavioural effects of adult rats concurrently exposed high doses of oral manganese and restraint stress. Toxicology 211: 59-69

Study Design

- Male Sprague Dawley rats (15 males/dose/restraint group) were administered $MnCl_2•4H_2O$ (analytical grade) via their drinking water at doses of 0, 275 or 550 mg/kg bw/day of $MnCl_2•4H_2O$ (analytical grade) for 19 weeks. Animals receiving manganese chloride had an initial 2 week period where it was received at a dose of 137.5 mg/kg bw/day to allow for habituation to the taste while control animals received only water.

- Animals from all treatment groups were sub-divided into those that were restrained for 2 hours/day and those that were free from restraint. Restraint stress was applied by placing the animals in metacrilate cylindrical holders.

- At the end of the treatment period, activity levels (exploratory behaviour, rearings and defaecation) were monitored in an open field test. Learning was evaluated by a water

maze task, where rats were subjected to 5 trials/day for 5 consecutive days. The time spent on the platform of the water maze was also assessed.

Valency of MnCl2: II

Study Findings

Water intake was significantly reduced in animals receiving $MnCl_2$. Food consumption was also reduced in animals at the top dose for both restrained and non-restrained animals. However at the lowest dose, a reduction in food consumption was only observed in the animals that were restrained. Bodyweight was significantly reduced in animals that received manganese chloride either alone or combined with restraint stress compared to controls. The total distance travelled was significantly increased with constraint stress and significantly decreased with Mn administration. In rats exposed to the top dose of manganese chloride, there was a significant increase in the latency and swam distance. There was a dose-dependent increase in Mn concentrations in the brain (apart from the cerebellum where concentrations were not significantly different between dose groups) while restraint stress did not modify Mn concentrations in the brain. The authors stated that restraint of animals can increase stress which has been demonstrated to increase the potential toxicity of certain chemicals. The authors concluded that the results from this study indicate that stress and Mn interact at common neurotransmitter levels while inducing opposite effects.

Klimisch Rating: 2

3.4.3 Vezér, T., Papp, A., Hoyk, Z., Varga., Náray, M., Nagymajtényi, L. (2005) Behavioural and neurotoxicological effects of subchronic manganese exposure in rats. Environmental Toxicology and Pharmacology 19:797-810

Study design

- Young male Wistar rats (16/dose) were administered manganese chloride ($MnCl_2$, minimum 99% purity) via oral gavage for 10 weeks at doses of 0, 14.84 or 59.36 mg/kg bw/day followed by a 12 week post-treatment period.

- Mn levels were determined in the blood, cortex and hippocampus in the 5th and 10th week of treatment and at the 12th week at the end of treatment period (22nd week).

- Density of glial fibrillary acid protein immunoreactive (GFAP-IR) astrocytes was determined in the hippocampus at the end of the 10th week.

- Behavioural effects were monitored by:

    - Testing of spatial learning and memory (maze, short and long term memory) performed at the 3rd, 5th, 9th, 10th, 14th and 15th weeks).

- Locomotor activity (spontaneous behaviour, amphetamine induced locomotor activity, acoustic startle response and pre-pulse inhibition (PPI) test performed at the 10th and 17th week).

Valency of $MnCl_2$: II

Study findings

Mn accumulation in the blood increased in a dose and time-dependent way and was apparent from the first assessment point at 5 weeks. Mn concentrations in the brain were not significantly different at 5 weeks from the control animals, but at 10 weeks, levels were statistically higher than the control animals in a dose-dependent manner.

Short and long term spatial memory and spontaneous open field activity was decreased in treated animals in a dose and time-dependent way and was apparent from the first test at the 3rd week. Spontaneous behaviour and activity was decreased in treated animals compared to controls in a time and dose-dependent manner. The acoustic startle response (ASR) was measured in the 10th week, when Mn was found to reduce the number of acoustic responses dose-dependently. There were no differences between the densities of GFAP-IR between the control and treated animals. By the end of the 12 week post treatment period, manganese levels in tissue and blood returned to control levels in both dose groups. After 7 weeks post-treatment (17th week), the difference between the control and treated animals spontaneous behaviour was not statistically significant. Long term spatial memory and the number of acoustic startle responses did not return to pre-treatment levels. The authors concluded that alterations in behaviour and learning were suggestive of a decrease of certain neurotransmitters (such as dopamine and glutamate) and the involvement of glutamatergic auditory pathway and nigrostriatal dopaminergic system and possibly to neuropathological changes in some components of the neural axis for behaviour.

Klimisch Rating: 2

3.4.4 Golub, M.S., Hogrefe, C.E., Germann, S.L., Tran, T.T., Beard, J.L., Crinella, F.M., Lonnerdal, B. (2005) Neurobehavioural evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese. Neurotoxicology and Teratology. 27: 615-627

Study Design

- Male newborn Rhesus monkeys (8/dose) were fed either cow's milk (control), soy milk (300 µg Mn/l) or soy milk with added $MnCl_2$ (to a final concentration of 1000 µg/l) (purity not specified) from birth to 4 months of age when they were transitioned to commercial non-human primate diet.

- Animal weight was monitored weekly for the first 14 weeks and then monthly until the conclusion of the experiment (at 18 months). Crown rump length was also measured monthly as an indicator of linear growth.

- Cerebrospinal fluid (CSF) samples were collected from the cisterna magna at 5, 10 and 12 months of age and analysed for the catecholamines, (homovanilic acid (HVA) and 5-hydroxy-indole acetic acid (5HIAA)).

- Behavioural evaluations (motor development, social interactions, spontaneous activity, learning, reward delay, performance tests (specifically to test for learning, memory and attention), stereotypy (a behaviour or action repeated two or more times in rapid succession) were performed till completion of the experiment at 18 months of age.

Valency of $MnCl_2$: II

Study Findings

The different milk formulas had no statistical difference on animal health, growth, developmental milestones, stereotypy and measured catecholamine concentrations. Monkeys administered soy milk and soy milk with added manganese engaged in less play behaviour, displayed more affiliative clinging in social group interactions, had shorter wake cycles and shorter periods of daytime inactivity than controls (although this was only statistically different to controls for the animals administered soy milk). Monkeys administered soy milk were sensitive to reward delay testing (were more impulsive), however there were no differences between animals administered the soy milk with added Mn compared to control animals. However these animals did not show any improvement in their performance compared to control and soy milk animals. Monkeys administered soy milk with added Mn had a blunted response to apomorphine, a dopamine agonist. The authors suggested that components of soy formula, including manganese, may adversely influence brain development.

Klimisch Rating: 2

3.4.5 Fordahl, S., Cooney, P., Qui, Y., Xie, G., Ji, W., Erikson, K.M. (2012). Waterborne manganese exposure alters plasma, brain and liver metabolites accompanied by changes in stereotypic behaviour. Neurotoxicology and Teratology 34: 27-36

Study Design

- Male weanling (post-natal day 21) Sprague-Dawley rats (12/dose) were administered manganese chloride ($MnCl_2$; purity not specified) via their drinking water at a concentration of 1000 mg manganese/l (approximately 95 mg/kg bw/day) for 6 weeks. The authors stated that based on average water consumption for rats (10-12 ml/100g bw), Mn ingestion was approximately 100 mg/kg bw/day.

- Control animals had access to deionised water and rat chow containing 10 mg/kg diet of Mn (valency not stated).

- Behaviour was monitored for the 4th, 5th and 6th week only for a continuous 24 hour period (using video surveillance).

- Animals were euthanised and trunk blood collected for manganese analysis.

Study Findings

No water avoidance was observed over the duration of the study. No significant changes in bodyweight between control and treated animals were observed. Mn-exposed rats had similar haematocrit levels compared to control animals, however, plasma transferrin levels were significantly increased, accompanied by a trend towards reduced ferritin levels, which the authors concluded was suggestive of the early stages of iron deficiency. Mn administration significantly increased liver, plasma and brain (particularly the globus pallidus) Mn concentrations compared to controls. Mn significantly altered 98 metabolites in the brain, liver and plasma, particularly increasing cholesterol and fatty acid metabolism in the brain and liver. Furthermore, Mn-altered plasma metabolites (the authors stated that this was reflective of amino acid breakdown), homogentisic acid, chenodeoxycholic acid and aspartic acid correlated significantly with striatal and globus pallidus Mn content. Behaviourally, no alterations were observed until the 6th week of Mn exposure. Treated rats had greater total activity, which was strongly correlated to globus pallidus, striatal and plasma manganese levels. Treated animals also showed a decrease in nocturnal stereotypic and exploratory behaviour (and this was performed largely during the light cycle) compared to control animals. The authors stated that alterations in the circadian rhythm are likely to be driven by alterations in striatal dopamine, GABA and/or glutamate. A Lowest Observed Effect Level (LOEL) of 1000 mg Mn/l (approximately 95 mg/kg bw/day) could be identified.

Klimisch Rating: 2

3.4.6 Shukakidze, A., Lazriev, I., Mitagvariya, N. (2003) Behavioural impairments in acute and chronic manganese poisoning in white rats. Neuroscience and Behavioural Physiology 33: 263-267

Study Design

Two studies were conducted. In the first study, white rats (9 and 10 animals in control and treatment group, respectively) were orally administered a single dose of 50 mg $MnCl_2$/kg bw (technical purity not stated) and a conditioned response to a stimulus measured. In the second study, white rats (12/dose) were orally administered either 20 or 50 mg $MnCl_2$/kg bw (technical purity not stated) with feed for one month, and learning and memory processes were studied using a multipath maze method

Valency of $MnCl_2$: II

Study Findings

The single dose of $MnCl_2$ resulted in significant and reversible decreases in total physical activity as measured by worsening of the acquisition of an avoidance reaction in response to unconditioned and conditioned stimuli, an increase in the latent period of conditioned reflex activity and a temporary worsening of the learning process. There was no effect on the rate at which this reaction was performed. Over the duration of the experimental period (16-17 days), there was no reversibility of the effects of Mn.

Animals administered $MnCl_2$ at both doses for a month had significant impairment of learning processes in a multipath maze, but had no significant effects on reproduction or previously acquired stereotypical behaviour. A LOEL of 1 mg/kg bw/day can be identified.

Klimisch Rating: 2

3.4.7 Lai, J.C., Chan, A.W., Leung, T.K.C., Minski, M.J., Lim, L. (1992) Neurochemical changes in rats chronically treated with a high concentration of manganese chloride. Neurochemical Research 17: 841-847

Study Design

- Pregnant female Wistar rats (number of animals not specified) were administered manganese chloride ($MnCl_2$) (technical grade) via their drinking water at a concentration of 20 mg $MnCl_2.4H_2O$/ml (approximately 1.9 mg/kg bw/day) from gestation until weaning of the pups. Male pups were exposed to manganese via their mothers *in utero*, postnatally via their mother's milk and from weaning onwards via their drinking water at the same concentration as the dams until they were 90 days old.

- At 90 days of age, the male pups were sacrificed and concentrations of acetylcholinesterase, monoamine oxidase A and B, trace metals (iron, copper and zinc) and electrolytes (calcium, magnesium and sodium) were determined in the hypothalamus, cerebellum, pons, medulla, striatum, midbrain and cerebral cortex (including the hippocampus).

Study Findings

Mn significantly decreased bodyweight gain compared to control animals, although the relative brain weight (ratio between brain weight: body weight) was not significantly different.

Treated animals showed marked increases in Mn content in all brain regions, especially in the hypothalamus (HY) and striatum (ST). This affected the relative distribution of Mn throughout the brain, with the highest levels in the hypothalamus, intermediate levels in the striatum and midbrain (MB), and lowest levels in the pons and medulla (PM), cerebellum (CB) and cerebral cortex (CC). Control animals had low levels of Mn equally distributed throughout the brain.

The changes in other metals are shown in Table 3.2.

Table 3.2 Increases (↑) and decreases (↓) of metal concentrations in brain regions of treated animals

Brain sections	Ca	Mg	Fe	Cu	Zn
HY	↓				
CB	↓	↓			
PM		↓		↑	↓
ST			↑	↑	
MB	↑	↓			
CC	↓		↓		

Type A monoamine oxidase activities were significantly decreased in the midbrain, striatum and cerebral cortex, although its distribution was the same as that in controls. Type B monoamine oxidase activity was significantly decreased in the hypothalamus, although its distribution was the same as that in controls. Acetylcholinesterase activity was significantly increased in the striatum and cerebellum, although its distribution was the same as that in controls. The authors concluded that these enzymes were "rather insensitive" to this chronic manganese exposure, as the changes observed, although significant, were "small".

The authors were not able to correlate regional changes in manganese levels to specific neurochemical or behavioural changes associated with manganese encephalopathy. The authors concluded that chronic manganese encephalopathy not only affects brain metabolism of manganese, but also that of other metals.

Klimisch Rating: 2.

3.4.8 Reichel, C.M., Wacan, J.J., Farley, C.M., Stanley, B.J., Crawford, C.A., McDougall, S.A. (2006) Postnatal manganese exposure attenuates cocaine-induced locomotor activity and reduces dopamine transporters in adult male rats. Neurotoxicology and Teratology 28: 323-332

Study Design

- Sprague Dawley male rats (8/9/dose) were orally administered manganese chloride ($MnCl_2$) (analytical grade) at concentrations of 250 or 750 µg in a 10% sucrose solution on post natal days (PD) 1 – 21. This dose was administered orally by a micropipette and is purported to mimic the amount of Mn consumed by human infants fed milk and soy formula. However, the weight of the rats was not given in the paper and is difficult to calculate, but for a 400 g rat, the dose would be approximately 0.625 and 1.875 mg/kg bw/day for 250 and 750 µg/day dose, respectively.

- Striatal manganese, iron accumulation and serum iron were measured on PD14, PD21 and PD90.

- Throughout the dosing period, animals observations were made for body weight, developmental landmarks (eye opening, pina detachment incisor eruption), sensory and motor development (latency to correct negative geotaxis, olfactory discrimination between bedding).

- On PD day 90, rats were tested for balance and coordination (balance beam).

- On PD day 91, rats were injected with saline or cocaine (10 or 20 mg/kg bw via i.p injection) and activity monitored for 60 minutes. Following this assessment, saline treated animals were decapitated and the striata removed for a dopamine transporter assay.

Valency of $MnCl_2$: II

Study Findings

Mn caused a dose-dependent relative decline in bodyweight compared to control animals. No effect on developmental landmarks or sensory development was observed, although Mn increased the latency on the negative geotaxis task at the top dose at day 8 only. Adult rats (PD 90) who had previously been treated with Mn during PD 1-21, did not display adverse balance or coordination or significantly different bodyweights compared to control animals. Adult rats treated with the top dose of Mn showed enhanced locomotor response when challenged with cocaine, which increased locomotor activity. The authors concluded that this was either because early exposure to Mn caused a long term alteration in the sensitivity of dopamine receptors or Mn exposure caused dopamine neurotoxicity. At the top dose Mn exposure enhanced striatal Mn accumulation on PD14 and 21, whilst depressing serum levels on PD 21. Mn exposure (both doses) did not affect striatal or serum Mn or iron levels on PD90. Therefore the authors concluded that the behavioural and neurochemical impact of early Mn exposure was still evident even after serum and brain levels of these minerals returned to normal. A LOEL of 250 µg approximately 625 µg/kg bw/day) could be identified based on decreased body weight, while the neurodevelopmental toxicity is observed at the higher dose of 750 µg which is approximately in the range, 1.8 mg/kg bw/day.

Klimisch Rating: 2

3.4.9 Lai, J.C., Leung, T.K., Lim, L. (1981) Brain regional distribution of glutamic acid decarboxylase, choline acetyltransferase and acetylcholinesterase in the rat: effects of chronic manganese chloride administration after two years. Journal of Neurochemistry 36:1443-8

Study Design

- Female Wistar rats (number of animals not specified) were administered manganese chloride ($MgCl_2$) (analytical grade) via their drinking water at 1000 mg/l conception for 2 years.

- At the end of the treatment period (2 months or 2 years), animals were decapitated and the brain dissected into six regions: medulla and pons, midbrain, hypothalamus, striatum, cerebellum and cerebral cortex.

- Concentrations of acetylcholinesterase, (AChE), choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) were determined in the different brain regions.

Valency of $MgCl_2$: II

Study Findings

Manganese concentrations in all brain regions were significantly higher in treated animals compared to controls from the first 2 months (the first study endpoint) through to 2 years of age. In the animals treated for 2 months, there were no statistical differences in the concentrations or the regional distribution of AChE, ChAT or GAD compared to control animals. Consistent with the aging processes, control animals showed a decrease in GAD in the hypothalamus, pons, medulla and midbrain, and AChE was decreased in all regions, the concentration of ChAT was only decreased in the striatum. Treatment with manganese partially reduced the aging process whereby the concentrations of AChE and GAD levels did not decrease and were similar to the animals at 2 months of age, however, ChAT levels were significantly higher in the striatum. The authors conclude that GAD and ChAT are markers for cholinergic neurons and changes are suggestive of functional lesions and disturbances in GABAnergic and cholinergic neurones.

Klimisch Rating: 3

3.4.10 Lai, J.C., Leung, T.K., Lim, L. (1982) The ontogeny of acetycholinesterase activities in rat brain regions and the effect of chronic treatment with manganese chloride. Journal of Neurochemistry 39: 1767-9

- Male Rats (4-11/dose) (species not stated) were administered $MnCl_2.4H_2O$ at 1000 or 10 000 mg/l via their drinking water from conception. No further details were given.

- At 5, 12, 20, 30 and 60 days old, animals were decapitated and tissue pooled from two to four rats.

- Acetycholinesterase activities were determined in the rat brain (specifically in the cerebral cortex, striatum, midbrain, pons and medulla, hypothalamus and cerebellum). No other biochemical/histological/behavioural parameters were reported.

Valency of $MgCl_2$: II

Study Findings

There were no differences in the levels of acetycholinesterase activity between treated and control animals over the duration of the study apart from in the cerebral cortex in animals treated with the top dose of manganese at day 5.

Klimisch Rating: 4

3.4.11 Leung, T.K., Lim, L., Lai, J.C. (1993) Brain regional distributions of monoamine oxidase activities in postnatal development in normal and chronically manganese treated rats. Metabolic Brain Disease 8: 137 – 49

Study Design

- The aim of this study was to determine the effect of manganese toxicity on the development of monoamine oxidase (MAO) in the brain, in particular the A (serotonin-oxidising) and B (benzylamine-oxidising) forms of MAO.

- Wistar rats (2-4/dose) were administered manganese chloride ($MnCl_2$) (analytical grade) via their drinking water at concentrations of either 1 or 10 mg/ml (approximately 0.095 and 0.95 mg/kg bw/day, respectively) from conception until the animals were required for experimental studies.

- Animals were sacrificed at 5, 12, 20, 30 and 60 days post-natal.

- Brains were dissected into regions and the concentrations of monoamine oxidase (MAO-A and MAO-B) determined.

Valency of $MnCl_2$: II

Study Findings

Chronic manganese treatment did not significantly alter the age-related changes in regional MAO-A and MAO-B activities. The authors state that previous studies have linked MAO activity to Mn toxicity, although the results from this study do not support this. A NOAEL of 10 mg $MnCl_2$/l (approximately 0.95 mg/kg bw/day) can be identified.

Klimisch Rating: 3

3.4.12 Eriksson, H., Lenngren, S., Heilbronn, E. (1987) Effect of long-term administration of manganese on biogenic amine levels in discrete striatal regions of rat brain. Archives of Toxicology 59: 426-431

Study Design

- Male Sprague-Dawley rats (number of animals not specified) were administered manganese chloride tetrahydrate ($MnCl_2.4H_2O$) (technical purity of manganese were not specified) via their drinking water at a concentration of 10 000 mg/l (approximately 950 mg/kg bw/day) from 20 days of age for either 60, 100 or 165 days.

- At 60, 100, 165 and 265 days, animals were sacrificed for neurochemical analysis. Concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5 hydroxyindoleacetic acid (5-HIAA) were measured in the caudate-putamen.

Valency of $MnCl_2.4H_2O$: II

Study Finding

Treated animals did not show any physiological signs of toxicity, and water intake and bodyweight were also not affected.

Rats exposed for 60 and 165 days showed statistically significant increased levels of DA and DOPAC in discrete regions of the dorsal caudate-putamen. These alterations were not found in rats exposed for 100 or 265 days. A “close to significant” increase in HVA was also seen at 165 days.

The authors suggested that these changes were from increased synthesis and turnover of dopamine in the dorsal region of the caudate-putamen, which is reversible even with continuous manganese exposure.

Klimisch Rating: 2

3.4.13 Lai, J.C., Minski, M.J., Chan, A.W., Leung, T.K., Lim, L. (1999) Manganese mineral interactions in brain. Neurotoxicology 20: 433-44.

Study Design

- The aim of this study was to determine if chronic manganese exposure could interact with the metabolism of other trace metals in the brain.

- Wistar Rats (6-10/dose, sex not specified) were administered manganese chloride ($MnCl_2$) (analytical grade) via their drinking water at concentrations of either 1, 10 or 20 mg/ml (approximately 0.095, 0.95 and 1.9 mg/kg bw/day, respectively) from

conception (*in utero* via their mothers), postnatally via their mother's milk and following weaning, via direct exposure in their drinking water. Control and Mn-treated animals were fed *ad libitum* rat chow containing 50 µg Mn/g dry weight for nutritional purposes.

- The regional distribution of manganese, iron, copper, zinc, magnesium, aluminium. selenium and calcium (Mn, Fe, Cu, Zn, Mg, Al, Se and Ca) in the brain were measured after the animals had been sacrificed by instrumental neutron activation analysis.

Valency of $MnCl_2$: II

Study Findings

All treated animals showed dose-related increases of Mn in the brain, with the highest levels accumulating in the striatum, hypothalamus and hippocampus. This resulted in a regional variation in Mn accumulation across the brain compared to control animals. Treated animals displayed the following effects compared to controls:

- increased Fe levels in the hypothalamus, cerebellum, hippocampus, pons and medulla, and striatum;
- increased Cu levels in the pons and medulla, hippocampus, midbrain and striatum;
- increased Zn levels in the hypothalamus and striatum;
- increased Ca levels in the midbrain but decreased levels in the cerebellum.

The regional distribution and levels of Mg and Al were not affected by treatment with Mn. The authors concluded that chronic Mn treatment induced regional changes in the distribution of manganese and several other metals and also induced changes in the subcellular distributions of trace metals and electrolytes.

Klimisch Rating: 2

3.4.14 Garcia, S.J., Gellein, K., Syversen, T., Aschner, M. (2006) A manganese-enhanced diet alters brain metals and transporters in the developing rat. Toxicological Sciences. 92: 516-525

Study Design

- Two dietary concentrations of Mn were formulated into standard rodent chow. The control diet consisted of 10 mg Mn/kg and 35 mg Iron/kg, the Mn-enhanced (measured as Mn, form not stated) diet consisted of100 mg Mn/kg and 35 mg iron/kg.

- Pregnant Sprague Dawley rats (5-7/dose) were administered manganese via the previously mentioned diets from gestational day 7.

- On post-natal (PN) day 4, the pups from the control animals and treated animals were cross-fostered so that initial mean litter weights were approximately equivalent. Pups were exposed to each of these diets via maternal milk and from direct ingestion of solid chow when capable.

- Maternal haemoglobin was assessed during lactation on PN4, 11 and 21.

- On PN21, dams and pups were euthanised. Pup brains were taken for analysis and trunk blood collected from both pups and dams.

- Metal (Cr, Co, Cu, Fe, Mn, Mo, V, Zn, Al, Cd, Mg) concentrations within brain regions were determined via ICP-MS.

- Glutamate and GABA concentrations from the brain regions were determined by HPLC and fluorescence detection.

- DMT1 and TfR (transporters of Mn and Fe in the brain) were determined by western blot analysis for each area of the brain.

Study Findings

There was no change in pup weight until PN4; however, by PN21 pups in the treatment group had significantly lower bodyweights and total brain wet weight.

There were no differences between control and treated pups with regards to haemoglobin content, although treated pups had decreased plasma Fe levels and increased transferrin levels.

Pups raised on the Mn-supplemented diet, had significantly increased concentrations of Mn in the cerebellum, midbrain and striatum, indicating that that the distribution of Mn differs between mature and immature animals. Concentrations of Fe decreased whereas Cr and Zn levels increased in the brains of treated pups. The increase in Mn and decrease in Fe was associated with an increase in the expression of DMT1 and TfR. Only the expression of TfR showed site specific increases, especially large increases in the hippocampus. Mn treated pups also showed a net increase in GABA leading to a more inhibitory action in the brains of Mn exposed pups, although motor activity was not tested in this study.

There were no gender differences between the pups regarding Mn exposure.

Klimisch Rating: 2

3.4.15 Anderson, J.G., Fordahl, S.C., Cooney, P.T., Weaver, T.L., Colyer, C.L., Erikson, K.M. (2008) Manganese exposure alters extracellular GABA, GABA receptor and transporter protein and mRNA levels in the developing rat brain. Neurotoxicology 29:1044-53

Study Design

- 21 Day old Sprague Dawley rats (6/male/diet) were administered four different diets for 6 weeks to determine the effects of iron deficiency and Mn exposure:
    - Control: 35 mg Fe/kg diet, 10 mg Mn/kg diet
    - Control + Mn: 35 mg Fe/kg diet, 10 mg Mn/kg diet, 1g $MnCl_2$/l in drinking water
    - Iron deficient: 4 mg Fe/kg diet, 10 mg Mn/kg diet
    - Iron deficient + Mn: 4 mg Fe/kg diet, 10 mg Mn/kg diet, 1g $MnCl_2$/l in drinking water
- At five weeks, animals were anaesthetised and fitted with a cannula into the striatum.
- At six weeks, brain microdialysate was collected, the animals were then sacrificed and the brains dissected into 5 regions (caudate putamen, globus pallidus, substantia nigra, hippocampus and cerebellum).
- The brain microdialysate was analysed for GABA expression.
- The brain regions were analysed for metals (Fe and Mn) by inductively coupled plasma - mass spectrometry (ICP-MS), proteins and mRNA.

Valency of $MnCl_2$: II

Study Findings

Plasma and brain Mn was significantly increased in animals receiving diets containing additional Mn. Fe levels were generally decreased in the brain of animals receiving iron deficient diets, although this was only significant in the globus pallidus; plasma Fe levels were also decreased in these animals. Extracellular concentrations of GABA were observed in the striatum with animals treated with Mn- and iron-deficient diets. The Mn- and iron-deficient diet also caused an increase in the expression of the mRNA of GAT-1, which is a GABA transporter protein, although increases in the GAT-1 protein were not observed. GABA protein expression was significantly decreased in the globus pallidus, substantia nigra and hippocampus; there was also a decline in GABA mRNA in these areas, but it was not statistically different from control animals. The decrease in expression of GABA is likely to

lead to marked changes in behaviour, although behavioural changes were not recorded in this study.

Klimisch Rating: 2

3.4.16 Anderson, J.G., Cooney, P.T., Erikson, K.M. (2007). Brain manganese accumulation is inversely related to GABA uptake in male and female rats. Toxicological Sciences 95:188-195

Study Design

- 21 day old Sprague-Dawley rats (48/sex/dose) were administered four different diets for 6 weeks to determine the effects of iron deficiency and Mn exposure:
    - Control: 35 mg Fe/kg diet, 10 mg Mn/kg diet
    - Control + Mn: 35 mg Fe/kg diet, 10 mg Mn/kg diet, 1g $MnCl_2$/l in drinking water
    - Iron deficient: 4 mg Fe/kg diet, 10 mg Mn/kg diet
    - Iron deficient + Mn: 4 mg Fe/kg diet, 10 mg Mn/kg diet, 1g $MnCl_2$/l in drinking water
- Haematocrit was measured weekly and plasma Mn levels determined by graphite furnace atomic absorption spectrometry (GF-AAS).
- At four and six weeks, animals were sacrificed and the brains dissected into four regions (caudate putamen, globus pallidus, substantia nigra and cerebellum).
- Brain Fe and Mn levels were determined by GF-AAS.
- Changes in the expression of GABA uptake were determined by in vitro measurements of ^{3}H-GABA uptake.

Valency of $MnCl_2$: II

Study Findings

The iron-deficient diets caused significant decreases in bodyweight compared to control animals, with the addition of manganese causing the most significant decrease in bodyweight. Mn supplementation in animals not receiving iron deficient diets did not have an effect on bodyweight, haematocrit or plasma Mn. Animals receiving the iron-deficient diet showed decreased brain Fe and plasma Fe, and the supplementation of the diet with Mn exaggerated this effect. Animals treated with supplementary Mn displayed an increase in Mn in the brain

regions, with significantly more Mn accumulating with an iron-deficient diet. The uptake of ^{3}H-GABA (reflective of the expression of GABA) was not significantly different between treatment groups and controls at four weeks, although the iron-deficient diets were associated with a decrease in uptake. At six weeks, Mn accumulation was associated with a significant decrease in ^{3}H-GABA uptake. The authors concluded that a decrease in GABA uptake would lead to increased inhibitory activity producing increased hypokinetic activity which is typical of Mn neurotoxicity, although in this study behaviour and activity levels were not determined.

Klimisch Rating: 2

3.4.17 Garcia, S.J., Gellein, K., Syversen, T., Aschner, M. (2007) Iron deficient and manganese supplemented diets alter metals and transporters in the developing rat brain. Toxicological Sciences 95: 205-214

Study Design

- Three diets were formulated to determine the effects of excess Mn with concurrent iron deficiency:
    - Control: 35 mg Fe/kg diet, 10 mg Mn/kg diet
    - Low Fe: 3 mg Fe/kg diet, 10 mg Mn/kg diet
    - Low Fe + Mn: 3 mg Fe/kg diet, 100 mg Mn/kg diet
- Sprague-Dawley rats (5-7/dose) were administered Mn via the aforementioned diets from gestational day 7.
- On post-natal (PN) day 4, the pups from the control animals and treated animals were cross-fostered so that initial mean litter weights were approximately equivalent. Pups were exposed to each of these diets via maternal milk and from direct ingestion of solid chow when capable.
- Maternal haemoglobin was assessed during lactation on PN 4, 11 and 21.
- Pups and dams were sacrificed on PN21, trunk blood was collected and analysed for anaemia, Plasma Fe, transferrin and total iron binding capacity. Pup brains were dissected into five regions (cerebellum, cortex, hippocampus, striatum and midbrain).
- Metal (Cr, Co, Cu, Fe, Mn, Mo, V, Zn, Al, Cd, Mg) concentrations within brain regions were determined via ICP-MS.
- Glutamate and GABA concentrations from the brain regions were determined by high pressure liquid chromatography (HPLC) and fluorescence detection.

- DMT1 and TfR (transporters of Mn and Fe in the brain) were determined by western blot analysis for each area of the brain.

Study Findings

At PN4, pup weights were equivalent, however by PN21, bodyweight was significantly lower in animals treated with iron deficient diets, this effect was enhanced with the addition of supplementary Mn. Animals administered Fe deficient diets displayed significantly lower brain Fe and increased expression of DMT1 and TfR. The addition of supplementary Mn in the diet caused these effects to be enhanced. GABA and glutamate concentrations were not significantly different for any of the treatment groups. There were no significant differences between animals from either sex apart from TfR expression which was increased in female animals treated solely with Fe-deficient diets.

Klimisch Rating: 2

3.4.18 Fordahl, S.C., Anderson, J.G., Cooney, P.T., Weaver, T.L., Colyer, C.L., Erikson, K.M. (2010) Manganese exposure inhibits the clearance of extracellular GABA and influences taurine homeostasis in the striatum of developing rats. Neurotoxicology. 31: 639-646

Study Design

- 21-day-old male Sprague-Dawley rats (14/male/dose) were administered manganese chloride ($MnCl_2$) in their drinking water at 1000 mg/l.
- At five weeks, animals were anaesthetised and fitted with a cannula into the striatum.
- At six weeks, brain microdialysate was collected, the animals were then sacrificed and the brains dissected into 5 regions (caudate putamen, globus pallidus, substantia nigra, hippocampus and cerebellum).
- The brain microdialysate was analysed for GABA expression.
- The brain regions were analysed for metals (Fe and Mn) by ICP-MS, proteins and mRNA.

Valency of $MnCl_2$: II

Study Findings

Tissue Mn levels were significantly higher in Mn exposed animals, other metal concentrations were not significantly different, although there was a significant reduction in the Fe:Mn ratio in treated animals. Analysis of the microdialysate also revealed a similar pattern of increased Mn, although Fe levels were significantly decreased compared to control animals. Mn

exposure increased baseline extracellular GABA concentrations compared to control animals, whilst extracellular taurine an amino acid neurotransmitter known to modulate GABA neurochemistry, was significantly reduced.

Klimisch Rating: 2

3.4.19 Erikson, K.M., Shihabi, Z.K., Aschner, J.L., Aschner, M. (2002) Manganese accumulates in iron deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biological Trace Element Research 87: 143-56

Study Design

- Male 21 day old Sprague Dawley rats (48/sex/dose) were administered three different diets for 6 weeks to determine the effects of iron deficiency and manganese exposure:
    - Control: 35 mg Fe/kg diet, 10 mg Mn/kg diet
    - Iron deficient: 4 mg Fe/kg diet, 10 mg Mn/kg diet
    - Iron deficient + Mn: 4 mg Fe/kg diet, 100 mg Mn/kg diet.
- Haematocrit was measured weekly and plasma Mn levels determined by GF-AAS.
- At six weeks, animals were sacrificed and the brains dissected into four regions (caudate putamen, globus pallidus, substantia nigra and cerebellum.
- Brain Fe and Mn levels were determined by GF-AAS.
- Changes in the expression of GABA uptake were determined by *in vitro* measurements of ^{3}H-GABA uptake.

Specific Mn speciation not specified.

Study Findings

The iron-deficient diets caused significant decreases in bodyweight compared to control animals, and animals were anaemic. Both iron-deficient diets caused a significant increase in Mn concentration in all brain regions except the substantia nigra which although higher, was not statistically significant. The iron deficient diet with added Mn also caused significantly higher Mn concentrations in the hippocampus compared to the iron deficient diet. The glutamate concentrations were significantly higher in the caudate putamen in animals treated with the iron deficient diet with added Mn compared to either the iron-deficient diet or control animals.

The Mn concentration was significantly correlated with increased taurine concentration in the substantia nigra. In the caudate putamen increased Mn was negatively correlated with GABA concentrations.

The authors concluded that even at normal dietary Mn concentration, iron deficiency may cause brain regional Mn accumulation as iron deficiency results in the increased expression of transferrin and transferrin receptor which can also carry Mn.

Klimisch Rating: 2

3.4.20 Summary and Conclusion

The studies on oral exposure of Mn in laboratory animals vary widely in their experimental conditions with a number involving prenatal exposure and differing concentrations of Mn (from approximately 1 to 750 mg/kg bw/day). Of the 19 studies considered, 16 used $MnCl_2$ as the form of Mn, while three dietary studies did not state the form of Mn added to the diet. As stated previously, these experiments were conducted to observe and study adverse effects so Mn(II) was widely used as the form most easily absorbed. However, there are a number of observations that can be drawn from the studies, although the weight of evidence is not such that firm conclusions can be drawn on the effects of oral exposure of Mn by drinking water and, as suggested above, it is the Mn(II) form that has been studied.

Oral exposure via drinking water does lead to an accumulation of Mn in the brain, and in several studies the Mn accumulated in the regions of the brain associated with other routes of exposure (such as inhalation) and subsequent toxicity, the striatal region and the globus pallidus.

Oral administration of Mn does have an effect on brain neurotransmitters. While it is only a single study, Mn has been shown to increase dopamine in the striatal caudate-putamen region. It also appears to affect GABA in the striatal region while there is no change in acetylcholine or monoamine oxidase (MAO) activity.

A combination iron-deficient diet and Mn administration via drinking water led to changes in the levels of transporter in the brain associated with Mn uptake. A deficiency in iron led to an increase in transferrin receptor (TfR) and DMT-1 and this was accompanied by Mn accumulation in the brain.

In a number of studies, oral exposure to Mn led to increased locomotor responses, and behavioural and learning difficulties. In one study on new-born rats (at a concentration of approximately 1.8 mg/kg bw/day, although the exact dose was difficult to calculate), an increase in locomotor response was correlated with striatal Mn levels at post-natal day 21, and behavioural effects, which persisted when blood and brain Mn levels returned to normal (Reichel *et al*., 2006). In monkeys and in rats, low doses of Mn (1 mg/kg bw/day) had effects on brain development (in monkeys) or led to learning difficulties (in rats).

3.5 Human Epidemiology Studies

Relevant journal papers were identified from the following sites:

Science Direct (http://www.sciencedirect.com)

Pub Med (www.ncbi.nlm.nih.gov/pubmed/)

Using the following terms:

Manganese, Mn, $MnCl_2$, manganese chloride, manganese II, manganese oxide, MnO, manganese III, manganese sulphate, drinking water, oral, diet, epidemiology, cross-sectional study, cohort, infant, child, children, adolescents, neurotoxicity, neurotoxic, behaviour, motor, recognition, intellect, hyperactivity, ADHD.

Terms were searched for in the title, abstract and keywords. The references from these papers were checked to identify other relevant papers. With this method a number of papers were discounted for the following reasons:

No of papers discounted	Reason for being discounted
5	Review/symposium/opinion papers
1	Not an epidemiology paper
6	Wrong or no route of exposure
4	No neurological or biological parameters measured
1	Paper modelled uptake

So 17 papers were discarded and 18 studies are outlined below. Papers are grouped by research group (or associated research affiliations) as in many cases the data presented are based on either the same or similar studies and are also conducted in a similar manner.

A number of these studies were made with groups of children in Bangladesh (Wasserman *et al*., 2006, 2011; Khan *et al*., 2011, 2012). These studies form part of a much larger ongoing project by health, environmental and social scientists in Araihazar, Bangladesh. Each of the studies described here appears to be separate with a different set of children with different exposures. The first described study (Wasserman *et al*., 2006) had a smaller group of children with high drinking water concentrations of manganese and low arsenic. In the other three studies, the drinking water manganese and arsenic levels were both high and the study

attempted to separate out the effects of manganese and arsenic or determined their combined effects. The developmental and behavioural endpoints were different for each study ranging from intellectual function, academic achievement to classroom behaviour.

For some details on the psychological tests used in the studies, a Neuropsychological Assessments Glossary is included in Appendix A.

A number of the studies use the measurement of Mn in hair as an alternative to blood and urine. A number of problems have been identified with this measurement concerning whether to wash the hair or not as there may be deposition of Mn from the air. Some difficulties in analysis have also been identified (Bader *et al*., 1999). The UK Committee on Toxicity's Expert Group on Vitamins and Minerals stated that hair measurement of Mn was useful for looking at exposed versus unexposed populations but of limited use in assessing individual exposure (EVM, 2003).

3.5.1 Bouchard, M., Laforest, F., Vandelac, L., Bellinger, D., Mergler, D. (2007) Hair manganese and hyperactive behaviours: pilot study of school-age children exposed though tap water. Environmental Health Perspectives. 115: 122–127

Aim

To evaluate the relationship between hyperactive behaviours and concentrations of Mn in hair in school children.

Study Design

Participants:

An area of Quebec, Canada, was chosen as it was known to have naturally high occurring concentrations of Mn in the public water supply. Schools in the local area were identified and teachers who agreed to participate distributed 175 letters to their pupils of which 46 pupils were eventually included in the trial, an uptake rate of 26%.

Socio-demographic data were collected from one parent per pupil by a questionnaire and included length of residence in the local community, tap water use and a dietary questionnaire focusing on foods that are rich in Mn.

Neuropsychological Analysis

Children's behaviour was assessed for attention deficit/hyperactive disorder (ADHD) using the Revised Conners' Teachers Rating Scale (CTRS-R) and Revised Conners' Parents Rating Scale (CPRS-R). The test consists of four subscales of behaviour (oppositional, hyperactivity, cognitive problems/inattention, ADHD) which are scored based on a short interview. For each subscale of the test the score was transformed into a sex and age-specific "T-score" based on

the scores of a reference population and this can be used to assess ADHD behaviour (see Appendix A).

Mn Analysis

Hair samples were collected from as close to the scalp as possible and only the 2 cm closest to the scalp were used. Hair samples were not washed before analysis and were digested using concentrated nitric acid (HNO_3). The digested samples were then analysed with Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The concentrations of Mn, Pb, Hg and Fe concentrations in the two well waters that supplied the local area were provided by the municipality and included some historical data regarding the concentrations of Fe and Mn.

Study Findings

Mn concentrations were significantly higher in one well than the other (610 v 160 µg/l).

Income and age were found to significantly affect T-scores, and were therefore considered to be confounders. Sex was also considered to be a co-variant as hair Mn concentrations were statistically higher in girls than boys, even though T-scores were not significantly different. When these were accounted for, the concentration of Mn in hair was significantly and positively associated with high scores in oppositional and hyperactivity behaviour as described by their teachers from the results in the CTRS-R scale. There was no correlation between the results in the CPRS-R scale from the parents and hair Mn concentrations. Parents also reported behavioural problems for approximately 20% of the group although only two of the children had received a diagnosis of ADHD and neither was receiving medication for it.

The authors concluded that higher concentrations of Mn in drinking water were significantly associated with hyperactivity in the classroom and that the findings warranted further investigation.

Limitation Factors of the Study

The authors admitted that the study was limited by the sample size which led to a poor statistical power. The participants were also self-selected, therefore parents who were concerned about their children's behaviour could have been more likely to volunteer for the study. No account was made for other confounders known to affect educational and behavioural parameters such as maternal education, familial stress and perinatal stress.

No blood samples were taken from the students to test for Mn or other metals/toxins which may affect neurological parameters. The hair was only tested for Mn concentrations, not other metals, such as Fe, which was also known to occur in high concentrations in the area. Hair Mn concentrations may not be the most appropriate biomarker of historical exposure to Mn

concentrations. Furthermore, the hair samples were not washed prior to analysis, so Mn may have been present on the hair shaft not from airborne particulates, as the authors were concerned about, but from washing hair with water containing high concentrations of Mn.

Despite collecting what would seem sufficient evidence, the authors made no attempt to evaluate the intake of Mn from water. This would have proven useful for the dataset, as not only did many families drink bottled water but also the two wells provided significantly different concentrations of Mn.

T-scores would not be expected to differ between girls and boys as they are normalised for age and sex against a reference population (as stated in the materials and methods section). It is therefore right to include it a co-variant. However the fact that girls had statistically higher concentrations of Mn in their hair but the T-scores were not significantly different implies that if there was an effect occurring, then the magnitude of effect was lower in girls. There is a possibility that girl's hair may retain Mn in a different way or simply that they wash their hair more in water containing Mn.

The T-scores adjusted for age and income were correlated with Mn in hair concentration; however, the correlation coefficient, r^2, values (0.106 and 0.156 for oppositional and hyperactive T scores, respectively) were very poor, implying no relationship between high concentration of Mn in hair and increased T-values (implying poor behaviour). However the authors stressed that the relationship between Mn in hair and T-scores was significant with p values of 0.020 and 0.002 for oppositional and hyperactivity scales, respectively. It is not overtly clear how these were calculated, but it is assumed that this was the chi-squared test where only hair Mn concentrations of greater than 3 µg/g were used for comparison. The authors only used values of greater than 3 µg/g as these concentrations was deemed to be elevated. However, all values should be used to demonstrate a relationship.

3.5.2 Bouchard, M.F., Sauve, S., Barbeau, B., Legrand, M., Brodeur, M-E., Bouffard, T., Limoges, E., Bellinger, D.C., Mergler, D. (2011) Intellectual Impairment in School-age Children Exposed to Manganese. Environmental Health Perspectives. 119: 138-143

Aim

To assess the relationship between exposure to Mn from drinking water and IQ of school-age children.

Study Design

Participants

Eight areas of Quebec (Canada) were identified which had a range of naturally occurring Mn concentrations in the groundwater which fed their drinking water supply. Elementary schools within this area were approached and the principals and teachers who agreed to participate,

distributed letters amongst their pupils. 362 children participated in the study, a participation rate of 52%. Children were only included if they had lived in the same house for a period of greater than 3 months.

Neuropsychological Evaluation

The Weschler Abbreviated Scale of Intelligence (WASI) was used to assess general cognitive abilities. The results of this test provide a verbal IQ, performance IQ and a full scale IQ score. Maternal non-verbal intelligence was assessed by the Ravens progressive matrices test, home cognitive stimulation was assessed with the Home Observation for Measurement of the Environment (HOME) and maternal symptoms of depression with the Beck Depression Inventory-II. Data on family income and other factors such as alcohol and tobacco consumption through pregnancy were assessed through a questionnaire.

Mn Analysis

A sample of the child's hair was taken as close to the root as possible. Nine children were excluded from hair analysis because of recent use of hair dye, which had been shown to lead to higher Mn concentrations. Only the first 2 cm of the hair was used for analysis, therefore representing the most recent exposure. In contrast to the previous study by the same authors where hair was unwashed (Bouchard *et al*., 2007), in this study hair was washed before analysis to remove particulates from the surface. The hair was also checked by electron microscopy to ensure that structural integrity had not been compromised. Mn, lead (Pb), iron (Fe), arsenic (As), Zinc (Zn) and copper (Cu) concentrations in the hair samples were analysed by ICP-MS. Hair samples from 302 children were used for analysis.

Water samples were collected once from homes during a home visit at the start of the study using a standardised procedure. Twenty families also had water collected on 3 occasions over a 1 year period to estimate the variability over time. Mn, Pb, Fe, As, Zn and Cu concentrations in the water samples were analysed by ICP-MS.

Estimation of Mn intake: A semi quantitative questionnaire on food frequency was conducted to estimate Mn intake from the diet as well as water sources. The questionnaire also included some use on water consumption from different sources (such as bottled water) and filtered water.

Mn exposure was assumed to be based on either Mn concentration in hair, Mn concentrations in water or estimated Mn intake from water consumption and diet.

Study Findings

The distribution of Mn concentrations in hair and water was not normally distributed and was therefore logged (Log10).

The range of Mn in tap water was found to be 1 – 2700 µg/l with a mean of 98 µg/l (geometric mean (GM) was 20 µg/l). Houses with private wells had a lower GM Mn water concentrations than those using the public well (8 and 55 µg/l).

The concentrations of other metals measured in the participants' tap water were not considered to be high. These analyses included lead (95 percentile of 2.8 µg/l), exposure to which has also been shown to cause developmental and behavioural problems, although the maximum concentration of 13.8 µg/l is above the new UK standard of 10 µg/l, which was introduced in December, 2013.

Estimated median Mn intake from direct consumption of water, from water incorporated into food and the median intake from total water consumption was 1.6, 1.9 and 8 µg/kg bw/month, respectively. The estimated median intake from dietary sources was 2335 µg/kg bw/month.

Factors that were found to affect IQ were adjusted for in two models. Model A accounted for the socio-demographic factors of maternal education, maternal non-verbal intelligence, family income, home stimulation score and family structure. Model B accounted for the same socio-demographic factors as A, but also included sex, age of child, time of IQ session (morning, lunchtime, or afternoon), source of well water (private or public) and Fe concentration.

Mn concentrations in hair were found to significantly increase with measured Mn concentrations in water and estimated total Mn intake from water, but not from estimated total diet concentrations.

Children's IQ was not associated with estimated total diet Mn concentrations.

When Mn concentrations in tap water (collected as described above) were divided into quintiles (0-2, 3-19, 20-66, 67-153, 154-2700 µg/l) to provide a range of doses, with increasing Mn concentration, there was a decrease in full scale IQ when using model B (results for model A and the unadjusted model were not reported), consistent with a dose-response relationship. Children exposed to the highest quintile of tap water Mn concentration (154-2700 µg/l) had on average a full IQ score of 6.2 points lower than children who were exposed to the lowest quintile of tap water Mn concentration (0-2 µg/l). There was no consistent pattern with full scale IQ and estimated Mn intake from water or Mn concentrations in the lowest four quintiles. However the full scale IQ was consistently lower in children from the fifth quintile compared to the other quintiles.

Full scale IQ score was found to decrease by 3.2 points with a 10-fold increase of Mn concentration in hair. Using model A and model B, full scale IQ was found to decrease 3.7 and 3.3 points, respectively. A 10-fold increase in the concentration of Mn in water resulted in a decrease in full scale IQ of 2.1, 1.9 and 2.4 points for the unadjusted model, model A and model B, respectively. A 10-fold increase in the estimated Mn intake from water consumption resulted in a decrease in full scale IQ of 1.3, 1.2 and 1.2 points for the unadjusted model, model A and model B, respectively.

The authors suggest that Mn in food may be absorbed or metabolised differently from Mn in drinking water and this can therefore lead to overload and subsequent neurotoxic effects in children exposed to Mn in drinking water.

In conclusion, the authors state that low-level exposure to manganese from drinking water is associated with significant intellectual impairments in children.

Limitations Factors of the Study

There is likely to be maternal recall bias, mothers are less likely to admit smoking or drinking during pregnancy.

Children were only included in the study if they had lived in the area for a minimum of 3 months. However length of residence (exposure time) might be an important co-variant and would have been useful to investigate.

The authors describe a positive relationship with the distribution of Mn concentrations in hair and the estimated intake from water consumption (when divided into quintiles). However the highest quintile shows a large variation in Mn hair concentrations, also no statistical evaluation is provided. From visual inspection, it is unlikely that there is a statistical difference between the first four quintiles.

The authors state that the recommended dietary intake for children between the ages of 6-13 years of age is between 1.5 and 1.9 mg/day and that the dietary intake of the children in this study was similar to these concentrations. From this study, the median estimated Mn concentration from dietary sources from direct consumption of food and drink was 2335 µg/kg bw/month and 8 µg/kg bw/month, respectively, a total of 2343 µg/kg bw/month (dietary sources were calculated from 346 children and total intake from water calculated from 362 children). Assuming a 10 kg child and an average of 30 days a month then the average median intake was 0.781 mg/day considerably below the recommended dietary intake that the authors state (1.5 – 1.9 mg/day, taken from the US Institute of Medicine, Food and Nutrition Board). Using the same assumption, the 75th percentile of total daily intake was 1.18 mg/day, and the 95th percentile 2.23 mg/day. This suggests that less than a quarter of the children in the study were receiving the recommended concentration of Mn a day. This assumption of using a 10 kg child is probably a conservative estimate and the actual intake is lower. This is suggested in the letter of Chen and Copes (2011) outlined below, which compares the recommended daily intake for 4-13 year-old children (1.5 - 1.9 mg/day) with an estimated intake of <0.43 mg/day from water,

3.5.3 Bouchard, M.F. (2011) Manganese in drinking water: Bouchard Responds. Environmental Health Perspectives; 119: A241

The authors H. Chen and R. Copes at the Ontario Agency for Health Protection and Promotion raised several questions about the study “Intellectual Impairment in School-age

Children Exposed to Manganese" Environmental Health Perspectives. 119(1):138-143. (2011).

- The concentration of Mn in tap water was significantly associated with IQ, however 33% of the study participants did not drink tap water at home.

- The children in the highest quintile of Mn concentration (median 216 µg/l), assuming a daily intake of 2 litres per day received <0.43 mg/day of Mn from water intake, which is below recommended daily intakes.

- The children's intake of Mn from food was more than two orders of magnitude higher than from water.

- There is no standardised biomarker test for Mn exposure. Bouchard excluded children who had dyed hair. However, there may be natural differences in Mn concentration in hair as a reflection of different hair colours.

Dr Bouchard responded to the first point by suggesting that although these children did not drink tap water, they were still exposed to the tap water through consumption of food and drink prepared with tap water. Dr Bouchard also hypothesised that children might be exposed by inhalation of aerosols containing Mn when showering and this could also induce neurotoxic effects. Regarding the relatively large intake of Mn from food compared to water, Dr Bouchard reasons that the dietary Mn intake was not associated with IQ and did not change the point estimates for water Mn concentration when included in the regression model. Bouchard acknowledges that a valid biomarker for Mn exposure would greatly help the understanding of this metal's toxic effect. Dr Bouchard did not address the point that Mn intake for many of the participants was lower than the recommended daily intake.

3.5.4 Communication with Dr Maryse Bouchard

Following publication of the study described in Section 3.5.2 and the start of this project, WRc contacted Dr Bouchard for further information on two topics and her replies are given below:

- The oxidative state of Mn present in the water supplies of the different groups in her study.

Speciation was not measured directly as several hundred houses were visited in the study, and it would have been necessary to analyse sample at the time of sampling. Therefore in relation to characterising the "type" of Mn present in water, a sample of filtered water (0.45 um) and unfiltered (total, including particulates) were taken. The ratio of filtered/unfiltered Mn was quite high in general (above 95%), but was lower in certain sites (around 70%). This indicated that most Mn was in a soluble form (therefore, likely to be Mn(II)).

The study was carried out at 8 different sites and each site was a community with their independent aqueduct and it was suspected that the chemical form of Mn varied depending on the water treatment used. For example, some communities add sequestrants to the water in order to reduce the some of the problems caused by the presence of Mn, such as bad taste and staining.

Dr Bouchard also kindly supplied a published paper on the removal of Mn from drinking water (Carriere *et al*., 2011) and this contained the following comment that the most common Mn^{4+} compound was manganese dioxide and that this is insoluble in water. In the Bouchard *et al*. (2011) study, as stated above, more than 90% of Mn in 8 municipal groundwaters was dissolved. However, the addition of chlorine in distribution systems oxidises Mn, and so the fraction of dissolved Mn found in household was highly variable (4-100%, average of 63%). For domestic wells, Mn appears to remain in a dissolved state.

- Follow-up studies that Dr Bouchard is currently conducting or planning to conduct.

Dr Bouchard is presently following up the children who participated in the initial study published in EHP in 2011. For approximately half the cohort, it is known that exposure levels, i.e. water concentration of Mn, have been dramatically reduced. Communities and/or individual families have installed water treatment to remove Mn from water.

Thus, Dr Bouchard now plans to investigate whether this exposure reduction is associated with improvement in cognitive function.

The exposure to Mn in drinking water has been reduced relative to previous levels. However, this accounts for only a small proportion of total manganese exposure (approximately 0.3%), so unless diet also has changed, total manganese exposure will not reduce to any significant extent.

3.5.5 Wasserman, G.A., Liu, Z., Parvez, F., Ahsan, H., Levy, D., Factor-Litvak, P., Kline, J., van Green, A., Slavkovich, V., Lolacono, N.J., Cheng, Z., Zheng, Y., Graziano, J.H. (2006) Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environmental Health Perspectives; 114:124-129

Aim

This study focused on a potential association between well water Mn concentrations and intellectual function in 142 children.

Study Design

Participant Selection

This study was part of a wider multidisciplinary study between health and drinking water quality in Bangladesh. 11 749 adults were enrolled in the wider cohort study, 142 of their children were identified for the study if they were between 9.5 to 10.5 years of age, were currently attending school and drank from well water with concentrations of arsenic (As) of less than 10 µg/l.

The selected children attended a clinic with their mothers and received a medical examination. Weight, height, and head circumference were measured, and urine and blood samples were taken. Of the 142 children, only 95 agreed to provide blood samples. The intellectual function of both the child and mother were determined. Information of family demographics was obtained in the initial enrolment of the wider cohort study. Information on the primary source of drinking water and birth order of the child was collected from the mother; details of parental age, education, occupation and social class were also collected.

Neuropsychological Evaluation

Children's intellectual function was measured by the Weschler Intelligence Scale for Children, Version III, of which 6 subtests (similarities, digit span, picture completion, coding, block design and mazes) were used that were most adaptable to account for cultural differences. Maternal intelligence was assessed with Raven's Standard Progressive Matrices.

Mn Analysis

Water samples were collected as part of wider survey of all wells in the study region. The samples were analysed by High Resolution Inductively Coupled Plasma-Mass Spectrometry (HR ICP-MS). Blood Mn concentrations were measured by ICP-MS.

Study Findings

The mean Mn concentration in the well water was found to be 795 µg/l (the range was from 4 to 3908 µg/l).

Higher scores in the tests for intellectual function were associated with better educated mothers, more adequate housing, access to televisions, taller height and larger head circumferences. When these socio-demographic factors were accounted for, water Mn was significantly inversely associated with intellectual function.

The concentrations of Mn in water were initially divided into quartiles; however, the range of concentrations for each quartile was found to be similar to policy guideline cut-off points (guidelines not stated). Consequently the authors used the cut-off points from the guidelines to represent the quartiles (<200 µg/l, ≥200 - <500 µg/l, ≥500 – <1000 µg/l, ≥1000 µg/l). Using

the average mean concentration for each quartile and estimating daily water intake to be 2.4 and 2.1 l/day for a 10 year old boy and girl, respectively, the Mn water intake quartile values were calculated to be: 0.25, 1.06, 1.92 and 4.37 mg/day for boys (estimated to be 0.011, 0.047, 0.09, 0.194 mg/kg bw/day, respectively, assuming an average bodyweight of 22.4 kg); and, 0.21, 0.93, 1.68 and 3.82 mg/day for girls (0.009, 0.041, 0.075 and 0.17 mg/kg bw/day, respectively, assuming an average bodyweight of 22.4 kg). There was a dose-response relationship between intellectual function scores and children's intelligence whereby, with increasing Mn concentration in water, intellectual function scores decreased. Children who were exposed to the highest concentrations of Mn in water (≥1000 µg/l) had significantly lower scores in all three functions of intelligence compared to children who were exposed to the lowest concentrations.

Blood Mn concentrations were not correlated with child intelligence.

The authors conclude that exposure to Mn in drinking water causes neurotoxic effects as they observed a decrease in intellectual function with increasing Mn concentration in water consistent with a dose-response relationship.

Limitations Factors of the Study

The authors acknowledge that the intelligence tests used were not standardised for use in Bangladesh. Furthermore, Mn in food and air was not monitored to provide an estimate of total Mn exposure, and equally the contribution from these sources cannot be calculated.

The children were identified from wells that contained low levels of As (≤10 µg/l) however, concentrations of other metals, which may contribute to neurotoxicity, were either not measured or reported.

The socio-demographics of the children in the study were not compared to children who were not included in the study but were part of the cohort study, therefore any selection bias in picking the children for this particular study cannot be identified.

Only children who had attended school regularly were included in the study. However, this may have led to selection bias as children with learning difficulties may not be regularly attending school.

3.5.6 Khan, K., Factor-Litvak, P., Wasserman, G., Liu, X., Ahmed, E., Parvez, F., Slavkovitch, V., Levy, D., Mey, J., van Geen, A. Graziano, J.H. (2011) Manganese exposure from drinking water and children's classroom behaviour in Bangladesh. Environmental Health Perspectives. 119: 1501-1506

Aim

The joint effects of Arsenic (As) and Mn (and possible synergistic effects) in drinking well water on children's behaviour was investigated.

Study Design

Participant selection

This study was part of a wider multidisciplinary study between health and drinking water quality in Bangladesh. 11 749 adults were enrolled in the wider cohort study and the children of these adults were used for the study. For recruitment purposes only, wells were grouped into four categories:

a) High As (>10 µg/l), High Mn (>400 µg/l);

b) High As (>10 µg/l), Low Mn (<400 µg/l);

c) Low As (<10 µg/l), High Mn (>400 µg/l); and,

d) Low As (<10 µg/l), low Mn (<400 µg/l).

The aim was then to recruit approximately 75 children per category. Children were identified from households that were within commuting distance of field clinics and were between the ages of 8 – 11 years of age, had regular school attendance, no chronic illnesses and those who did not share a home well with other children in the study. The selection of children was further refined by identifying those that attended schools close to field clinics and whose principal agreed to allow their teachers to evaluate the participating child's behaviour. Consequently 201 children participated in the study.

A field team collected information regarding socio-demographic information and blood and urine samples for the children were collected at the field clinic.

Neuropsychological evaluation

The children's teachers completed a child behaviour checklist Teachers Report Form (TRF) based on the Achenbach System of Empirically Based Assessment (ASEBA) which provides two subscales for determining internalising (anxious/depressed/withdrawn) and externalising (attention problems/aggressive) behaviour.

Manganese Analysis

Well water samples were analysed for Mn and As by ICP-MS. Blood samples were analysed for blood Mn, As and Pb concentrations using ICP-MS.

Study Findings

To avoid selection bias, children who participated in the study were compared to those who were not part of the study but were included in the cohort. No significant differences were found for child's sex, age, BMI or maternal education or intelligence.

The average water Mn concentration was approximately 900 µg/l (range: 40 – 3443 µg/l).

Boys were found to have significantly higher externalising behaviour (showing extrovert and under-controlled behaviour to others, which may include physical aggression and verbal bullying) than girls; children who mothers had received more schooling also had lower problem behaviour scores. When adjustments were made for socio-demographic, maternal factors and covariates including sex and BMI, the concentration of Mn in water was significantly correlated with externalising, internalising and total scores of behaviour. The authors observed no statistical interaction between the concentrations of As and Mn in water and behaviour.

The concentrations of Mn in the well waters were then categorised into quartiles (0 – 264 µg/l, 265 – 641 µg/l, 642 – 1279 µg/l, ≥1280 µg/l) and the adjusted means compared to the behaviour scores to allow for the expression of a dose-response relationship. A monotonic dose-response relationship was then observed between dose and externalising behaviour. A similar dose-response was observed for internalising behaviour and Mn concentration; however, behavioural scores were only significantly different between the lowest and highest quartiles.

The total TRF scores were significantly higher for children in the three highest quartiles compared to the lowest quartile with water Mn concentration, however this was largely driven by the data from the externalising score. Internalising behaviour displayed a poor association with water Mn concentrations; only the children in the highest quartile had a significantly higher TRF score compared to the lowest quartile.

The authors concluded that they found a significant positive association between water Mn concentrations and externalising and internalising behaviour with increasing problematic behaviour with increased water Mn. Externalising behaviour was significantly affected by concentrations greater than 265 µg/l. Internalising behaviour was significantly affected at concentration of Mn greater than 1280 µg/l.

Limitations Factors of the Study

The children participating in the study were all regular attendees at local schools; however, this may have led to selection bias as children with behavioural problems may not have been regular attendees at school and therefore not included in the study.

The authors admit that unmeasured teacher bias cannot be discounted.

Only Mn and As concentration were reported, potentially other neurotoxic agents could have been present in the water supply.

No account was made for total dietary Mn intake which was not measured.

3.5.7 Wasserman, G., Liu, X., Parvez, F., Factor-Litvak, P., Ahsan, H., Levy, D., Kline, J., van Green, A., Mey, J., Slavkovitch, Siddique A.B., Islam, T., Graziano, J.H. (2011) Arsenic and manganese exposure and children's intellectual function. NeuroToxicology. 32:450-457

Aim

To investigate the possible synergistic impact of simultaneous exposure to Mn and As on children's intellectual function.

Study Design

Participants

This study was part of a wider multidisciplinary study between health and drinking water quality in Bangladesh. 11 749 adults were enrolled in the wider cohort study and the children of these adults were used for the study. All eligible children between the ages of 8 to 11 were identified on the basis of the concentrations of As and Mn in their local well waters (above and below 10 µg/l for As and above and below 500 µg/l for Mn). 772 families were visited as part of the eligibility review resulting in 304 children included on the study, a participation rate of 39%. Children were excluded if they suffered from a chronic illness/disability, were a multiple birth, if their families had switched wells or the child shared a well with another participant in the study. The children also had to attend school regularly.

Neuropsychological evaluation

Children's intellectual function was measured by an adapted Weschler Intelligence Scale for Children, Version IV (WISC-IV). Maternal intelligence was measured with an adapted Weschler Abbreviated Scale of Intelligence (WASI). Both tests were adapted to account for cultural differences, equally not all parts of the test were administered as no standardised IQ test had been developed or fully adapted for Bangladesh.

Socio-demographic characteristics were assessed during a home visit and included information relating to the child's health and parental lifestyle variables. The home environment was also assessed to determine educational material and toys at the child's home.

Manganese Analysis

Water samples were collected as part of wider survey of all wells in the study region. The samples were analysed by High Resolution (HR) ICP-MS for As and Mn. Blood As, Mn, Pb and selenium concentrations were measured by ICP-MS. The Mn concentration in urine was measured by GF-AAS.

Study Findings

When adjustments were made for socio-demographic features and ferritin levels, blood Mn was significantly associated with a reduction in perceptual reasoning and working memory.

When blood Mn concentrations were divided into quartiles (range of values in quartiles not stated, but the average blood Mn for all children was 14.8 µg/l), there was a decrease in all measures of IQ from lowest to highest quartile. Comparing the lowest and highest blood Mn quartiles, full scale IQ (adjusted for confounders) decreased by 9.71 points, and perceptual reasoning and working memory scores decreased by 4.89 and 2.57 points, respectively.

There were no significant correlations with water Mn concentrations and IQ.

Blood As was not associated with a reduction in any of the measured parameters of intellectual function. However urinary As concentrations were negatively correlated with verbal comprehension scores, even after adjustment of confounders. There was no significant relationship between Mn and As concentrations on intellectual function; however, only two children drank from wells that were extremely high in both Mn and As concentrations.

The authors concluded that higher blood Mn concentrations in children were associated with poorer scores in intellectual function tests.

Limitations Factors of the Study

Only children who attended school regularly were included in the study, therefore children who have difficulty at school because of intellectual or behavioural problems may have been excluded from the study.

There is no suggestion from the paper that other metals or neurotoxic agents were analysed in the well water that might be responsible for the effects on neurological function. Furthermore, no account was made for total dietary Mn intake and other sources of exposure.

The authors divide the range of blood Mn concentrations into quartiles but do not state characteristics (range, mean) for the individual quartiles, therefore, it is not possible to attribute a change in intellectual function with a specific increase in blood Mn.

The authors did not compare water Mn concentrations to blood Mn levels; this would have been useful for identifying the value of blood Mn as a biomarker of Mn exposure (if they did not correlate, either blood Mn was not a good biomarker, or total Mn exposure was not predominately from water). As blood Mn was found to correlate with intellectual function, but water Mn concentration did not, it is perhaps likely that the two factors are not associated, although without further mathematical support this suggestion is purely hypothetical.

3.5.8 Khan, K., Wasserman, G.A., Liu, X., Ahmed, E., Parvez, F., Slavkovich, V., Levy, D., Mey, J., van Geen, A., Graziano, J.H., Factor-Litvak, P. (2012) Manganese exposure from drinking water and children's academic achievement. NeuroToxicology 33: 91-97

Aim

This study aimed to examine possible associations between Mn and As concentrations in well waters and academic achievement among 8 - 11 year old children. It also aimed to identify any possible joint effect (synergism) of the two metals on academic achievement.

Study Design

Participants

This study was part of a wider multidisciplinary study between health and drinking water quality in Bangladesh. 11 749 adults were enrolled in the wider cohort study and the children of these adults were used for the study. Children were identified for the study if their school was deemed to be within a reasonable travelling distance from the field clinic and the class had a minimum of 10 children between the ages of 8 - 11. Children were included if they had no known physical disability or chronic illness, were not twins and attended school frequently.

A field team collected information regarding socio-demographic information and urine samples for the children were collected at the field clinic along with height, weight, head and arm circumference.

Neuropsychological evaluation

Intellectual function was measured by the performance records of each child in the most recent annual school-wide district test in both languages (English and Bangla) and mathematics.

Manganese Analysis

Well water samples were analysed for Mn and As by ICP-MS. Urine samples were analysed for As concentrations by GF-AAS.

Study Findings

Socio-demographic factors such as parental education and school grade were found to affect school performance. When account was made for these factors (along with correlations for specific teachers), there was a statistically significant inverse association between water Mn concentration and mathematics achievement test scores. There was an association between language scores and water Mn concentrations; however, this was not statistically significant. The authors stated that language is taught by reading-memorisation-writing approach and tests; therefore, they have very little dependence on working memory, which has been shown to affect mathematics ability.

The authors found no correlation between measures of As exposure and academic achievements.

The concentrations of Mn in the well waters was categorised into quintiles, one below the WHO standard (400 µg/l), and a further four higher exposure levels with approximately equal numbers of subjects in each (≤400 µg/l, 401 – 1000 µg/l, 1001 – 1440 µg/l, 1441 – 2000 µg/l, 2001 – 6000 µg/l). The adjusted means from each quintile were compared to the behaviour scores to allow for the expression of a dose-response relationship. However, the test scores for the adjusted mean Mn water concentrations were similar above concentrations of >400 µg/l indicating that there was a threshold effect. Consequently the authors grouped the higher four groups together so that there were two groups: ≤400 µg/l and >400 µg/l.

Well water Mn concentrations >400 µg/l were associated with a 6% decrease in mathematics scores and a 1% and 3% decrease in Bangla and English scores, respectively, although the differences in Bangla and English were not statistically different from well water concentrations <400 µg/l.

The authors concluded that there was a statistically significant negative association between water Mn and mathematics scores.

Limitations Factors of the Study

No biomarker of Mn exposure was taken, only educational attainment. Furthermore, total dietary Mn intake was not calculated.

There is no suggestion from the paper if any account was taken of the concentrations of other metals or neurotoxic agents.

The children participating in the study were all regular attendees at local schools, however this may have led to selection bias as children with behavioural problems or learning difficulties may not have been regular attendees at school and therefore not included in the study.

3.5.9 He, P., Liu, D.H., Zhang, G.Q. (1994) Effects of manganese on learning abilities in school children. Zhonghua Yu Fang YiXue Za Zhi 29: 156-8

Abstract only (Article in Chinese)

Aim

The neurobehavioural status of school pupils aged 11-13 in an area with high level Mn sewage irrigation and a control area were compared.

Study Design

Participants

The neurobehavioural status of 92 matched-pair pupils aged 11-13 in an area with high level Mn sewage irrigation and a control area were compared.

Neuropsychological evaluation

Hair Mn concentration were determined from the children in the area with a high level of Mn sewage irrigation and from control children.

Mn analysis not stated in abstract.

Study Findings

The Mn concentrations in the drinking water were found to range from 0.241 - 0.346 mg/l and 0.030 - 0.040 mg/l for the area with high levels of Mn and the control area, respectively. Average hair Mn concentrations in the children who were exposed to the high concentration of Mn was found to be statistically higher at 1.252 µg/g compared to 0.961 µg/g for the children in the control area.

Negative scores in the neurobehavioural tests were significantly correlated with high concentrations of hair Mn.

The authors concluded that high levels of manganese in drinking water might be an important factor affecting children's neurobehavioural changes.

Limitations Factors of the Study

From the abstract it is not possible to tell if analysis for other metals was conducted.

Not enough information was available from the abstract to determine the reliability of the study.

3.5.10 Zhang, G., Liu, D., He, P. (1995) Effects of manganese on learning abilities in school children. Zhonghua Yu Fang Yi Xue Za Zhi 29: 156-8

Abstract only (article in Chinese)

Aim

To determine the effects of high concentrations of manganese in drinking water on the learning capacity of school children.

Study Design

Participants

Details were not available in the abstract but it is assumed to be the same participants as the students from the study by He *et al*., (1994) wherein 92 matched-pair pupils aged 11-13 in an area with high level Mn sewage irrigation and a control area were compared.

Levels of serum 5-hydroxytryptamine (5-HT), dopamine, norepinephrine, acetycholinesterase, were also determined in the school children.

Neuropsychological evaluation

Intellectual function was determined by school records for Chinese language and mathematics.

Manganese Analysis

Hair and blood samples were taken from the students.

Mn analysis not stated in abstract.

Study Findings

Hair and blood Mn concentrations averaged 1.242 µg/g and 3.39 µg/dl, respectively. The school records of the children with high concentrations of Mn in blood and hair were poorer than those of the children with low concentrations of Mn. The four neurotransmitters were also found to be lower in children who had high concentrations of hair and blood Mn.

Limitations Factors of the Study

The abstract does not indicate whether analysis for other metals was conducted.

Not enough information was available from the abstract to determine the reliability of the study.

3.5.11 Wright, R.O., Amarasiriwardena, C., Woolf, A.D., Jim, R., Bellinger, D.C. (2006) Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. NeuroToxicology 27: 210-216

Aim

To evaluate the associations between hair levels of As (As), Mn and Cadmium (Cd) and neuropsychological function and behaviour in school-aged children.

Study Design

Participants

The study was conducted among children residing in an area of Oklahoma, USA, with a 100-year history of Pb and Zn mining. Children were recruited from two science classes with a 40% uptake rate of participation with 31 children in total participating. Each child was paid $50 for participation.

Neuropsychological evaluation

The children were assessed with a variety of neuropsychological tests as potential effects on neurological parameters were unknown.

The tests included:

- Weschler Abbreviated Intelligence Scale (WASI).
- Wide Range Assessment of Visual Motor Ability (WRAVMA).
- The 3 receptive scales of the Clinical Evaluation of Language Fundamentals - Third Edition (CELF 3).
- Children's Category Test Level II (CCT).
- California Verbal Learning Test-Children (CVLT-c),

- Story memory subtest of the Wide Range Assessment of Memory and Learning (WRAML).
- Children's Depression Inventory (CDI).
- Behaviour Assessment System for Children.

The Children's parents completed the following tests:

- Behaviour Rating Inventory of Executive Functions (BRIEF).
- The Conners' ADHD/DSM-IV Scales (CADS-IV).
- A questionnaire on ethnicity, primary language, family structure, parental education, the child's medical and education history.

Teachers additionally completed:

- Behaviour Rating Inventory of Executive Functions (BRIEF).
- The Conners' ADHD/DSM-IV Scales (CADS-IV).
- BASC.

Manganese Analysis

Hair samples (approx. 30 – 40 strands) free of hair gel were collected from each child and cleaned by sonication with triton X-100, the samples were digested with nitric acid and analysed by DRC ICP-MS. Samples were analysed for Mn, Pb, As and Cd.

Study Findings

Average Mn concentrations were found to be 471.5 (range 89.1 – 2145.3) ppb and concentrations were not significantly different between girls and boys. The average IQ across the cohort as a whole was 94.5.

Hair As and Mn concentrations were inversely associated with full scale and verbal IQ, but not to performance IQ. Because of the demonstrated relationship between Pb and IQ, Pb concentrations were also used as co-variant as well as maternal education and sex. After adjustments were made for these confounders, relationships were still statistically significant. Hair Cd concentrations were not significantly related to any IQ score before or after adjustment for confounders.

There was a significant interaction between hair As and Mn and full scale and verbal IQ. Children who had high concentrations of both metals in their hair had up to 10 full scale IQ points lower than children who low concentrations of both metals in their hair (the authors do not state what the effect on IQ is for As and Mn individually). High exposure to As has been shown to affect IQ.

There were no significant correlations between behavioural assessments or metal concentrations in hair.

The authors concluded that in this cross-sectional pilot study that significant inverse associations between children's neuropsychological function and both hair Mn and As levels were observed. Children's verbal skills including memory were most affected.

Limitations Factors of the Study

The interaction between As and Mn was not statistically evaluated for IQ and due to the small sample size, it is unlikely that they would be significantly different.

The sources of exposure have not been detailed in the study and although high concentrations of metals have been measured in local ground water, concentrations in drinking water have not been explicitly measured or evaluated. Furthermore, other routes of exposure and possible sources have not been evaluated.

The sample size was very limited, only 31 children were included in the study, and there could have been a self-selection bias, whereby families who consented to participate may already have had concerns about pollution in the area or about the educational development of their child.

The hair Mn concentrations in this study are given as ppb, which is presumably equivalent to µg/kg. The hair Mn in the Canadian study (Bouchard et al., 2007) was measured as µg/g with the mean of the two groups being 3.3 and 6.2 µg/g. The mean and range of the hair Mn in this study are equivalent to 0.47 (range 0.09 – 2.15) µg/g, which appears to lower, although water Mn levels were not measured in this study,

The authors admit that they cannot evaluate the role that past Pb exposure has had on the children's test score, as although Pb levels in the children's hair did not appear to modify any associations with Mn and As on the test scores, hair is a poor biomarker for Pb.

3.5.12 Kim, Y., Kim, B-N., Hong, Y-C., Shin, M-S., Yoo, H-J., Kim, J-W., Bhang, S-Y., Cho, S-C. (2009) Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. NeuroToxicology. 30: 564-571

Aim

To investigate the association between the intelligence of school-aged children and co-exposure to Pb and Mn.

Study Design

Participants

Study participants were identified from four Korean cities, one school near each city centre was selected and a letter of participation sent to third and fourth grade school children (8 – 11 year of age). Of 434 children contacted, there was an uptake rate of 66%, however only 261 actually participated due to exclusions; not all the participants completed both sets of blood sampling and IQ measurements, and other were then excluded owing to medical conditions.

Neuropsychological Evaluation

The Korean Educational Development Institute-Weschler Intelligence Scales were used to assess each child's cognitive function. The results of the intelligence scales were age adjusted and provided two scores: verbal IQ and performance IQ, the combination of these two results provided a full scale IQ.

The parents completed an extensive questionnaire recording socio-demographic information.

Manganese Analysis

Pb and Mn concentrations were determined from blood samples. Samples were assayed using a GF-AAS.

Study Findings

The mean blood concentration of Pb and Mn were 1.73 µg/dl (range: 0.42 – 4.91 µg/dl) and 14.3 µg/dl (range: 5.30 – 29.02 µg/dl), respectively. Blood Pb levels were significantly higher in females than males. Paternal and maternal educational years correlated significantly with children's IQ. Both metals were associated with a negative effect on IQ. Furthermore, there was a significant association between the two metals and IQ indicating a synergistic effect. When blood Pb levels were divided into quartiles (0 - 0.18, 1.18 – 1.54, 1.55 – 2.17, 2.18 - 4.19 µg/dl) there was a statistically significant difference amongst the quartile groups and for the mean total IQ score. When Mn blood levels were divided into quartiles (5.3 – 11.7, 11.7 – 14.0, 14.0 – 16.4, 16.4 – 29.0 µg/dl) there was no difference between quartiles groups

and IQ score. However, linear regression, with and without adjustment for confounders, was inversely associated with total and verbal IQ scores; there was no association for performance IQ.

To test for synergistic effects between co-exposure between Pb and Mn, multiple linear regression was performed for all scores of IQ using predictive variable for IQ. There was an increase in the percentages of the variances explained when blood Pb and Mn levels were entered as predictive variables, demonstrating an additive association between Pb and Mn concentrations and verbal and total IQ. Children were divided into two groups: children with blood Mn levels lower than 14 µg/dl and those higher (14 µg/dl was the median Mn level of the study population). There was no difference between Pb concentration in blood and the two Mn groups. Linear regression analysis was modelled for the three scores of IQ (full scale, verbal and performance IQ) as outcome variables and the measured blood Pb concentrations used as the predictor variable for the low and high-level blood Mn groups. Verbal and full scale IQ for children with Mn concentrations above 14 µg/dl were significantly associated with Pb levels in the regression analysis, although there was no association for children with Mn concentrations below 14 µg/dl.

The authors conclude that in this study Mn was not found to correlate with any scores of IQ, it may, however worsen the effects of Pb, a toxicant known to affect children's neurological development.

Limitations Factors of the Study

The authors acknowledge that socio-demographic data were collected by questionnaires and no direct assessment of the home environment or caregivers IQ was collected, therefore potentially leading to either self-reported bias or unaccounted variants which may affect IQ. Furthermore, this was a cross-sectional study, which limits interpretations of the results for a causal relationship. Parents who were concerned about their child's behaviour could also have been more likely to volunteer for the study.

No source or route of exposure to Mn has been suggested.

3.5.13 Said, Y., Abdu, A., Taoufik, Z., Melita, K., Abdel, M., Azim ,A., Valsamma, E. (2011) Attention Deficit Hyperactivity Disorder and environmental toxic metal exposure in the United Arab Emirates. Journal of Tropical Paediatrics. 57: 457-460

Aim

To investigate the effects of toxic metals on childhood behaviour in the Gulf Region including the United Arab Emirates (UAE).

Study Design

Participants

Participants were selected by "… *a gender stratified random sample consisting of four females and five males from public elementary schools from Al Ain Educational Zone, UAE, children were enrolled using class lists…*" this resulted in a total of 92 participants. Eighteen children were identified with Attention Deficit Hyperactivity Disorder (ADHD) diagnosed using the DSM-IV criteria (4th Edition). These children were compared with 74 children without ADHD for blood levels of heavy metals. Children were excluded if they had mental retardation or autism.

Neuropsychological evaluation

ADHD was initially screened by the short ten-item Conners' Rating Scale for ADHD (parent and teachers version) and through a child behaviour check list. Data were collected by the school nurses who had had prior training in surveying. Data collected were subjected to quality control (5% random check). Children who were identified as having behavioural problems in the screening phase were further clinically assessed by a paediatrician and a child psychologist and a consensus diagnosis of ADHD was made using the DSM-IV criteria.

Socio-demographic and psychosocial variables were ascertained by questionnaire to the parents and collection by the school nurse.

Manganese Analysis

Blood samples were collected and heavy metal determination was carried out using ICP-MS for Pb, Hg, As, C, Cu, Zn, Co, Mn, Cr, antimony (Sb), nickel (Ni) and molybdenum (Mo).

Study Findings

Eighteen children were identified with ADHD.

The change in odds ratio of ADHD for a 1 ppb (µg/l; 0.1 µg/dl) increase in blood concentrations for all the metals analysed, was calculated. There was a statistically higher blood levels of Pb, Zn and Mn in the ADHD groups compared with the controls. Additional analyses showed that the odds ratio increased by 5.2% when Pb concentrations increased by 1 ppb, by 30% when Zn concentrations increased by 1000 ppb and by 80.1% when Mn concentrations increased by 1 ppb. The metal with the strongest association with ADHD was determined by stepwise multi-logistic regression and was found to be Zn.

The authors conclude that blood levels of several metals (Pb, Mn, and Zn) were significantly associated with the occurrence of ADHD in children.

Limitations Factors of the Study

It is not clear how children were selected for the study and why the number of boys and girls was not the same. There is no indication of the uptake for participation in the study.

It is not clear from the study details if children with ADHD were identified initially and then the control group assigned by matching with gender and age or whether the study group were identified prior to assessment for ADHD.

It is not clear if socio-demographic information was used to assess for confounders.

The actual values of the blood metal levels are not provided and no statistical evaluation of the blood metal levels between control children and those with ADHD were included in the research paper. There appears to be extremely small changes in low levels of blood metal levels which are associated with measurable increases in ADHD.

The relationship and synergism between different metal concentrations were not explored.

No route or source of exposure to the heavy metals has been suggested.

3.5.14 Collipp, P.J., Chen, S.Y., Maitinsky, S. (1983) Manganese in infant formulas and learning disability. Ann. Nutr. Metab. 27:488-494

Aim

The aim of the study was to determine if there was a positive correlation between high Mn levels in hair and hyperactivity in children who have been fed infant formulas containing Mn compared to breast-fed babies.

Study Design

Participants

Hair samples were taken from newborn infants and from children up to age 10 when they were seen for routine check-ups at a paediatric clinic and from normal 7 – 10 year olds (n = 44) at a local prepaid health care centre. Hair from hyperactive children was taken when they attended a Child Development Centre (n = 16). The paediatric clinic, health care centre and child development centre were all based in New York state (USA).

Neurological Assessment

The children at the Child Development Centre were all referrals to the centre from schools, doctors or social agencies because the child had a learning problem and was hyperactive. A report written by the parent or guardian describing how they saw the child's problem was requested as well as a structured interview with the parent detailing the child's early

development and behaviour. Teachers were also interviewed addressing the child's problems. Previous medical, psychological and school reports were also reviewed. The children were assessed with a series of tests to determine their neurological development and a detailed psychological test to determine the cognitive and psychoeducational evaluation. The duration of infant formula feeding and the brand of formula were reported.

Manganese Analysis

Hair samples were washed with 0.1% Triton X100 and then digested with nitric acid (3:1). Mn was assayed using a flameless AAS.

Study Findings

The average concentration of Mn in hair at birth was 0.19 µg/g (S.D = 0.11). There was a significant increase in the concentration of Mn in hair of formula-fed normal infants between birth and age 6 weeks (0.965 µg/g), which then gradually declined at 4 and 9 months and at 3 years. Breast-fed infants had hair Mn concentrations significantly lower than formula-fed infants at 4 months (0.330 µg/g, S.D = 0.21) and concentrations were not significantly different from newborns. The children with learning disability between the ages of 7 and 10, had a significantly higher hair Mn concentration (0.434 µg/g, n= 16, S.D. not stated) than the age-matched controls (0.268 µg/g, n=44, S.D. not stated).

The authors concluded that formula-fed infants have a marked increase in concentration of Mn in their hair during the first few months. This supports the concept that young infants can absorb considerable Mn from their dietary intake. The authors speculate that children with learning disabilities either absorb Mn more readily or excrete it more slowly than children without learning disabilities.

Limitations Factors of the Study

Total Mn intake was not taken into account. Furthermore, other sources of neurotoxic agents which may cause behavioural problems such as Pb are not reported.

The study is based on two cohorts: newborn babies up to three years of age, and school aged children up to the age of 10. There is an assumption that the increase in Mn concentration in the hair of babies who were fed formula milk, is due to the presence of Mn in the formula directly or the liquid to make up the formula, although this is not measured. Secondly, while children with learning disabilities have higher concentrations of Mn in their hair in this study, there is insufficient evidence to assume that the two findings are related as implied but not explicitly stated in the paper.

The authors make the assumption that learning disability is caused by problems with Mn uptake and excretion, as opposed to a higher exposure to Mn.

3.5.15 Kondakis, X.G., Makris, N., Leotsinidis, M., Prinou, M., Papapetropoulos, T. (1989) Possible health effects of high manganese concentration in drinking water. Archives of Environmental Health. 44: 175- 178

Aim

The aim of this study was to determine the health effects of Mn in drinking water by studying the health of residents in three areas of northwest Greece where varying concentrations of Mn in drinking water are found.

Study Design

Participants

A 10% random sample of all households registered in the Civil Registry Office was contacted to ask if they would like to participate. If they refused another household was drawn randomly and contacted. The study was limited to people over the age of 50 years of age and who had lived in the same residence in the same area for more than 10 years. There were 77 participants in the study.

Neurological Analysis

A thorough clinical examination was performed by a trained neurologist who identified neurological signs and rated them from 0 (absent) to 3 (strong). Each symptom was assigned a score based on its diagnostic value for parkinsonism and the score number for each subject was the sum of the diagnostic value multiplied by the level of appearance.

Social-demographic data were collected on a home-visit and included information regarding profession, education, and food items bought or produced.

Manganese Analysis

Blood samples were taken and whole blood Mn concentrations determined (method not stated). Blood samples were only taken from areas A and C. Hair samples were taken (by the participants respective hairdresser) and washed in 10% SDS. The hair samples were dried and digested with nitric acid and hypochlorous acid. Mn concentration was determined by flameless AAS.

Study Findings

The authors state that the social data between the three areas showed remarkable similarities, although no statistical evaluation was provided. The concentration of Mn in the water for the three areas A, B and C were 3.6 – 14.6 µg/l, 81.6 - 252.6 µg/l and 1800 - 2300 µg/l, respectively. The concentrations of blood Mn did not statistically differ between the areas A (1.583 µg/100 ml, S.D. = 0.054) and C (1.647 µg/100 ml, S.D. = 0.080) which had the lowest and highest concentration of Mn in the drinking water, respectively. There was no

statistical differences between blood Mn concentrations between the two sexes and there was no correlation between blood and hair Mn concentrations. Hair Mn concentrations were significantly higher for Area C, which had higher water concentrations of Mn. Neurological symptoms were significantly different between the three areas, with increasing symptoms associated with increasing concentrations of Mn in the water.

The authors conclude that progressive increases in Mn concentration in drinking water are associated with progressively higher incidences of neurological signs. Higher hair Mn concentrations (means of areas: A 3.51 µg/g, B 4.49 µg/g, C 10.99 µg/g) were observed in areas with higher Mn concentration in the drinking water, however blood Mn concentrations were found not to be significantly different.

Limitations Factors of the Study

No account of dietary intake of Mn was considered, it was assumed that differences in Mn concentrations were solely from different concentrations in drinking water. It is not apparent if the authors took into account all the confounders such as age which may influence the appearance of Parkinson-like symptoms.

The authors did not attempt to correlate neurological symptoms or hair and blood concentrations of Mn to water Mn concentrations.

The samples of hair were taken by the participants' hairdressers to instructions rather than in a defined method, which would lead to the same length of hair being taken from the same place in each participant.

3.5.16 Vieregge, P., Heinzow, B., Korf, G., Teichert, H-M., Schleifenbaum, P., Mosinger, H-U. (1995) Long term exposure to manganese in rural well water has no neurological effects. Canadian Journal of Neurological Sciences. 22: 286-289

Aim

To determine whether long-term exposure to well-water with high Mn concentration may carry a risk of neurological impairment and whether Mn intake could be a co-factor that might have some kind of trigger function in the onset and development of Parkinson's Disease.

Study Design

Participants

An area in the north of Germany was identified based on well water Mn concentrations. The survey was combined with a cross-sectional investigation of a randomly selected group of right handed residents above 40 years of age who had used their wells as a principal drinking water source for at least 10 years. Participants were divided into two groups, those who

consumed water from wells where concentrations of Mn were higher than 300 µg/l (Group A) and those where their well waters were below 50 µg/l who acted as a control group (Group B). The individuals from the study group were matched with members from the control group. Subjects were excluded if they had dietary restrictions, occupations in the steel industry or had ever taken central nervous system-acting drugs, or had a serious neurological illness or diabetes. 164 subjects were eligible for participation, however 49 participants were excluded on the basis of either insufficient information relating to the well water or did not meet the eligibility terms. The control group had 41 subjects and the study group 74.

Subjects were extensively interviewed to determine dietary habits, lifestyle factors and drug use.

Neurological Analysis

Each participant underwent a neurological examination by the same neurologist. Signs of parkinsonism were evaluated by the Columbia University Rating Scale (CURS). Fine motor abilities of both hands were tested in randomised sequence between participants using conventional apparatus. The results were adjusted for age.

Manganese Analysis

Blood samples were analysed for Mn by AAS. Liver function was assessed by determination of bilirubin, alkaline phosphatase, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase and gamma glutamyltransferase.

Study Findings

The mean ages between the two groups were not significantly different, and there were no dietary differences. There were no significant differences between scores on the symptom list and from the CURS between the study and control group. Three subjects had Parkinson's disease; however, they all belonged to control Group B. There were no significant differences between the groups and blood Mn concentrations (mean Mn concentration in groups A and B were 8.5 +/-2.3 µg/l and 7.7 +/- 2.0 µg/l, respectively) and the results from the liver function tests were within normal ranges. When possible account was made for confounders (demographic and dietary factors); however, there was still no statistical differences between blood Mn and liver function tests in the two exposure groups.

The authors concluded that there were no significant differences in any of the neurological parameters measured in middle-aged adults exposed to low (< 50 µg/l) or high concentrations (300 – 2160 µg/l) of Mn in drinking water.

Limitations Factors of the Study

The authors acknowledge that people who were sick could have moved away from the area and thus not been included in the study. Furthermore, the authors could not account for intake

of Mn from other sources such as diet or sources of drinking water from sources other than the well waters. The authors did not correlate external exposure with internal parameters of Mn metabolism.

The authors state that the well waters were part of an extensive surveillance programme, however they do not report the concentrations of other metals or chemicals that may have been present.

There is no indication of why only right handed people were included in the study.

There were three incidences of parkinsonism, all of which were in the control group which had 74 subjects. The study group had 41 participants but no incidence of Parkinson's. Although this is a very small study, it would appear that possibly the study group may have had a lower incidence of Parkinson's Disease than the control group.

3.5.17 Roels, H.A., Bowler, R.M., Kim, Y., Henn, B.C., Mergler, D., Hoet, P., Gocheva, V.V., Bellinger, D.C. and 7 others. (2012) Manganese exposure and cognitive deficits: a growing concern for manganese neurotoxicity. NeuroToxicology 33: 872-880

Aim

This publication was a review of the proceedings of a symposium on Mn exposure and cognitive deficits and summarised five oral presentations, of which only one was concerned with manganese and children's cognitive performance. In this presentation, six studies on children in Mexico and Brazil (exposed to airborne Mn via mining or processing), China, Bangladesh and Canada (Quebec) were summarised (also reviewed in this report) and the results of three studies were combined and re-analysed.

Study design

The results were combined from 617 children in the published Mexico, Brazil and Canada studies, using hair Mn as a marker of exposure and IQ as a marker of cognitive development. The results were analysed for association taking into account study, age and sex. The results were stratified for sex and re-analysed.

Study findings

The results showed an overall decrease in full IQ of 2.62 points for a 10-fold increase in hair Mn, with a greater loss estimate for girls compared with boys. The latter result was considered interesting as a recent study in mice showed long-lasting neuronal morphological changes in female but not male mice, although striatal Mn accumulation and decrease were similar.

The presentation concluded that the results of the studies indicated a "*negative impact of excess environmental Mn on children's cognitive development…*".

3.5.18 Summary and Conclusions

Studies in experimental animals suggest that Mn is bioavailable by exposure through drinking water. This can lead to accumulation in the brain and changes in neurotransmitters levels, with evidence for effects on locomotor responses and behaviour and learning. Not all of these effects are seen only at high dose levels of Mn. Absorption through the gastrointestinal tract is poor in adults (< 5%) but greater in infants and children (40%). Biliary excretion is the main means of disposal but again this is reduced in children. Therefore, studies in experimental animals indicate that absorption of Mn and accumulation in the brain is possible, and effects on behaviour and learning were observed at doses of approximately 1 mg/kg bw/day and above.

A total of 14 human epidemiological studies assessing the effects of oral intake of Mn on behaviour and development are summarised and assessed in this report. The available information on the conduct of these studies varies considerably but taking the conclusions from these studies, there appear to be 12 studies reporting effects on behaviour and development (measured using a whole range of neuropsychological tests) and two studies (one in adults) finding no effects. From this it might be concluded from a weight of evidence approach, that oral uptake of Mn from drinking water has an effect on development and cognitive behaviour. However there are a number of limitations to each of these studies which were outlined in the summaries above and some more generalised limitations as follows:

The studies may be based on limited actual datasets as the four studies from Bangladesh may be based on the same population of children, although this is not clear.

In a number of papers the study population clearly comes from an area where there is industrial or mining pollution, and while some studies try to separate out the effects of other neurotoxins such as arsenic and lead, others do not address their potential presence.

In general, the data are limited by the fact, admitted by some authors, that there is not, at present, a standardised method for estimating manganese exposure, and although hair manganese has been used in a number of studies, blood and urine Mn have been measured. It has been suggested that blood Mn may represent recent exposure. At present, there is no standard method for the determination of hair Mn. There has been some discussion as to the preparation of the hair in terms of washing, in order to separate out any Mn particles obtained from the air, while the presence of hair dye appears to increase Mn levels and has led to the exclusion of participants in studies.

In some studies other routes of exposure, such as food have not been considered. Even in the more robust, recent studies, there are difficulties in identifying and estimating the exposure. In the Bouchard *et al*. (2011) study, Mn concentrations in hair were found to significantly increase with measured Mn concentrations in water and estimated total Mn intake from water, but not from estimated total diet concentrations. Children's IQ was not associated with estimated total diet Mn concentrations. Dr Bouchard hypothesised that the children's

intake from water may also include inhalation of aerosols from showering, which might have a neurotoxic effect. The early study of Collipp *et al*., (1983) suggested that children could absorb Mn from being fed infant formula. It has also been suggested that the total intake of Mn for some of the children with effects on IQ may not have reached the recommended daily intake for Mn as an essential trace element.

The most recent best-conducted studies of Bouchard *et al*. (2007, 2011) were cross-sectional, i.e. they estimated exposure data, the Mn concentrations in water, and the effects on cognitive function, were measured on only one occasion. Other studies on exposure to Mn, for example, occupational exposure via inhalation, suggests that long-term exposure and accumulation of Mn in the brain is probably necessary to induce neurotoxicity. In the Bouchard *et al*., (2011) study, children need only to have been exposed to the drinking water for three months to be included in the study, and this introduces the problem of length of exposure which might also be an important variable in terms of Mn accumulation in the brain together with relative changes in brain chemistry and damage. There may also be exposure prenatally that may impact upon development. The future study of this group includes returning to the same areas of Quebec, where remediation has taken place to remove Mn from the drinking water, in order to observe whether any of the neuropsychological effects are reversible.

There is little information on the species of Mn which are involved in the drinking water. Mn in the environment is present in Mn(II) and Mn(IV) forms, but in the body, the active forms are Mn(II) and Mn(III). Therefore, although there are limited data, the soluble form of Mn(II) is likely to more bioavailable and this is the form present in over 90% of the municipal groundwater sources investigated in the Bouchard *et al*. (2011) study. Addition of chlorine in distribution may well oxidise Mn and the fraction of dissolved Mn would then decrease. In the Bouchard study the fraction of dissolved Mn was much more variable (4-100%, average of 63%). Additional chlorine stages are often used to decrease Mn concentrations in drinking water.

The work on experimental animals and on the mechanism of Mn neurotoxicity suggests that Mn in drinking water could be absorbed through the gastrointestinal tract, especially in children and accumulate in the brain, leading to changes in brain chemistry. There have been a number of human studies looking at neuropsychological changes mainly in children exposed to Mn via drinking water. Although the majority have found an effect, there have been limitations to the findings even in the most recent studies, which have been conducted efficiently, especially in the estimation of exposure to Mn. Accurate exposure to the active substance is always the most difficult estimation to make in a human epidemiological study.

4. Behaviour of Manganese and its Removal from Water

4.1 Aqueous manganese chemistry

Mn normally exists in two redox states in aqueous solutions, Mn(II) and Mn(IV), where Mn(II) is the more stable form. Mn(II) salts are generally more soluble than Mn(IV) salts; this is particularly true for the oxides, where MnO_2 readily precipitates. Other oxidation states are possible but Mn(III) is only stable as a complex, Mn(V) and Mn(VI) are not stable in neutral solutions and Mn(VII), in the form of the purple permanganate ion (MnO_4^-), is strongly oxidising and does not form in most natural waters (Hägg, 1984).

4.2 Removal of manganese

Most of the reported work relates to Mn removal during treatment and leads to the following general conclusions:

1. Removal during treatment depends on capture of manganese that can appear to be colloidal but fundamentally depends on the conversion on Mn(II) to Mn(IV).

2. The conversion of Mn(II) to Mn(IV) can be slow, taking hours unless the conditions are alkaline pH with a positive redox potential, generally the presence of oxygen.

3. The rate is increased by having an oxidant present, e.g. chlorine, chlorine dioxide, permanganate, and by elevating the pH to 8.0.

4. The presence of a layer of MnO_2 has a catalytic effect, increasing the rate of surface deposition of manganese. Optimum conditions are achieved when a layer of MnO_2 has built up, when the pH and concentration of oxidant can then be reduced.

5. The presence of humic material can make oxidation difficult.

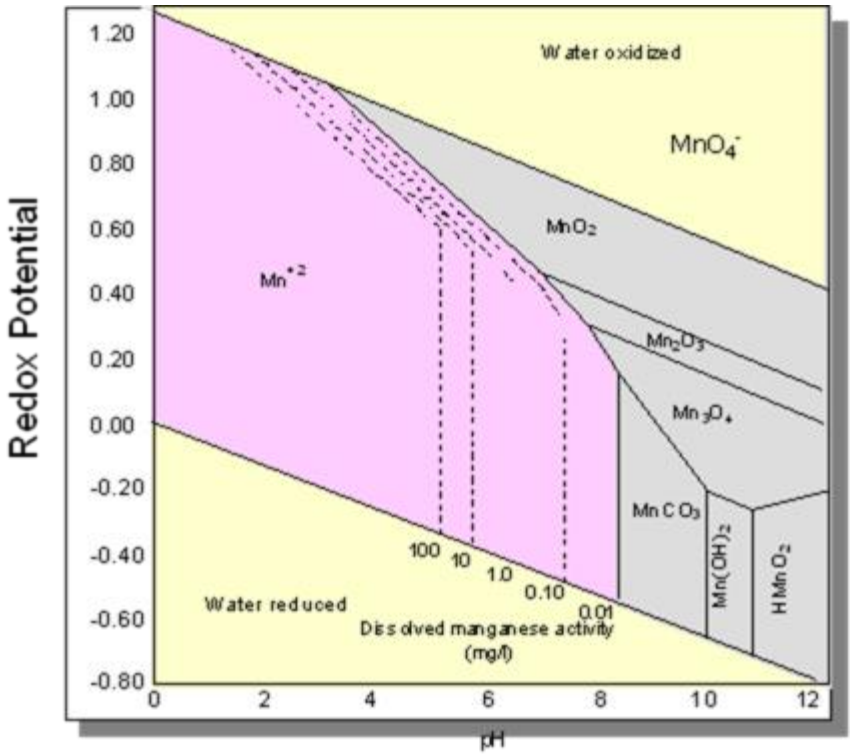
6. Low temperature reduces the rate.

7. MnO_2 does not form a settleable precipitate; it requires a surface on which to deposit.

A “phase diagram” is shown in Figure 4.1; this shows the form which manganese takes under a range of pH and redox in water that contains carbonate. In soft waters with very low carbonate, the area for Mn(II) extends to the right and a higher pH is required to achieve oxidation to Mn(IV).

From an operating perspective, the area of importance, where control can be effected, is approximately between pH 6 and 9 and redox of -0.4 v to +0.4 v. For Mn(II) to convert to Mn(IV) the pH either needs to be greater than *circa* 8.5 or the redox needs to be greater than *circa* + 0.2 volts (below this, there is practically no control at normal pHs). By managing pH and oxidant (chlorine) concentration treatment can be optimised for a given water; often this is a pH of 7.5-8.0 and a free chlorine concentration of 0.2-0.5 mg/l.

Figure 4.1 Phase diagram for manganese in water that contains carbonate (0.5 mg/l of free chlorine at a pH of 7.5 has a redox potential of approximately + 0.65 V)



Removal technologies for manganese removal can be categorised into six general groups:

- Oxidation-precipitation-filtration.
- Sequestration.
- Adsorption.
- Ion exchange.
- Membrane technologies.
- Biological removal.

The techniques and their main advantages and disadvantages are summarised in the following sections.

4.2.1 Oxidation-precipitation-filtration

Coagulation using aluminium sulphate (alum) and ferric (Fe(III)) is widespread practice in the water industry and the use of coagulants for Mn removal has been reviewed by e.g. Welch (1963) and Lloyd *et al*., (1983). Mn(II) is not readily removed by the coagulation process as Mn^{2+} is only weakly adsorbed to iron and aluminium (hydr)oxides. The removal is due to the oxidation of Mn(II) to Mn(IV) and the subsequent precipitation of MnO_2 and thus, an oxidant needs to be present. The precipitated MnO_2 acts as an adsorbent, catalyst for further oxidation and as a powerful coagulant aid (Welch, 1963). The effect of MnO_2 as a coagulant aid is likely to be the precipitation of small particles that increase the density of the floc blanket.

Lloyd *et al*., (1983) found that oxidation takes place at adsorption sites and Mn removal is favoured by a high surface area adsorbent. In floc blanket clarifiers, this condition is equivalent to high suspended solids concentration. Oxidation of adsorbed manganese becomes significant when pH approaches 8.5. This may cause problems in all but the softest waters, as it could cause the solubility product of calcium carbonate $CaCO_3$ to be exceeded. Precipitation of $CaCO_3$ in filters may cause cementing of particles and deteriorating performance. Precipitation of $CaCO_3$ in the floc blanket will reduce the number of sites available for Mn(II) oxidation and precipitation and also increase the amount of sludge produced.

Welch (1963) emphasised the use of permanganate as an oxidant as it is more efficient than aeration and it requires little equipment and capital investments. The compound itself is however, more expensive than e.g. chlorine, and an excessive dose will cause a pink colouration even at low concentrations.

The use of other oxidants such as chlorine, chlorine dioxide and ozone can be used for Mn oxidation and precipitation at a pH between 7 and 7.5 to reduce $CaCO_3$ precipitation and chlorine is widely used for this purpose. Matthews (1947) found that oxidation of Mn by chlorine was relatively slow in homogenous solution but was greatly accelerated in filters. This is likely to be a catalytic effect of precipitated MnO_2 in the filter bed. Chlorination is a well proven technique that is fairly inexpensive and requires simple equipment, but potential problems are the possible formation of trihalomethanes (THMs) and possibly the need for de-chlorinating the treated water. Manufacturers of chlorine dioxide claim that chlorine dioxide reacts more readily with Mn compared to chlorine and that it also reacts with organically bound Mn. The risk for THM formation is eliminated but chlorite formation can cause problems. The main problems with ozone are the reduced performance in highly coloured waters, the risk of bromate formation, and formation of permanganate and pink colouration at excessive ozone doses.

4.2.2 Sequestration

Sequestration is a process that prevents dissolved Mn (or other ions) from precipitating out of the solution and causing discolouration and will thus not remove Mn. A previous review (AWWA, 1990) found that the combination of polyphosphate and chlorine worked best and produced the least turbidity and colour. Other combinations with orthophosphate, polyphosphate, sodium silicates, chlorine dioxide and chlorine did not result in satisfactory performance. Manganese sequestration is adversely affected by the presence of calcium as a high polyphosphate dose is required and calcium phosphate is precipitated increasing turbidity. Sequestration as a treatment process is only suitable for Mn concentrations below the concentration prescribed in Water Supply (Water Quality) Regulations, i.e. <0.05 mg/l, and is, to the best of our knowledge, not used in the UK.

4.2.3 Adsorption

Iron oxides, such as ferrihydrite and goethite, and iron oxide coated sand (IOCS) are effective in removing both anions and cations but the removal is highly pH-dependent; cations are generally more readily removed at high pH and anions more readily at low pH. Mn^{2+} is generally not readily removed by adsorption process.

Some filter media have been developed for the removal of Mn, e.g. Manganese Greensand (MnO_2 coated glauconite), ANTHRA/SAND (MnO_2 coated “granular media”) and CalMedia GSR Plus (granular MnO_2). The MnO_2 surface works as a catalyst and enhance the oxidation of Mn(II) and the formed MnO_2 accumulates on the surface of the filter media. All of the mentioned filter media need to be regenerated using permanganate or chlorine, either by batch regeneration or by continuous dosing and sometimes chlorine is needed to boost the performance. Some of the accumulated MnO_2 on the filter media surfaces may be removed by backwashing of the filter, but much of the material remains on the filter media, increasing the sand particle size over time.

4.2.4 Ion exchange

Ion exchange can be used for Mn removal but is generally only used for the removal of low concentrations due to the risk of rapid clogging if oxidation of removed Mn(II) occurs. The removal is effective at neutral to alkaline pH conditions.

4.2.5 Membrane technologies

As with ion exchange, membrane technologies such as reverse osmosis (RO) and nanofiltration (NF) can be used for the removal of low concentrations of Mn but higher concentrations can lead to rapid clogging if oxidation of Mn(II) occur.

An alternative method is the electrodialysis reversal (EDR) process. It works in a similar way to RO but instead of using pressure over a membrane, it pushes the contaminants through the membrane by applying a current. By reversing the polarity, the membrane fouling is

minimised but cannot be ruled out at high Mn concentrations. The Mn is concentrated in a waste stream. As with RO, EDR is typically more expensive than traditional water treatment but can be suitable for smaller installations.

4.2.6 Biological removal

As an alternative to physico-chemical treatment, biologically mediated oxidation and removal of manganese (0.5 mg/l) has been demonstrated in rapid sand filtration of groundwater (Frischherz *et al*., 1985).

The bacteria involved are aerobic and include *Gallionella*, *Leptothrix* and *Hyphomicrobium sp* (Czekalla *et al*., 1985). The mechanisms are complex and could involve reactions with bacterial exocellular polysaccharide ("slime") material (Frischherz *et al*., 1985; Czekalla *et al*., 1985).

The benefits of biological manganese removal are that much smaller volumes of sludge are produced compared with physico-chemical treatment, as well as avoidance of the need for chemical dosing.

Biological oxidation of manganese requires higher redox conditions than those for iron. The application of these conditions can lead to physico-chemical oxidation of iron, and the benefits of biological oxidation are lost. For waters high in both iron and manganese, a two stage process may therefore be needed, the first stage for biological iron removal followed by aeration to produce redox conditions suitable for biological manganese removal in the second stage.

The Vyredox process (Braester and Martinell, 1988) relies on the removal of iron and manganese *in situ* within the aquifer, through a mechanism thought to be at least in part microbiological, induced by injection of aerated water to establish a zone of oxidation and precipitation around an abstraction well. Removal of iron and manganese from 0.5 mg/l to below 0.05 mg/l has been demonstrated without problems of aquifer blockage. Removal of manganese by biological activity in slow sand filters has been demonstrated, with reductions from 0.39 to 0.02 mg/l (Hatva *et al*., 1984).

Later studies using bioreactors have found up to 95% removal achieved at 22 m/h and a Mn concentration of 0.2-1.1 mg/l (Correa, 2011). Although this is promising, there are a number of potential issues, e.g. scaling to a full treatment works, local microbes might out-compete the Mn oxidising strains, blocking of filter requiring backwashing, aeration is needed, the success rate is variable, and a conditioning time of up to 8 months might be needed before fully mature.

5. Monitoring of Manganese in Drinking Water

5.1 Development of a method for the determination of total Mn and Mn(IV) concentrations in drinking water samples using inductively coupled plasma- mass spectrometry (ICP-MS)

This chapter describes the development of a method for the measurement of total dissolved and particulate or colloidal manganese concentrations in drinking waters. ICP-MS is used to measure two concentrations of Mn in water, the first representing the total manganese present in the sample and the second representing the dissolved manganese fraction following filtration of the water sample through a suitable filter. The difference between the two measured values represents the concentration of particulate or colloidal manganese in the sample of water. The method uses direct addition of a yttrium internal standard to the manganese standards, blanks and samples to afford a final concentration of 1 ppb of the internal standard. This approach was found to be superior to the alternative of introducing the yttrium internal standard via an in-line addition process as it resulted in a more robust assay as a result of less drift occurring during the course of each analytical run. The method has been shown to be reproducible and precise, to have a linear range up to 100 ppb (w/v; 100 µg/l) and a minimum detectable amount for manganese of 0.1 ppb (0.1 µg/l) and a limit of quantitation of 0.5 ppb (0.5 µg/l). During development of the method, account was taken of Technical Guidance Note (Monitoring) M18, Environment Agency, version 2, April 2009, Performance Standard for Organisations Undertaking Sampling and Chemical testing of Water, Part 1 – Sampling and chemical testing of untreated sewage, treated sewage effluents and trade effluents, Environment Agency, Version 1, July 2008, NS30 – A Manual On Analytical Quality Control For The Water Industry, Water Research Centre plc, 1989 and The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and related Topics, EURACHEM Guide, V1, December 1998.

5.1.1 Introduction

Inductively-coupled plasma-mass spectrometry (ICP-MS) is a highly sensitive technique capable of determining the concentration of a range of metals at levels approaching 1 ng/l (1 ppt). ICP-MS utilises an inductively-coupled plasma as a means of producing ions that are then separated and quantified in the mass spectrometer. The method has the advantage of speed, precision and sensitivity although the potential does exist for specific analytes for there to be interferences which may require additional effort to support validation of the analysis. The US Environmental Protection Agency have a published method for the determination of trace elements in waters and wastes by ICP-MS (Method 200.8 Revision 5.4) and this was adopted as the starting point for the establishment of the manganese analytical method which is described in this report. A specific objective of this study was to attempt to measure both the total Mn and Mn(IV) concentrations in drinking water samples and this was possible by the incorporation of a filtration step to remove any colloidal or particulate Mn(IV) species from the samples prior to analysis.

5.1.2 Method development - results and discussion

Calibration

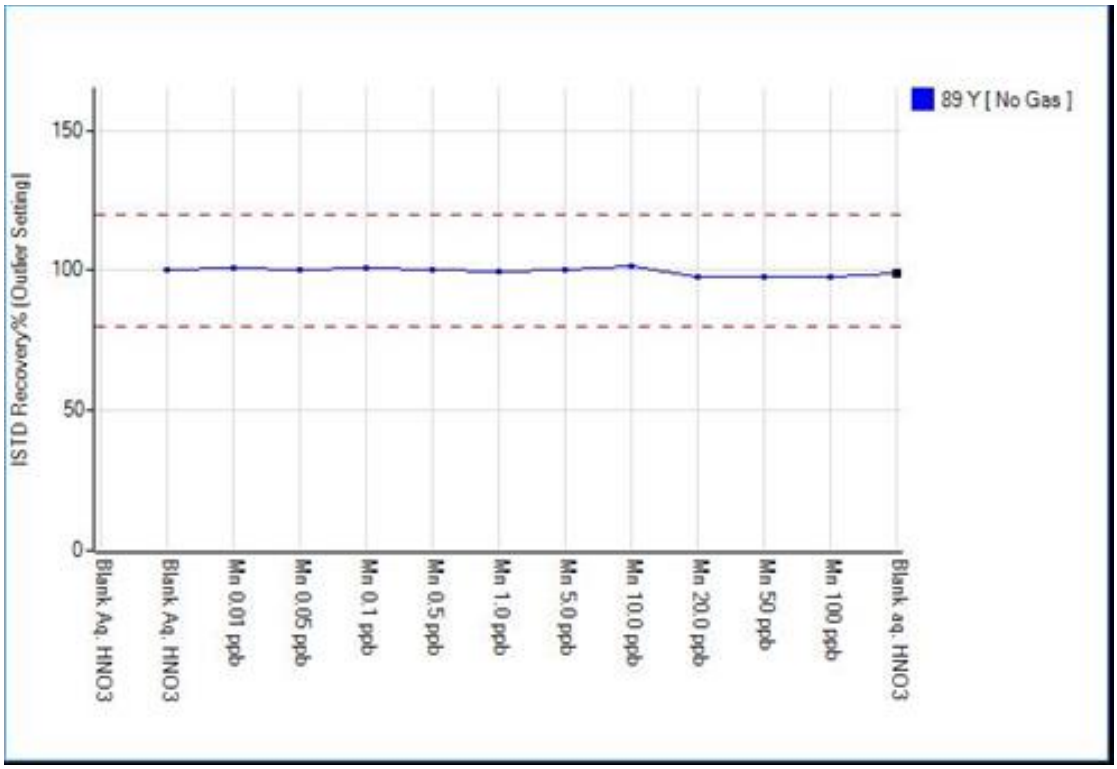
Identifying a suitable calibration range for this method was a challenge as the concentration of Mn in the samples to be analysed was unknown. However, in selecting a range of 0.01 – 100 ppb it was anticipated that this would be sufficient to analyse the majority of samples received without the need for dilution. The use of a direct addition approach for the introduction of the internal standard into the standards, samples and blanks made the protocol for preparing these standards critical. As the concentration of the yttrium internal standard in each one had to be identical, it was essential that a single stock solution of the yttrium internal standard was used throughout. This was a 100 ppb yttrium stock standard solution which was used to make up the 1% v/v aqueous nitric acid stock solution containing 1 ppb yttrium for preparing the standards and to spike the samples and blanks. The protocol for preparing the standards is set out in Appendix C.

Regression analysis of the calibration data generated using this protocol was performed using the Agilent Mass Hunter software employing a standard linear fit algorithm with the contribution from the blank, which determined the Y intercept, being given no special significance through the selection of the “ignore” option in the software. If no weighting factors are applied to the data, the curve gave a very good fit over the concentration range 0.5 – 100 ppb (R = 1). However, due to the influence of the standards at the high end of the concentration range, the curve fit for concentrations < 0.5 ppb was less good. It was possible to overcome this deficiency by the application of a weighting factor which ensured that greater emphasis was placed upon the standards at the low end of the concentration range. Application of either 1/SD2 or 1/Y weighting factors to the data did improve the curve fit at the lower end of the calibration range with the latter affording the best overall linear fit over the entire range of interest (Table 5.1). The benefits of direct addition of the internal standard are also demonstrated in the ISTD recovery plot (Figure 5.1) which shows that a very stable response was observed over the course of the run.

Table 5.1 Mn calibration standards using yttrium internal standard (1 ppb) and direct addition

Standard	55 Mn (No weighting)		55 Mn (Weighting 1/SD^2)		55 Mn (Weighting 1/Y)	
	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD
Blank aq. HNO3	<0.000	N/A	<0.000	N/A	<0.000	N/A
Mn 0.01 ppb	<0.000	N/A	0.012	8.533	0.011	9.457
Mn 0.05 ppb	0.020	6.088	0.048	2.511	0.047	2.575
Mn 0.1 ppb	0.075	4.007	0.102	2.903	0.102	2.937
Mn 0.5 ppb	0.502	2.301	0.526	2.178	0.529	2.183
Mn 1.0 ppb	0.992	0.551	1.012	0.536	1.018	0.536
Mn 5.0 ppb	5.064	0.378	5.050	0.375	5.083	0.376
Mn 10.0 ppb	10.031	0.721	9.977	0.719	10.042	0.719
Mn 20.0 ppb	20.029	2.243	19.894	2.240	20.025	2.240
Mn 50.0 ppb	50.014	0.284	49.634	0.284	49.962	0.284
Mn 100.0 ppb	99.981	0.607	99.193	0.607	99.850	0.607

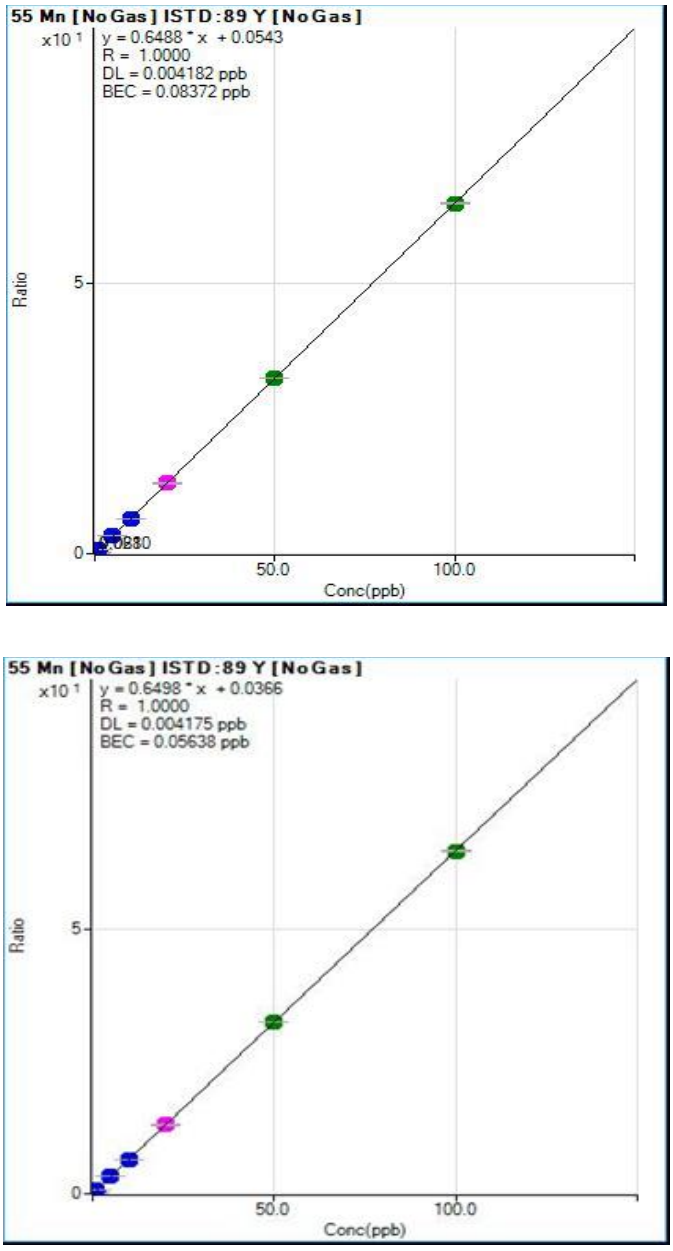
Figure 5.1 ISTD recovery using the direct addition of an yttrium-89 internal standard



Linearity

Over the calibration range selected, the method exhibited excellent linearity with regression analysis giving a value of R = 1 (Figure 5.2). This was achieved by eliminating some of the instrument performance variables that can impact upon the raw data obtained. Specifically, three replicate data points were obtained for each sample using an integration time of 1 second as opposed to the more usual 0.1 second and a mean of the three data points taken for the sample. By using this extended integration time much greater precision was obtained, as reflected by the conc. RSD% values (typically < 5%) when compared with those obtained using an integration time of 0.1 second (conc. RSD < 10%).

Figure 5.2 Mn calibration curves using no weighting and 1/Y weighting corrections



Precision of measurements, limits of detection and quantitation

The limit of detection for the method was set at 0.1 ppb based upon a signal to noise ratio of 3:1. The use of 1% v/v aqueous nitric acid as the matrix for all samples, standards and blanks being primarily responsible for the majority of the noise observed during analysis. The limit of quantitation was set at 0.5 ppb based upon a signal to noise ratio of 10:1. The precision of the measurements was very good and above the limit of quantitation the coefficient of variation (conc. RSD%) is < 3%. Below the limit of quantitation the precision appeared to still be good

(conc. RSD% < 5%) and so all data above 0.1 ppb were reported. Data below the limit of detection were reported as < 0.1 ppb (µg/l).

Reproducibility of the measurements

A set of manganese standards prepared on Day 0 was analysed on four separate occasions over the course of approximately one month to establish the reproducibility of the method. Good agreement was obtained for each individual analysis with no evidence of drift or loss of Mn from solution (Table 5.2).

Table 5.2 Repeat analysis of seven Mn calibration standards on three separate occasions

Standard	Concentration Mn [ppb] (No weighting applied)			
	Day 1	Day 7	Day 19	Day 26
Mn 0.01 ppb	0.009	0.016	0.018	0.017
Mn 0.05 ppb	Not analysed	0.058	0.065	0.064
Mn 0.1 ppb	0.088	0.083	0.083	0.079
Mn 0.5 ppb	0.482	0.503	0.506	0.509
Mn 1.0 ppb	0.979	0.994	1.003	1.002
Mn 5.0 ppb	4.934	4.994	5.005	5.012
Mn 10.0 ppb	9.839	10.004	9.997	9.994

The additivity of the method was demonstrated by spiking both samples and a blank with a 200 ppb manganese standard to achieve a final nominal concentration of 2 ppb of Mn in the sample when volumes were adjusted to 10 ml. When corrections were made for the dilutions to compensate for the additions of conc. nitric acid, the Mn standard and the Y internal standard to each sample, the contribution of the spiked Mn was found to be identical for each sample (Table 5.3). Moreover, the analysis performed for the duplicate samples gave identical results highlighting the reproducibility of the method. Repeat analysis of a laboratory reagent blank gave a mean manganese concentration of -0.019 ppb (SD ± 0.00161) following 10 consecutive analyses.

Table 5.3 Reproducibility and additivity of the method

Sample Name	Conc. [ppb]	Conc. RSD	Conc. adjusted for dil. factor	Concentration of added Mn [ppb]
Blank aq. HNO_3 – 1st sample	-0.011	N/A		
Blank aq. HNO_3 – 2nd sample	-0.017	N/A		
Blank aq. HNO_3 - spiked 2 ppb Mn	1.863	0.057	1.882 (1.01)[a]	1.866
Coalville - 1st sample	1.990	0.210	2.030 (1.02)[b]	
Coalville - 2nd sample	1.973	0.446	2.012 (1.02)[b]	
Coalville - spiked 2 ppb Mn	3.747	0.080	3.859 (1.03)[c]	1.878
Loughborough - 1st sample	1.924	0.215	1.962 (1.02)[b]	
Loughborough - 2nd sample	1.975	0.554	2.015 (1.02)[b]	
Loughborough - spiked 2 ppb Mn	3.735	0.488	3.847 (1.03)[c]	1.898

a 100 µl 200 ppb Mn spike added

b 100 µl each of conc. nitric acid and 100 ppb Y internal standard added

c 100 µl each of conc. nitric acid, 100 ppb Y and 200 ppb Mn added

Instrument performance and potential interferences

ICP-MS is an ideal technique to measure concentrations of trace metals in aqueous matrices. However, the potential for interferences in using this technique must be recognised and corrected for and should include compensation for isobaric elemental interferences as well as those arising from polyatomic ions derived from the plasma gas, reagents or sample matrix.

The use of an internal standard in ICP-MS method development was essential to ensure that any instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix were corrected. Selection of an appropriate metal to serve as an internal standard required two main considerations. Firstly, the metal selected as the internal standard should not be present in the samples being analysed and secondly, the mode of introduction for the internal standard to the sample should not detract from the performance of the assay i.e. in-line addition vs. direct addition. Ideally, the metal selected as the internal standard should be of a similar atomic weight to the metal being analysed.

Manganese possesses only one stable isotope at atomic weight 55. Potential isobaric interferences can arise from polyatomic species such as: $^{40}Ar^{14}N^{1}H^{+}$, $^{39}K^{16}O^{+}$, $^{37}Cl^{18}O^{+}$, $^{40}Ar^{15}N^{+}$, $^{38}Ar^{17}O^{+}$, $^{36}Ar^{18}O^{1}H^{+}$, $^{38}Ar^{16}O^{1}H^{+}$, $^{37}Cl^{17}O^{1}H^{+}$, $^{23}Na^{32}S^{+}$, $^{36}Ar^{19}F^{+}$ and these were checked for using deionised water as a blank. No mass interferences were observed at m/z 55 when operating the ICP-MS in No Gas mode during the course of this method development and subsequent sample analyses. During our initial investigations scandium, which has an atomic weight of 45, was selected as a possible internal standard for

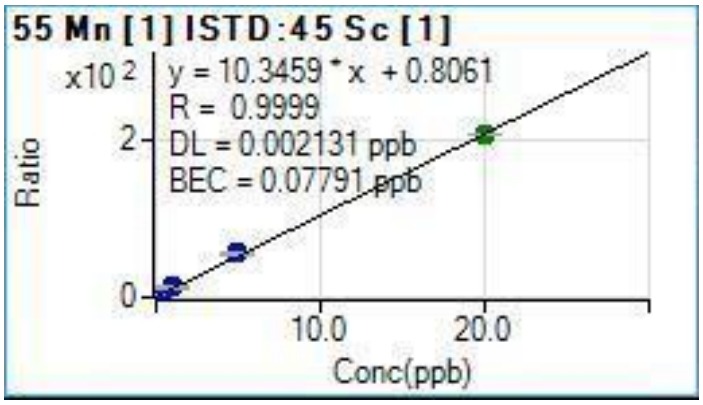
manganese (atomic weight 55) used in conjunction with the in-line addition mode for the introduction of the internal standard. This approach represented the simplest and least resource intensive way of trying to achieve satisfactory instrument performance and obtaining good reproducibility. Indeed, the initial data obtained gave a good calibration curve (Table 5.4; R = 0.9999) and the instrument performance was acceptable (Figure 5.3).

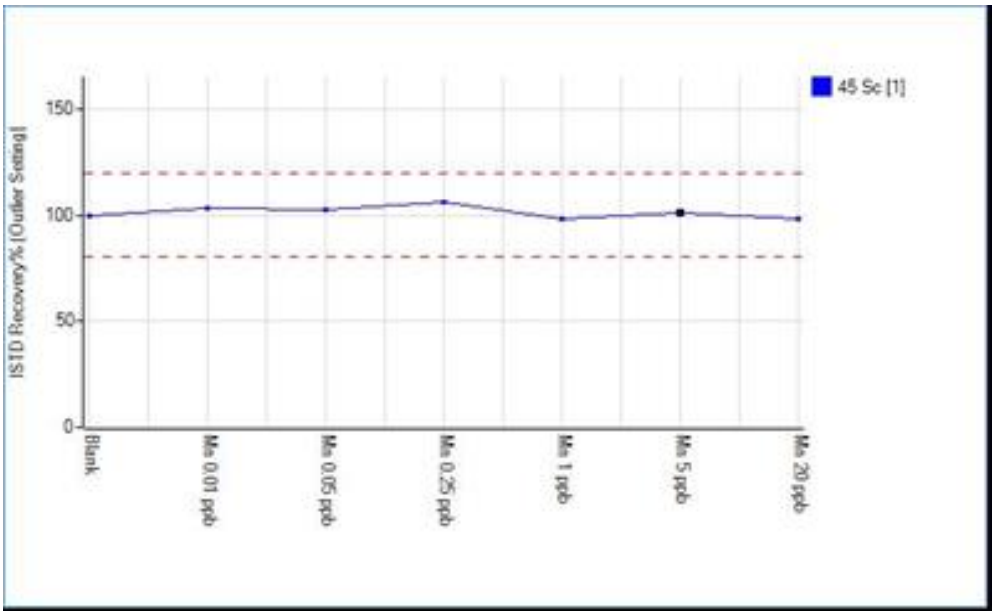
Table 5.4 Mn calibration standards using 1 ppb scandium as internal standard

Standard	55 Mn	
	Conc. [ppb]	Conc. RSD
Blank	0.00	N/A
Mn 0.01 ppb	0.06	0.81
Mn 0.05 ppb	0.12	3.18
Mn 0.25 ppb	0.34	0.85
Mn 1 ppb	1.17	8.90
Mn 5 ppb	5.29	1.26
Mn 20 ppb	19.92	0.68

However, during the first analyses of water samples, it became apparent that there were two problems that needed to be resolved.

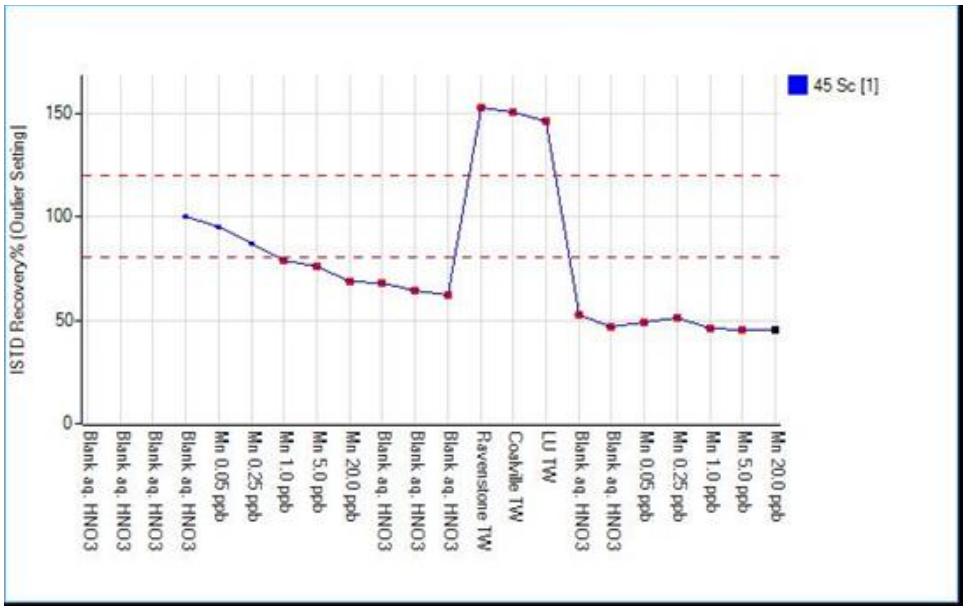
Figure 5.3 Mn calibration curve and Sc-45 internal standard stability plot





Firstly, there was an interference present in the drinking water samples that had not previously been observed and which threatened the use of scandium as an internal standard (Figure 5.4). Secondly, during the course of the analytical run, there was a tendency for the ISTD recovery to drift downwards such that eventually it fell outside the acceptable range of ± 20% which had been set. The interference was believed to arise from the presence of dissolved carbon dioxide ($CO2 + H^+$) in the drinking water samples which produced an isobaric interference at m/z 45. The possibility of overcoming the interference problem using the collision cell capabilities of the ICP-MS instrument and operating in helium gas mode was explored, however, this was found to be at the cost of overall sensitivity of the method. The cause of the drift observed was less clear.

Figure 5.4 ISTD recovery using Sc-45 and in-line addition of the internal standard



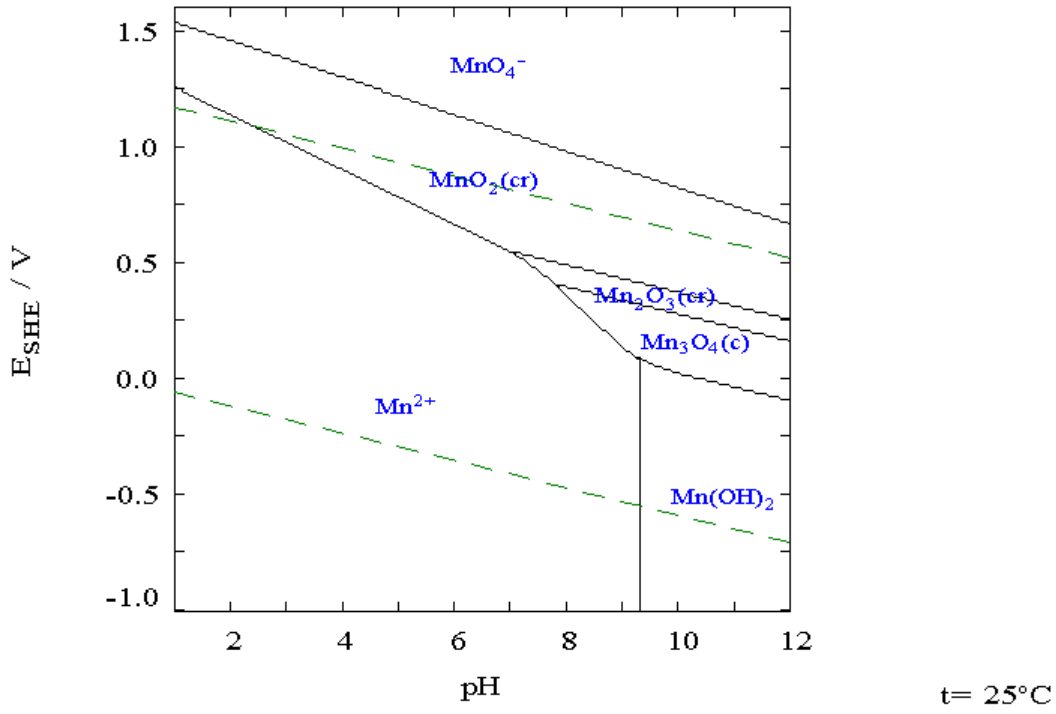
Instead both issues were resolved by switching to a direct addition approach for the introduction of the internal standard into samples, blanks and standards and substituting yttrium in place of scandium.

Germanium is often used as an internal standard as it is rarely found in drinking water. However, it possesses a number of stable isotopes (five in total) which makes its choice as an internal standard far from ideal. Yttrium possesses just one stable isotope at m/z 89 and has similar ionisation potentials to manganese making it a better choice. Moreover, its occurrence in water is very rare and only one instance of naturally occurring yttrium in a sample was encountered during the course of this study.

Measurement of Mn(IV) species in drinking water samples

Of the 11 different oxidation states that manganese can exist in, the most common are Mn(II), Mn(IV) and Mn(VII). Dependent upon the pH and redox potential (Eh) of the matrix in which these species are found, there is the potential for the oxidation states to interconvert and this can be represented in a Pourbaix diagram (Figure 5.5).

Figure 5.5 Pourbaix diagram of manganese in drinking water



Typically tap water in the UK has an Eh of between +150 and 600 mV which means that at low pH, Mn is likely to exist predominantly as Mn(II) with possibly some Mn(IV) present. These Mn(IV) salts are mainly insoluble and as a consequence are present either as very fine particulates or colloids. Section 5.5.3 in Performance Standard for Organisations Undertaking Sampling and Chemical testing of Water, Part 1 – Sampling and chemical testing of untreated sewage, treated sewage effluents and trade effluents, Environment Agency, Version 1, July 2008 states “For some determinands on some samples it may be required that the dissolved portion of the determinand in the sample is analysed and reported on. The dissolved portion of the determinand in the sample shall be defined as that which will pass through a 0.45 µm membrane filter. Filtration shall take place immediately at the point of sample collection. Any deviation from this prescribed procedure shall be justified and reported with results”. The validity of this approach has been assessed in the development of the method described in this report and the effect of filter size, storage of samples and timing of filtration step has been examined.

Samples of drinking water, taken from two sources locally, were assayed for Mn content both before filtration and after passing separately through 0.45 µm, 0.22 µm and 10 kD filters. The filters allow the dissolved manganese i.e. Mn(II) to pass through and remove any particulate or colloidal manganese i.e. Mn(IV) from the water sample. By measuring the manganese concentrations both before and after filtration, the difference in the two measured concentrations is equivalent to the concentration of the particulate of colloidal manganese in the drinking water.

Before filtration	Mn total (a)	=	Mn(II) + Mn(IV)
After filtration	Mn total (b)	=	Mn(II)
Therefore	Mn total (a) - Mn total (b))	=	Mn (IV)

Table 5.5 Use of different filter media to remove particulate and colloidal manganese

Sample	Manganese conc. [ppb]		
	Total Mn before filtration (a)	Total Mn after filtration (b)	Mn(IV) by difference
Blank aq. HNO_3	0.073	-	-
Source L - unfiltered	1.261	-	-
Source L – 0.45 µm	-	0.726	0.535
Source L – 0.22 µm	-	0.706	0.555
Source L – 10 kD	-	1.471	-0.210
Source C - unfiltered	0.135	-	-
Source C – 0.45 µm	-	0.153	-0.018
Source C – 0.22 µm	-	0.118	0.017
Source C – 10 kD	-	0.551	-0.416

The measurements show that there is little difference between the 0.22 and 0.45 µm filters with respect to the removal of any colloidal or particulate Mn species in the sample (Table 5.5). The measurements for Source C sample are below the limit of quantitation for the method and hence show slightly greater variability in the measurements. Interestingly the use of a Whatman Vectaspin 3 10 kD centrifuge filter revealed a significant increase in the manganese content for both samples following filtration. This is despite having first pre-flushed the filter with deionised water and then the actual sample itself. This increase has been attributed to contamination arising from the presence of wetting agents within the filter which are being stripped off by the water samples.

Further work was conducted using tap waters taken from Sources L, C and Q to test this approach and the measurements obtained confirmed that the method was working correctly. A number of drinking water samples together with blanks were processed by filtration through a 0.22 µm filter and then three sequential 10 ml aliquots collected. Following the addition of conc. nitric acid and the Y-89 internal standard to each of the samples, the manganese concentrations in each were measured; the original unfiltered sample was measured on three separate occasions and a mean value obtained. This provided confirmation that the filtration process was consistently removing an equivalent amount of colloidal and particulate

manganese each time and that the dissolved manganese component was essentially remaining constant (Table 5.6).

Table 5.6 Removal of Mn(IV) species by filtration

	Mn Concentration (ppb)				
	Unfiltered	Mean ppb	Filter 1st 10 ml	Filter 2nd 10 ml	Filter 3rd 10 ml
DI water	0.020, 0.020, 0.020	0.020	0.028	0.022	0.018
1% aq. HNO_3	0.042, 0.042, 0.043	0.042	0.072	0.068	0.051
C TW	1.312, 1.309, 1.308	1.310	1.299	1.288	1.275
L TW	0.938, 0.936, 0.937	0.937	0.508	0.454	0.449
Q TW	1.305, 1.290, 1.286	1.294	0.637	0.626	0.631

Therefore, assuming only Mn(II) and Mn(IV) species exist in the water samples and that the Mn(IV) species is colloidal or particulate and therefore removed by a 0.22 µm filter, the following results are obtained (Table 5.7).

Table 5.7 Total Mn versus Mn(IV) concentrations in tap water samples

Replicates	Concentration Mn [ppb]					
	Mn Concentration [ppb]					
	Tap water – C		Tap water - L		Tap water - Q	
Total Mn	1.310		0.937		1.294	
	Mn(II)	Mn(IV)	Mn(II)	Mn(IV)	Mn(II)	Mn(IV)
1st	1.299	0.011	0.508	0.429	0.637	0.657
2nd	1.288	0.022	0.454	0.483	0.626	0.668
3rd	1.275	0.035	0.449	0.488	0.631	0.663

Stability of manganese species

At low pH, Mn(II) is relatively stable in solution and, this has been demonstrated during the course of this investigation. However, if the pH is raised to above 9, the potential exists for Mn(II) to convert to Mn(IV). A series of Mn(II) standards were prepared in 1% v/v aqueous

nitric acid at nominal concentrations of 1, 20, 200 and 10000 ppb and the pH of these measured; all were found to be at pH < 2. The pH of each standard was then adjusted by the addition of 4M aqueous sodium hydroxide solution until it was around 10. The total Mn concentration was measured both prior to basification and following filtration of the sample after standing for one and six days. Samples were processed in the usual manner prior to analysis by first diluting with deionised water to bring the concentration down to within the working range of the assay and then the addition of conc. nitric acid and the Y-89 internal standard.

The data confirmed that at high pH there were rapid losses of dissolved manganese from solution, presumably through conversion to the less soluble Mn(IV) species. In the case of the 10 000 ppb standard there was a visible precipitate formed although for the rest of the samples there was no obvious change in physical appearance. After 6 days, all dissolved Mn present had effectively been converted to particulate and colloidal manganese and removed by filtration. In the case of the 1 ppb sample, the analysis was performed on the neat solution. Due to the high salt content of the sample arising from the basification and subsequent re-acidification prior to analysis, the ISTD recoveries were significantly reduced as a result of ion suppression occurring in the ICP-MS instrument and so making these measurements unreliable.

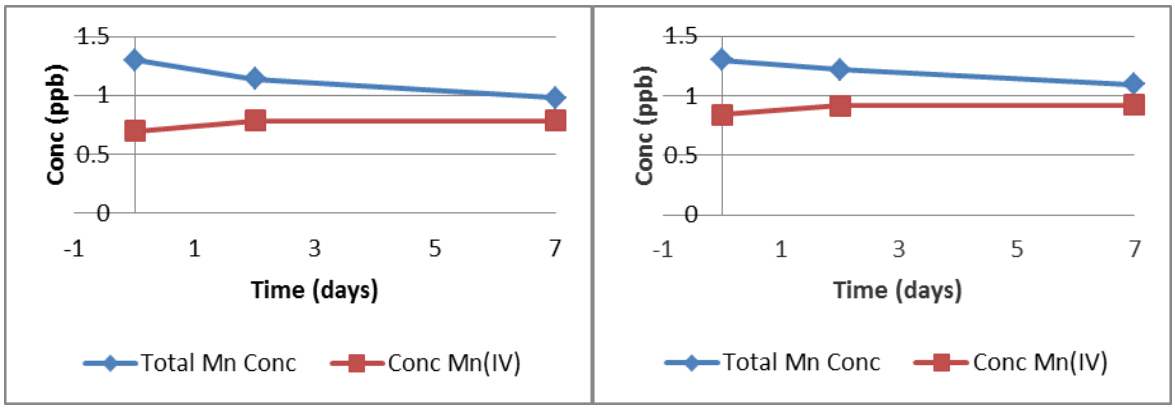
Table 5.8 Effect of pH 10 on manganese concentration of standards following filtration

Mn sample nominal conc. (ppb)	Diluted down for analysis to (ppb)	Before filtration (ppb)	Filtered after 1 day (ppb)	Filtered after 6 days (ppb)
1	1	0.897	0.505	0.476
20	4	3.603	0.148	0.003
200	4	3.599	0.090	-0.025
10000	10	8.701	0.077	-0.035

Further work was conducted on the stability of both Mn(II) and Mn(IV) species in drinking water samples to allow a suitable sampling protocol to be devised for the purpose of this study. The time frames between sampling and analysis were likely to be variable and it was therefore important to provide confidence that the analytical data generated actually reflected the concentrations of both Mn species present at the time of sampling.

For samples left untreated there was a slow reduction in total Mn concentration levels over time with the ratio of Mn(IV) to Mn(II) increasing (Figure 5.6). However, acidification of the samples by the addition of concentrated nitric acid to afford a nominal 1% v/v solution reduced the pH to < 2. At this pH, the concentration of total Mn present remained unchanged even over extended periods.

Figure 5.6 Total Mn and Mn(IV) concentrations versus time in untreated samples of L and Q tap waters respectively



The effect of pH was explored further and its impact upon dissolved Mn(II) species versus particulate/colloidal Mn(IV) species was examined. As highlighted above, untreated there was a slow reduction in the total manganese present in the sample with a small increase in the Mn(IV) species. This would suggest that not only was absorption taking place with the material of the storage container but that the Mn(II) species present was oxidising to Mn(IV) over time. However, following acidification to a pH of < 2, the absorption process was effectively halted as the total manganese content remained essentially the same. Moreover, filtration of the acidified samples through a 0.22 µm filter revealed no removal of any colloidal or particulate manganese species (Table 5.9). This suggested that at pH < 2, any Mn(IV) species that was present in the sample was slowly reduced to the more soluble Mn(II) species which remained stable until analysed.

Table 5.9 Effect of pH on manganese species

Time (days)	Sample	Non-acidified (ppb)			Acidified (ppb)		
		Unfiltered (Total Mn)	Filtered (Mn(II))	By diff. (Mn(IV))	Unfiltered (Total Mn)	Filtered (Mn(II))	By diff. (Mn(IV))
0	DI water	-0.034	-0.031	0.003			
	Blk. HNO_3	0.001	0.012	-0.011			
	C	0.064	0.029	0.035			
	L	1.299	0.694	0.605			
	Q	1.301	0.842	0.459			
2	DI water	-0.041	-0.037	0.004			
	Blk. HNO_3	0.017	0.012	0.005			
	C	0.075	0.054	0.021	0.110	0.151	-0.041

Time (days)	Sample	Non-acidified (ppb)			Acidified (ppb)		
		Unfiltered (Total Mn)	Filtered (Mn(II))	By diff. (Mn(IV))	Unfiltered (Total Mn)	Filtered (Mn(II))	By diff. (Mn(IV))
	L	1.138	0.784	0.354	1.318	1.335	-0.017
	Q	1.219	0.917	0.302	1.305	1.305	0.000
7	DI water	-0.035	-0.002	0.033			
	Blk. HNO_3	0.015	0.029	-0.014			
	C	0.084	0.056	0.028	0.108	0.122	-0.014
	L	0.976	0.787	0.189	1.319	1.343	-0.024
	Q	1.096	0.919	0.177	1.305	1.322	-0.017

These findings have implications for the protocol that needed to be employed when collecting samples. Accurate quantitation of both total manganese and the Mn(IV) levels present in the samples required some initial processing at the time of collection. Specifically two samples would need to be provided, the first of which would need to be collected in a suitable vessel containing sufficient concentrated nitric acid to afford a 1% v/v aqueous nitric acid solution when filled i.e. a 100 ml polypropylene bottle containing 1 ml concentrated nitric acid. For the second sample, which was needed to measure the dissolved Mn content and hence by difference determine the amount of Mn(IV) present, the sample needed to be passed through a 0.22 µm filter into a similar container as above. By following this sampling protocol, data generated during the course of these investigations, together with stability data contained in the EPA 200.8 document afforded a window of up to several months between sampling and analysis without detriment to the analytical outcome.

Quality controls

A range of quality control procedures have been employed during the development of this method and have included the analysis of blanks, spiked samples and analysis of replicates. In the absence of any suitable external reference material for this purpose, an internally prepared reference standard comprising 5 ppb Mn has been used and this has been sampled at appropriate points in the sample sequence during each analytical run to confirm the absence of drift. In addition, at the end of each run, the set of calibration samples was rerun to confirm the stability of instrument performance. A laboratory blank was also prepared in the same way as the samples and run at various points in the sample sequence to demonstrate the absence of any contamination arising during either the sample preparation or during the analysis.

Errors in the measurements

Grade A Volumetric flasks, glass pipettes, graduated sample containers and air displacement pipettes were calibrated using water to demonstrate that the errors on any measurements are within ± 5%.

5.2 Monitoring sites and procedures

The choice of drinking water treatment sites to sample for manganese speciation was not random, but made by a combination of suggestions from drinking water quality managers for sites where manganese had been detected even at low concentrations and areas where sources such as the British Geological Survey had detected manganese in streams, such as North Wales, Yorkshire and Northumberland. A number of these sites had historically high levels of Mn but had been equipped with rapid gravity filtration (RGF) processes for the removal of Mn. A total of 19 sites were sampled at least once in the project and details of the source water and the treatments undertaken at these sites are shown in Table 5.10.

For the drinking water treatment work sites now equipped with RGF for the removal of Mn. It was considered of interest to observe the oxidation state of remaining Mn, as in some cases, prechlorination was in place to generate the precipitable Mn(IV) form.

Each site was sampled four times as far as possible (some sites were sampled less often owing to operational difficulties, such as temporary shut downs), each sampling regime representing the four seasons and taking place in June/July 2012, September/October 2012, January/February 2013 and April/May 2013. Sampling kits consisting of polypropylene tubes containing nitric acid, 0.22 µm disposable filter and 50 ml syringe and the samples taken according to the protocol issued by the laboratory with one sample filtered on site according to the procedure outlined above in Section 5.1. The samples were collected, delivered to the laboratory and analysed well within the tested stability of the water samples.

Table 5.10 Details of the sites monitored

Site Number	Source Water	Treatment
1	Surface water - reservoir, a highly coloured soft upland source which has been known to stratify seasonally	Coagulation with aluminium sulphate, clarification through DAF, filtration through sand RGFs, pH elevation using kalic and oxidation with chlorine gas for manganese removal through second stage sand filters and finally further pH conditioning with kalic, disinfection with chlorine gas and plumbosolvency control using orthophosphoric acid.
2	Surface water - reservoirs, lakes, streams	Preozonation, pH adjustment, PAC, Coagulation, DAF, RGF, pH correction, super- and de-chlorination.
3	Surface water - river	Coagulation/clarification, filtration, GAC adsorption and aeration. Disinfection at the site is achieved using a combination of chlorine and UV followed by chloramination. Plumbosolvency using phosphoric acid.
4	Groundwater - greensand boreholes	Aeration and chlorination prior to filtration, super-chlorination and then chloramination. Plumbosolvency using phosphoric acid.
5	Surface water - upland reservoir water, which is highly coloured and contains high concentrations of manganese. Can be very high organic loading	Pretreatment with MIEX before coagulation with ferric sulphate, and then DAFs. pH correction pre RGF, and further pH correction and sodium hypochlorite prior to Mn Contactors. Final dose of sodium hypochlorite for disinfection, and contact in a relatively large (but variable) volume clean water tank.
6	Surface water mainly - Impounding Reservoir (IR) which receives river water and also a supply from a number of other impounding reservoirs, an upland River source and a Borehole	Pre-treatment with ferric sulphate for coagulation and sodium hydroxide for pH correction, then treated with a polyelectrolyte coagulant aid prior to entering the accelerators for clarification. The water then enters the primary RGF. Primary filtered water will be treated with sodium hypochlorite and pH corrected with sodium hydroxide before entering the second stage filters (RGFs). Water is pH corrected with sodium hydroxide

Site Number	Source Water	Treatment
		and dosed with orthophosphate prior to the contact tank and onto distribution.
7	Groundwater - two wells	Pressurised membranes followed by marginal chlorination.
8	Groundwater - combination of greensand and chalk boreholes	Chlorination, aeration, softening and coagulation with lime and ferric, filtration, superchlorination and then chloramination. Plumbosolvency using phosphoric acid.
9	Surface water – river with high colour and turbidity when in spate, generally a high quality source	Coagulation with ferric sulphate followed by direct filtration through a set of sand pressure filters. Filtered water is dosed with lime, carbon dioxide and chlorine (hypo) before filtration through secondary sand pressure filters for manganese removal and finally ultrafiltration through a membrane plant.
10	Surface water - upland reservoir water, which is highly coloured and contains high concentrations of manganese	Blended and then coagulated at pH 4.5 with ferric sulphate and dosed with polyelectrolyte, followed by upflow clarification. There is further pH correction to approx pH 6.2 on RGFs, and then approx pH 8.0 and 0.5 mg/l Cl on Mn Contactors, before final treatment with sodium hypochlorite and the a dedicated two compartment relatively small (fixed) volume contact tank.
11	Surface water - river	The process consists of two separate streams, each with clarification and rapid gravity filtration stages. They differ by the type of clarifier, flat-bottomed and Passovant Turbo LME lamellas, using alum and ferric salts respectively. Some minor pH adjustment is made upstream of the RGFs for manganese removal. The two partially treated streams combine after RGFs and then pass through GAC adsorbers before chlorine, phosphoric acid and fluoride is added ahead of a combined contact / treated water storage tank.

Site Number	Source Water	Treatment
12	Surface water – river	Three-stage treatment plant. After abstraction, the water is pumped into three parallel streams and the pH of the raw water is adjusted with either sulphuric acid or lime and dosed with aluminium sulphate and polyelectrolyte for coagulation. The water passes to Accentrifloc (Streams 1 and 2) or Superpulsator clarifiers (Stream 3). The clarified water then passes through GAC primary filters. The filtered water from streams 1, 2 and 3 combines and flows via the Interstage Pumping Station (IPS) to the 2nd stage sand RGFs. At this point the water is pH adjusted using lime. Chlorine is dosed downstream of the lime to aid manganese removal by oxidation on the 2nd stage RGFs. The filtered water is chlorinated for final disinfection and flows into two contact tanks. De-chlorination with sulphur dioxide occurs on the outlets of the contact tanks. The water is then pumped into distribution.
13	Surface and Ground water – Impounding reservoir (IRs) receiving supplies from springs, two boreholes and other IRs	Raw water entering the works is pH adjusted with sodium hydroxide or sulphuric acid and dosed with a ferric sulphate coagulant. The dosed water then enters the Dissolved Air Flotation (DAF) tank for clarification. Following clarification, the partially treated water is pH adjusted with sodium hydroxide before entering the primary filters (sand filters). Following filtration the water is pH adjusted and dosed with sodium hypochlorite for manganese removal. The water is then filtered through the second stage RGFs. Filtered water is then disinfected with sodium hypochlorite solution before

Site Number	Source Water	Treatment
		entering the Contact Tank. Water is dosed with Sodium dihydrogen orthophosphate for plumbosolvency control on the inlet to the contact tank and disinfected with chlorine gas to reach final set point on the outlet main.
14	Groundwater - private borehole	
15	Groundwater - the current site has 2 boreholes. Issues with cryptosporidium, manganese and nitrate.	Manganese first appeared in the late 1990s and is thought to be due to geochemical reactions in the aquifer, possibly associated with the formation of a permanent lake on adjacent land in the 1990s due to a rise in groundwater level associated with a drop in output of the boreholes at that time. A Mn removal plant was installed on site, but the boreholes are severely coated and encrusted with manganese particles and these have caused blinding of the filters when the boreholes have been operated at higher rates. I and M filters, UV, disinfection, blending (nitrate)
16	Groundwater - private borehole	
17	Surface water - river	Flat-bottom clarification using aluminium sulphate, followed by rapid gravity filtration (sand / polarite), GAC adsorbers, chlorination, plumbosolvency using phosphoric acid, contact and storage, and artificial fluoridation.
18	Surface water - impounding reservoirs under normal operation, also river if required	The works applies ferric sulphate ahead of flat-bottomed clarification. Lime is then added for manganese removal and the water then passes through rapid gravity filters (sand/anthracite) before being chlorinated. Phosphoric acid and fluoride is also added at this point.
19	Groundwater - greensand boreholes	Aeration and chlorination prior to filtration, superchlorination and then chloramination. Plumbosolvency using phosphoric acid.
20	Groundwater - two deep chalk boreholes	Treatment required is minimal so there is a UV unit for disinfection and then a small chlorine residual is added.

6. Monitoring Results

The seasonal results for all the sampling sites are shown in Table 6.1 and graphically in Figure 6.1. To investigate further the oxidative state of the Mn detected at each site, Figures 6.2 to 6.11 show the individual concentrations for each site for the four seasons, together with the percentage of each oxidative state (Mn(II) and Mn(IV)) present.

Generally low levels of total Mn (from <0.1 to 11 µg/l, except for one sample from site 5, see below) were detected in the final water samples, with the exception of the samples from two private supplies (37-541 µg/l; sites 14 and 16), situated in a site of naturally high levels of Mn according to a map of stream levels of Mn produced by British Geological Survey (BGS). The Mn detected in the water from these two sites was in the soluble form - Mn(II). This is in agreement with the Mn detected in boreholes and used for consumption, described in the Quebec studies, where the Mn were very high and predominantly in the Mn(II) form (95%) (Bouchard *et al*., 2011; Carriere *et al*., 2012). The treated water from the treatment works (site 2) which was located close to these two private supplies had much lower concentrations of Mn, and the Mn detected was in the Mn(IV) form.

The other high level of Mn which was detected was at site 5 in the autumn sampling (142.5 µg/l). This was much higher than other samples taken from the site and proved difficult to analyse. There is some suggestion that this sample might have been taken from a raw water tap by error rather than the final water. The samples from this site taken in other seasons did contain some of the highest levels seen (3-11 µg/l), but the works is equipped with RGF to remove Mn.

There does not appear to be any seasonal pattern in either the total concentrations of Mn or the oxidative states of the Mn. Some changes in oxidative states were detected but these were not consistent. Studies on Mn levels and oxidative states in Quebec (Bouchard *et al*., 2011; Carriere *et al*., 2011) showed that although borehole Mn was predominantly as Mn(II) (95%), when the water went into a public supply, the oxidative state was much more variable (4-100%). The total Mn level and its oxidative states can be determined by a range of natural geological, physical and chemical properties, as well as the oxidation of Mn(II) to Mn(IV) by drinking water processes such as chlorination. Therefore the average percentage of Mn(II) species detected in the final drinking waters for each season showed a wide variation: Summer, 55% (SD ± 27); Autumn, 31% (SD ± 21); Winter, 55% (SD ± 27); Spring, 40% (SD ± 29).

Figure 6.1 Manganese concentrations (µg/l) detected in final waters from sites in England and Wales

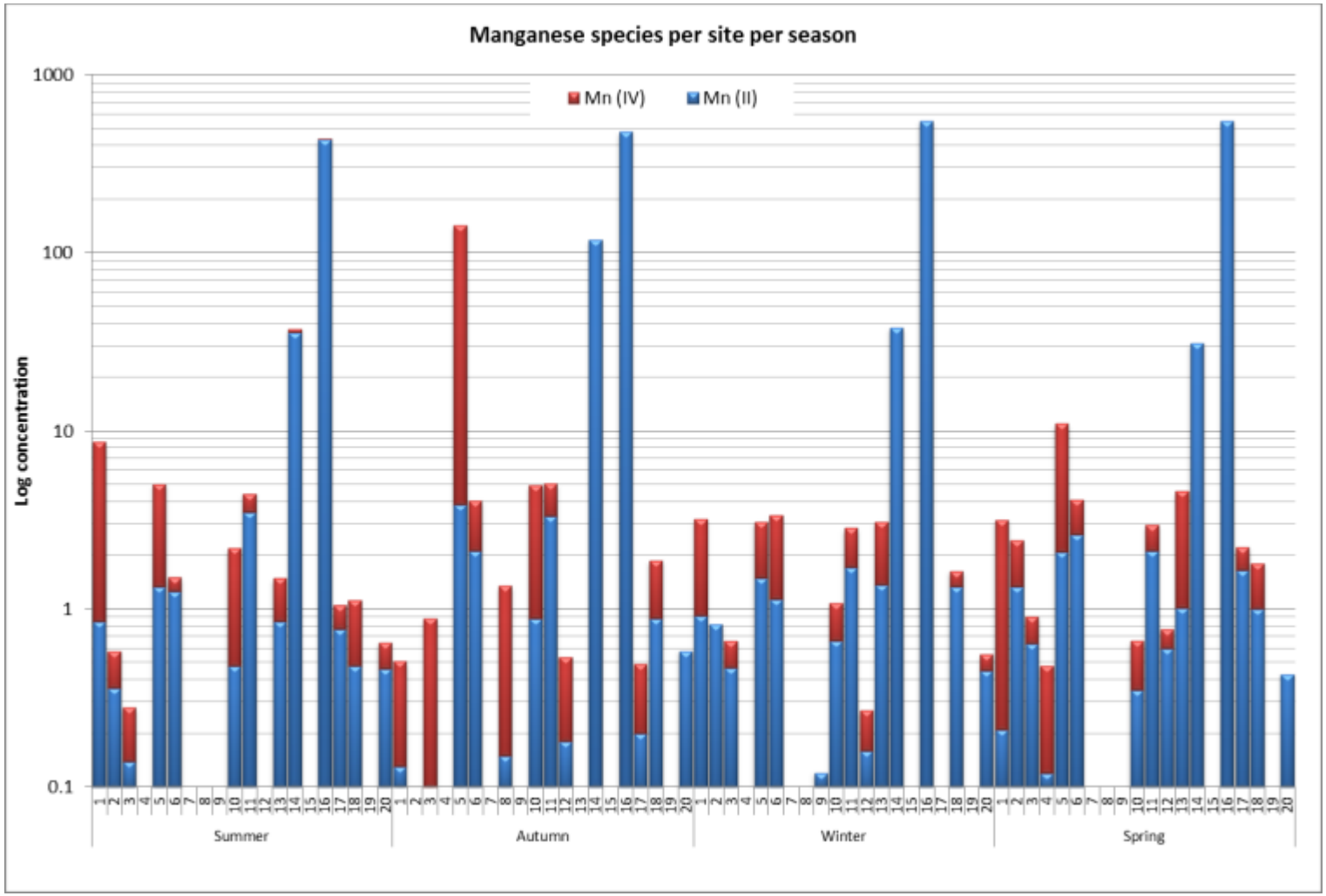
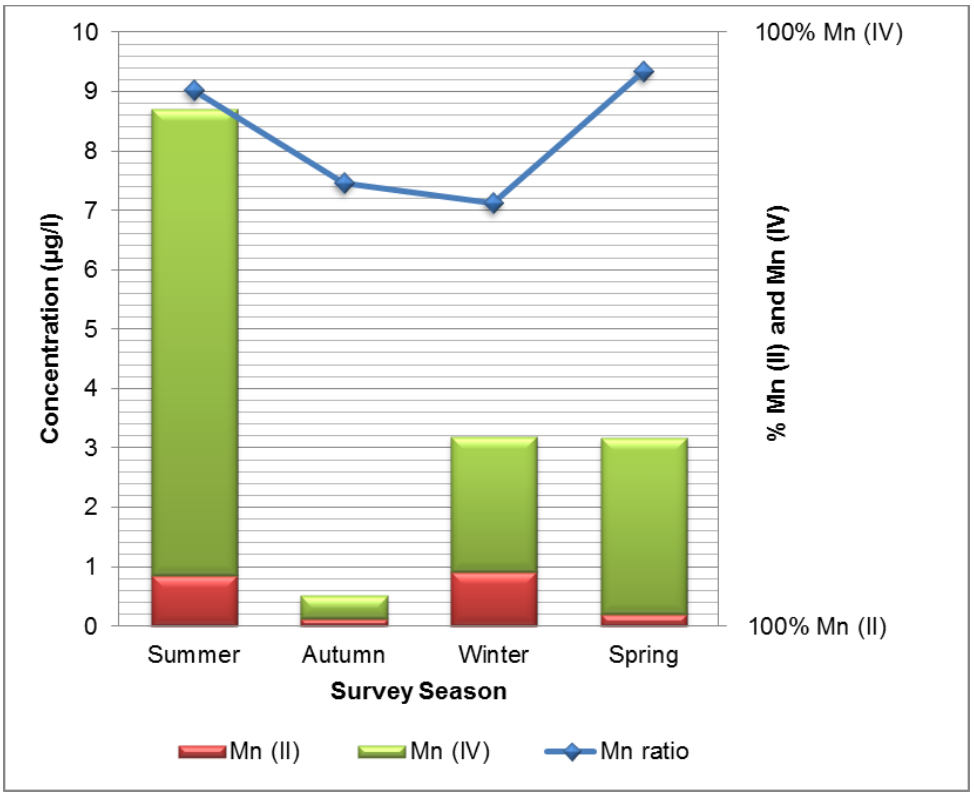


Table 6.1 Manganese concentrations (µg/l) detected in final waters from sites in England and Wales

Site	Summer (µg/l)			Autmn (µg/l)			Winter (µg/l)			Spring (µg/l)		
	Total Mn	Mn(II)	Mn(IV)	Total Mn	Mn(II)	Mn(IV)	Total Mn	Mn(II)	Mn(IV)	Total Mn	Mn(II)	Mn(IV)
1	8.71	0.85	7.86	0.51	0.13	0.38	3.19	0.92	2.27	3.17	0.21	2.96
2	0.58	0.36	0.22	0.18	0.10	<0.10	0.84	0.83	<0.10	2.42	1.34	1.08
3	0.28	0.14	0.14	0.88	0.10	0.78	0.66	0.47	0.19	0.91	0.64	0.27
4	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.48	0.12	0.36
5	5.03	1.34	3.69	142.5*	3.86	138.64*	3.10	1.50	1.60	11.04	2.10	8.94
6	1.51	1.26	0.25	4.07	2.13	1.94	3.36	1.14	2.22	4.14	2.61	1.53
7	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.28	<0.10	0.28
8	0.13	0.10	<0.10	1.35	0.15	1.20	N/S	N/S	N/S	0.70	<0.10	0.70
9	<0.10	<0.10	<0.10	0.12	<0.10	0.12	<0.10	0.12	<0.10	<0.10	<0.10	<0.10
10	2.19	0.48	1.71	4.96	0.89	4.07	1.08	0.66	0.42	0.66	0.35	0.31
11	4.46	3.50	0.96	5.06	3.34	1.72	2.85	1.72	1.13	2.97	2.12	0.85
12	0.40	<0.10	0.40	0.54	0.18	0.36	0.27	0.16	0.11	0.77	0.66	0.17
13	1.49	0.85	0.64	N/S	N/S	N/S	3.10	1.37	1.73	4.60	1.02	3.58
14	37.5	35.9	1.6	112.2	118.1	<0.10	37.0	38.0	<0.10	31.0	31.0	<0.10
15	<0.10	<0.10	<0.10	N/S	N/S	N/S	0.69	<0.10	0.69	2.70	<0.10	2.70
16	435.5	434.0	1.5	474.5	480.0	<0.10	527.0	552.0	<0.10	541.0	549.0	<0.10
17	1.05	0.77	0.28	0.49	0.20	0.29	<0.10	<0.10	<0.10	2.23	1.66	0.57
18	1.12	0.48	0.64	1.88	0.89	0.99	N/S	N/S	N/S	1.80	1.00	0.80
19	0.36	<0.10	0.36	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
20	0.65	0.46	0.19	0.37	0.58	<0.10	0.56	0.45	0.11	0.38	0.43	<0.10

*Raw water ample taken in error

Figure 6.2 Seasonal manganese concentrations and oxidative states – Sites 1 and 2



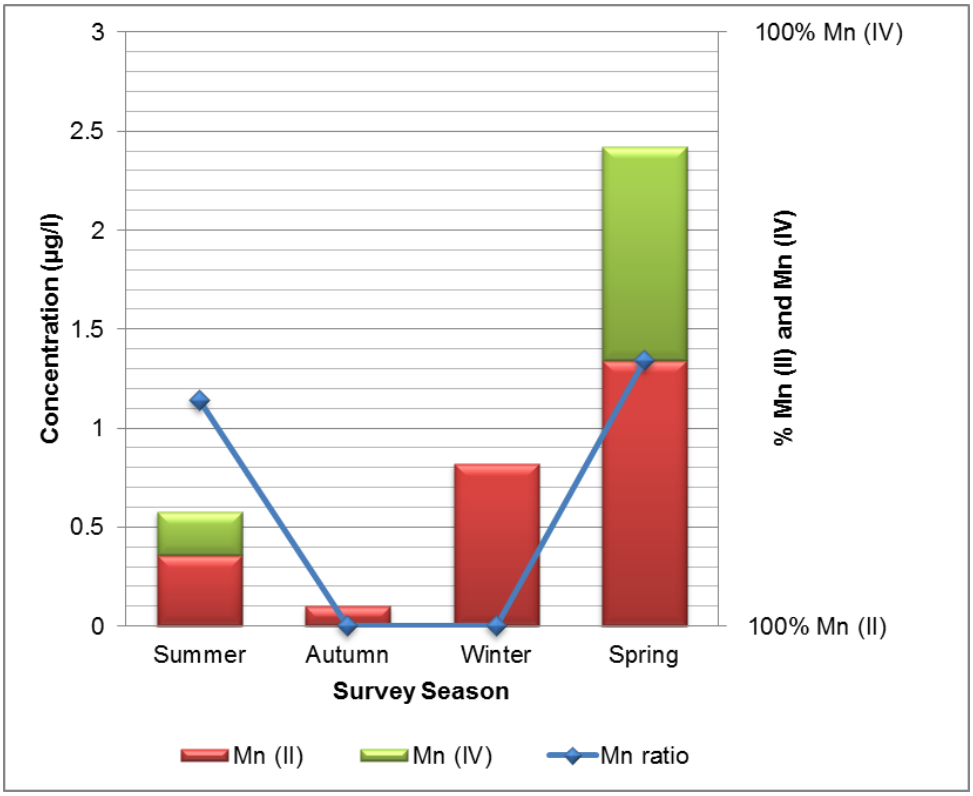
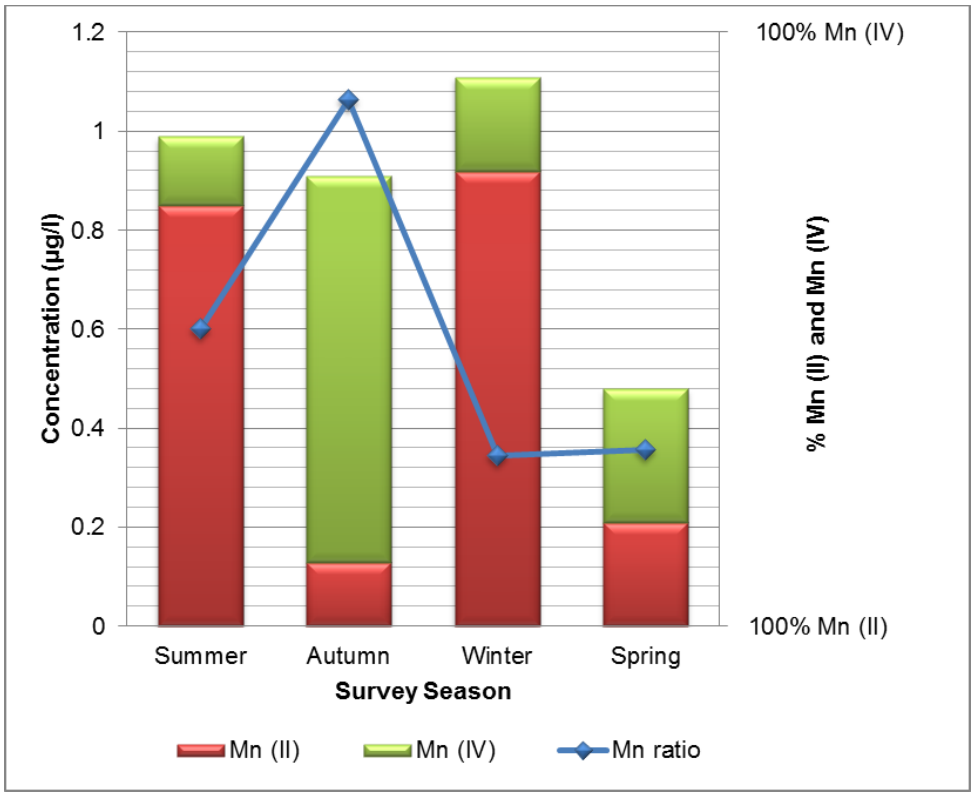


Figure 6.3 Seasonal manganese concentrations and oxidative states – Sites 3 and 4



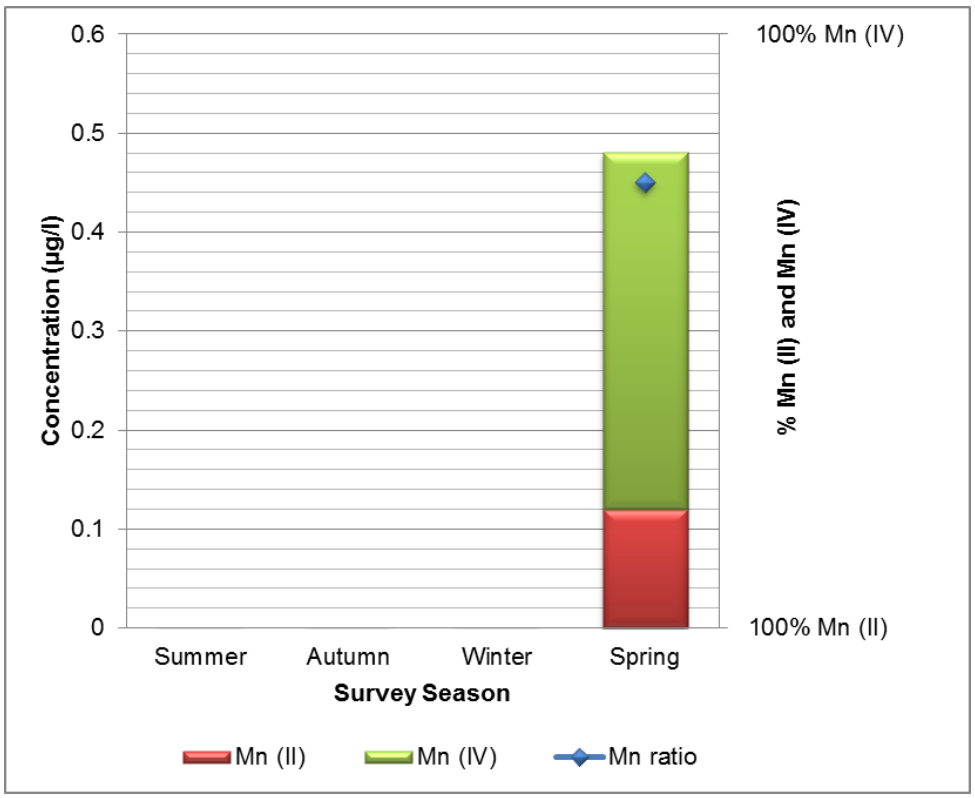
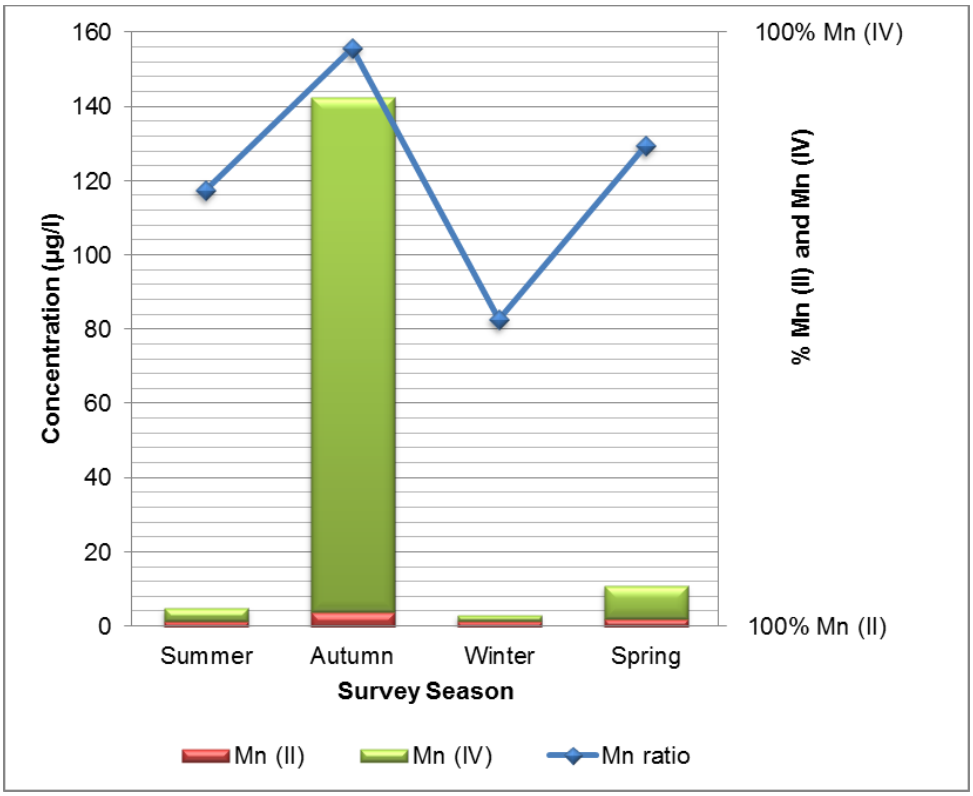


Figure 6.4 Seasonal manganese concentrations and oxidative states – Sites 5 and 6



The autumn sample was raw water taken in error.

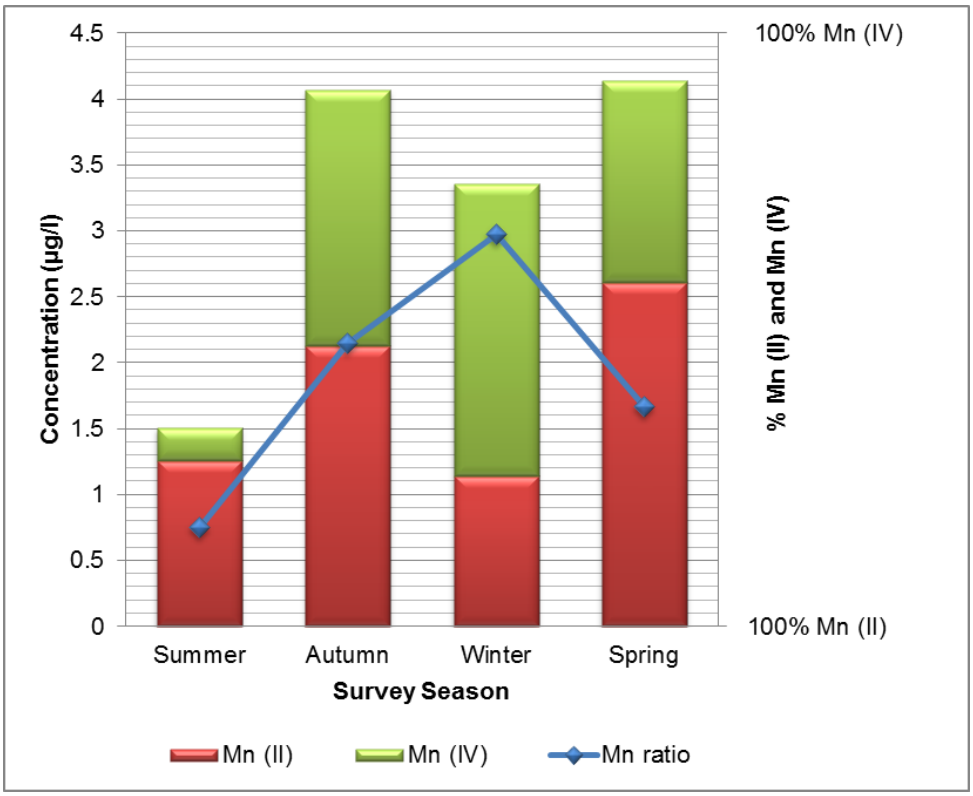
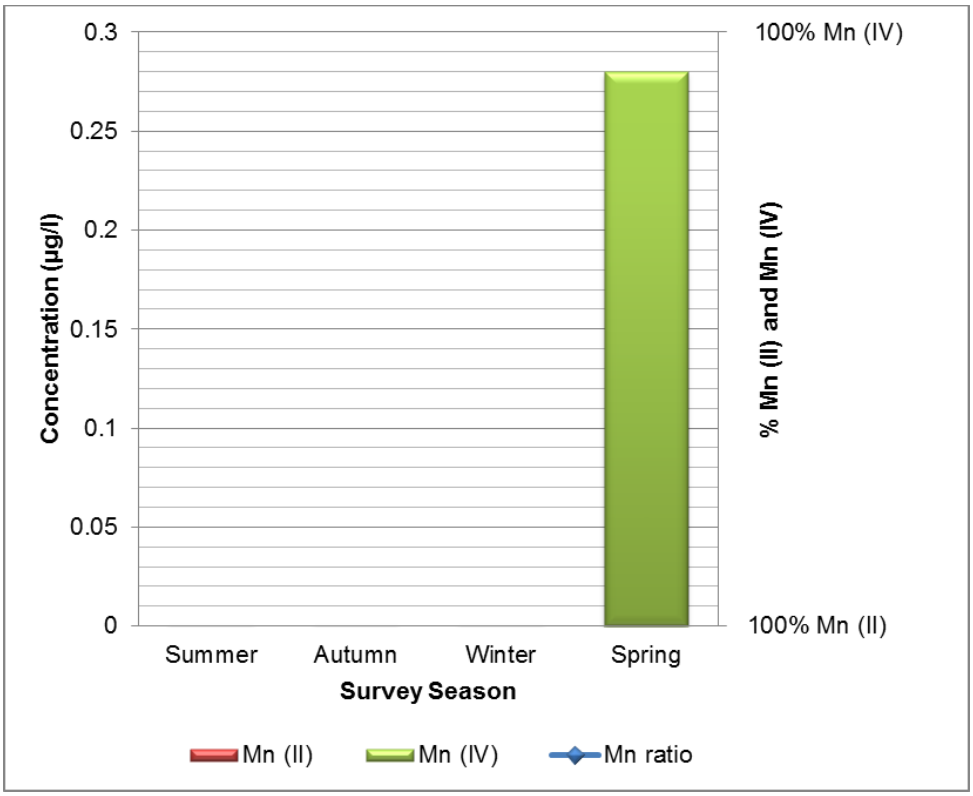


Figure 6.5 Seasonal manganese concentrations and oxidative states – Sites 7 and 8



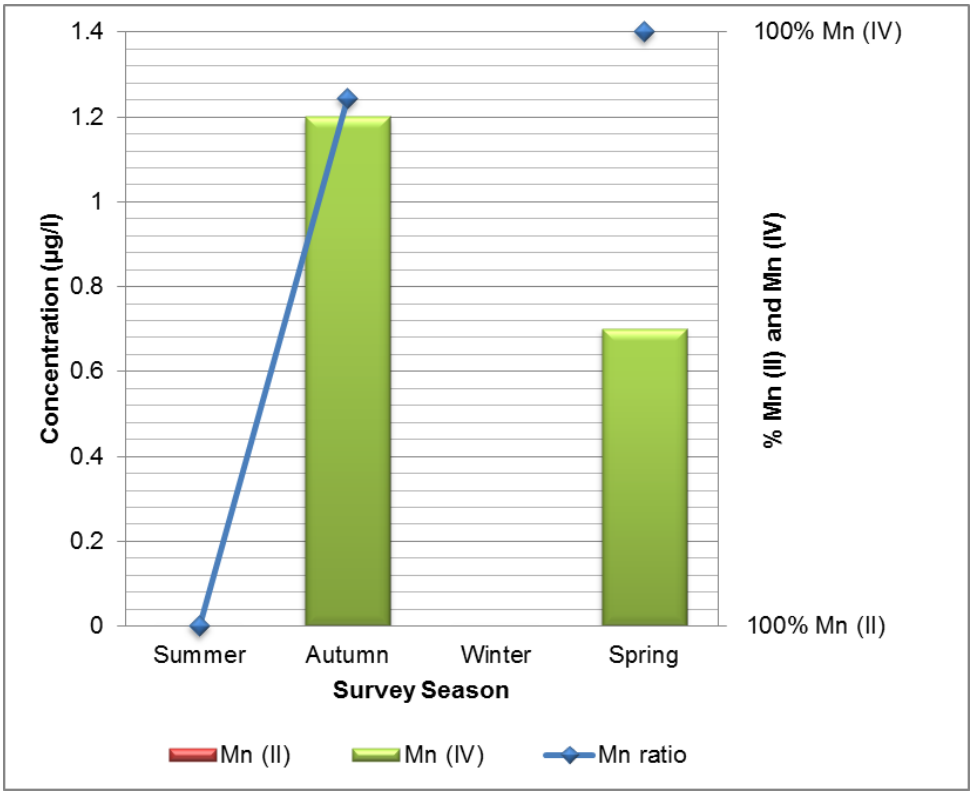
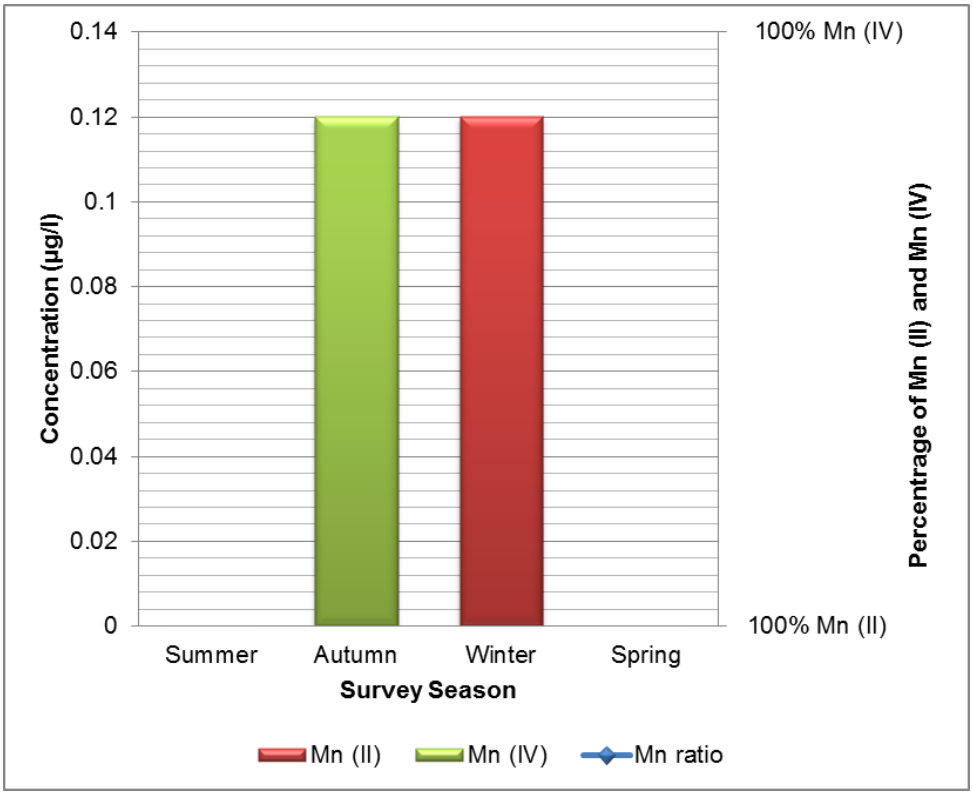


Figure 6.6 Seasonal manganese concentrations and oxidative states – Sites 9 and 10



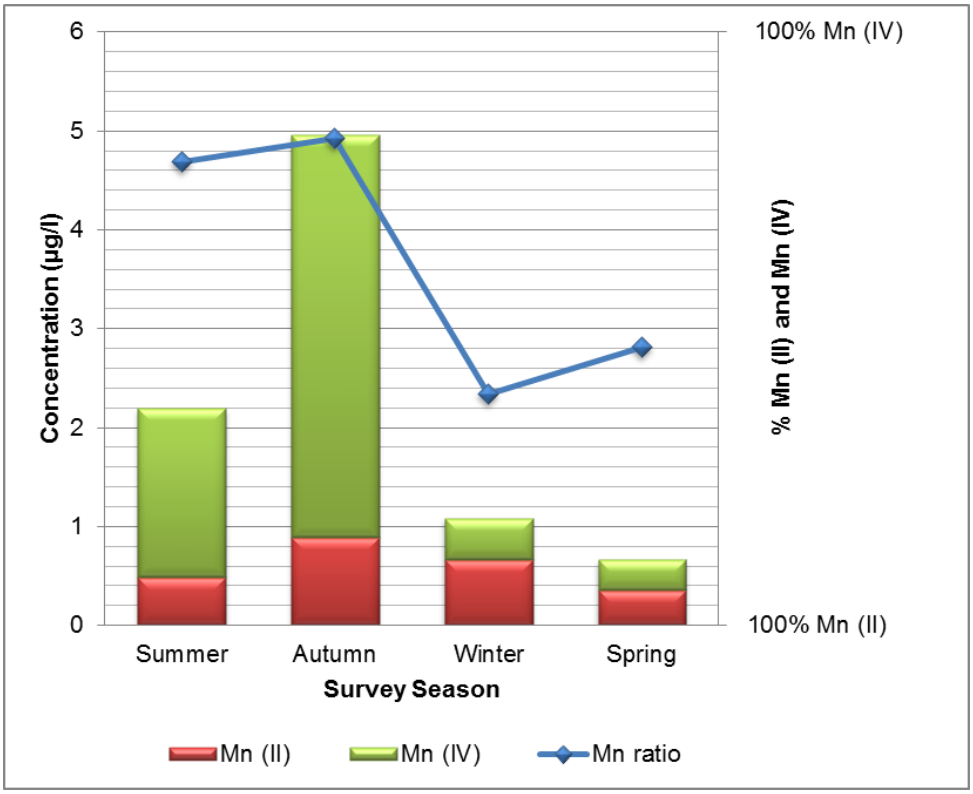
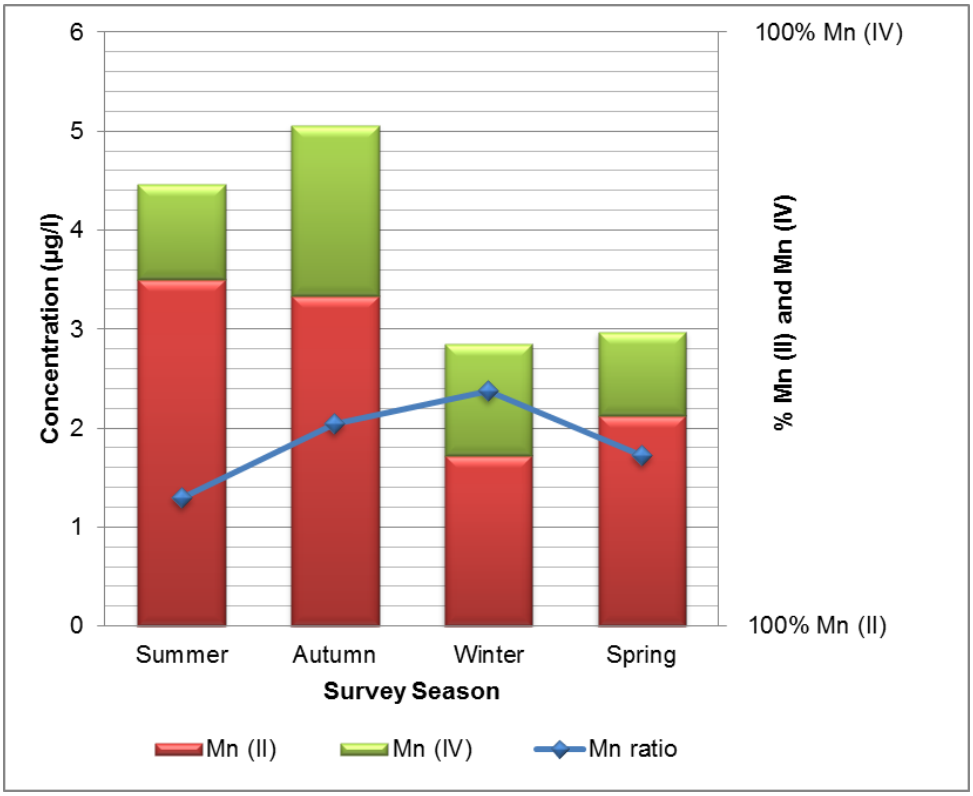


Figure 6.7 Seasonal manganese concentrations and oxidative states – Sites 11 and 12



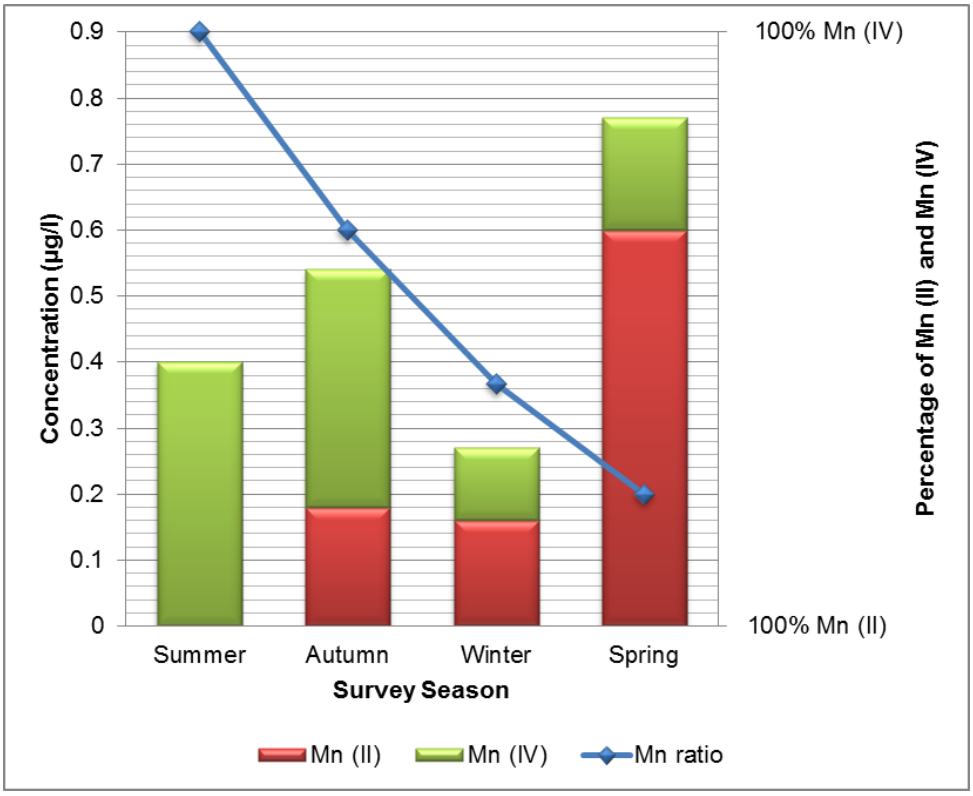
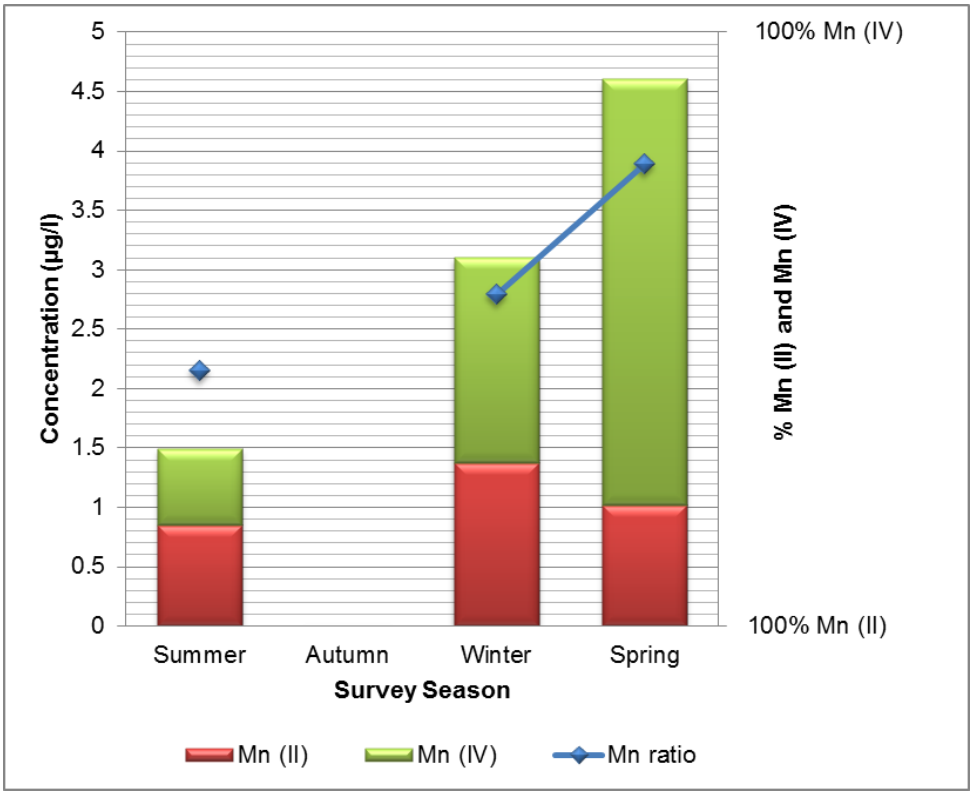


Figure 6.8 Seasonal manganese concentrations and oxidative states – Sites 13 and 14



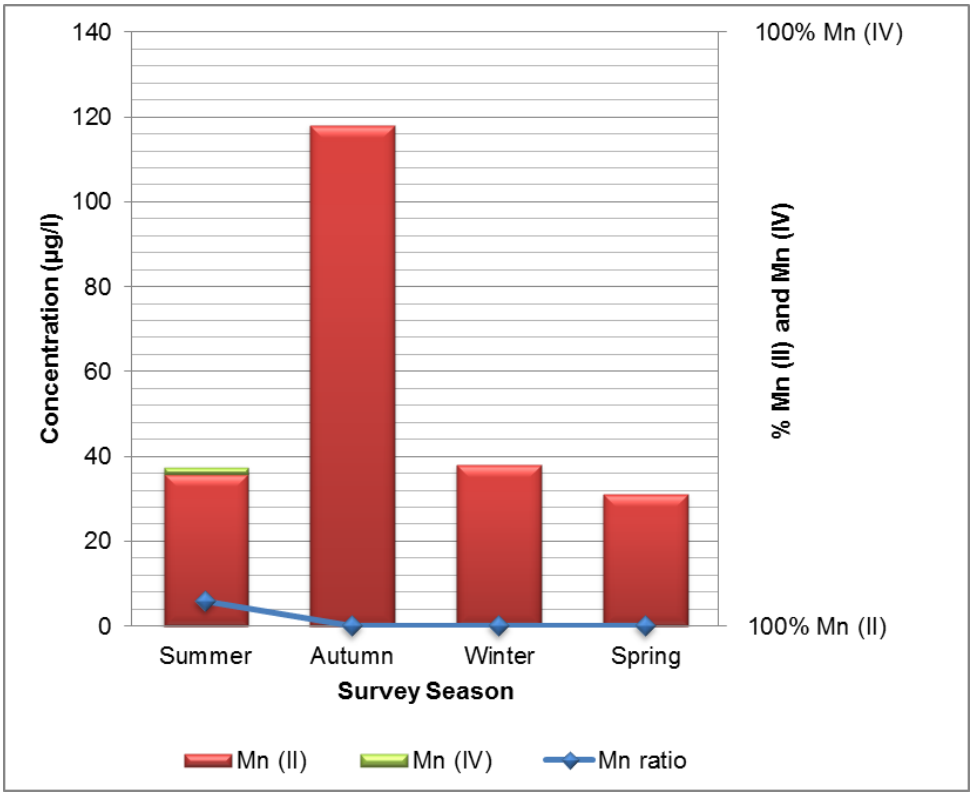
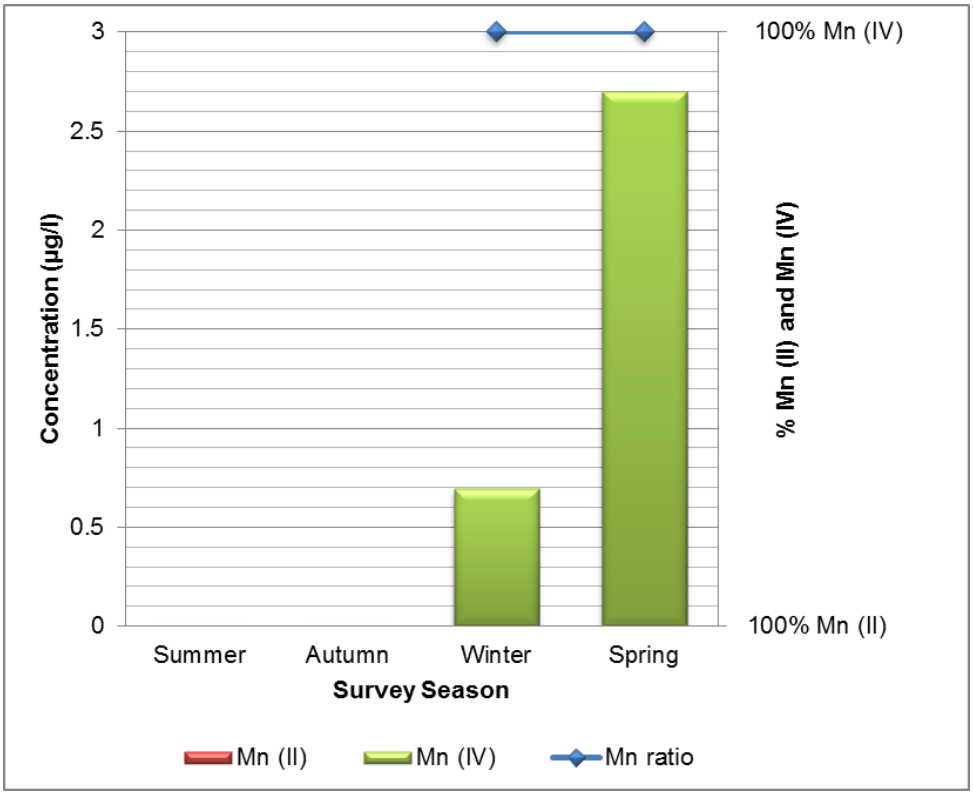


Figure 6.9 Seasonal manganese concentrations and oxidative states – Sites 15 and 16



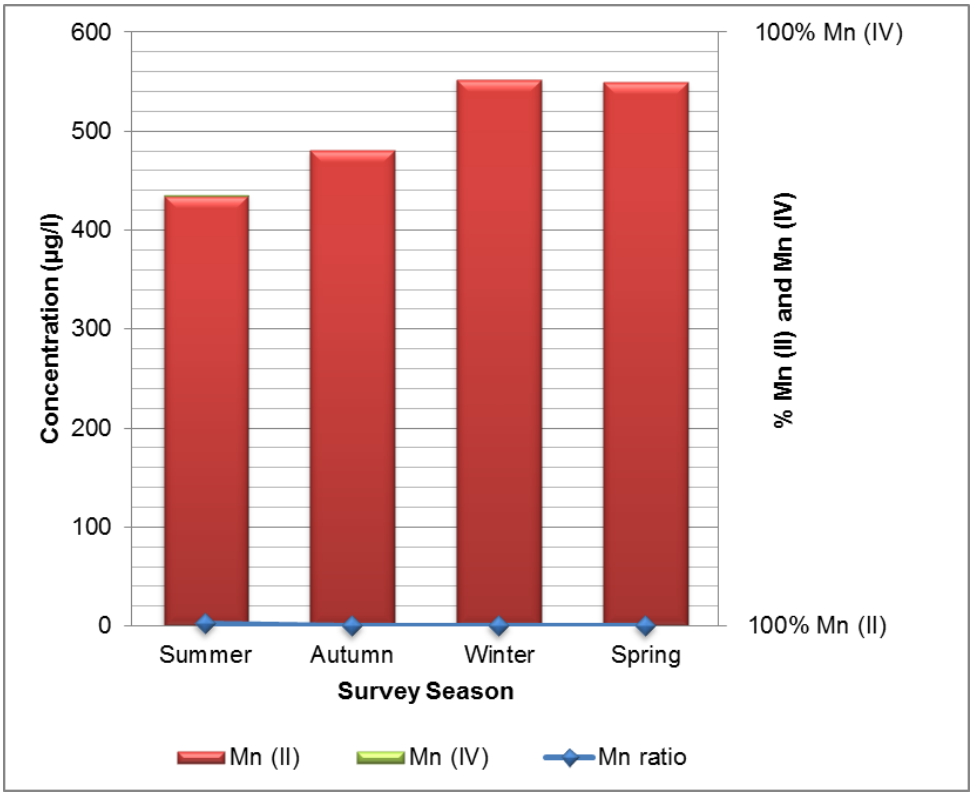
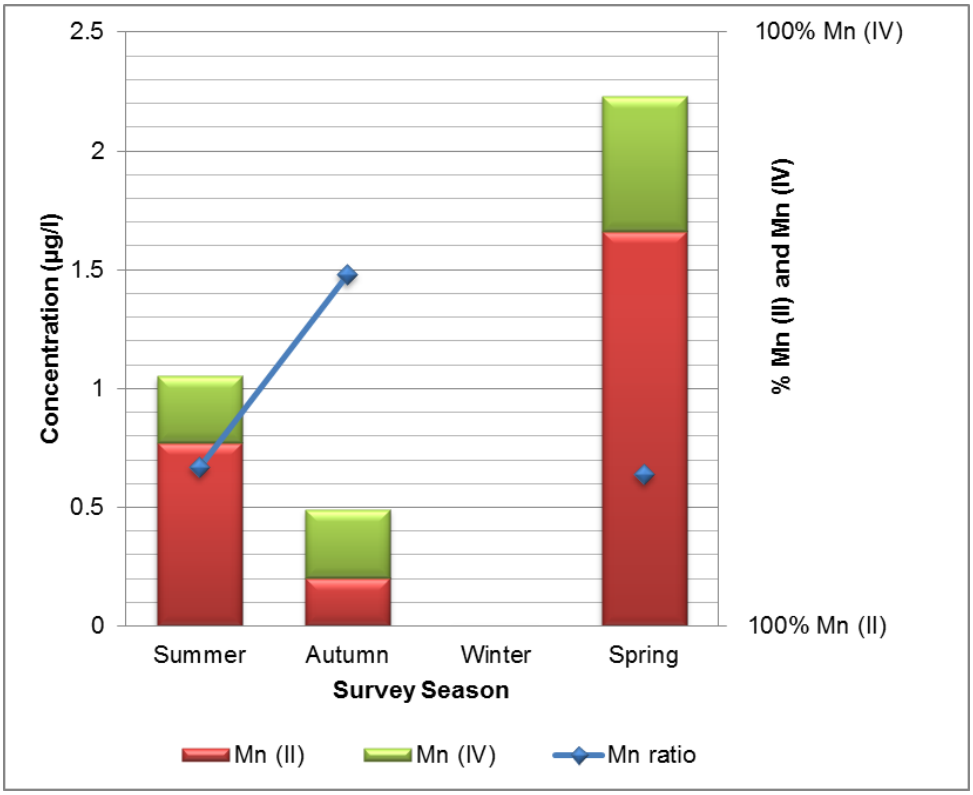


Figure 6.10 Seasonal manganese concentrations and oxidative states – Sites 17 and 18



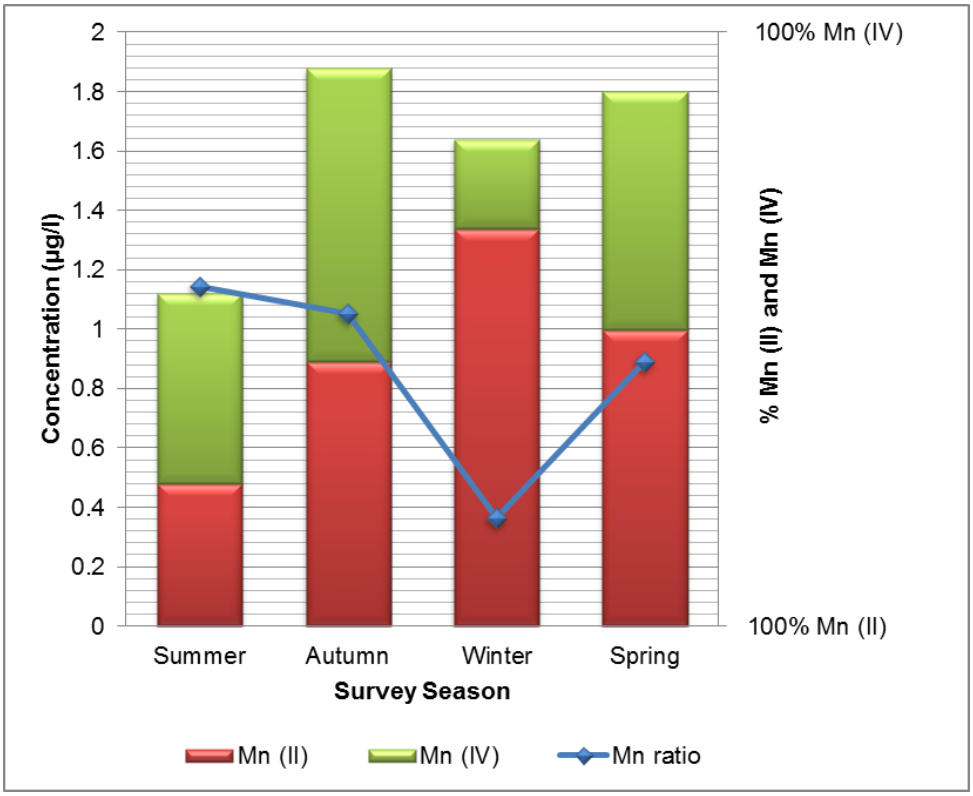
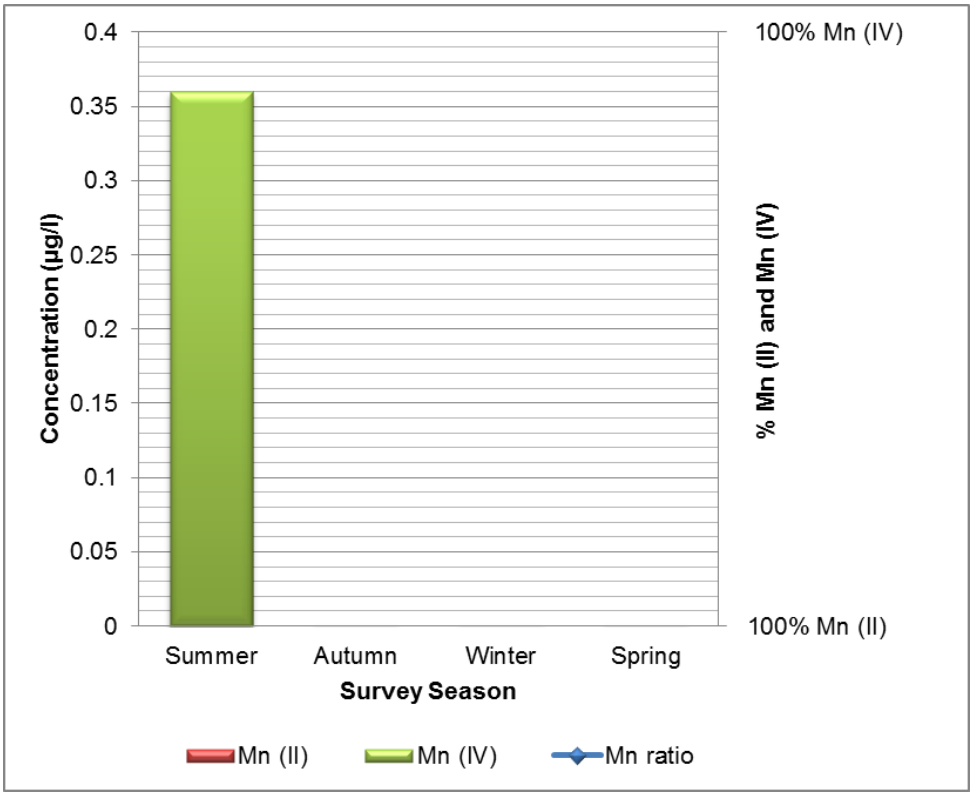
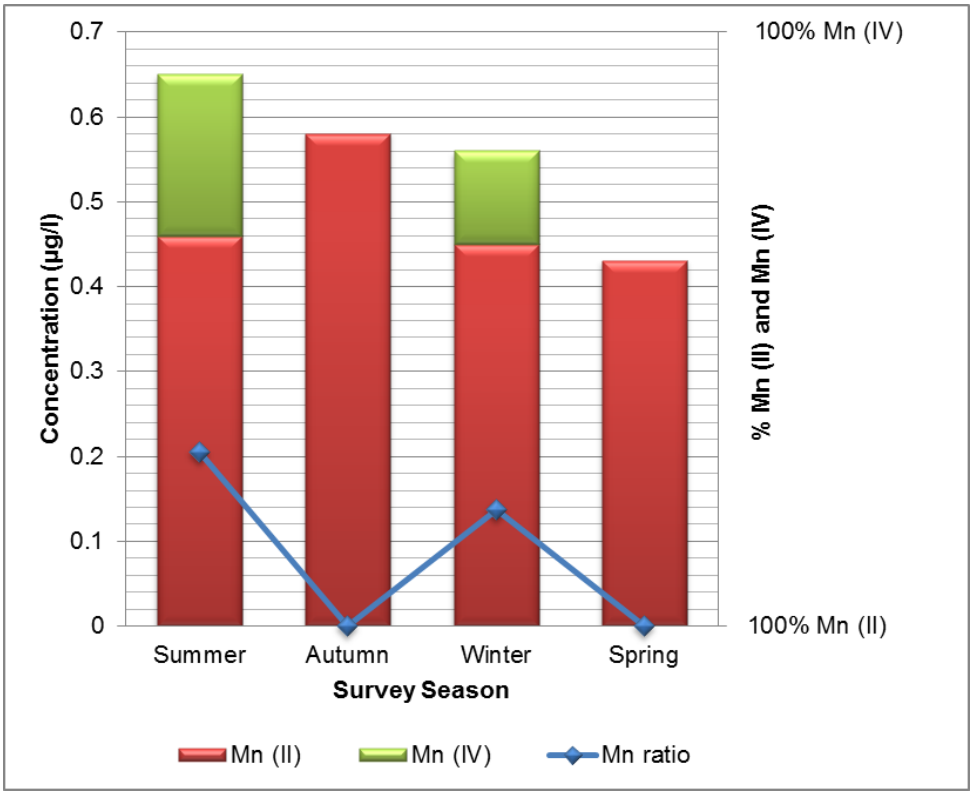


Figure 6.11 Seasonal manganese concentrations and oxidative states – Sites 19 and 20





6.1 Conclusions

The monitoring of Mn generally indicated very low concentrations of Mn were present in final waters from the treatment works that were sampled, except for a single sample at site 5 where there appears to be a sampling error as other samples taken from this site during the year were much lower. The speciation of Mn detected was variable with no consistent results at any site, and no consistent variations with the seasons. The exception to these conclusions is the results of the water samples from two private supplies coming from boreholes, that both had high levels of Mn, all of which was soluble and hence probably the bioavailable Mn(II) form.

7. General Discussion and Conclusions

Mn(II) is the oxidation form of Mn that is absorbed by the gastrointestinal tract when consumed orally. Mn(IV) is generally insoluble and so less likely to be absorbed to any extent. In adults, oral absorption is low (3-5%), but in infants and children, this is higher (40%) and excretion, which is via the biliary system, is lower. Therefore, the potential for uptake into the body is higher in children. In the body there is some exchange of oxidative states between Mn(II) and Mn(III), with Mn(II) more likely to be excreted.

Studies in experimental animals indicate that Mn administered orally as Mn(II) can be pass the blood brain barrier and accumulate in the brain. This accumulation occurs in those regions, such as the substantia nigra and globus pallidus, which are associated with the neurotoxicity (including the Parkinson's Disease-like syndrome, manganism) seen after exposure by inhalation. There is also evidence in experimental animals that this accumulation can be accompanied by adverse effects on neurological development, even at dose levels not considered particularly high in experimental animals (approximately 1 mg/kg bw/day), although it should be pointed out that these experiments were conducted in order to study adverse effects caused by Mn. Therefore, there is a biologically plausible hypothesis for an adverse effect on neurological development of Mn taken in orally via drinking water.

There have been a number of studies in humans which conclude that there is an association between the intake of drinking water containing high levels of Mn and adverse effects on intellectual and cognitive development in children (including a decrease in IQ in a number of studies). These studies are cross-sectional in nature, relying on a measurement of Mn in drinking water, and neuropsychological testing at a single point in time. The present hypotheses on the effects of Mn on the brain and the subsequent neurotoxicity, suggest that accumulation is important, as there is active uptake into specific brain regions and into mitochondria in these cells, and no specific efflux transportation mechanisms. Therefore, it is difficult for these time-bound type of studies to adequately account for accumulation of Mn. The control of bias and confounders in intellectual and cognitive development is obviously important including many socio-economic factors and this is variable in these studies. The Quebec studies, particularly the most recent study (Bouchard *et al.*, 2011), does include consideration of these potential confounders. This study finds an association with the highest Mn exposure group from drinking water and a decrease in IQ, despite the intake from drinking water being a small fraction of the total daily intake, with much of the rest coming from food. Why there is a difference in effect from Mn taken in from these two sources has yet to be resolved. From the figures given in these studies, it appears that for some of the children, their total daily intake of Mn may be lower than that recommended as essential.

Although one of the smaller Bangladesh studies (Wasserman *et al*., 2006) does measure effects on children exposed to high levels of Mn and low As in drinking water, the other studies attempt to separate the effects of the two chemicals. Although the Canadian studies

do take exposure of other metals in drinking water into consideration, others do not consider the exposure to metals such as lead which has been shown to affect development and behaviour in children.

Because of the difficulties of cross-sectional studies in considering accumulation of Mn over time and estimating accurate exposure measurements, the human experimental studies are not currently conclusive in indicating that Mn taken in orally via drinking water can cause adverse effects on intellectual and cognitive development in children, i.e. although association have been described, there is no proof of causation. However, their results cannot be dismissed. Some of the shortcomings in the studies have been admitted by the authors of the studies, and the work of the Canadian group in particular is continuing. Their studies are being repeated in other parts of Canada, and there are continuing studies in the Quebec area, where measures have been taken to reduce Mn in drinking water and retesting is taking place to ascertain any reversibility of adverse effects. These additional studies should yield important information on any potential association between oral intake of Mn and adverse effects on intellectual and cognitive development.

The total concentrations of Mn detected in treated waters in England and Wales (maximum 11.04 µg/l) was much lower than those seen in Quebec. Both the Quebec waters and the monitoring sites in England and Wales were chosen as being potentially high sources of Mn. It is also important to note that Mn is an essential element and most of the exposure is from the diet. The intake from water at this maximum concentration detected in public water supplies (approximately 22 µg/day for an adult drinking 2 litres of water) represents only a small proportion of an adequate dietary intake of about 3 mg/day for an adult (EFSA, 2013). The exception to these monitoring results was the two borehole sources of private drinking water which contained much higher concentrations of Mn (31-541 µg/l) than the public supplies and almost all Mn was present in the Mn(II) form, i.e. similar concentrations and oxidation state to the Quebec boreholes. It may be of importance to further investigate sources of private drinking water in England and Wales, not only for Mn but other metals and potential contaminants.

Information on the drinking water from boreholes in the Quebec study indicate that the high concentration of Mn detected was generally in the Mn(II) form (95%), i.e. the form more likely to be absorbed from the gastrointestinal tract (Carriere *et al*., 2011). In the public system In Quebec, treatment processes and chlorination (leading to a greater likelihood of oxidation) means that the level of Mn(II) is much more variable (4-100%, average 63%). This wide range was also observed in the measurement of Mn in final drinking water in public supplies in England and Wales, where the percentage of the bioavailable, Mn(II) form varied from 0-100% (average 31-60% over the four seasonal samplings). This indicates that the concentration of the bioavailable Mn(II) species is less in the drinking waters of England and Wales as compared to the boreholes in Quebec. In the UK, public supplies with high levels of Mn present have been identified by systematic monitoring and if above the standard for Mn of 50 µg/l, then treatment has been put in place to lower Mn in the final drinking water. These treatment methods include rapid gravity filtration (RGF) and/or oxidation to insoluble Mn(IV)

by an extra stage of chlorination. The final drinking water monitored in this survey were not chosen randomly but as being at potential risk of increased levels of Mn as identified by water companies or in areas of high natural Mn. At a number of these sites either no or very low concentrations of Mn were detected.

In summary, the experimental studies that have been conducted, do suggest that there is a biologically plausible hypothesis for an adverse effect on neurological development of Mn taken in orally via drinking water. The human epidemiological studies particularly on children are suggestive of an effect on intellectual and cognitive development; however, the type of studies conducted are not the most appropriate for measuring the longer-term effect such as those which may occur after accumulation of Mn in the brain. There are also problems in the accurate estimation of exposure via drinking water, and the detection of Mn in the body (through the measurement of blood or hair). Therefore, these studies do not, at present, give definite proof of an effect of Mn, but they will be continued. In the recent Canadian study, populations were exposed to borehole drinking water with variable, but with some groups high levels of Mn, mainly in the bioavailable Mn(II) form (95%). A monitoring survey was conducted in England and Wales to ascertain whether these final waters were similar to those in the Quebec region of Canada. The four seasonal sampling exercises indicated that public water supplies were low in Mn, and variable in the level of Mn(II) present, which was on average approximately 50% Mn(II). Therefore, the Quebec studies do not represent the typical situation in the public supplies of England and Wales, as the concentrations of Mn are low and less is in the bioavailable Mn(II) form. The exception to these findings in England and Wales was in two private borehole supplies, which had high concentrations of Mn nearly all in the Mn(II) form, i.e. similar to the water in the Quebec boreholes.

8. Future Work

Work is continuing world-wide studying the potential adverse intellectual and cognitive effects of Mn on the development of children by both oral exposure and by inhalation and the comparison with the known toxicity of occupational inhalation of Mn. The Canadian studies on the development of children are being repeated in the same areas where efforts have been made to reduce the Mn concentrations in order to see if the effects are reversible, and in other areas where the concentrations and potentially the oxidation states of Mn may be different.

Efforts are constantly being made to improve the exposure estimates in studying the effects of chemicals on population. It is always difficult to estimate water concentrations of a chemical over time, and subsequent consumption by populations especially when, like Mn, it is considered that accumulation of the chemical may be important to its toxicity. Estimation of the actual body-load of chemicals, such as Mn, measured by hair or blood levels is also being improved. While more sensitive tests of intellectual and cognitive skills are being developed, the effects of exposure to ever lower concentrations of chemicals are more difficult to detect, with other factors being more important to children's development, such as socio-economic factors.

In the UK, the low concentration of Mn and less bioavailable Mn(II) being present in public supplies, indicates that more immediate study may not be necessary. However, the results from just two private water supplies from borehole sources suggest that there may be a high concentrations of Mn(II) in such supplies. Therefore, a further study looking at private water supplies is suggested. Private supplies are subject to statutory regulatory regime enforced by local authorities. It may be wise to review existing data before deciding whether such a monitoring survey might be warranted.

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Appendix A Neuropsychological Assessments Glossary

A1 Achenbach System of Empirically Based Assessments (ASEBA)

ASEBA is an evidence based assessment which assesses competencies, adaptive functioning and behavioural, emotional and social problems from the ages of 1½ to over 90 years of age. Tests for pre-school children and school children include a child behaviour checklist filled in by the primary care-giver and a parallel form completed by day care providers, preschool teachers and teachers. Children aged 6 – 18 years also fill out an additional self-report form. ASEBA identifies children at risk of eight syndromes: anxiety, depression, somatic complaints, social problems, cognitive problems, attention difficulties, rule breaking behaviour and aggressive behaviour and relate to DSM-IV scales (Aseba, 2012).

A2 Beck Depression Inventory

21 Questions, self-reporting multiple choice which measures the severity of depression as described by the criteria of the DSM-IV, designed for people 13+ years. It focuses on symptoms relating to depression including sleep loss and changes in appetite as well as cognitive thought relating to depression (Pearson Education Inc, 2012a).

A3 Behaviour Assessment System for Children (BASC)

BASC is a multi-method, multi-dimensional approach to evaluating behaviour and self-perception of children from 2½ years to 18 years. The test offers assessment from three vantage points: teacher, primary carer and self. The teachers evaluate the students behaviour using a Teacher Rating Scales (TRS) which evaluates behaviours in the school setting, A Student Observation System (SOS) where the child's behaviour is monitored during three second intervals spaced 30 seconds apart. The parents rate their child's behaviour in the community and home settings. The Self-Report of Personality (SRP) perspective component measures a child's personality through an interview (Pearson Education Inc, 2012b)

A4 Behaviour Rating Inventory of Executive Function (BRIEF)

The BRIEF is based on two evaluations from a parent and a teacher which are designed to assess executive function in the home and school environments and is used to evaluate children with a wide range of developmental and neurological conditions such as learning difficulties, Attention deficit/hyperactivity disorder, pervasive developmental disorders/autism, lead exposure, traumatic brain disorders and Tourette's disorder.

The executive functions are mental processes that direct a child's thought, action and emotion particularly during active problems solving. These include skills such as selecting appropriate goals for a task, planning and organising, initiating a plan and blocking out distractions. Executive functions are also responsible for controlling a child's emotional responses thereby allowing for more effective problem solving.

Each questionnaire contains questions in eight non-overlapping clinical scales and two validity scales. The normative data are based on a child ratings from 1419 parents and 720 teachers from rural, suburban and urban areas reflecting ethnicity and gender distribution from the US 1999 census data (Gladman and Lancaster, 2003).

A5 California Verbal Learning Test – Children's version

Assessing verbal learning and memory in children and adolescents, it can be used to identify learning and memory difficulties, to isolate deficient learning strategies, it measures multiple aspects of how verbal learning occurs or fails to occur as well as the amount of verbal material learned. It assesses verbal learning through an everyday memory task in which is the child is asked to recall a list, an interference task is given followed by a short delay and then free recall and cued recall trials (Pearson Education Inc., 2012c; O'Jile *et al*., 2005).

A6 Clinical Evaluation of Language Fundamentals (CELF)

The Clinical Evaluation of Language Fundamentals tests for a language disorder or delay in students aged from 5 to 21 years. The tests include evaluations of concepts, following directions, word structure, recalling and formulating sentences, word definitions, expressive vocabulary and phonological awareness. The CELF uses a standardisation sample of 2650 students representative of the US population in age, gender race/ethnicity, socio-economic status based on the educational level of the primary parent, geographic region and children with identified conditions including language disorders (Pearson Education Inc., 2012d).

A7 Children's Category Test Level II

The Children's Category Test (CCT) measures complex aspects of intellectual functioning and nonverbal abilities which incorporates concept formation, memory and learning from experience measures nonverbal abilities. It can be co-administered with the California Verbal Learning Test (Children's Version) to measure verbal abilities. There are two versions of the test: Level 1 for children between the ages of 5 to 8 years and Level 2 for children aged between 9 to 16 years old. The CCT was stratified on a national sample with stratification variables including age, sex, race/ethnicity region and parent education level (Pearson Education Inc., 2012e). The Children's Category Test has been reported to be less sensitive to measuring structural brain damage and to neurodevelopmental disorders such as ADHD (Bello *et al*., 2008).

A8 Children's Depression Inventory (CDI)

The CDI evaluates the presence and severity of specific depressive symptoms in youths between 7 and 17 years of age. It measures emotional and functional problems, including negative mood, physical symptoms, negative self-esteem, interpersonal problems, and ineffectiveness. The test consists of 28 questions of which there are three statement answers. A shortened version of 10 questions is also available as well as a parent and teacher version. The CDI may be used to monitor changes in symptoms over time. The tests are evaluated against a norm sample of 1100 children from the 26 states in America with proportional representation of racial/ethnic groups (Pearson Education Inc., 2012f).

It is generally recommended as a screening device and considered less reliable for diagnostic purposes. It is based on self-assessment and can therefore result in participants modifying their answers to what they think are the desired answer as opposed to what they actually feel (CEBC4CW, 2012).

A9 Conners' ADHD/DSM-IV Scales

The Conners' ADHD/DSM-IV scales consist of the items on the CRS-R that best differentiate children with Attention-Deficit/Hyperactivity Disorder and can be used as a screening method or when there are time constraints. The output scales directly relate the DSM-IV criteria for ADHD diagnosis. The CADS scales consist of three versions: parent, teacher and an adolescent form. The Conners' Scales are standardised using a normative database compiled from over 200 data collection sites (Psychological Assessments Australia, 2012).

A10 Conners' Rating Scale- Revised (CRS-R)

The CRS-R can assess for attention-deficit/hyperactivity disorders in children and adolescents aged 3 – 17 years. The CRS-R are composed of three scales: parent, teacher and adolescents, all of which are available as a long or short version, taking between 15 – 20 or 5 – 10 minutes to complete, respectively.

Behaviour is rated on a 4 point Likert scale (not true at all, just a little true, pretty much true and very much true)

Scores can be calculated for the following areas: oppositional, cognitive problems/inattention, hyperactivity, anxious/shy, perfectionism, psychosomatic and social problems. The test can be used to identify ADHD and the following DSM-IV symptoms: inattention and hyperactivity-impulsive (Pearson Education Inc., 2012g).

A11 Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV)

The American Psychiatric Association publish a manual called the Diagnostic and Statistical Manual of Mental disorders (4th edition) which covers all mental health disorders for both children and adults It lists the known causes of the disorders, statistics in terms of gender, age prognosis and research concerning the optimal treatment approaches (American Psychiatric Association, 2000).

A12 Ravens Progressive Matrices Test

The Ravens Progressive Matrices Test is a non-verbal multiple choice test which measures the reasoning aspect of intelligence only. It is useful for ethnically diverse populations where language maybe a barrier to the implementation of other intelligence tests based on language understanding or where an intelligence test has not been transcribed to a language or local dialect. Tests involve the identification of a missing element which completes a pattern. The test is available in three different forms: standard, coloured and advanced (Pearson Education Inc. 2012h).

A13 Weschler Abbreviated Scale of Intelligence (WASI)

The WASI assessment if part of a family of Weschler intelligence tests. This particular test is a shortened (abbreviated) form of the full Weschler Intelligence Scale. WASI is a measure of intellectual ability used in clinical educational and research setting suitable for ages 6 to 89 years. It is not designed as a detailed intelligence test but rather to provide an estimate for quickly measuring an individual's cognitive functioning. WASI contains four sub-tests on Vocabulary, Similarities, Block Design and Matrix Reasoning which results in two scores: Verbal IQ (VIQ) and Performance IQ (PIQ), these combine to provide a Full Scale IQ (FSIQ). VIQ is a measure of crystallised abilities (learnt knowledge and verbalisation), and is provided for by the results from the Vocabulary and Similarities subtest. PIQ is a measure of perceptual organisation and non-verbal intelligence and is provided for by the results from the Block Design and Matrix Reasoning. The results are standardised against a highly representative population of the English speaking US population with a range of abilities and ages (from 6 – 89 years) (Pearson Education Inc, 2012i).

A14 Weschler Intelligence Scale for Children (WISC-IV)

The Weschler Intelligence Scale for children is part of a family of Weschler intelligence tests, this one in particular focuses on measuring the IQ score for children between the ages of 6 and 16 years of age. The test was last updated in 2004 and is currently the fourth edition. The test generates a Full Scale IQ (FSIQ) which represents overall ability and is composed of the scores from four other areas: Working memory index (WMI), Verbal comprehension index

(VCI), Perceptual Reasoning index (PRI) and Processing Speed Index (PSI) (Pearson Education Inc., 2012j).

A15 Wide Range Assessment of Visual Motor Ability (WRAVMA)

WRAVMA provides an assessment of a child's visual-motor skills (visual spatial, fine motor and integrated visual-motor skills) and is suitable for children between the ages of 3 and 17 years old. The assessment is composed of three subtests which can be used individually or in combination: The drawing test which measures visual motor integration by asking the child to copy designs of increasing difficulty. The matching test assesses visual-spatial skills by asking the child to look at a visual standard and select the option that goes best with it. The pegboard test evaluates fine motor skills by asking the child to insert as many pegs as possible within 90 seconds into a waffle pegboard. The results can be compared to norms based on a nationally representative sample of more than 2600 US children (Hogrefe, 2012).

A16 Wide Range Assessment of Memory and Learning (WRAML)

WRAML evaluates an individual's memory functioning (immediate and delayed memory ability). WRAML scores on two levels: working memory index and general memory index. Working memory is evaluated on symbolic working memory and verbal working memory. General memory is evaluated on two levels: a verbal memory index and attention/concentration index which in turn is based on two verbal, two visual and two attention/concentration sub-tests. The test has been normalised for a US population aged between 5 to 90 years old using a national stratified sampling technique to provide a representative population (Western Psychological Services, 2012).

Appendix B Standard Operating Procedure for the Determination of Total Manganese and Manganese(IV) Concentrations in Drinking Water

B1 Equipment and Supplies

Agilent 7700x ICP-MS

Argon gas supply

Displacement pipettes capable of delivering from 0.01 to 1 ml

Glassware – volumetric flasks, pipettes, graduated cylinders

Graduated polypropylene ICP-MS tubes

Disposable 0.22 µm Luer-lock filters

Disposable 10 ml Luer-lock syringes

B2 Reagents and Standards

Concentrated nitric acid (≥69%; Fluka TraceSELECT grade, Mn ≤ 0.5 µg kg-1)

Concentrated nitric acid (≥69%; AR grade)

Manganese ICP-MS standard solution (1000 ± 3 ppm; SPEX Certiprep Lot No. 16-65MN)

Yttrium ICP-MS standard solution (1000 ± 3 ppm; SPEX Certiprep Lot No. 17-04Y)

Deionised water (ASTM Type I water, 18.2 MΩcm)

B3 Preparation of aqueous nitric acid solutions

Solutions of aqueous nitric acid are required at concentrations of 1% v/v, 2% v/v and 1% v/v containing a 1 ppb Y-89 internal standard.

1% v/v aqueous nitric acid – 10 ml of concentrated nitric acid (TraceSELECT) is added to deionised water and the volume made up to 1 litre.

2% v/v aqueous nitric acid – 20 ml of concentrated nitric acid (TraceSELECT) is added to deionised water and the volume made up to 1 litre.

1% v/v aqueous nitric acid containing 1 ppb Y-89 internal standard – 20 ml of concentrated nitric acid (TraceSELECT) is added to deionised water followed by an aliquot (20 ml) of a 100 ppb stock solution of Y-89 internal standard in 1% v/v aqueous nitric acid and the volume made up to 2 litres with deionised water.

B4 Preparation of a 100 ppb yttrium-89 internal standard stock solution

An aliquot of yttrium-89 (SPEX Certiprep; 1000 ppm, 0.5 ml) is taken and diluted 500-fold using 1% v/v aqueous nitric acid (TraceSELECT) to afford a stock solution (250 ml) at a concentration of 2 ppm. A further 20-fold dilution (5 ml to 100 ml) is performed using 1% v/v aq. nitric acid to afford the final yttrium-89 internal standard stock solution at a concentration of 100 ppb.

B5 Preparation of manganese standards

A stock solution of manganese (SPEX Certiprep; 1000 ppm) is used to prepare the standards to establish the calibration curve for this method. An initial dilution of 100-fold (5 ml down to 500 ml) using 1% v/v aqueous nitric acid is carried out to afford a stock solution at a Mn concentration of 10 ppm. All subsequent dilutions are performed using the 1% v/v aqueous nitric acid solution containing 1 ppb Y-89 internal standard:-

10 ppm Mn std. (2.5 ml) diluted to 250 ml	100 ppb Mn + 1 ppb Y int. std.
100 ppb Mn std. (50 ml) diluted to 100 ml	50 ppb Mn + 1 ppb Y int. std.
100 ppb Mn std. (20 ml) diluted to 100 ml	20 ppb Mn + 1 ppb Y int. std.
100 ppb Mn std. (10 ml) diluted to 100 ml	10 ppb Mn + 1 ppb Y int. std.
50 ppb Mn std. (10 ml) diluted to 100 ml	5 ppb Mn + 1 ppb Y int. std.
10 ppb Mn std. (10 ml) diluted to 100 ml	1 ppb Mn + 1 ppb Y int. std.
5 ppb Mn std. (10 ml) diluted to 100 ml	0.5 ppb Mn + 1 ppb Y int. std.
1 ppb Mn std. (10 ml) diluted to 100 ml	0.1 ppb Mn + 1 ppb Y int. std.

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0.5 ppb Mn std. (10 ml) diluted to 100 ml	0.05 ppb Mn + 1 ppb Y int. std.
0.1 ppb Mn std. (10 ml) diluted to 100 ml	0.01 ppb Mn + 1 ppb Y int. std.

The shelf life of these standards has been demonstrated to be at least two months from the date of preparation with no degradation in the performance of the assay.

B6 Preparation of blanks

Two types of blank are required for this method. A calibration blank which is used to establish the analytical calibration curve and a laboratory reagent blank which is carried through the same preparation scheme as the samples.

Calibration blank – consists of 1% v/v aq. nitric acid containing 1 ppb Y-89 internal standard

Laboratory reagent blank – deionised water is added to conc. nitric acid and 100 ppb Y-89 internal standard (100 µl of each) and made up to 10 ml volume in a graduated ICP-MS tube.

Tuning solution:-

This comprises of a solution containing the following metals: Li, Mg, Y, Ce, Tl and Co at a concentration of 1 ppm (Agilent Part No. 5185-5959).

Quality control samples:-

A sample containing 5 ppb Mn and 1 ppb Y is analysed together with a laboratory reagent blank at various points during sample sequence of the analytical run. The frequency is determined by the total number of unknown samples being analysed but is no less than every fifth sample. The calibration set of standards is rerun at the end of each analysis to demonstrate the absence of significant drift having occurred during the run.

Laboratory fortified blank:-

A sample of deionised water is spiked with a 10 ppm Mn stock standard (50 µl) to give a final concentration of 50 ppb when processed in the same way as the rest of the samples and made up to 10 ml.

B7 Sample collection, preservation and storage

Two samples of approximately 100 ml are required for the total manganese and soluble manganese determinations. For total manganese content, the sample of drinking water (approx. 100 ml) should be collected into a suitable plastic container (e.g. polypropylene) containing concentrated nitric acid (1 ml; TraceSELECT grade). For soluble manganese

determinations, the sample should first be passed through a 0.22 µm disposable filter at the time of collection with the first 20 ml being used to rinse the filter and discarded. A further 100 ml sample should then be collected into a suitable plastic container (e.g. polypropylene) containing concentrated nitric acid (1 ml; TraceSELECT grade). When correctly acid preserved, these samples can be held for several months at ambient temperature prior to analysis. The pH of samples should be measured prior to analysis and the pH recorded to confirm that it is <2. This will provide further assurance as to the validity of all subsequent manganese concentration determinations.

A field blank using deionised water should be generated at the same time as the samples are collected and using an identical process to eliminate the possibility of contamination occurring during the sampling process.

B8 Preparation of samples for analysis

The pH of all samples should first be measured and recorded to confirm that the sample has been properly preserved (pH <2) and thereby the validity of all subsequent determinations.

An aliquot (100 µl) of a 100 ppb yttrium internal standard is added to a 15 ml polypropylene ICP-MS tube and the sample used to make the volume up to the 10 ml mark on the tube. A dilution factor of 1.02 is applied to correct for the manganese concentration in the original sample (corrects for the addition of concentrated nitric acid to the sample when taken plus the addition of the internal standard). This is repeated for all other samples together with field blanks, laboratory reagent blanks and the laboratory fortified sample.

The concentration of Mn(IV) in each sample is determined by difference by subtracting the manganese concentration found in the filtered sample away from the total manganese concentration of the unfiltered sample.

B9 Linear calibration range

A series of manganese standards covering the range 0.01 – 100 ppb has been selected for this analytical method. A linear regression fit is applied to the data and the line is not force fitted to the calibration blank standard (via the “ignore” option in the software). The limit of quantitation of the method is 0.5 ppb and for concentrations of unknowns in the range 0.5 – 100 ppb the use of the “no weighting” option gives a very good curve fit over this range. It is, however, possible to generate reasonable data above the limit of detection and so for concentrations of between 0.1 and 0.5 ppb, a weighting factor (1/SD2 or 1/Y) will be applied to the curve to enhance the fit at the lower end of the calibration range and this data will also be reported. Concentrations below the limit of detection will be reported as < 0.1 ppb.

B10 Instrument set up

ICP-MS set up:-

Lift the lid cover on the MS to allow access to the cones. Hold down the maintenance button for 2 seconds to allow the nebuliser assembly to relocate and to allow access to the cones. There are dedicated cones for the Agilent 7700x ICP-MS which have been acquired for both the chromium and manganese analysis and these should be fitted in place of the standard cone assemblies.

Turn the cooling system on and wait until temperature reaches approximately 15°C. Check the argon pressure is >6 bar.

Remove internal standard delivery tubing from the T-mixing piece and fit the blanking plug in its place. Fit drain tubing and sample delivery tubing around the rotator, ensuring that the directions of flow are correct, and clamp into position.

Ensure that the containers holding the 1% v/v aqueous nitric acid rinse solution, the 2% v/v aqueous nitric acid sample line solution and the tuning solution have adequate supplies for the duration of the analytical run and that the respective drain collection vessels are empty.

Start up the Online ICP-MS Mass Hunter Instrument Control software on the PC and select “Acquire New Data” from the Welcome menu. Check the box against “Existing Batch” in the Create from menu and then select the most recent “Mn_in_WaterAE” folder; these are suffixed sequentially i.e. AA, AB, AC….. Input the name for the new batch folder e.g. Mn_in_WaterAF and in the “Select Batch Contents” menu ensure that the contents boxes for Acq Method, DA method, Sample List and QC Configuration are all checked. This will create a new batch folder for your planned analysis and import all the necessary instrument settings (Figure B.1 and Figure B.2), quantification parameters and sample sequence tables.

Figure B.1 Acquisition parameters for Mn_in Water method

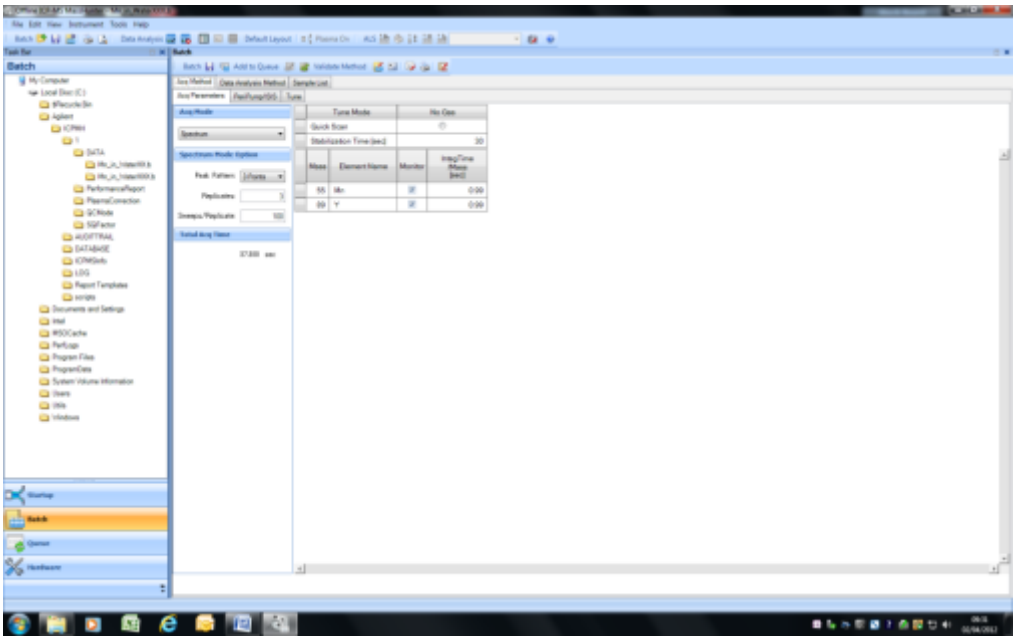
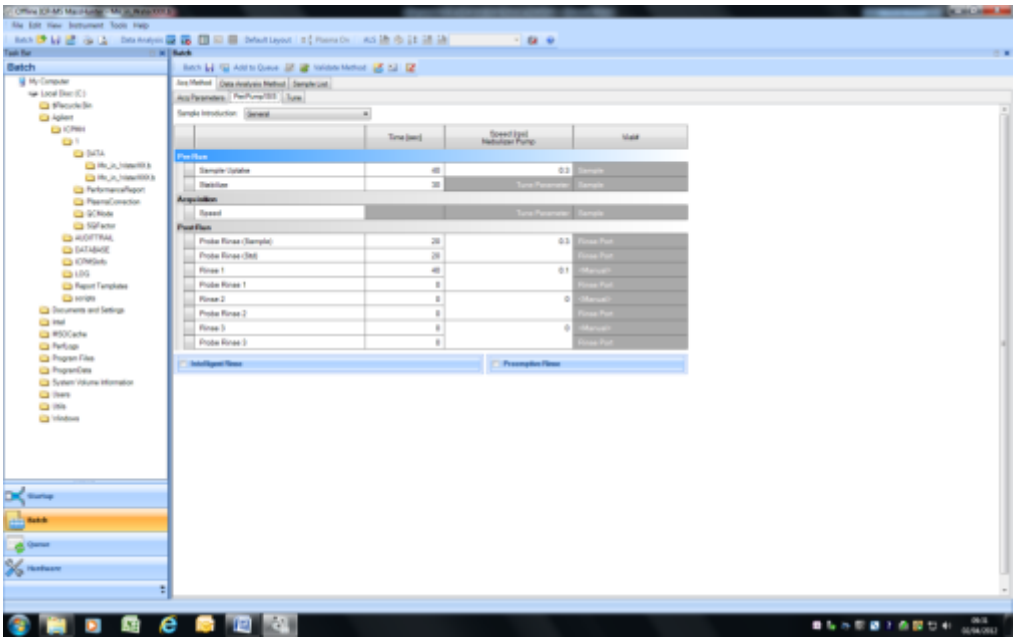
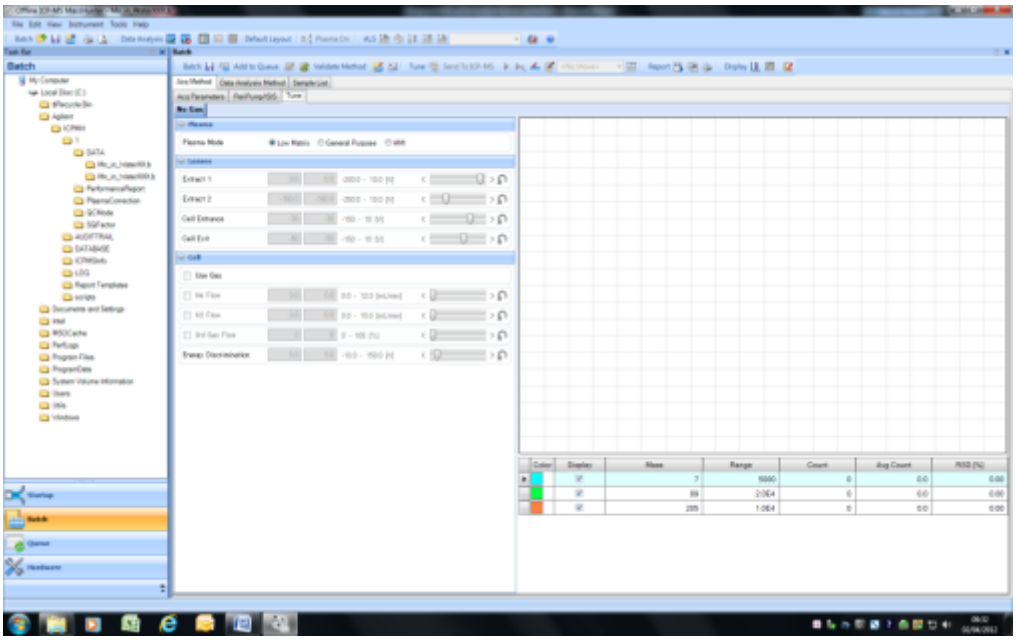


Figure B.2 Peripump settings for Mn_in_Water acquisition method



Click on the “Acq. Method” tag and select the “Tune” option (Figure B.3). Ensure that the autosampler probe is in the tune solution and run the “Start Autotune” option for the instrument. Once completed typical readings for the instrument should be as follows: mass/count, 7/3000; 89/10,000; 205/8000; oxide ratio 156/140 <1%; doubly charged ratio <1%. If there is a significant difference consult with the laboratory steward for advice

Figure B.3 Tune menu for the Mn_in_Water acquisition method



Select the “Data Analysis Method” tag check that the information in the three highlighted tags is correct (Figure B.4, Figure B.5 and Figure B.6).

Figure B.4 Basic information for Mn_in_Water acquisition method

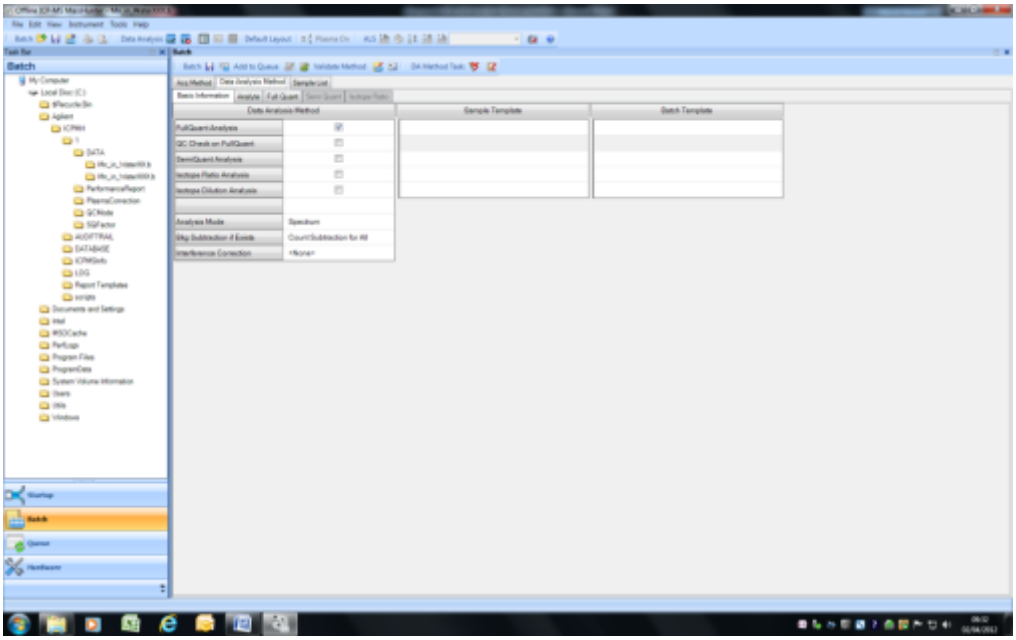


Figure B.5 Analyte information for Mn_in_Water acquisition method

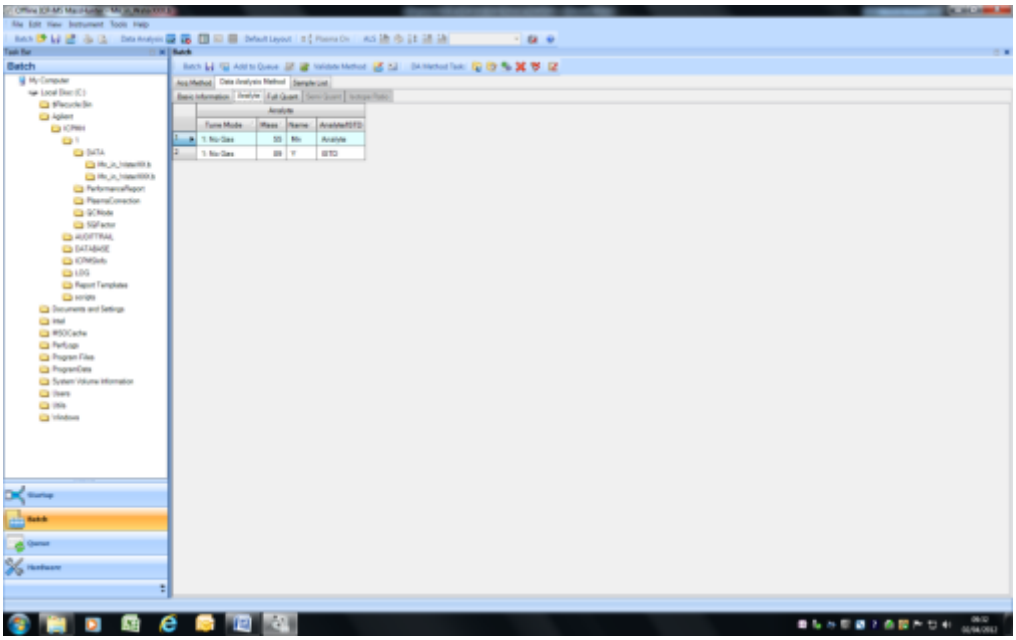
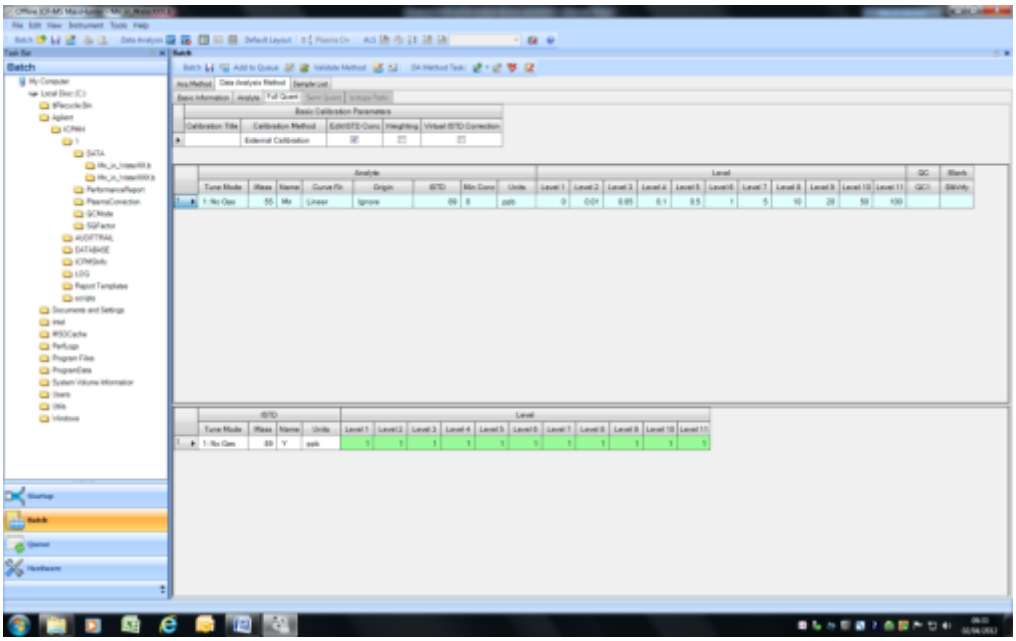
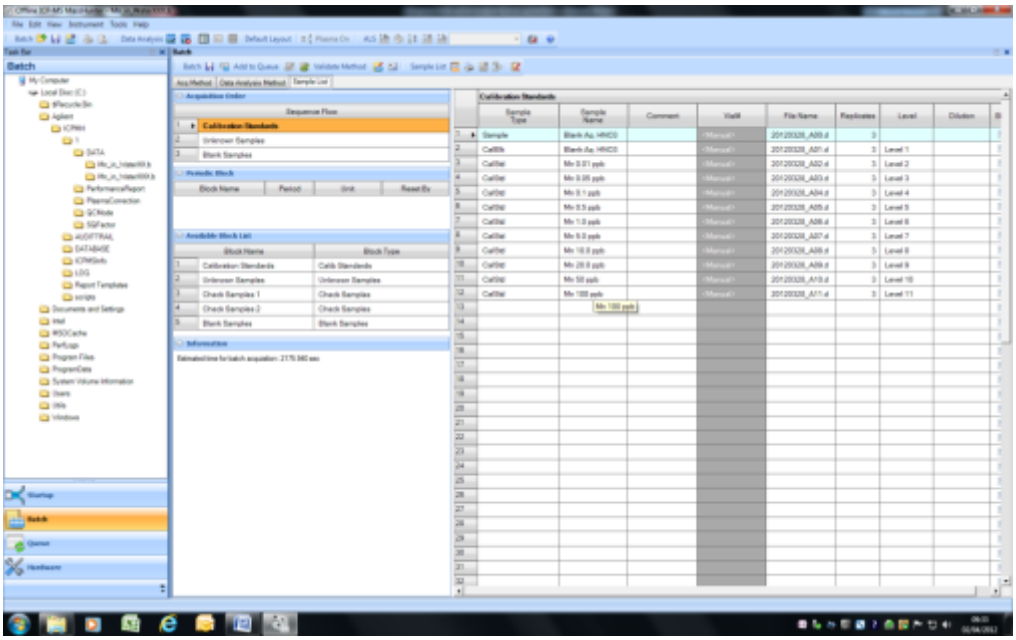


Figure B.6 Quantitation parameters for Mn_in_Water acquisition method



Following completion of autotune, it is necessary to edit the sample list. This can be done by clicking on the “Sample List” tag (Figure B.7). The order of acquisition of the data is determined by the sequence flow. Check that the sample order in the calibration standards is correct with an initial sample blank being followed by the calibration blank and then the 10 manganese standards covering the range 0.01 -100 ppb.

Figure B.7 Calibration standards from the Mn_in_Water Sample List



The unknown sample list should then be completed followed by the blank sample list. The use of the periodic block option allows for either QC's or blanks to be interspersed with the unknown samples at regular intervals e.g. every 5th sample. Alternatively, these can included as part of the sample sequence when setting up the unknown sample list.

When complete ensure that the batch file is saved and click upon the "Add to queue" button. If there are no remaining samples to run of a previously queued batch the batch will commence running immediately.

Upon completion of the run, the batch file folder can be saved to a USB memory stick and taken away for Offline Data Analysis using a standalone version of the Mass Hunter software.