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ÉTUDE DE COHORTE SUR LES FONCTIONS NEUROCOMPORTEMENTALES DE TRAVAILLEURS 14 ANS APRÈS LA FIN DE L'EXPOSITION AU MANGANÈSE

THÈSE

PRÉSENTÉE

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PAR

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AVANT-PROPOS

La thèse est composée de six chapitres, dont trois sont des articles publiés ou sous presse dans des journaux scientifiques et desquels la candidate est première auteur. Le premier chapitre introduit la pertinence du travail effectué en présentant l'état des connaissances quant aux aspects suivants : les utilisations du manganèse (Mn), les populations à risque d'exposition, les mécanismes d'absorption et de régulation du Mn dans le corps, les aspects neuropathologiques de l'intoxication au Mn, et les signes et les symptômes de toxicité chez des personnes exposées à ce métal. Le deuxième chapitre présente les aspects méthodologiques des études, en commençant par le contexte général de la recherche, le design de recherche et la cueillette des données. Le troisième chapitre est un article intitulé *Manganese exposure and age: Neurobehavioral performance among alloy production workers*, publié dans *Environmental Toxicology and Pharmacology* en 2005 (volume 19, pages 687-694). Pour cet article, la première auteure (la candidate, Maryse Bouchard) a fait les analyses statistiques et a rédigé l'article; la co-auteure Donna Mergler a supervisé la recherche (directrice de recherche de Maryse Bouchard), et la co-auteure Mary Baldwin a effectué les mesures d'exposition au Mn dans l'usine et a calculé les indices cumulés d'exposition au Mn. Le quatrième chapitre est un article intitulé *Neurobehavioral functioning after cessation of manganese exposure: A follow-up after 14 years*, sous presse dans *American Journal of Industrial Medicine* (doi:10.1002/ajim.20407). Pour cet article, la première auteure (Maryse Bouchard) a élaboré le design de l'étude, mené l'ensemble de la collecte de données, fait les analyses statistiques et rédigé l'article. Les co-auteurs sont, dans l'ordre, Donna Mergler (supervision de l'ensemble des travaux), Mary Baldwin (mesures d'exposition), Michel Panisset (contribution à la rédaction pour les aspects neurologiques), Rosemarie Bowler (contribution à la rédaction pour les aspects neuropsychologiques) et Harry A. Roels (contribution à l'élaboration du protocole de recherche, à la collecte de données et à la rédaction de l'article). L'article qui constitue le cinquième chapitre est intitulé *Neuropsychiatric symptoms and past manganese exposure in a ferro-alloy plant*, sous presse dans *Neurotoxicology* (doi:10.1016/j.neuro.2006.08.002). Les auteurs de cet article sont, dans l'ordre, Maryse Bouchard, Donna Mergler, Mary Baldwin, Michel Panisset, et Harry A. Roel; leur rôle respectif est le même que pour l'article précédent. Le dernier chapitre discute de l'ensemble des résultats de la thèse et présente les conclusions générales. Finalement, un article sur le calcul des indices cumulés d'exposition au Mn des travailleurs de l'usine à l'étude a été placé en appendice car ces travaux sont directement reliés à la thèse. Cet article a été rédigé par Mary Baldwin, avec les co-auteurs Maryse Bouchard, Fabrice Larribe et Donna Mergler; il a été soumis pour publication en septembre 2006 à la revue *Journal of Occupational & Environmental Hygiene.*

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RÉSUMÉ

L'inhalation de particules de manganèse (Mn) peut causer des effets neurotoxiques qui se manifestent par des altérations des fonctions neurocomportementales, nommément des symptômes psychologiques et des déficits cognitifs et moteurs. Une première étude fut menée en 1990 sur les fonctions neurocomportementales de travailleurs exposés au Mn dans une usine de production d'alliages ferro-Mn et silico-Mn; des hommes cols bleus non-exposés au Mn servirent de groupe de comparaison (référents). Pour chaque travailleur, l'exposition aux poussières et aux fumées de Mn dans l'air au moment de l'étude a été estimée et un indice d'exposition cumulée sur toute la durée d'emploi dans l'usine a été calculé. L'usine a arrêté ses opérations quelques mois après la fin de cette étude, mais cette fermeture n'était pas liée aux résultats de l'étude.

Dans la première partie de la thèse, l'objectif était d'examiner l'effet modificateur de l'âge dans les altérations neurocomportementales. L'analyse des données appariées (74 paires) a montré que les différences de scores aux tests neurocomportementaux entre les travailleurs exposés au Mn et les référents (ces différences représentant l'effet du Mn) augmentaient significativement avec l'âge pour la mémoire, la concentration, la flexibilité cognitive, la stabilité des mains et la sensibilité tactile. L'âge n'était pas associé à la concentration de Mn dans l'air au moment de l'étude, ni à l'exposition cumulée, indiquant que les déficits plus prononcés les travailleurs plus âgés ne peuvent pas être expliqués par une exposition supérieure chez ces derniers. Ces résultats indiquent que, à exposition égale, les travailleurs plus âgés sont davantage affectés que les plus jeunes par les effets du Mn sur certaines fonctions nerveuses.

Pour la seconde partie de la thèse, nous avons mené une étude de suivi auprès de 78 anciens employés de l'usine et 81 référents afin de vérifier le degré de réversibilité ou d'aggravation des atteintes neurofonctionnelles 14 ans après la fin de l'exposition. Les travailleurs ayant été exposés au Mn ainsi que les référents ayant accepté de participer à l'étude de suivi ont complété une batterie de tests similaire à celle utilisée en 1990. Nous avons émis l'hypothèse que ces atteintes s'aggraveraient avec le temps, et qu'elles seraient donc plus marquées au moment du suivi (2004) que lors de l'étude initiale (1990). En effet, la progression des signes neurologiques a été rapportée lors d'études longitudinales portant sur des travailleurs gravement intoxiqués au Mn, ainsi que des travailleurs exposés à d'autres substances neurotoxiques, notamment les solvants organiques et le plomb. Globalement, les résultats indiquent que la performance pour plusieurs des tests que les travailleurs exposés avaient moins bien réussi en 1990 n'était pas différente entre les exposés et les référents en 2004. Néanmoins, les déficits de vitesse motrice (membres supérieurs) observés en 1990 étaient toujours présents en 2004. Par ailleurs, les travailleurs ayant été exposés au Mn et qui étaient âgés de plus de 45 ans lorsque l'exposition a cessée ont obtenu une performance significativement inférieure aux

référents d'âge similaire à trois tests de flexibilité cognitive. Ensuite, pour certains tests moteurs et cognitifs, la performance était significativement et inversement associée à l'exposition cumulée au Mn. En somme, les résultats révèlent deux trajectoires possibles : i) un maintien des déficits, ou ii) une récupération des fonctions, particulièrement pour les moins exposés. L'hypothèse d'aggravation des déficits n'a donc pas été retenue, mais un autre suivi de la cohorte à un âge plus avancé (ici, l'âge moyen était 57 ans au moment du suivi) serait souhaitable pour connaître l'évolution des fonctions neurocomportementales en fonction de l'exposition au cours du vieillissement.

Ensuite, un questionnaire portant sur les symptômes neuropsychiatriques a été ajouté à la batterie de tests administrée en 2004 afin d'examiner plus profondément ce domaine neurofonctionnel. En effet, certaines données dans la littérature indiquent que les questionnaires de symptômes neuropsychiatriques sont particulièrement sensibles aux effets du Mn sur le système nerveux. Le résultat des analyses a montré une relation significative entre l'exposition cumulée au Mn et les scores aux échelles Somatisation, Dépression, Anxiété et Hostilité. Les travailleurs dans les deux tertiles supérieurs d'exposition cumulée avaient un risque significatif d'avoir un score élevé aux échelles Hostilité, Dépression et Anxiété. Ces résultats suggèrent que l'exposition cumulée au Mn est associée à la gravité des symptômes neuropsychiatriques jusqu'à 14 ans après la fin de l'exposition.

Nos travaux ont mis en évidence la vulnérabilité des travailleurs plus âgés aux effets neurotoxiques du Mn. Le suivi de cette cohorte de travailleurs plusieurs années après la fin de l'exposition a montré un portrait complexe de changements des atteintes neurofonctionnelles, où certaines atteintes sont partiellement réversibles, particulièrement chez les moins exposés, alors que d'autres atteintes persistent, particulièrement chez les plus âgés. Nous estimons que la possibilité de récupération neurofonctionnelle après la fin de l'exposition devrait encourager les mesures de réduction des concentrations de Mn dans les milieux de travail concernés par ce risque. En même temps, la persistance de certaines atteintes associées à l'exposition au Mn, et cela jusqu'à 14 ans après la fin de l'exposition, montre l'importance de prévenir celles-ci. Il semble que l'exposition *cumulée* à ce métal soit déterminante dans l'apparition des signes de neurotoxicité. La concentration de Mn dans l'air de l'usine où étaient employés les travailleurs à l'étude ici respectait la norme d'exposition, norme toujours en vigueur aujourd'hui. L'exposition cumulée au Mn était en deçà de 100 $mg/m^3 \times$année et a néanmoins été associée à des effets néfastes durables sur certaines fonctions neurocomportementales. Or, un travailleur exposé à la valeur maximale légalement admise pendant 35 ans aurait un indice d'exposition cumulée encore plus élevé, soit de 175 $mg/m^3 \times$année, ce qui suggère que la norme actuelle d'exposition ne protège probablement pas la santé de tous les travailleurs.

Mots clé : manganèse, travailleurs, neurotoxique, neurocomportemental, exposition cumulée

CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Utilisations du manganèse et les populations à risque d'exposition

1.1.1 Milieu de travail

Le manganèse (Mn) est connu depuis la préhistoire. On en a retrouvé dans les pigments de peintures réalisées il y a plus de 17 000 ans. Les Égyptiens et les Romains utilisaient des composés de Mn dans la fabrication du verre pour colorer ou décolorer celui-ci. On a trouvé du Mn dans les minerais de fer utilisés par les Spartiates, ce qui pourrait expliquer la légendaire qualité de leurs armes, lesquelles étaient fabriquées avec un alliage « accidentel » fer-manganèse. Le premier témoignage écrit relatif aux propriétés toxiques du Mn remonte à 1837, dans un article scientifique écrit par un médecin anglais, James Couper, rapportant l'étrange syndrome d'intoxication dont souffraient des travailleurs préposés au broyage du minerai de Mn.

L'intérêt pour l'étude de la toxicité de cet élément a beaucoup augmenté au cours des dernières années en raison de son utilisation grandissante dans de nombreuses applications industrielles. Mondialement, le Mn est le quatrième métal le plus utilisé, après le fer, l'aluminium et le cuivre; plus de huit millions de tonnes sont extraites chaque année. Le Mn est rarement utilisé seul, mais constitue une matière première nécessaire à une multitude productions, en particulier sidérurgiques. Ainsi, la plus grande partie du Mn utilisé entre dans la production d'acier : 95% de la quantité totale est utilisée dans cette industrie (Institute for Environment and Health/Institute of Occupational Medicine, IEH/IOM, 2004). Le Mn confère de la dureté au métal; par exemple, il participe à hauteur de 12 % dans la composition des alliages nécessaires à la fabrication de coffres-forts, mais seulement à 1,2% pour les rails de chemin de fer. Les autres utilisations incluent la production de fongicides et de fertilisants, d'allumettes, de verre, de pigments, de batteries sèches et de composantes électroniques. Ainsi, les travailleurs des manufactures où l'on fabrique ces produits sont à risque d'être exposés au Mn, surtout via des particules aériennes.

Par ailleurs, des fumées de Mn peuvent être émises lors des procédés où des matériaux contenant du Mn sont chauffés, fondus ou brûlés, notamment dans le secteur des fonderies, lors de la préparation des alliages comme le ferro-Mn, les aciers et certains alliages d'aluminium, de cuivre ou de nickel. Des fumées de Mn seront également émises lors des opérations de soudage ou de coupage de matériaux contenant du Mn comme les alliages ferreux, ou certains alliages de cuivre, d'aluminium ou de nickel. Les soudeurs représentent un grand bassin de travailleurs à risque d'exposition au Mn, puisque les opérations de soudure font partie d'un grand nombre de procédés industriels et sont souvent nécessaires à la maintenance des équipements industriels (IEH/IOM, 2004).

Le secteur des mines de Mn et la transformation du minerai constituent évidemment des milieux de travail à risque élevé d'exposition; les conditions d'hygiène de travail peuvent varier grandement d'un pays à l'autre, ce qui se reflète dans les niveaux d'exposition. Les ressources minières de Mn sont de deux types : celles de haute qualité, soit celles qui ont une forte teneur en Mn (35-60%), sont concentrées essentiellement en Afrique du Sud, au Gabon, au Brésil et en Australie, et celles de basse qualité (35% de Mn), au Ghana, en Inde, au Mexique et en Chine (IEH/IOM, 2004).

1.2.2 Environnement général

Parmi les nombreux métaux qui forment la couche terrestre, le Mn est le 12^{e} plus abondant et se retrouve sous diverses formes minérales aux noms surprenants : rodochrosite, psilomélane, franklinite et pyrolusite. Il abonde particulièrement dans certaines régions, mais on le retrouve en plus ou moins grande concentration presque partout : sol, air, eau, nourriture. Il est si répandu qu'il semble être un constituant presque obligé de la matière vivante (Rodier, 1955). C'est d'ailleurs un élément essentiel pour le bon fonctionnement des organismes végétaux et animaux, y compris l'être humain.

Le Mn est avec le fer le contaminant le plus couramment retrouvé dans les eaux souterraines. Des concentrations extrêmement élevées peuvent même être retrouvées dans les nappes phréatiques de certaines régions, particulièrement là où l'eau reste en contact prolongé avec une roche-mère riche en Mn (U.S. EPA, 2004). Cette contamination naturelle se produit parfois dans des conditions de faible concentration en oxygène, ce qui permet la solubilisation du Mn et du fer dans l'eau (Santé Canada, 1996). Certaines études épidémiologiques suggèrent que les populations qui dépendent de ces sources aquifères peuvent subir des effets neurotoxiques suivant l'exposition au Mn (Kondakis *et al.*, 1989; Wasserman *et al.*, 2006).

Ensuite, certaines activités anthropiques entraînent la dispersion de particules de Mn dans l'environnement, et augmentent ainsi le niveau d'exposition des populations. Dans une région minière du Mexique, des concentrations élevées de Mn ont été mesurées dans le sang de personnes vivant à proximité d'une usine de raffinage du minerai brut émettant des poussières de Mn, et cette concentration était associée à un accroissement du risque de déficits cognitifs (Santos-Burgoa *et al.*, 2001). Une seconde étude a montré une association entre des déficits moteurs et l'exposition au Mn dans l'air (Rodriguez-Agudelo *et al.*, 2006).

La dernière estimation de l'apport relatif de différentes sources aux émissions totales de Mn dans l'air au Canada remonte à 1984. On a ainsi estimé que les émissions de Mn attribuables à des activités humaines totalisaient 1 225 tonnes; 79 % de ces émissions étaient d'origine industrielle et liées principalement à la fabrication d'alliages métalliques (Environment Canada, 1987). Les émissions dues aux véhicules à moteur à essence comptaient pour 17 % du total. En effet, le Mn a été ajouté à l'essence au Canada entre 1977 et 2003 sous forme d'un composé organique, le methylcyclopentadienyle Mn tricarbonyle (MMT), qui sert d'agent antidétonant (Davis *et al.*, 1999). On a d'ailleurs montré que la concentration de Mn dans l'air augmente avec la densité de la circulation (Thibault *et al.*, 2002). Finalement, les 4 % restant proviennent de la combustion de charbon pour la production d'électricité, de l'incinération des déchets solides et de l'application de pesticides.

Au Québec, on a montré que les personnes vivant à proximité d'une usine de production d'alliages de Mn avaient des niveaux plus élevés de Mn sanguin lorsque leur maison était située en aval des vents dominants (Baldwin *et al.*, 1999). De plus, une association significative entre le Mn sanguin et des désordres moteurs, cognitifs et psychologiques a été trouvée chez cette population (Bowler *et al.*, 1999; Mergler *et al.*, 1999). En 2005, la concentration de Mn dans le sol et dans l'air a été mesurée à différentes distances du site de l'usine, fermé depuis 1990 mais toujours pas décontaminé (Boudissa *et al.*, 2006). Ces travaux ont montré de très hautes concentrations de Mn à 10 mètres du site de l'usine (sol: 266 000 ± 45 000 ppm, air: 21,9 ± 13,7 μg/L), et celles-ci demeuraient beaucoup plus élevées que les niveaux mesurés en l'absence d'une source de contamination, même à une distance de 800 mètres (sol: 3 079 ± 843 ppm, air: 0,37 ± 0,39 μg/L).

1.3 Mécanismes d'absorption et de régulation du manganèse

Le Mn est un élément essentiel au bon fonctionnement de l'organisme; il joue un rôle de premier plan dans la constitution d'enzymes, en particulier la pyruvate carboxylase et la superoxide dismutase (Goldhaber, 2003). On a souligné son rôle dans le métabolisme des carbohydrates, des lipides et des stérols et dans la phosphorylation oxydative. Les besoins en Mn sont facilement comblés par l'alimentation : un seul cas de carence a été rapporté dans la littérature, et celle-ci avait été provoquée lors d'une étude clinique sur la vitamine K où le Mn avait été omis par erreur dans la diète d'un des participants (Keen, Zidenberg-Cherr et Lonnerdal, 1994).

Comme pour la plupart des éléments essentiels, la concentration du Mn dans l'organisme est contrôlée par des mécanismes d'homéostasie. Seule une petite fraction du Mn ingéré est absorbée par le corps, soit entre 3% et 5%, et la variation de ce taux permet de maintenir la quantité adéquate aux processus physiologiques (Davidsson *et al.*, 1989). Le taux d'absorption peut augmenter si les réserves en fer sont basses, ce qui explique que les personnes anémiques sont vulnérables à l'intoxication au Mn (Mena *et al.*, 1969). Le tractus gastro-intestinal et le foie agissent de concert pour contrôler l'absorption nette du Mn dans le corps en modifiant les caractéristiques de l'absorption et de l'excrétion selon l'apport alimentaire (Andersen, Gearhart et Clewell, 1999). L'homéostasie du Mn est maintenue principalement par des mécanismes hépatiques où l'excrétion joue un rôle primordial (Britton et Cotzias, 1966). En effet, le Mn entré dans la circulation sanguine est concentré dans le foie puis est excrété dans les fèces via la bile (Cotzias *et al.*, 1968).

Très peu de cas d'intoxication ont été rapportés suite à l'ingestion de Mn chez des personnes aux fonctions homéostatiques normales (Goldhaber, 2003), quoique deux études aient montré une association entre l'exposition à de l'eau à forte teneur en Mn et des altérations neurofonctionnelles (Kondakis *et al.*, 1989; Wasserman *et al.*, 2006). Dans la vaste majorité des cas où l'exposition au Mn a entraîné des effets neurotoxiques, c'est l'exposition à des particules aériennes de Mn qui était en cause. L'inhalation de particules de Mn résulte en une grande biodisponibilité du Mn dans l'organisme car le Mn évite alors les contrôles homéostatiques destinés à réguler les concentrations de Mn provenant de l'alimentation. En effet, l'absorption des particules aériennes de Mn peut se faire par trois voies : 1) absorption par les alvéoles et entrée dans la circulation sanguine; 2) absorption par les cellules de l'épithélium olfactif et entrée dans les capillaires sanguins de la muqueuse; et 3) absorption par les cellules de l'épithélium olfactif et translocation antérograde le long des axones olfactifs jusqu'au bulbe olfactif.

La taille des particules a une influence sur l'absorption pulmonaire du Mn. En effet, les petites particules, « respirables » (inférieures à 5 microns), peuvent entrer profondément dans les voies respiratoires et atteindre les bronchioles et les alvéoles. Le Mn sera alors absorbé par les poumons et entrera dans la circulation sanguine. Les particules plus grandes n'entreront pas aussi profondément dans les voies respiratoires et seront arrêtées par la barrière mucociliée, remontées hors des voies respiratoires, et finalement avalées dans le système digestif où elles subiront le même sort que le Mn alimentaire (Andersen, Gearhart et Clewell, 1999).

La voie d'absorption passant par le système olfactif a été découverte récemment et a seulement pu être démontrée chez le rat. Ces travaux ont une importance particulière car ils indiquent que l'absorption des particules aériennes de Mn dans le cerveau peut être beaucoup plus importante que ce qui était jusqu'alors soupçonné (Tjalve et Henriksson, 1999; Dorman *et al.*, 2004). En effet, l'étude par autoradiographie d'un isotope radioactif de Mn ($^{54}Mn^{2+}$) administré par instillation nasale à des rats a permis d'observer l'absorption du métal par la muqueuse olfactive et son transport le long des neurones olfactives jusqu'au bulbe olfactif où une grande concentration a été mesurée. Ensuite, on a observé que le Mn a diffusé dans d'autres aires cérébrales, évitant ainsi les contrôles homéostatiques (Tjalve *et al.*, 1996). Récemment, on a aussi démontré ce phénomène de translocation dans le système nerveux central chez des rats exposés à des particules ultrafines non solubles d'oxyde de Mn (Elder *et al.*, 2006).

Dans un autre ordre d'idées, certaines conditions cliniques peuvent prédisposer à l'intoxication au Mn. Ainsi, des niveaux élevés de Mn dans le sang ont été mesurés chez des patients souffrant de conditions cliniques caractérisées par la perte des fonctions hépatiques (cirrhose au stade final) (Krieger *et al.*, 1995; Hauser *et al.*, 1996). Ceci s'explique par l'incapacité du foie à remplir sa fonction d'excrétion du Mn provenant de l'alimentation. Des études d'imagerie par résonance magnétique sur des patients cirrhosés ont aussi permis d'observer l'accumulation du Mn dans le cerveau (Malecki *et al.*, 1999); Hauser et al. (1994) et Krieger et al. (1995) ont montré une corrélation entre la concentration de Mn sanguin et l'intensité du signal T1 indicatif de la présence du Mn dans certaines zones cérébrales. La perte des fonctions hépatiques est associée au développement d'un syndrome, l'encéphalopathie hépatique, dont certains des symptômes et des signes neurologiques sont similaires à ceux de l'intoxication au Mn, dont des changements dans la personnalité, le comportement et la cognition, de même que des dysfonctions du système moteur extrapyramidal (Hauser *et al.*, 1994; Krieger *et al.*, 1995).

Des cas d'intoxication au Mn ont également été observés chez des personnes nourries par voie parentérale (nutrition artificielle nécessaire aux personnes aux fonctions digestives compromises). On a observé des niveaux élevés de Mn dans le sang, une accumulation dans certaines zones cérébrales, ainsi que des symptômes d'atteinte des ganglions basaux chez certains de ces patients 36 jours après le début de la nutrition parentérale (Fitzgerald *et al.*, 1999).

1.4 Aspects neuropathologiques et neurobiochimiques

La cible toxique du Mn est le cerveau, et plus particulièrement le *globus pallidus*, une structure sous-corticale faisant partie d'un ensemble du nom de « noyaux gris centraux ». Ceci a pu être constaté suite à des études animales (rats, lapins, singes), des examens post-mortem menés sur les personnes ayant été exposées au Mn, et plus récemment, par des études d'imagerie par résonance magnétique (IRM) (Lucchini *et al.*, 2000). Chez l'humain, la persistance du Mn dans le cerveau serait d'environ six mois après la fin de l'exposition, durée après laquelle l'accumulation de Mn dans les noyaux gris centraux n'est plus détectable par IRM (Lucchini *et al.*, 2000).

Les noyaux gris centraux sont constitués par le noyau caudé, le putamen, le *globus pallidus*, le noyau sous-thalamique et la substance noire. Ils sont situés au cœur de la substance blanche cérébrale de chaque hémisphère, dans la partie ventrale du cortex cérébral (Napier *et al.,* 1991). Ces noyaux sont les centres majeurs du système moteur extrapyramidal, agissant dans la régulation et le contrôle de la motricité, ce qui expliquerait les symptômes de dysfonctions motrices manifestés suite à l'intoxication (Barbeau *et al.*, 1976). Le système moteur extrapyramidal est formé de boucles rétroactives dont la principale, la boucle dopaminergique nigrostriée, relie la substance noire et le striatum. Le fonctionnement des noyaux gris centraux est fort complexe et encore mal compris, mais on sait néanmoins qu'ils sont impliqués dans nombre de fonctions cognitives, dont la motivation, les émotions, l'apprentissage et la mémoire (Zaborsky *et al.*, 1991).

L'accumulation du Mn chez les organismes intoxiqués s'accompagne d'une détérioration neuronale touchant surtout le *globus pallidus*, et, dans une moindre mesure, le striatum et la substance noire (Bernheimer *et al.*, 1973; Barbeau, 1984; Yamada *et al.*, 1986; Eriksson *et al.*, 1987; Eriksson *et al.*, 1992; Shinotoh *et al.*, 1995). Les fibres dopaminergiques de la voie nigrostriée sont préservées lors de l'intoxication au Mn (Wolters *et al.,* 1989, Shinotoh *et al.*, 1997). Toutefois, les changements anatomiques n'ont pas été observés dans toutes les études; les effets toxiques du Mn entraîneraient des perturbations biochimiques avant l'apparition de changements pathologiques (Neff, Barrett et Costa, 1969; Pal, Samii et Calne, 1999).

L'altération neurobiochimique la plus souvent observée dans les modèles animaux d'exposition au Mn et dans les cas d'humains intoxiqués est la diminution des catécholamines, plus particulièrement de la dopamine, dans les ganglions basaux (Bernheimer *et al.*, 1973; Bonilla et Diez-Ewald, 1974; Barbeau, Inoue et Cloutier, 1976; Bird, Anton et Bullock, 1984; Yamada *et al.*, 1986; pour une revue, voir Dobson, Erikson et Aschner, 2004). De plus, des études sur des rats ont montré que le fonctionnement de la GABA (acide γ-aminobutyrique) serait aussi altéré suivant l'exposition au Mn (Erikson *et al.*, 2002; Gwiazda *et al.*, 2002). En effet, la structure où s'accumule le plus le Mn, le *globus pallidus*, est particulièrement riche en projections GABAergiques. Étant donné les relations étroites entretenues entre les différents ganglions basaux, il est probable que leur fonctionnement résulte des interactions entre les différents systèmes de neurotransmetteurs (Fitsanakis *et al.*, 2006).

Des changements dans la concentration sérique de prolactine ont été rapportés chez des travailleurs exposés au Mn (Alessio *et al.*, 1989; Mutti *et al.*, 1996; Ellingsen *et al.*, 2003), quoique d'autres n'aient pas fait cette observation (Roels *et al.*, 1992; Myers *et al.*, 2003b). Cette hormone a été proposée comme bioindicateur d'effet dans l'étude de la toxicité du Mn (Smargiassi et Mutti, 1999). En effet, le Mn affecte la voie dopaminergique du système dopaminergique tubéroinfundibulaire, lequel influence l'axe hypothalamo-hypophysaire en inhibant la sécrétion de prolactine. En diminuant l'activité dopaminergique, l'exposition au Mn aurait comme conséquence l'augmentation de la sécrétion de prolactine par la levée de l'inhibition tonique de cette sécrétion. Dans un autre contexte, une relation positive a été trouvée entre la concentration de Mn dans le sang et la concentration plasmatique de prolactine dans le cordon ombilical (Takser *et al.*, 2004).

1.5 Signes et symptômes de l'intoxication au Mn

1.5.1 Effets cliniques : Manganisme

En 1837, Couper fut le premier à publier dans une revue scientifique la description des désordres neurologiques affectant des mineurs préposés au broyage du minerai de Mn. Par la suite, des centaines d'autres cas d'intoxication au Mn (*manganisme*) ont été rapportés, toujours chez des travailleurs intoxiqués après avoir été exposés chroniquement à des poussières de Mn (Penalver, 1955). Différents milieux industriels ont été associés au développement du manganisme chez certains travailleurs : mines et traitement du minerai de Mn, fabrication d'alliages métalliques, manufactures de batteries sèches et ateliers de soudure. Des cas ont aussi été rapportés chez des travailleurs agricoles utilisant des fongicides contenant du Mn (Ferraz *et al.*, 1988; Meco *et al.*, 1994). Outre l'exposition professionnelle, l'ingestion de permanganate de Mn (Holzgraefe *et al.*, 1986) et celle de comprimés d'herbes traditionnelles chinoises (Kao, Chen et Liu, 1999) ont chacune causé un cas de manganisme.

Le manganisme est un syndrome parkinsonien caractérisé par une atteinte des noyaux gris centraux de la voie motrice extrapyramidale. La maladie de Parkinson est la cause la plus répandue d'atteinte des noyaux gris centraux, d'où le nom de syndrome parkinsonien qui sert à regrouper les autres syndromes similaires. Différentes causes peuvent être à l'origine des syndromes parkinsoniens, dont l'exposition au monoxyde de carbone et l'auto-administration d'un analogue de synthèse d'héroïne (le 1-methyl-4-phenyl-4-propionoxypiperidine ou MPPP) contaminé au 1-methyl-4-phenyl-1,3,6-tetrahydropyridine (MPTP). En 1982, un groupe de jeunes drogués californiens utilisant cette drogue fabriquée de façon artisanale dans des laboratoires clandestins développèrent un syndrome parkinsonien progressant de façon rapide (Langston *et al.*, 1983). Différents modèles animaux du parkinsonisme ont ensuite été développés, notamment chez les rongeurs et primates non humains, en administrant du MPTP (Przedborski *et al.*, 2001).

Les signes et les symptômesdu manganisme ont été décrits maintes fois dans la littérature et peuvent survenir après seulement quelques mois ou encore après plusieurs dizaines d'années d'exposition (Penalver, 1955; Rodier, 1955; Schuler *et al.*, 1957; Abdel-Naby et Hassanein, 1965; Chandra, Seth et Mankeshwar, 1974; Huang *et al.*, 1989). Les cas de manganisme seraient toujours constatés chez des travailleurs exposés à une concentration de plus de 5 mg/m^3 de Mn dans l'air, et de rares cas suivant une exposition entre 1 et 5 mg/m^3 de Mn (Levy et Nassetta, 2003). Certains facteurs conféreraient une vulnérabilité à développer le manganisme, dont l'anémie, l'alcoolisme et les maladies respiratoires (Hudnell, 1999).

La description clinique de Rodier (1955), sans aucun doute la plus reprise dans la littérature scientifique, établit la progression du manganisme en trois phases :

1) Les premières manifestations du manganisme sont aspécifiques : fatigue, asthénie, apathie, léthargie, faiblesse des membres inférieurs, changements de l'humeur (irritabilité, nervosité, agressivité), anorexie, insomnie ou somnolence, pertes de mémoire, troubles de concentration, douleurs musculaires, arthralgie, céphalées, troubles de la sexualité (libido).

2) La seconde phase représente le début de la maladie. On note alors paresthésie, trouble de la parole (ton monocorde, bégaiement) et de la démarche (perte d'équilibre, difficulté à marcher à reculons), tremblements légers, difficulté à écrire, diminution de la dextérité manuelle, faciès figé, instabilité émotionnelle, troubles de la mémoire et du jugement, mouvements lents et maladroits, rires et pleurs incontrôlés. On peut également observer, à ce stade ou au suivant, une psychose maniaco-dépressive.

3) Dans la troisième phase, lorsque la maladie est bien établie, on note une dystonie sévère du tronc et des extrémités; les tremblements lors des mouvements intentionnels sont plus fréquents et sont associés à des mouvements ralentis et plus raides. On peut également observer une démarche hésitante et caractéristique (démarche du coq), une dysphagie, une incoordination motrice, des mouvements musculaires spasmodiques de la musculature crânienne qui peuvent donner des tics, des grimaces ou le torticolis.

Chez certains groupes intoxiqués, particulièrement dans les villages miniers des Andes, une phase psychiatrique, appelée *locura manganica* (folie du manganèse), précède l'apparition de signes neurologiques. Mena (1967) s'est attardé à décrire le comportement caractéristique de cette phase de perturbations psychologiques en se basant sur ses observations de 14 mineurs chiliens hospitalisés pour cause d'intoxication au Mn. Chez ces patients, la nature et l'intensité des manifestations comportementales de l'intoxication étaient variables, mais tous rapportaient de la nervosité et de l'irritabilité, provoquant des frictions et des disputes, et menant parfois même à des comportements violents. La plupart des mineurs intoxiqués manifestaient des comportements compulsifs et aberrants, des rires ou des pleurs incontrôlés, et certains ont rapporté des hallucinations. Tous les mineurs étaient conscients de la nature anormale de leurs agissements sans toutefois pouvoir les contrôler. Cette phase dure environ un mois, que le malade soit retiré ou non de l'exposition, puis les signes neurologiques prennent le pas sur les perturbations mentales.

Le manganisme se distingue de la maladie de Parkinson par certains aspects cliniques et neuropathologiques (Olanow, 2004). Les tremblements du manganisme sont présents surtout lors des mouvements volontaires (tremblement d'action), alors que ceux du Parkinson sont présents au repos. Dans le manganisme, on observe des difficultés de stabilité posturale dès le début de la maladie, alors que ceci apparaît seulement à un stade avancé de la maladie de Parkinson. Le manganisme est

CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Utilisations du manganèse et les populations à risque d'exposition

1.1.1 Milieu de travail

Le manganèse (Mn) est connu depuis la préhistoire. On en a retrouvé dans les pigments de peintures réalisées il y a plus de 17 000 ans. Les Égyptiens et les Romains utilisaient des composés de Mn dans la fabrication du verre pour colorer ou décolorer celui-ci. On a trouvé du Mn dans les minerais de fer utilisés par les Spartiates, ce qui pourrait expliquer la légendaire qualité de leurs armes, lesquelles étaient fabriquées avec un alliage « accidentel » fer-manganèse. Le premier témoignage écrit relatif aux propriétés toxiques du Mn remonte à 1837, dans un article scientifique écrit par un médecin anglais, James Couper, rapportant l'étrange syndrome d'intoxication dont souffraient des travailleurs préposés au broyage du minerai de Mn.

L'intérêt pour l'étude de la toxicité de cet élément a beaucoup augmenté au cours des dernières années en raison de son utilisation grandissante dans de nombreuses applications industrielles. Mondialement, le Mn est le quatrième métal le plus utilisé, après le fer, l'aluminium et le cuivre; plus de huit millions de tonnes sont extraites chaque année. Le Mn est rarement utilisé seul, mais constitue une matière première nécessaire à une multitude productions, en particulier sidérurgiques. Ainsi, la plus grande partie du Mn utilisé entre dans la production d'acier : 95% de la quantité totale est utilisée dans cette industrie (Institute for Environment and Health/Institute of Occupational Medicine, IEH/IOM, 2004). Le Mn confère de la dureté au métal; par exemple, il participe à hauteur de 12 % dans la composition des alliages nécessaires à la fabrication de coffres-forts, mais seulement à 1,2% pour les rails de chemin de fer. Les autres utilisations incluent la production de fongicides et habituellement utilisées pour mettre en évidences ces effets chez des cohortes de travailleurs (Levy et Nassetta, 2003). Les altérations neurofonctionnelles associées à l'exposition au Mn peuvent être détectées en comparant les performances d'un groupe de travailleurs exposés au Mn avec celles de référents non exposés (Mergler et Baldwin, 1997). Ceci diffère des effets cliniques qui sont détectables à l'examen individuel.

Siegl et Bergert (1982) furent les premiers à rapporter des effets sous-cliniques de l'exposition au Mn chez des soudeurs d'Allemagne de l'Est, lesquels avaient un temps de réaction à un stimuli plus long que le groupe référent. Ensuite, Roels *et al.* (1985) menèrent la première étude épidémiologique chez des travailleurs asymptomatiques exposés au Mn en utilisant une batterie de tests comportementaux administrés de façon systématique à tous les travailleurs d'une usine. Plus tard, environ une dizaine d'études sur différents groupes de travailleurs actifs documentèrent les effets neurotoxiques de l'exposition au Mn au moyen de tests neurocomportementaux (Roels *et al.*, 1987; Ferraz *et al.*, 1988; Iregren, 1990; Wennberg *et al.*, 1991; Roels *et al.*, 1992; Chia *et al.*, 1993a; Chia *et al.*, 1993b; Mergler *et al.*, 1994; Lucchini *et al.*, 1995; Sjögren *et al.*, 1996; Lucchini *et al.*, 1997; Sinczuk-Walczak, Jakubowski et Matczak, 2001; Bast-Pettersen *et al.*, 2004; Park *et al.*, 2005). Une variété d'instruments ont été utilisés pour mesurer les fonctions comportementales, et bien que les résultats diffèrent légèrement entre les études, globalement, les effets touchent les fonctions suivantes :

- Fonctions neuromotrices : stabilité des mains, coordination motrice (particulièrement pour les mouvements rapides alternés), rigidité musculaire, habilités grapho-motrices, stabilité posturale.

- Fonctions cognitives : mémoire, temps de réaction, flexibilité cognitive.

- Équilibre psychologique : sentiments dépressifs, irritabilité, anxiété, agressivité, confusion et désordres affectifs.

Une autre façon d'obtenir une indication de l'état du système nerveux est d'enquêter sur les symptômes ressentis, lesquels sont des indicateurs des dommages organiques qui peuvent avoir été causés par l'exposition au Mn. En effet, certains chercheurs estiment que les symptômes constituent les premières réponses de l'organisme face aux effets toxiques (Hanninen *et al.*, 1979; Hawkins, 1990; Iregren et Gamberale, 1990). Plusieurs des études épidémiologiques citées plus haut incluaient des questions sur les symptômes ressentis, ceux qui étaient les plus souvent rapportés étant la somnolence, une perturbation de la libido, une fatigue générale, des crampes, une apathie, de l'insomnie, une perte d'appétit, des pertes d'équilibre et des maux de tête (Ferraz *et al.*, 1988; Mergler *et al.*, 1994; Deschamps, Guillaumot et Raux, 2001).

La diversité des fonctions touchées et des symptômes ressentis s'expliquerait par le fait que la cible toxique privilégiée du Mn serait les ganglions basaux, lesquels sont impliqués dans un grand nombre de processus cérébraux (Dubois, Funkiewiez et Pillon, 2005; Saint-Cyr, 2005).

Bien que les résultats des études sur les effets du Mn soient généralement similaires, Myers *et al.* ont publié deux études sur les fonctions neurocomportementales et les symptômes chez des cohortes de travailleurs sud-africains qui ne s'accordent pas avec la littérature. Une des études portait sur 489 employés de mines (Myers *et al.*, 2003a) dont l'exposition moyenne était de 0,21 mg/m^3 (± 0,14) et variait entre 0 et 0,99 mg/m^3; aucune relation significative entre l'exposition au Mn, y compris la concentration de Mn sanguin et l'exposition cumulée, et les performances aux tests neurocomportementaux ou les symptômes n'a été trouvée. L'autre étude portait sur 509 employés d'usines de production d'alliages ferro-Mn exposés à une moyenne de 0,82 (± 1,04); l'étendue des valeurs n'est pas présentée (Myers *et al.*, 2003c). Dans les deux cas, l'évaluation de l'exposition et des fonctions neurocomportementales ainsi que le contrôle des covariables d'importance semblaient adéquats. Plusieurs facteurs pourraient contribuer à expliquer ces résultats divergents d'avec la littérature sur le Mn : taille et forme chimique des particules (spéciation, solubilité) ou caractéristiques génétiques des groupes à l'étude.

1.5.3 Effets autres que sur le système nerveux central

Des changements dans les paramètres cardiaques, soit une diminution de la variabilité temporelle des battements cardiaques, ont été rapportés chez des hommes travaillant à la réparation de rails de chemin de fer en acier à forte teneur en Mn (Barrington *et al.*, 1998). Le système respiratoire peut aussi être affecté par l'exposition à des particules inhalables de Mn (Halatek *et al.*, 2005). En effet, le Mn serait un irritant respiratoire et a été associé à l'augmentation de la fréquence d'infections (Roels *et al.*, 1987; Saric, 1992; Boojar et Goodarzi, 2002). Le Mn pourrait aussi avoir des effets sur le système reproducteur; on a notamment rapporté un nombre moindre d'enfants chez des travailleurs pendant la période d'exposition au Mn, en comparaison avec un groupe référent (Lauwerys *et al.*, 1985).

1.5.4 Les études de suivi après la fin de l'exposition

Plusieurs cliniciens ont observé que les patients souffrant de manganisme peuvent voir leur condition continuer de se détériorer même après l'arrêt de l'exposition. L'évolution de la condition de six travailleurs taïwanais intoxiqués au cours des dix années après le développement de leur maladie a été décrite par Huang et al. (Huang *et al.*, 1993). Des signes neurologiques peuvent même se développer après la fin de l'exposition chez certains travailleurs qui étaient asymptomatiques lorsqu'ils étaient encore exposés (Penalver, 1955). Par ailleurs, la disparition partielle ou complète des signes neurologiques après l'administration d'agents chélateurs a été rapportée (Smyth *et al.*, 1973; Herrero Hernandez *et al.*, 2005).

Il est courant, dans l'étude des cas cliniques de manganisme, d'effectuer un suivi de l'évolution de la condition des malades, puisque ceux-ci font l'objet d'une attention médicale. Par contre, un tel suivi est beaucoup moins courant dans le cas d'altérations neurofonctionnelles subcliniques. Deux études seulement ont porté sur ce sujet, celles de Roels et collègues (1999) et de Lucchini et collègues (1999). Roels et collègues (1999) ont suivi des travailleurs d'une usine de production de batteries sèches exposés au dioxyde de Mn. Une première évaluation avait été menée en 1987, portant sur trois fonctions : coordination visuo-motrice, stabilité des mains et temps de réaction (Roels *et al.*, 1992). Après l'évaluation initiale, des mesures de contrôle hygiénique ont été mises en place et l'exposition au Mn a diminué progressivement. Les trois fonctions nommées plus hauts ont été réévaluées annuellement chez les travailleurs pendant huit ans; l'effectif a diminué de 92 travailleurs en 1987 (âge moyen : 31 ans) à 34 à la fin de la période de suivi en 1995 (âge moyen : 39 ans). Chez ces travailleurs, la diminution de l'exposition était significativement associée à une amélioration de la coordination visuo-motrice. À la fin de la période de suivi, la performance du groupe le moins exposé s'était normalisée (performance égale aux référents). Pour les travailleurs plus fortement exposés, une amélioration des performances de coordination visuo-motrice a été constatée en relation avec la diminution de l'exposition, mais un déficit partiel subsistait par rapport aux référents. Cependant, la diminution de l'exposition n'a pas eu d'effet bénéfique sur la stabilité des mains ni sur le temps de réaction, pour lesquels les déficits ont été conservés.

Une partie de la cohorte de Roels (1999) a cessé d'être exposée au Mn au cours de la période de suivi, et les performances de ces 24 travailleurs ont été réévaluées en 1996 (durée moyenne depuis cessation de l'exposition : 5 ans; étendue :

3 - 7 ans). Le portrait de l'évolution des performances neurofonctionnelles chez ces travailleurs était semblable à celui de la cohorte toujours exposée au Mn, soit une amélioration de la coordination visuo-motrice et un maintien des déficits de stabilité des mains et du temps de réaction. Cependant, il y avait trop peu de travailleurs dans chaque sous-groupe d'exposition pour effectuer des analyses plus détaillées.

Dans leur étude, Lucchini *et al.* (1999) rapportent les changements des fonctions neurocomportementales chez des travailleurs d'une usine de production d'alliages de ferro- et de silico-Mn dont l'exposition au Mn diminuait progressivement. Une évaluation initiale avait montré des relations significatives entre le taux de Mn sanguin et les scores à plusieurs tests neuropsychologiques (Lucchini *et al.*, 1995). L'évaluation de suivi menée six ans plus tard portait sur 30 travailleurs toujours employés dans la même usine et a permis de constater que les performances des tests moteurs et cognitifs étaient inchangées. De plus, les performances étaient associées au niveau d'exposition cumulé (Lucchini *et al.*, 1999).

Finalement, Hochberg (1996) a rapporté des déficits de coordination motrice et de stabilité des mains chez 59 mineurs chiliens âgés de plus de 50 ans ayant été exposés au Mn cinq ans auparavant. Comme ces travailleurs n'avaient pas été évalués précédemment, on ne peut conclure sur le type de changement dans le temps de ces habilités (amélioration, persistance ou dégradation).

1.6 Problématique

L'exposition au Mn peut causer des effets neurotoxiques qui se manifestent par des altérations neurofonctionnelles et dont la gravité varie en fonction de la dose d'exposition et de facteurs individuels. Il existe actuellement une controverse quant au niveau d'exposition sécuritaire pour la santé de tous les travailleurs. Plusieurs organismes réglementaires travaillent actuellement à l'établissement de nouvelles normes d'exposition tant aux États-Unis et en Europe qu'au Québec (IEH/IOM, 2004). Au Québec, la norme maximale d'exposition (valeur d'exposition moyenne pondérée sur une période de 8 heures-VEMP) est de 5 mg/m^3 pour les poussières et 1 mg/m^3 pour les fumée de Mn (RSST, 2003). La dernière recommandation de l'*American Conference of Governmental Industrial Hygienists* sensiblement plus basse, à 0,2 mg/m^3 pour les poussière ou les fumées de Mn (ACGIH, 2004).

Certains facteurs peuvent modifier la relation entre l'exposition au Mn et les effets neurotoxiques, dont la consommation excessive d'alcool, les déficiences hépatiques et un statut du fer bas (Hudnell, 1999). Une meilleure compréhension de ces facteurs est nécessaire afin de comprendre les risques posés par l'exposition au Mn et ainsi identifier les mesures adéquates pour limiter ces risques.

Certaines données indiquent que l'âge pourrait être un autre facteur de vulnérabilité aux effets neurotoxiques du Mn. On sait que la neurotoxicité du Mn serait liée à des changements de l'activité des systèmes dopaminergiques (Dobson, Erikson et Aschner, 2004). Des études expérimentales ont montré que les effets de l'exposition au Mn sur l'activité dopaminergique dépendent de l'âge des animaux (Erikson *et al.*, 2004); ils sont peu prononcés à un jeune âge et beaucoup plus marqués à un âge plus avancé. Une diminution de l'activité dopaminergique, plus particulièrement dans les ganglions basaux, est également observée au cours du vieillissement et celle-ci expliquerait le déclin de certaines fonctions neurocomportementales (pour une revue, voir Kaasinen et Rinne, 2002). Ainsi, l'effet combiné de ces deux éléments, vieillissement et exposition au Mn, pourrait entraîner une diminution accrue de l'activité dopaminergique et des performances neurofonctionnelles qui lui sont associées.

Par ailleurs, lors d'une étude menée auprès d'une population du sud-ouest du Québec exposée au Mn provenant de diverses sources environnementales (dont l'usine où étaient employés les travailleurs ayant participé à la présente étude), on a observé que les personnes âgées de plus de 50 ans étaient davantage affectées par un taux élevé de Mn sanguin (Mergler *et al.*, 1999). Les résultats d'une batterie de tests neurocomportementaux ont montré une interaction entre l'âge et le Mn sur les plans de l'apprentissage verbal, des performances motrices (coordination main-yeux et dextérité manuelle fine), du tremblement et des symptômes neuropsychiatriques, dont l'anxiété, l'irritabilité, les désordres affectifs, l'agressivité et la confusion (Beuter *et al.*, 1999; Bowler *et al.*, 1999; Mergler *et al.*, 1999).

Ces données recueillies auprès de populations exposées au Mn dans l'environnement suggèrent que les personnes plus âgées sont vulnérables aux effets neurotoxiques du Mn. Ces résultats devraient être reproduits dans une autre population afin d'en confirmer la portée. En particulier, on ignore si cela est applicable également aux personnes exposées au Mn en milieu de travail, qui sont pourtant les plus susceptibles d'être exposées à des niveaux élevés de Mn. Ainsi, le premier problème sur lequel la présente thèse se penchera est l'effet modificateur de l'âge sur les altérations neurofonctionnelles chez des travailleurs exposés à des particules de Mn.

Le profil d'atteinte des fonctions neurocomportementales associé à l'exposition au Mn a été amplement décrit (Levy et Nassetta, 2003), mais très peu d'informations sont disponibles sur la santé des travailleurs après la fin de l'exposition. Plusieurs cliniciens ont rapporté que la condition des patients atteints de manganisme peut continuer de s'aggraver même après la fin de l'exposition (Huang *et al.*, 1998), mais l'évolution des atteintes neurofonctionnelles moins prononcées que celles observées dans les cas de manganisme est beaucoup moins bien connue. Certaines données suggèrent que certains déficits neurocomportementaux pourraient s'atténuer alors que d'autres persisteraient après la réduction ou la fin de l'exposition (Roels *et al.*, 1999). Bien que l'étude de Roels *et al.* ait apporté des informations importantes sur la réversibilité des effets du Mn, certains éléments limitent la portée de l'étude pour comprendre les effets à long terme de l'exposition passée au Mn sur l'état des fonctions neurocomportementales : 1) leur effectif était réduit à la fin de la période de suivi, soit 34 travailleurs encore exposés et 24 travailleurs dont l'exposition avait complètement cessé (temps moyen depuis cessation : 5 ans; étendue : 3 – 7 ans); 3) l'âge moyen à la fin de la période de suivi était relativement bas (moyenne : 39 ans; étendue : 31 – 52 ans); 4) seulement trois tests de fonctions neurocomportementales ont été utilisés. Le second problème abordé dans la présente thèse est le degré de réversibilité des fonctions neurocomportementales chez des travailleurs ayant été exposés au Mn par le passé.

Finalement, des troubles de l'humeur sont souvent rapportés par les travailleurs exposés au Mn. Des symptômes psychiatriques s'apparentant à la psychose peuvent même survenir lors de la phase aiguë du manganisme. Comme cela a été dit plus haut, peu d'études ont été menées sur la santé de travailleurs après la fin de l'exposition au Mn, et celles qui ont porté sur ce sujet n'ont pas évalué les symptômes psychologiques; ceci constituera donc le troisième problème abordé dans la présente thèse.

Le vieillissement de la population, y compris de la population active, renforce l'intérêt de mieux connaître les risques neurotoxiques spécifiques aux travailleurs de différents groupes d'âge. Ce vieillissement résulte de l'accroissement de l'espérance de vie conjugué à la baisse de la natalité, de sorte que la proportion des personnes âgées de plus de 65 ans augmente sans cesse. D'ici l'an 2031, cette proportion sera de 20% (Statistique Canada, 2006). Pour l'individu comme pour la société, l'une des principales préoccupations associées à la vieillesse est la dégradation éventuelle de la santé, surtout si elle entraîne une plus grande dépendance personnelle. Bien que la santé se détériore avec l'âge, cette détérioration ne survient que progressivement chez la plupart des personnes âgées, et celles-ci apprennent à s'adapter. Avec l'augmentation des dépenses en soins de santé, il importe de maintenir l'autonomie de notre population vieillissante. La prévention des altérations neurofonctionnelles associées à l'exposition à des substances neurotoxiques au cours de la vie professionnelle peut contribuer à maintenir une population vieillissante en santé.

1.7 Hypothèses de travail

La revue de la littérature scientifique effectuée nous a permis d'élaborer trois hypothèses de travail :

- Pour une exposition similaire, les déficits sont plus grands chez les travailleurs plus âgés.
- Les performances neurocomportementales après l'arrêt de l'exposition au Mn sont associées au niveau d'exposition cumulé au Mn.
- Les altérations neurofonctionnelles observées alors que les travailleurs étaient exposés peuvent s'aggraver après la fin de l'exposition chez les travailleurs ayant été les plus exposés.

1.8 Objectifs

Afin de vérifier ces hypothèses, nous avons posé trois objectifs principaux correspondants :

- Examiner le rôle de l'âge dans la vulnérabilité aux effets de l'exposition au Mn sur les fonctions neurocomportementales chez des hommes exposés dans une usine de production d'alliages métalliques.
- Estimer la relation entre le niveau cumulé d'exposition passée au Mn et les performances neurocomportementales et les symptômes 14 ans après la fin de l'exposition.

- Évaluer le degré de réversibilité, ou d'aggravation, des altérations neurofonctionnelles 14 ans après la fin de l'exposition.

CHAPITRE II

MÉTHODOLOGIE

2.1 Contexte de la recherche et description générale des études

En 1990, une étude a été menée sur l'exposition au Mn et les fonctions nerveuses chez des travailleurs d'une usine de production d'alliage de ferro-Mn et de silico-Mn. Ce projet de recherche avait été entrepris par Donna Mergler, suite à la demande du Comité conjoint de santé au travail de l'usine regroupant des représentants du syndicat et de la direction. Les résultats de cette étude ont été publiés dans *Environmental Research* en 1994. Les travailleurs exposés au Mn avaient une performance inférieure dans le cas de plusieurs fonctions neurocomportementales : flexibilité cognitive, mémoire, stabilité des mains, mouvements simples et alternés des mains. De plus, ils rapportaient davantage de troubles de l'humeur ainsi que différents symptômes (Mergler *et al.*, 1994). Quelques mois après la fin de l'étude, l'usine a fermé ses portes suite à une décision de la maison-mère de la compagnie; tous les employés ont été mis à pied. Soulignons que la fermeture de l'usine n'avait aucun lien avec les résultats de l'étude de Mergler et collègues.

L'étude de Mergler *et al.* (1994) a eu un grand impact sur l'avancement des connaissances sur les effets du Mn, notamment à cause de la variété des tests utilisés pour évaluer le fonctionnement du système nerveux et du grand nombre de travailleurs ayant pris part à l'étude. Dans le cadre de cette étude, l'exposition aux particules aériennes de Mn dans l'usine avait fait l'objet d'une évaluation par une hygiéniste industrielle (Mary Baldwin), par un échantillonnage à la fois personnel et par aires de travail (Appendice A). Ces travaux ont montré que la quantité totale de Mn dans la poussière était de 0,225 mg/m^3 (moyenne géométrique), alors que la fraction respirable des poussières contenait 0,035 mg/m^3 (moyenne géométrique). Notons que ces valeurs sont en deçà de la norme d'exposition en vigueur en 1990 et aujourd'hui encore, soit 5 mg/m^3. Un index cumulatif d'exposition a été calculé à partir des histoires de travail et des estimations d'exposition présente et passée (Baldwin *et al.*, 1993).

L'étude 1 (Chapitre III) a été entreprise afin vérifier si l'âge jouait un rôle dans les déficits neurocomportementaux chez les travailleurs de l'usine présentés plus haut. Pour ce faire, nous avons utilisé la banque de données constituée en 1990 et avons conduit des analyses statistiques supplémentaires. Suite à l'obtention d'une subvention des Instituts de recherche en santé du Canada (IRSC), nous avons effectué le suivi de cette cohorte de travailleurs et de leurs référents à l'été 2004. Ceci constitue les études 2 (Chapitre IV) et 3 (Chapitre V). L'étude 2 présente les résultats des tests neurocomportementaux qui ont été utilisés lors de l'étude initiale (1990) et de l'étude de suivi (2004). L'étude 3 présente les résultats portant sur les symptômes neuropsychiatriques, lesquels ont été évalués seulement en 2004, et leur relation avec la dose d'exposition cumulée. Les méthodes relatives à ces études sont résumées dans les chapitres IV et V; toutefois, nous présenterons ici des détails méthodologiques complémentaires.

2.2 Méthodologie générale

2.2.1 Étude 1 : Réanalyse des données de l'étude initiale de 1990

Cette étude est basée sur des données de l'étude de Mergler *et al.* (1994); aussi, les détails méthodologiques étant disponibles dans la publication originale, ils ne seront pas présentés ici. Néanmoins, nous présentons les informations essentielles à la compréhension du travail effectué.

Le devis de recherche est transversal; un groupe de travailleurs exposés au Mn et un groupe d'hommes référents sont à l'étude. Les travailleurs exposés au Mn étaient employés dans une usine de production d'alliages de ferro-Mn et de silico-Mn située à Beauharnois, au sud-ouest de Montréal. Cent quinze travailleurs exposés au

Mn ont participé à l'étude, soit 95% des travailleurs de l'usine. Un groupe de 145 travailleurs référents, non exposés, ont été recrutés dans des entreprises de la même région du sud-ouest du Québec afin de servir de groupe de comparaison. Les référents ont été choisis parmi les travailleurs actifs de cette même région géographique; ils n'étaient pas exposés à des substances neurotoxiques. Les analyses statistiques ont été réalisées sur les données appariées : chaque travailleur est apparié à un référent et la différence de score pour chaque test neurocomportemental est calculée. Les critères d'appariement étaient les suivants : âge (± 3 ans), éducation (± 2 ans), tabagisme et nombre d'enfants. Les analyses statistiques effectuées étaient des régressions entre les différences de scores (indiquant l'effet du Mn) et l'âge des paires. De plus, nous avons vérifié l'influence de l'indice d'exposition cumulée sur l'effet du Mn.

2.2.2 Études 2 et 3 : Suivi de la cohorte en 2004

Le suivi de la cohorte de travailleurs exposés au Mn et des référents a résulté en la rédaction de deux études originales. Comme ceci constitue des études originales, nous présentons ici les détails de la réalisation des études.

2.2.2.1 Design de recherche et recrutement

L'usine dans laquelle les travailleurs exposés au Mn ont été recrutés a cessé ses opérations depuis un peu plus de 14 ans, de sorte que la première étape consistait à en retracer le plus grand nombre possible. L'un des facteurs les plus importants pouvant affecter la validité des résultats d'une étude de cohorte est le taux d'attrition de la cohorte. Ainsi, nous avons déployé les efforts nécessaires afin de recruter le plus grand nombre possible de participants à l'étude, à la fois dans le groupe exposé et le groupe référent. L'adresse de tous les participants à l'étude initiale a été vérifiée dans l'annuaire téléphonique sur le web. Nous avons envoyé une lettre à ceux dont l'adresse n'avait pas changé depuis l'étude initiale. Nous avons tenté de retrouver ceux dont l'adresse n'était plus valide, et nous avons téléphoné afin de confirmer l'identité de la personne retrouvée avant de lui faire parvenir la lettre de recrutement. Finalement, nous avons fait faire des recherches à l'état civil afin de savoir si les personnes qui n'ont pas pu être retrouvées étaient décédées. La figure 2.1 présente les résultats du recrutement à l'étude de suivi. Le taux de participation a été de 78% dans le groupe exposé et de 67% chez les référents (les personnes non retrouvées ne sont pas comprises dans le dénominateur). Les participants ont reçu un dédommagement monétaire de 75$.

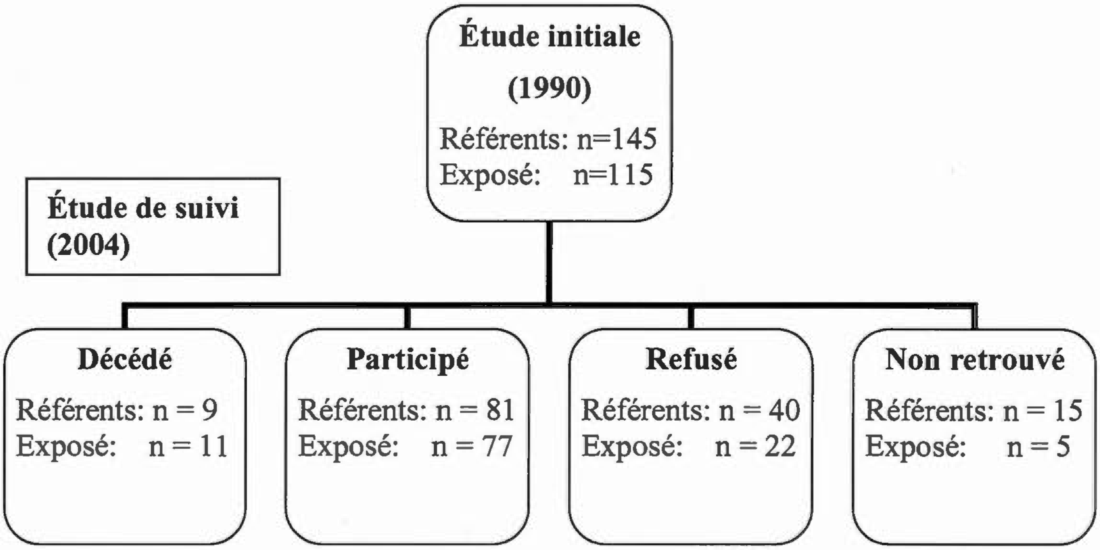

Figure 2.1: Résultats du recrutement à l'étude de suivi de 2004.

2.2.2.2 Déontologie

Le protocole de l'étude a été soumis au Comité institutionnel d'éthique de la recherche avec des êtres humains de l'Université du Québec à Montréal, lequel l'a jugé conforme aux règles déontologiques et a délivré un certificat de déontologie. Tous les participants à l'étude ont signé une formule de consentement éclairé. De plus, nous avons fait une demande à la Commission d'accès à l'information du Québec afin d'obtenir, via le Ministère de la Santé et des Services Sociaux (MSSS) du Québec, la date et la cause de décès des participants à l'étude initiale de 1990 qui étaient décédés en 2004. La Commission nous a accordé cette autorisation après que nous eûmes satisfait aux exigences de confidentialité des informations, et le MSSS nous a transmis les informations demandées pour 20 des 21 hommes décédés.

2.2.2.3 Informations générales sur les participants

Comme les résultats obtenus dans le cadre d'études longitudinales sont susceptibles d'être influencés par des facteurs qui pourraient masquer ou amplifier l'effet des agents neurotoxiques, une attention spéciale a été portée à identifier les facteurs de confusion possibles, notamment les expositions à des neurotoxiques qui pourraient être survenues après 1990 et les problèmes de santé pouvant affecter les performances neurocomportementales. Un expert en santé au travail (Harry Roels, Université Catholique de Louvain) a recueilli, au moyen d'une entrevue semi-dirigée, les informations socio-économiques et celles concernant l'histoire médicale et professionnelle. Dans l'ordre, les sujets suivants étaient abordés : socio-démographie (âge, nombre d'années de scolarité, revenu et statut marital); histoire médicale (maladies chroniques et conditions médicales particulières, accident, médication); histoire professionnelle (emploi(s) occupé(s) depuis 1990); exposition à des produits toxiques au travail et dans les passe-temps depuis 1990; style de vie (tabagisme, consommation d'alcool, consommation de drogues récréatives). De plus, certaines questions sur des variables susceptibles d'affecter les performances aux tests ont été posées, comme la consommation récente de café, le nombre d'heures de sommeil et les blessures aux membres.

2.2.2.4 Évaluation des fonctions neurocomportementales

Une maison située proche du lieu de résidence des participants a été louée afin d'y accueillir les participants et de procéder à l'administration des questionnaires et des tests. Les tests ont été effectués durant 6 semaines, du 6 juin au 14 juillet 2004. Trois cases-horaires étaient disponibles pour les participants : 9h00-12h00, 13h00-16h00 et 18h00-21h00. Chaque test était toujours administré par la même personne. Les administrateurs des tests ne connaissaient pas le statut d'exposition des participants.

Les tests ont été choisis parmi la batterie utilisée dans l'étude initiale afin d'avoir des mesures longitudinales (répétées). De plus, les tests ayant été sensibles à l'interaction Mn×âge ont été préférentiellement inclus dans la batterie pour l'étude de suivi. Finalement, étant donné l'importance des symptômes neuropsychiatriques, nous avons ajouté un questionnaire d'évaluation de ces symptômes (Inventaire bref des symptômes-BSI), bien que ceci n'ait pas été évalué lors de l'étude initiale.

Voici la liste des tests utilisés en 2004 :

- Parallel Line Drawing : Ce test mesure les habiletés graphomotrices. Il consiste à dessiner des lignes parallèles dans un rectangle tracé sur une feuille de papier; deux essais sont effectués. Le nombre de lignes tracées ainsi que le temps pour compléter le test sont notés.
- CancellationH : Ce test évalue l'attention et le repérage visuel par le biais d'une tâche de reconnaissance visuelle. Une feuille présente une série de lettres et le

participant doit mettre un trait sur toutes les lettres « H » le plus rapidement possible. Le temps pris pour effectuer la tâche est noté, ainsi que le nombre d'erreurs et d'omissions.

- Trail Making A & B : Ce test évalue le repérage visuel et les habiletés graphomotrices; il est constitué de deux parties (Reitan, 1958). Dans la première partie (Trail Making A), le participant doit relier des chiffres successifs qui apparaissent dans des cercles distribués de façon aléatoire sur la feuille. Dans la deuxième partie (Trail Making B), le participant doit relier les cercles mais en alternant les chiffres et les lettres qui se suivent (1-A-2-B-3-C, etc.); cette deuxième partie procure une évaluation de la flexibilité cognitive. Ces deux tâches doivent être faites le plus rapidement possible et les scores consistent en le temps pris pour compléter les tests.

- Symbol Digit Modalities Test : Ce test évalue l'attention visuelle, la concentration et la vitesse graphomotrice. Des paires correspondantes de symboles et de chiffres (1 à 9) sont présentées au participant sur une feuille. Dans chaque case sous les symboles, le participant doit écrire le chiffre correspondant au symbole. Le score correspond au nombre de chiffres corrects pour une durée de 90 secondes, et le nombre d'erreurs est noté. Une deuxième partie consiste à se rappeler les chiffres correspondant aux symboles; le nombre de chiffres corrects est noté.

- Delayed Word Recall : Ce test évalue la capacité d'apprentissage d'une liste de mots (Knopman et Ryberg, 1989). Lors de ce test, le participant doit apprendre une liste de dix mots qu'il devra se rappeler immédiatement et suivant un délai de 30 minutes. Le nombre de mots rappelés, le temps de rappel et le nombre d'erreurs sont notés.

- Digit Span : Ce test évalue la capacité de concentration soutenue et la mémoire à court terme par le biais d'une tâche verbale (Weschler, 1981). Le participant doit répéter une séquence de chiffres progressivement plus longue, d'abord dans l'ordre donné (de 3 à 9 chiffres), puis dans l'ordre inverse (de 2 à 8 chiffres).

- Stroop Color and Word Test : Ce test évalue l'habileté à nommer les couleurs et les mots, ainsi que l'interférence dans la tâche (Golden, 1978). Dans le test Word, le participant se voit présenter une feuille contenant cinq colonnes de vingt mots (« rouge », « vert » ou « bleu ») imprimés à l'encre noire et il doit les lire à voix haute le plus rapidement possible. Dans le test Color, la feuille est arrangée de la même manière mais les 100 cent items sont remplacés par des « XXXX » imprimés à l'encre rouge, verte ou bleue et le participant doit nommer les couleurs. Dans le test Color/Word, la feuille présente les mots « rouge », « vert » ou « bleu », mais ceux-ci sont imprimés dans des couleurs différentes de celles qu'ils désignent. Le participant doit nommer les couleurs dans lesquelles sont imprimés les mots, en faisant abstraction du sens des mots eux-mêmes.

- Fingertapping : Ce test mesure le taux maximal d'oscillation de l'index (vitesse motrice). Le Fingertapping manuel est utilisé (Lafayette Psychological Instruments) : il est formé d'un levier placé à 3,5 cm au-dessus de la base et d'un compteur; le participant doit presser sur le levier le plus rapidement possible. Le test dure dix secondes et il est répété trois fois pour la main dominante et non dominante, alternativement, et la moyenne des résultats est utilisée.

- None-Hole Steadiness Test (Tremometer) : Ce test permet de mesurer la coordination du mouvement ainsi que les tremblements statiques et cinétiques. Le participant doit maintenir un stylet dans un trou dont la taille diminue progressivement sans en toucher le pourtour; le nombre de contacts avec le pourtour est enregistré par l'appareil (Lafayette Psychological Instruments).

- Dynamomètre : Cet appareil mesure la force de préhension (Lafayette Psychological Instruments). Le test est administré deux fois, et si les résultats sont dissemblables, un troisième essai est effectué. La moyenne des essais est retenue.

- Échelle motrice de la batterie Luria-Nebraska : Ceci est une batterie de tests neuromoteurs qui évaluent différents domaines fonctionnels à l'aide de tâches chronométrées (Golden, Hammecke et Purisk, 1980). L'habileté d'exécution est notée sur une échelle de 0 à 2.

- Profil des humeurs (POMS) : L'humeur et l'état émotif seront évalués avec ce questionnaire standardisé et auto-administré qui est la version française du *Profile of Mood States* (POMS) (McNair, Lorr et Droppleman, 1992). Il est constitué de 65 items descriptifs cotés sur une échelle à cinq niveaux, allant de « pas du tout » à « extrêmement ». Les répondants indiquent comment ils se sont sentis au cours de la dernière semaine. Le POMS permet l'évaluation de six facteurs de l'humeur et de l'état émotif, au moyen des échelles suivantes : *Tension-anxiété*, *Dépression-découragement*, *Agressivité-colère*, *Vigueur-activité*, *Fatigue-inertie* et *Confusion-désorientation*. Un score est dérivé pour chacune des six échelles selon des directives du manuel, puis transformé en score-T, un score standardisé selon les normes dérivées d'une population de référence (McNair, Lorr et Droppleman, 1992).

- Inventaire bref des symptômes (BSI) : La symptomatologie neuropsychiatrique a été évaluée au moyen de ce questionnaire standardisé et auto-administré, une version française (Fortin, Coutu-Wakulczyk et Engelsmann, 1989) du Brief Symptom Inventory (BSI) (Derogatis et Melisaratos, 1983). Ce questionnaire permet de faire un inventaire de la santé mentale par une auto-évaluation de symptômes ou de comportements physiques ou psychiatriques. Le BSI comporte 53 énoncés couvrant neuf dimensions de symptômes : *Somatisation, Obsession-compulsion, Sensibilité interpersonnelle, Dépression, Anxiété, Phobie, Hostilité, Paranoïa* et *Psychotisme*. De plus, trois indices de détresse globale reflétant le statut psychopathologique peuvent être calculés. Les normes pour une population dite « normale » sont utilisées pour la standardisation des scores.

CHAPITRE III

MANGANESE EXPOSURE AND AGE: NEUROBEHAVIORAL PERFORMANCE AMONG ALLOY PRODUCTION WORKERS

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Abstract

Manganese (Mn) is associated with neurotoxic effects under certain conditions of exposure. A recent study on environmental Mn exposure showed a Mn×age interaction for several neurobehavioral functions. The objective of the present study was to examine the neurobehavioral test results in relation to age and Mn exposure, using an existing data set on 74 workers from a Mn alloy production plant and referents pair-matched for age (± 3 years), educational level (± 2 years), number of children and smoking status. The pair differences between Mn-exposed workers and referents increased significantly with age for scores on Delayed Word Recall, Trail Making B, Cancellation H, Nine-Hole Hand Steadiness Test, and Vibratometer. These results suggest that for certain neurobehavioral functions, and in particular for information processing, Mn-related deficits increase with age. This outcome could not be explained by higher cumulative Mn exposure.

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Résumé

Le manganèse est associé avec des effets neurotoxiques dans certaines conditions d'exposition. Une étude récente sur les effets de la pollution environnementale au Mn a montré une interaction Mn×age pour plusieurs fonctions neurocomportementales. L'objectif de la présente étude était d'examiner les résultats à des tests neurocomportementaux en relation avec l'âge et l'exposition au Mn, en utilisant une banque de données existante portant sur 74 travailleurs d'une usine de production d'alliages de Mn appariés à de référents sur la base de l'âge (± 3 ans), niveau d'éducation (± 2 ans), nombre d'enfants, et statut de tabac. Les différences inter-paires entre les travailleurs exposés au Mn et les référents augmentaient significativement ave l'âge pour les scores au Delayed Word Recall, Trail Making B, Cancellation H, Nine-Hole Hand Steadiness Test, et Vibratometer. Ces résultats suggèrent que pour certaines fonctions neurocomportementales, et en particulier pour le traitement de l'information, les déficits associés à l'exposition au Mn augmentent avec l'âge. Ces résultats n'étaient pas reliés à une plus grande dose cumulée d'exposition.

Mots clés: manganèse, exposition, âge, interaction, neurofonctions

3.1 Introduction

Manganese (Mn) exposure has been associated with neurobehavioral disturbances, mostly of the extrapyramidal system and most often in relation to workplace exposure in industries such as ferro-Mn production, iron and steel smelters, dry-cell battery manufacture, and welding. Mn exposure can also occur during Mn mining and ore processing, and application of Mn-based fungicides. Great variability is observed in the occurrence and gravity of signs and symptoms of Mn intoxication in relation to exposure; some workers heavily exposed for a long period show no signs of intoxication while others demonstrate signs and symptoms of intoxication following a moderate and short exposure (Mergler and Baldwin, 1997). Concentrations as low as 1 mg/m^3 have been reported to cause subclinical neurological signs of intoxication (Roels et al., 1987; Iregren, 1990; Roels et al., 1992; Chia et al., 1993; Mergler et al., 1994; Lucchini et al., 1995; Sjögren et al., 1996). Dose-effect relationships have rarely been reported, although Roels et al. (1992) and Lucchini et al. (1995) have observed such relations. Many factors have been related to susceptibility to Mn neurotoxicity, including iron deficiency, gender, genetics, co-exposures, respiratory and hepatic diseases or dysfunctions (for a review see Hudnell, 1999), and high alcohol intake (Bouchard et al., 2003).

Based on experimental animal studies, Donaldson (1987) suggested that Mn might accelerate the central nervous system aging. In an early report of 25 cases of Mn intoxication following consumption of contaminated well water (Kawamura et al., 1941), the authors observed a much higher degree of severity in the intoxication symptoms in older persons than in younger. Another report of older persons affected by Mn exposure is from Kondakis et al. (1989) who studied a group of persons over 50 years exposed to Mn through drinking water, and showed a positive relation between Mn content in water using a neurological examination rating scale. A recent population-based study on environmental Mn exposure showed a Mn×age interaction for certain cognitive and neuromotor performances, with the poorest performance observed among those over 50 years of age and in the higher blood Mn category (Mergler et al., 1999). Hochberg (1996) reported that former Mn-exposed Chilean miners who were previously asymptomatic, had more tremor and were slower than controls on tasks that require pointing movements and eye-hand coordination when aged over 50 years. Weiss has suggested that Mn could accelerate the normal aging process through neuronal attrition (1999), particularly in the basal ganglia and may contribute to earlier onset of parkinsonian syndrome in susceptible persons (2001).

Magnetic resonance imaging investigations suggest Mn accumulation in basal ganglia structures (for a review see Lucchini et al., 2000). Selective neuronal loss in basal ganglia has also been reported (Yamada et al., 1986) and mechanisms of neurotoxicity likely involve oxidative stress (Hazell, 2002). It is believed that Mn neurotoxic effects are related to disturbances in the activity of the dopaminergic system. Long term exposure to Mn has been associated with downstream of the nigrostriatal pathway in primates (Shinotoh et al., 1995) and humans (Wolters et al., 1989; Shinotoh et al., 1997). Animal studies have shown that subchronic oral exposure to $MnCl_2$ induces changes in markers of monoaminergic systems activity in the brainstem of aged rats considerably more than in young rats (Lai et al., 1984; Desole et al., 1994).

Many neurobehavioral performances are known to decrease with age. Recent functional imaging findings indicate that certain cognitive deficits associated with normal aging are modulated by changes in the brain dopamine system (Backman and Farde, 2001; Kaasinen and Rinne, 2002). Since Mn affects the dopaminergic system, which is likewise vulnerable to aging, the combination of the two factors could be additive or even synergistic. The objective of the present study was to examine the neurobehavioral performance with respect to age and Mn exposure, using an existing data set (Mergler et al., 1994) on a group of workers from a Mn alloy production plant and a group of pair-matched referents.

3.2 Participants and Methods

The raw data used in the present study are derived from a large investigation carried out in 1991 on nervous system functions of workers from a ferro- and silico-Mn alloy production plant. Socio-demographic information, Mn exposure, blood Mn, and data from a neurobehavioral test battery were available. Only the methods directly related to the present study are presented here, the complete study methods are extensively described in the original paper by Mergler et al. (1994). An extensive battery of tests was used in order to evaluate a wide range of neuromotor, cognitive, mood, and sensory nervous system functions (Table 3.1).

Table 3.1: Description of the neuromotor, cognitive, mood, and sensory domain of nervous system functions involved in the test battery

Tests	Nervous system functions
Neuromotor domain	
Motor scale of the Luria Nebraska Neuropsychological Battery	Simple motor movement
Finger tapping (manual)	Maximum oscillating speed of the index
Nine Hole Steadiness Test	Hand steadiness and tremor
Purdue Pegboard	Manual dexterity, concentration
Santa Ana	Manual dexterity
Dynamometer	Grip strength
Parallel Line Drawing	Graphomotor speed and tremor
Cognitive domain	
Simple Reaction Time Test	Reaction time, sustained concentration
Choice Reaction Time Test	Reaction time, sustained concentration, stimulus discrimination
Trail Making A	Visual conceptual and visuomotor tracking
Trail Making B	Visual conceptual, visuomotor tracking, cognitive flexibility
Cancellation H	Visuomotor tracking, concentration
Digit Span Test	Memory, mental tracking and sustained concentration
Serial Threes	Serial addition, concentration, mental control
Delayed Word Recall	Learning and recall, attention
Symbol Digit Modalities Test	Graphomotor speed, visual attention, concentration
Digit Naming	Speech initiation and regulation
Controlled Oral Word Association	Verbal fluency
Stroop Color and Word Test	Speech initiation and regulation, interference in verbal processing
Mood	
Profile of Mood Scale (POMS)	Mood and affect state (6 factors)
Sensory domain	
Lanthony D-15 desaturated panel	Chromatic discrimination
Vistech 6000	Near visual contrast sensitivity
Olfacto-labs Kit No. 11	Olfactory perception threshold
Vibratometer	Vibrotactile perception threshold of the index and toe

3.2.1 Study Group

A total of 115 workers, representing 95% of the plant's workforce, agreed to participate in the 1991-study. A matched-pair design was used; 144 actively working men, with no history of workplace exposure to neurotoxicants, were recruited from the region as referents. A total of 74 pairs were created using the following matching criteria: age (± 3 years), educational level (± 2 years), smoking status, and number of children. Characteristics of the Mn-exposed workers and referents in these pairs are presented in Table 3.2. Workers had been employed in the alloy plant on average 19.3 years (ranging from 1 to 27 years) and 71 of the 74 workers had been employed in the plant for over 10 years; the Mn production was introduced 17 years prior to the study.

Table 3.2: Characteristics of the Mn-exposed workers and referents from the 74 pairs in the study

	Mean ± SD (range)	
	Mn-exposed workers	**Referents**
Age (years)	43.4 ± 5.4 (32-58)	43.2 ± 5.6 (32-58)
Educational level (years)	11.0 ± 1.8 (6-16)	10.9 ± 2.0 (6-16)
Alcohol intake (g/week)	186 ± 24 (0-767)	180 ± 22 (0-772)
Smoking status	Pairs (n)	
Smokers	29	
Non-smokers	45	
Blood Mn (µg/L)	11.3 ± 5.3 (4.4-25.9)	7.2 ± 0.3 (2.8-15.4)

3.2.2 Manganese Exposure

The plant produced ferro- and silico-Mn alloys, containing respectively 79% and 67% Mn. Workers were exposed to airborne Mn particulates from the crushing and screening of the raw materials (pyrolusite ore, with a content of 32–50% Mn) and alloy products, and to the fumes from furnace tapping and cooling of the product in open bays. The sampling included full-shift personal monitoring in the breathing zone for total dust and Mn content, and parallel full shift stationary environmental sampling for total dust, respirable dust and their Mn content at representative locations across the plant (Baldwin et al., 1992).

The 8 h time-weighted average environmental measurements of Mn levels in total dust ranged from 0.014 to 11.48 mg/m^3 (geometric mean: 0.225 mg/m^3) while Mn levels in respirable dust ranged from 0.001 to 1.273 mg/m^3 (geometric mean: 0.035 mg/m^3). For each worker the cumulative Mn exposure indices for total and respirable dust were computed using several sources of information on exposure, i.e. current area sampling, personal air sampling, company records, and historical sampling data (Baldwin et al., 1993). Blood samples were collected before the last workday of the week and kept frozen until analysis of blood Mn concentration by flameless atomic absorption spectroscopy (Mergler et al., 1994).

3.2.3 Statistical Analysis

Every exposed worker was paired on several variables with a referent, therefore analyses were performed on the differences between the test scores obtained for the exposed worker and the referent of each pair. A study design including pair-matching between exposed and referents subjects is known to be very robust to account for confounders. The pair differences were normally distributed and regression analyses were used to assess the relationships between these differences in neurobehavioral performance and age or Mn exposure variables. Figure 3.1 (a, b, c) illustrates the proper interpretation of regression analysis results on pair differences with respect to the pairs' age.

Since a large number of tests were used to assess specific neurological domains, multivariate analyses of variance (MANOVA) were performed on the pair differences of the series of tests within a particular domain to account for the potential correlation between the dependent variables (neurobehavioral tests results). The Hotelling-Lawley statistic, which tests the significance of the difference between centroids of the matched samples, was applied. StatView 5.0.1 (SAS Institute, 1998) was used and a probability of 0.05 was adopted for statistical significance.

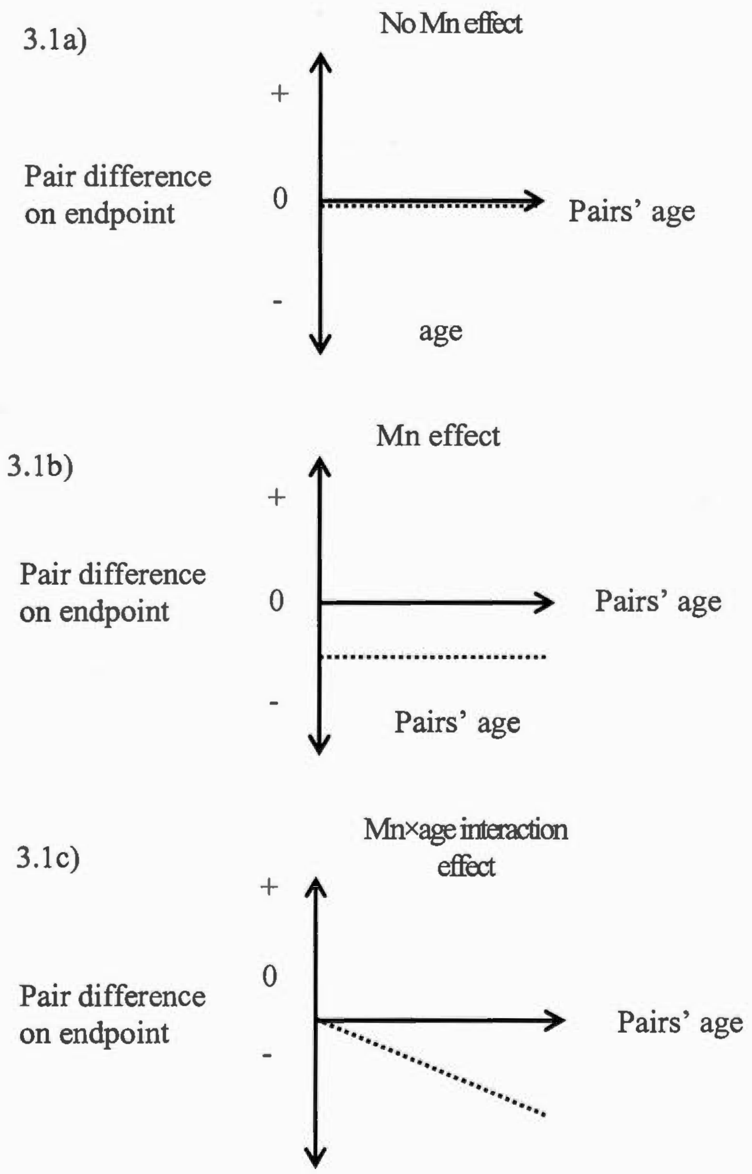


Figure 3.1: Interpretation of the regression analyses results of pair differences on nervous system endpoints and pairs' age. Figure 3.1a) The regression line (dotted line) indicates that there is no Mn effect at any age, since there are no pair differences (in test score) between exposed and referent scores (pair difference=0). Figure 3.1b) The regression line (dotted line) indicates a Mn effect, which is constant at all ages. Figure 3.1c) The regression line (dotted line) indicates a Mn×age interaction, since pair differences increase as pairs get older.

3.3 Results

The regression analysis did not show significant association between age and blood Mn, exposure to total or respirable Mn, either current or cumulated over the entire work history. Pair differences on test scores did not correlate with the exposure variables.

Then, we performed regression analyses between pair differences of the neurobehavioral tests results and age of pairs. Age was found to be significantly associated with an increase in pair differences on many neurobehavioral tests results, showing that being exposed to Mn results in a poorer performance with increasing age. Significant relationships were found for Delayed Word Recall, Trail Making B, Cancellation H, Nine-Hole Hand Steadiness Test and Vibratometer, while borderline significance ($0.05 < p < 0.9$) was observed for several other test scores including Choice Reaction Time, Finger tapping, Symbol Digit Modalities (written version), and Dynamometer (Table 3.3). These results indicate a significant Mn×age interaction effect that is due to the little or no pair differences at the lower age range, followed by increasing differences as pairs' age grows.

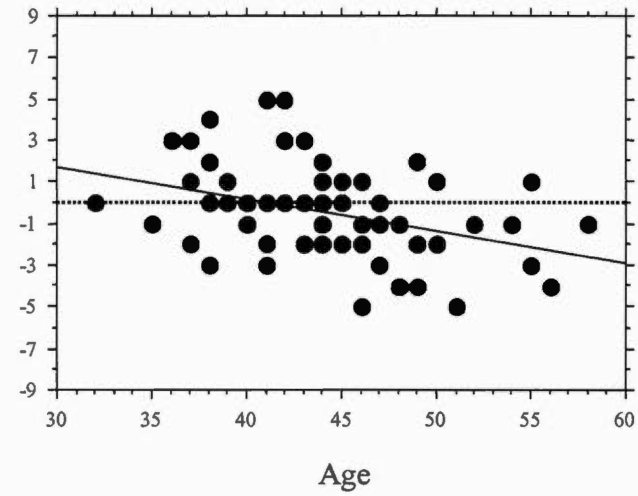
Table 3.3: Regression analyses results on pair score differences and age (n = 74)

Test	R^2	**F-value**	**Slope**
Delayed Word Recall (number)	0.14	9.93	-0.15**
Trail Making B (sec)	0.07	5.49	1.95*
Cancellation H (sec)	0.06	4.91	1.04*
Nine Hole Hand Steadiness Test (cumulated time)	0.09	7.20	19.44**
Vibratometer-toe (μm)	0.07	5.63	16.36*
Choice Reaction Time (msec)	0.04	3.13	7.98[1]
Finger tapping (manual) (number)	0.05	3.38	-0.33[1]
Symbol Digit Modalities Test written version (number)	0.04	3.05	-0.41[1]
Dynamometer (kg)	0.05	3.45	-0.39[1]

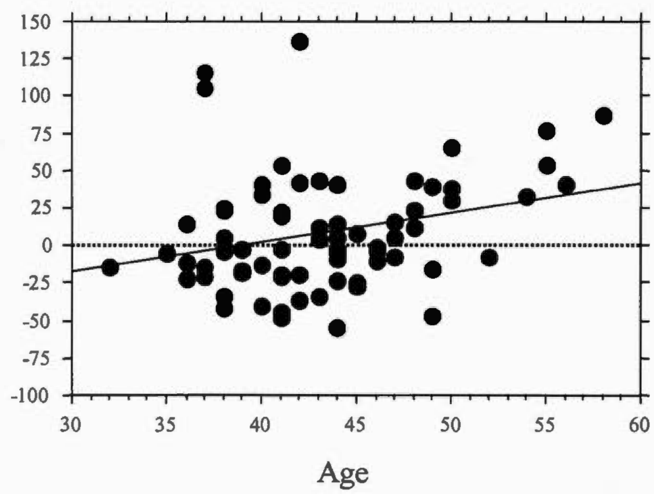
[1] Marginally significant ($0.05 < p < 0.09$)
* $p < 0.05$
** $p < 0.01$

Figure 3.2 (a, b, c, d) illustrates for some neurobehavioral endpoints the changes in pair differences as a function of pair' age. Figure 3.2a shows that the number of words correctly remembered on Delayed Word Recall declined significantly faster with age among the Mn-exposed workers as compared to pair-matched referents. Pair differences increased with age on the Trail Making B test (Figure 3.2b), which evaluates cognitive flexibility, and on Cancellation H (Figure 3.2c), a task involving visuomotor tracking and attention, indicating that the time required to complete these tests was longer for Mn-exposed workers compared to their pair-matched referents. On the Nine-Hole Hand Steadiness Test (Figure 3.2d), the results showed that with increasing age the exposed workers held the stylus for a longer period on the sides of the holes. Finger tapping and grip strength

(Dynamometer) showed a tendency of Mn-mediated worsening by age. For the sensory tests, only pair differences for vibrotactile perception threshold of the big toes (mean of both feet) was significantly associated with age, and increased more markedly among Mn-exposed workers.

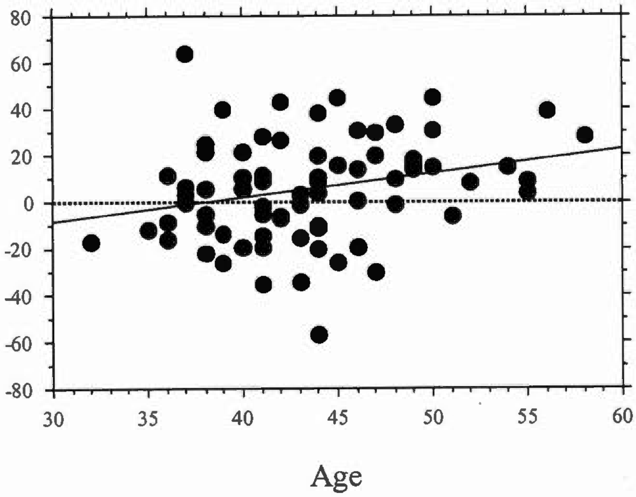


3.2.a)



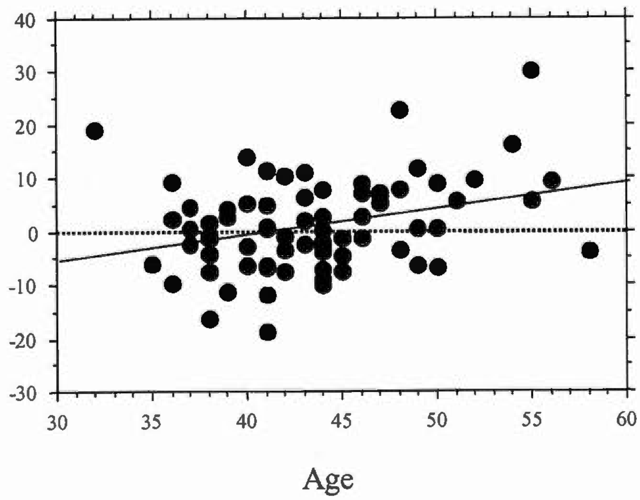
3.2.b)

Pair difference on Cancellation H (sec)



3.2.c)

Pair difference on Nine-Hole Hand Steadiness Test (sec)



3.2.d)

Figure 3.2. Regression plot of the pair differences on test scores and age; regression equation. a) Number of words recalled on Delayed Word Recall = 6.3–0.15×age; R^2 =0.14. b) Score on Trail Making B = -76.6+1.95×age; R^2=0.07. c) Cancellation H = -39.4+1.04×age; R^2=0.06. d) Nine-Hole Hand Steadiness Test (total time side is touched-mean for both hands) = -39.4+0.48×age; R^2=0.09.

For cognitive tests, the most significant relation of pair differences by age was for Delayed Word Recall. In addition, performance on several other tests requiring complex information processing likewise diminished with age. Since pair differences increased with age for several tests of information processing, they were entered into a MANOVA model with age dichotomized at 50 years. This cut-off was chosen because a previous paper showed a significant interaction between blood Mn concentration and age at 50 years on nervous system outcomes among a population-based study (Mergler et al., 1999), while others also reported increased effect of Mn exposure above 50 years of age (Hochberg et al., 1996, Kondakis et al., 1989). The model, which included Stroop Color and Word test (interference part), Trail Making B, Cancellation H, Symbol Digit Modalities (written version) and Choice Reaction Time was significant (Hotelling-Lawley = 0.249; p = 0.02). This result indicates that overall, pair-matched differences on these tests were significantly greater for those over 50 years of age.

3.4 Discussion

This pair-matched study showed a pattern of age-dependant aggravation in performance on several neurobehavioral tests in the presence on Mn exposure. Generally, there was little score difference between Mn-exposed workers and referents at the lower end of the age range (around 32 years), whereas the divergence increased with age. These Mn×age interaction results were consistent for tasks involving information processing and control of tremor. Since there was no relation between exposure variables and age, the decreased neurobehavioral performances at higher age cannot be explained by elevated cumulative exposure. The present results may underestimate the effect of age because older Mn-exposed workers with adverse health effects may have already left the workplace before the study was carried out (healthy worker effect). It should be noted that, the above results were observed in a group of relatively young workers, the oldest was 58 years of age.

The results of the initial study by Mergler et al. (1994), who looked at the differences between the same 74 pairs of Mn-exposed workers and referents, showed poorer scores among the exposed workers for several neuromotor functions, especially those involving rapid alternating coordinated movements. A number of other studies on workers exposed to similar levels of Mn have demonstrated neurobehavioral impairment mainly related to neuromotor functions, while cognitive effects have been inconsistently reported (Iregren, 1999). The data re-analysis presented here shows that for certain neurobehavioral functions, mostly cognitive in nature, the deficits increased as the workers aged. Thus, certain functions that were not found to be affected by Mn exposure in the initial study were shown to be affected in older workers.

These results were reinforced by the results of the multivariate analysis, a rigorous procedure to assess multiple effects. The tests results involving information processing were entered in a MANOVA model and resulted in a significant relationship, indicating that age has an effect on the performance on these tests taken together. However, the results are more informative when taken separately. For example, the slope of the regression line on the results of Delayed Word Recall indicated that Mn-exposed workers would remember two to three less words (total of 10 words) than referents at 55 years of age.

The results on the Nine-Hole Hand Steadiness Test suggest that the aging Mn-exposed workers were less capable of controlling their position on the apparatus and touched the side of the holes for a prolonged period of time. In addition, overall the Mn-exposed workers had higher levels of tremor (number of times that they touched the sides of the hole) as compared to the referents (Mergler et al., 1994). The results of the present study suggest that with age, tremor parameters may change. In a community based study, Beuter et al. (1999) noted in subjects with higher levels of blood Mn an age×Mn interaction for frequency dispersion and harmonic index on a tremor test, as well as for the Fitt's Constant (a measure, which takes into account both speed and precision) on the eurythmokinesimeter, an instrument that quantifies eye-hand coordination in a pointing task

The results of the present study support a previous report of increased neurotoxic Mn effects with aging among an environmentally exposed population (Mergler et al., 1999). In this community based study, persons over 50 years with higher blood Mn concentrations had poorer performance for learning and recall and certain motor tasks evaluating the ability to perform rapid and precise pointing movement, and manual dexterity. Test results of fine movement precision and regularity, as well as tremor analysis parameters were more strongly related to age among those with higher blood Mn than those with lower Mn levels (Beuter et al., 1999). The subgroup of older men with higher blood Mn concentration also exhibited higher scores in the anxiety, nervousness, irritability, emotional disturbance, as well as aggression and hostility dimensions relative to those in the same age group but who had lower levels of Mn (Bowler et al., 1999).

The issue of age in population studies of neurotoxic effect has rarely been treated. The matching of Mn-exposed workers and referents on age and years of education made it possible to explore the role of age, since education and age are often associated. The role of age is most often considered as a confounding factor, and standard statistical corrections are applied. However, these corrections may not account for the differential effect of age among the exposed and non-exposed group. In cases where no significant correlation is found between age and neurobehavioral test results, age may be ruled out from the analyses although it may be an explanatory variable among the exposed group. By investigating neurobehavioral performance among an exposed group without a control group, the effects of exposure may be underestimated by over-adjusting for age-related decline in neurobehavioral test performance.

Mn is an essential nutrient, and complex homeostatic mechanisms regulate the body systemic concentration. Differences in individual physiological characteristics could be related to changes in Mn absorption and/or production of neurotoxic effects. For example, less efficient biliary excretion among older individuals may play a role in the adverse nervous system effects observed. Other factors could contribute to explain these results; it is known that the nervous system has a great capacity to compensate for toxic insult, but this capacity could be reduced with aging (Grandjean, 1991). Since both aging and long-term exposure to Mn are known to reduce the activity of the dopaminergic system, the more marked effect among older Mn-exposed workers could occur via changes in this neurotransmitter system. Recent research findings of functional imaging investigations have shown that certain neuromotor and cognitive deficits observed in normal aging are mediated by changes in the striatal dopamine system (for a review see Backman and Farde, 2001). PET examinations on healthy volunteers indicated significant correlations between dopamine receptor availability (D2 type) and neurobehavioral test performance, such as Finger tapping, measures of abstraction and mental flexibility (Wisconsin Card Sorting Test), attention and response inhibition (Stroop Color/Word Test) (Volkow et al., 1998). D2 receptors in the caudate and putamen declined with age, but these relationships remained significant after controlling for age effect suggesting that dopaminergic activity may be more a predictor of neurobehavioral performance than age.

Exposure to other neurotoxicants that affect the dopaminergic system was also found to accelerate aging of many nervous system functions. On a follow-up study 18 years after the end of exposure, the memory, perceptual speed and attention decrement were related to heavy solvent exposure among a group of floor layers, while previous investigation of neuropsychological impairment revealed only slight exposure effect (Nilson et al., 2002). Past occupational exposure to lead has been found to be related to prospective decline in cognitive functions, including visuoconstructive ability, and verbal memory and learning, among men with a mean age of 55 years and a mean duration of cessation of lead exposure of 16 years (Schwartz et al., 2000). The results of the present cross-sectional study do not provide direct information on the long term combined effect of previous Mn exposure and nervous functions aging. A follow-up of the Mn-exposed workers as well as their referents would help determine if past exposure to Mn will further accelerate the decline of nervous system functions.

3.5 Acknowledgements

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CHAPITRE IV

NEUROBEHAVIORAL FUNCTIONING AFTER CESSATION OF MANGANESE EXPOSURE: A FOLLOW-UP AFTER 14 YEARS

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Abstract

Background: Little is known about the long-term course of early Mn neurotoxic effects. We followed up manganese (Mn) alloy workers 14 years after exposure ceased. Methods: The same battery of neurofunctional tests used in the initial examination in 1990 was administered to 77 Mn-workers and 81 referents in 2004. Results: Mn-workers had poorer scores compared to referents both in the initial and follow-up examinations for several motor functions. At follow-up, older Mn-workers (>45 years at cessation of exposure) had poorer scores than referents of similar age for tests of cognitive flexibility. Mn-workers reported more confusion on a mood scale at follow-up, but not in the initial examination. Cumulated exposure was associated with poorer test scores. Differences on tests for attention and memory observed in the initial examination were not present at follow-up. Conclusions: Manganese exposure was associated with persistent deficits for certain neuromotor functions, cognitive flexibility and adverse mood states, while there was recovery for other functions.

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Résumé

Introduction : On sait peu de chose sur l'évolution des effets neurotoxiques précoces associés à l'exposition au manganèse (Mn). Nous avons suivi des travailleurs ayant été exposés au Mn 14 ans après la fin de l'exposition. Méthodes : Une batterie de tests neurofonctionnels utilisé lors d'une étude initiale menée en 1990 a été ré-administrée en 2004 à 77 travailleurs ayant été exposés au Mn et 81 référents. Résultats : Les travailleurs ayant été exposés au Mn ont eu des scores inférieurs à plusieurs tests des fonctions motrices lors de l'examen initial ainsi qu'à l'examen de suivi s. À l'examen de suivi, les travailleurs plus âgée (>45 ans au moment de la fin de l'exposition) ont eu des scores inférieurs à des tests de flexibilité cognitive. Les travailleurs ayant été exposés au Mn ont rapporté plus de sentiments de confusion à une échelle d'humeur lors de l'examen de suivi, mais pas à l'examen initial. L'indice d'exposition cumulé était associé à des scores inférieurs pour plusieurs tests. Les différences entre les travailleurs exposés et les référents aux tests d'attention et de mémoire observées lors de l'étude initiale n'étaient plus présentes à l'examen de suivi. Conclusion : L'exposition au Mn a été associée à des déficits persistant de certaines fonctions neuromotrices, la flexibilité cognitive et les problèmes d'humeur, alors qu'un rétablissement a été observés pour d'autres fonctions.

Mots clés: manganèse, exposition cumulée, longitudinale, neurofonctions

4.1 Introduction

Manganese (Mn) is an essential element, but it is neurotoxic when exposure increases, or when homeostatic mechanisms fail to prevent its accumulation [Hauser et al., 1996]. High exposure to airborne Mn has been associated with neurotoxic effects, with the worst cases displaying an extrapyramidal syndrome (manganism) characterized by gait dysfunction with a propensity to fall backward, postural instability, bradykinesia, rigidity, micrographia, masked facies, speech disturbances, and muscle tremors. Many of these clinical features are shared with Parkinson's disease (PD), but manganism differs from PD on several clinical, neurobiochemical and pathological aspects [Barbeau 1984; Barbeau et al., 1976; Calne et al., 1994]. Manganese neurotoxic action is likely to damage neuronal structures downstream from the nigrostriatal dopaminergic pathway [Shinotoh et al., 1997]. Alterations in neurotransmitter content and metabolism, notably dopamine, GABA and glutamate are concurrent with these neurological signs [Eriksson et al., 1987].

Manganism has been documented in workers exposed to high levels of Mn dust or fumes in mines, smelters, welding and in the manufacture of dry batteries [Abdel-Naby and Hassanein 1965; Cook et al., 1974; Josephs et al., 2005; Wang et al., 1989; Whitlock et al., 1966]. The prognosis of manganism is uncertain. An early report of cases of Mn intoxication among miners stated that the evolution of neurological signs of manganism is generally progressive: some cases reach a complete paralysis, others are stationary for years, while partial recovery may appear in a few cases [Penalver 1955]. The progression of neurological symptoms of manganism, documented in five alloy-workers nine to ten years after cessation of exposure, revealed continuing deterioration in gait, speed of foot tapping, rigidity, and handwriting [Huang et al., 1993]. In other studies of workers with high exposure who were only mildly or non-symptomatic while exposed, neurological signs developed several months after cessation of exposure [Jonderko et al., 1971; Meco et al., 1994; Penalver 1955; Rodier 1955]. In contrast, recovery from neurological signs was reported for intoxicated workers after treatment with $CaNa_2$ EDTA [Discalzi et al., 2000; Herrero Hernandez et al., 2005; Whitlock, et al. 1966].

Exposure to lower levels of Mn exposure (< 1 mg / m^3) can also cause early neurotoxic effects. Neurobehavioral tests, used in a number of studies, have shown that moderate but long term exposure to Mn in the workplace, is associated with neuromotor and cognitive deficits as well as mood changes [Chia et al., 1993; Iregren 1990; Lucchini et al., 1995; Mergler et al., 1994; Roels et al., 1987; Sjögren et al., 1996], although Myers et al. did not observe effects in South African mineworkers [Myers et al., 2003]. The most characteristic neuromotor signs of Mn intoxication are poor hand steadiness, difficulty in performing fast alternating movements, muscular rigidity, and postural instability. Cognitive deficits consist of poor memory, slow reaction time, and decreased cognitive flexibility [Iregren 1999]. Mood disorders, characterized by symptoms of depression, irritability, anxiety, aggressiveness, confusion, and emotional disorders, have also been reported [for a review, see Bowler et al., 1999]. Little is known, however, on the long-term course of early neurotoxic deficits following cessation of exposure to Mn.

In 1990, our research team conducted a matched-pair, cross-sectional study on nervous system functions of workers from a Mn-alloy production plant [Mergler, et al. 1994]. The results revealed deficits in neuromotor, cognitive functions, and adverse mood states in the Mn-exposed workers when compared with paired referents [Mergler, et al. 1994]; furthermore, these deficits increased with workers' age [Bouchard et al., 2005]. Shortly after completion of the study, the plant closed. The objective of the present study was to examine the changes in neurobehavioral performances among the cohort of Mn-alloy workers and their referents, 14 years after cessation of exposure.

4.2 Methods

4.2.1 Recruitment of Study Groups

In the 1990-initial investigation, most employees of the Mn ferro-alloy production plant participated (115 workers representing 95% of the total workforce). A group of 145 men group-matched for age, education and alcohol consumption were recruited as referents among blue collar workers from the same geographical region. Further details on selection and recruitment of initial study participants are reported elsewhere [Mergler, et al. 1994]. For enrolment into the 2004 follow-up study, several strategies were used to recruit as many participants as possible from the initial study. Addresses of all participants were verified in different telephone and address directories. Various means were used to locate those who had moved: telephone directory search, contact information from other participants or persons with the same family name (most of the participants were from the same region with close community linkages). For those who could not be found, the civil registry was reviewed to determine if the person had died since the initial study. Of the 115 former Mn-exposed workers and 145 referents who participated to the initial study, 11 (10%) Mn-workers and 9 (6%) referents had died. We were able to obtain the date of death from the civil registry for 19 out of the 20 deaths (data missing for 1 former Mn-exposed worker). For those remaining, contact addresses were obtained for 99 (95%) Mn-workers and 121 referents (92%), to whom letters were sent inviting them to participate into the follow-up study.

Seventy-seven formerly Mn-exposed workers and 81 referents agreed to participate, resulting in a participation rate of 78% and 67% respectively (deceased and individuals not found were not included in the denominator). A monetary compensation of $75 was offered at the completion of the examination cycle. Of the 22 former Mn-exposed workers who refused to participate, we were able to reach 18 to investigate their reasons for refusal and medical diagnoses: none reported PD or other nervous system ailments. The following reasons were given for refusal: seven were not interested, four had no time, two were living too far to come, and the five remaining were not available at that time.

The study protocol was approved by the Institutional Ethics Board of the Université du Québec à Montréal, and informed consent was obtained from each participant.

4.2.2 Nervous System Assessment

Testing was carried out in a centrally located, rented residential building. Information on work and medical history, medication, lifestyle habits, and exposure to neurotoxicants was obtained using a questionnaire-based interview conducted by an occupational health expert (H.R.).

The following tests were administered according to the procedure used in the initial examination and described elsewhere [Mergler, et al. 1994]: Motor Scale of the Luria-Nebraska Neuropsychological Battery (total score), Fingertapping (mean of three trials of two hands), Dynamometer (kg), Nine-Hole Hand Steadiness Test (total number of contacts and total duration of contacts in sec for holes 1 to 9), Cancellation H (time in sec), Trail Making A & B (time in sec), Stroop Color-Word Test (number of words), Digit Span (forward and backward trial scores were added), Delayed Word Recall (number of words), and the written version of the Symbol Digit Modalities Test (number of digits). Finally, the Profile of Mood States (POMS) was administered, which provide scores on six scales, and raw scores were transformed into T-scores. Two neuropsychologists blinded to the exposure status of the participants administered the tests, and the same examiner always administered the same series of tests.

4.2.3 Manganese Exposure

The plant where the Mn-exposed group had worked began operations in 1973 and produced silico- and ferro-Mn alloys, containing 67% and up to 79% Mn respectively. The process used a sealed submerged electric arc furnace; workers were exposed to airborne Mn particulates (mixed oxides and salts) from the crushing and screening of the raw materials (pyrolusite ore with a content of 32-50% Mn) and from the alloy products, and to Mn fume from furnace tapping and cooling of the product in open bays. The exposure history of each worker was reconstructed and a Cumulative Exposure Index (CEI) to Mn in total dust was computed using several sources of information: personal and environmental sampling data, historical dust sampling data, company records relating to payroll and production, work history questionnaires completed for the 1990-study and interviews with long-term employees [Baldwin et al., 1993]. The average length of exposure to Mn in the alloy plant was 15.3 years, ranging from 1.6 to 17.3 years.

During the 1990-investigation, blood samples were collected and analyzed for Mn content. No additional blood samples were taken at follow-up since blood Mn is rapidly cleared after cessation of exposure and is reported to return to normal concentration within less than 30 days [Cotzias et al., 1968].

4.3.4 Exclusions from the Statistical Analysis

The following criteria were used as post hoc exclusions for the present analyses: 1) diagnosed medical condition or accident affecting the nervous system, but unlikely to be related to past Mn exposure: seven referents (two strokes, two severe concussions, one senility, one multiple sclerosis, one essential tremor) and four exposed (three strokes, one multiple sclerosis) ; 2) significant exposure to neurotoxicants after the 1990-study: three referents; 3) high alcohol consumption (>500 g / week): three referents and three exposed. In addition, one former Mn-exposed worker who was employed less than one year in the plant was excluded from the analyses. After exclusion, data from 68 referents and 69 former Mn-exposed workers were analysed. Four referents and five former Mn-workers reported taking antidepressive or antianxiety drugs; these individuals were not excluded *a priori*, but their influence on the results was verified. Figure 4.1 provides an outline of recruitment outcomes and exclusions from the analyses.

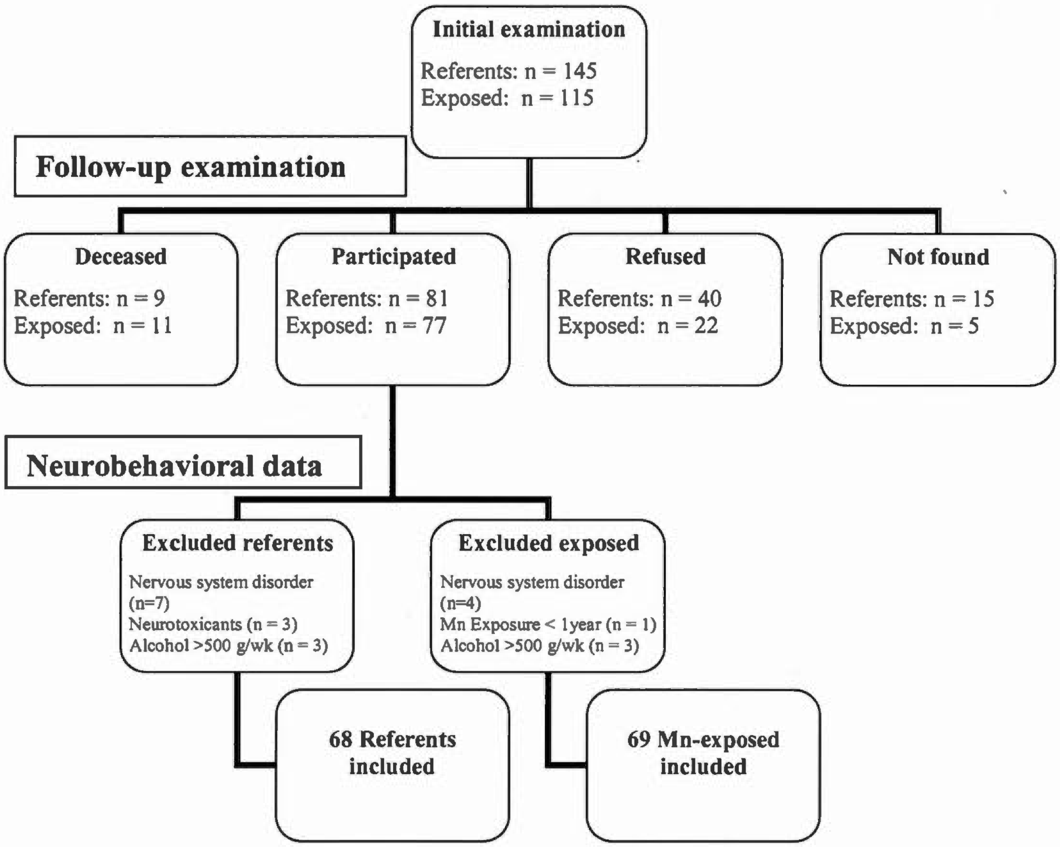


Figure 4.1: Outline of the recruitment and the inclusion in the neurobehavioral data analysis of the population groups (referents and Mn-exposed workers) from the 1990-initial to the 2004-follow up study

4.2.5 Statistical Analysis

SPSS 13.0 was used and the limit for statistical significance was set at $p < 0.05$, while p values between 0.05 and 0.1 are reported as trends. All significance testing was two-sided. Statistical comparison of general characteristics between exposure groups was performed with unpaired t test, or Pearson's *chi*-square test (for continuous and categorical data respectively). Multivariate GLM was used to compare neurobehavioral test scores with respect to Mn exposure groups, while controlling for covariates age, education (number of years of schooling), alcohol (g / week), and smoking (smoker / non-smoker). Since the calculation of retrospective cumulated exposure indices (CEI) implies several assumptions, the effect of CEI on neurobehavioral test scores was assessed using an ordinal dummy variable (dummy variable: 0 = referents, 1 = lowest CEI tertile, 2 = middle CEI tertile, 3 = highest CEI tertile). Table 4.1 presents CEI values corresponding to each tertile. Finally, the effect of cumulative exposure on changes in test scores was assessed using GLM and testing whether each CEI tertile differed significantly from the referents' mean change in test scores.

Table 4.1: Mean (±SD), Median and Range of Manganese Cumulated Exposure Indices (CEI) with Respect to Tertiles

	Cumulated Exposure Indices (mg Mn / m^3 ×year)	
Tertiles of Cumulative Exposure Indices	Mean (± SD) Median	Range
Lowest tertile (n = 23)	5.97 (± 4.28) 6.18	0.31 – 11.83
Middle tertile (n = 23)	19.50 (± 4.98) 19.03	11.98 – 30.40
Highest tertile (n = 23)	58.15 (± 21.68) 54.95	31.17 – 100.24
All (n = 69)	27.38 (± 24.73) 19.03	0.31 – 100.24

SD, standard deviation

4.3 Results

4.3.1 Descriptive Statistics

Mortality rate did not differ significantly between former Mn-exposed workers and referents. Deceased individuals were older and less educated than survivors ($p < 0.01$), and age at death did not differ significantly between former Mn-workers (53.7 ± 6.1 years) and referents (55.5 ± 8.0 years); mean CEI of deceased and individuals who were not found were similar to follow-up participants.

Follow-up participants and refusals were compared for differences on socio-demographic variables, initial examination neurobehavioral test results, blood Mn and workplace exposure variables. Few differences were observed. Former Mn-exposed workers who accepted to participate in the follow-up examination had significantly higher mean CEI than those who refused ($p < 0.05$), and those who refused to participate in the follow-up had obtained significantly poorer results on the Luria Motor Scale at initial examination than follow-up participants, after controlling for age and education ($p = 0.01$). This latter difference between participants and non-participants to follow-up was similar for both former Mn-workers and referents.

Table 4.2 presents the characteristics of the follow-up study population. There was no difference between former Mn-workers and referents for mean age, education, alcohol consumption, and distribution of smoking habits and marital status. However, former Mn-exposed workers had significantly decreased their alcohol consumption between initial and follow-up examinations (mean: 186 to 118 g / week; paired-samples t test, $p < 0.01$) but not the referents (mean: 143 to 116 g / week, paired-samples t test, $p > 0.1$).There was a trend for differences in occupational status: a higher proportion of former Mn-workers were unemployed or in disability and fewer were working or retired. Former Mn-workers had a significantly lower income than referents ($p < 0.01$).

Examination of the distribution of cases for various health problems investigated during the medical history interview showed that the former Mn-exposed workers reported more respiratory problems than referents (OR = 10.6 [95% CI = 1.3 - 85.7]). Inclusion of smoking habits, past and present, in the analysis did not modify this result. The other health problems investigated, namely cardiac problems, hypertension and diabetes, did not significantly differ between former Mn-exposed workers and referents.

Table 4.2. Characteristics of the participants to follow-up examination with respect to exposure group

	Mean (± SD) Range		
	Referents (n = 68)	Mn-exposed (n = 69)	p
Age (years)	56.9 (± 7.3) 43.5 – 74.1	58.1 (± 5.6) 45.2 – 71.4	ns [1]
Education (years)	11.1 (± 2.4) 6.0 – 17.5	11.1 (± 1.8) 6.0 – 15.5	ns [1]
Alcohol consumption (g / week)	116 (± 125) 0 – 462	118 (± 129) 0 – 450	ns [1]
	Frequency (%)		
Tobacco			
Current	16 (24)	16 (23)	ns [2]
Former	35 (52)	40 (58)	
Never	17 (25)	13 (19)	
Marital status			
Married	52 (77)	45 (65)	ns [2]
Divorced/Widowed	10 (15)	18 (25)	
Single	6 (9)	6 (9)	
Occupation			
Full time work	44 (65)	42 (61)	
Unemployed	1 (2)	5 (7)	0.06 [2]
Disabled	0 (0)	4 (6)	
Retired	23 (34)	18 (26)	
Income			
≤ 40 000$	28 (41)	44 (64)	< 0.01 [2]
> 40 000$	40 (59)	25 (36)	

SD, standard deviation
[1] Unpaired *t* test
[2] Chi-square test

4.3.2 Exposure Group Differences in Neurobehavioral Test Scores

The average scores of neurobehavioral tests with respect to exposure groups are presented for 1990-initial and 2004-follow up examinations in Table 4.3; significant group differences after controlling for age, education, alcohol consumption and smoking are indicated. At the initial examination, the mean total score on the Luria Motor Scale was significantly higher in Mn-workers than in referents ($p < 0.01$), indicating poorer performance with Mn exposure. Former Mn-workers' performance remained significantly poorer at follow-up ($p < 0.05$), although the difference between groups was less marked. Further analysis of the Luria Motor Scale items which were significantly affected by Mn exposure at the initial examination showed that poorer performance was still present at follow-up for slowing of speed of touching each finger in sequence with the thumb ($p < 0.05$) and hand clench/extension ($p < 0.05$), and there was a tendency for slowing of alternating hand tap ($p = 0.06$) in Mn-workers. At follow-up, former Mn-workers also showed poorer quality of drawing ($p < 0.01$), while this difference was not present at the initial examination.

Table 4.3. Comparisons of mean scores between exposure groups at the initial and follow-up examinations

	Better perfor-mance	1990-Initial Examination Referents (n = 68)	1990-Initial Examination Mn-Exposed (n = 69)	P^1	2004-Follow up Examination Referents (n = 68)	2004-Follow up Examination Mn-Exposed (n = 69)	P^1
Neuromotor Tests							
Luria Motor Scale (total score)	↓	6.1 (3.9)	9.8 (4.4)	***	8.6 (4.6)	10.6 (5.1)	*
Hand Steadiness Test (time of contacts in sec)	↓	8.02 (5.45)	9.25 (5.80)	-	28.75 (8.19)	30.95 (9.47)	-
Hand Steadiness Test (number of contacts)	↓	90 (57)	116 (82)	†	191 (58)	189 (54)	-
Fingertapping (number)	↑	50.5 (6.1)	49.0 (5.4)	-	45.9 (7.2)	45.9 (6.6)	-
Dynamometer (kg)	↑	52.2 (6.2)	52.1 (7.0)	-	45.9 (7.2)	46.5 (7.3)	-
Cognitive Tests							
Cancellation H (sec)	↓	77.9 (15.5)	84.4 (14.5)	*	88.6 (18.4)	89.7 (15.8)	-
Digit Span (total score)	↑	17.6 (4.2)	15.6 (3.9)	*	14.0 (4.2)	13.0 (3.7)	-
Delayed Word Recall (number)	↑	6.7 (1.5)	6.2 (1.6)	†	6.3 (1.4)	6.0 (1.5)	-
Trail Making A (sec)	↓	28.7 (8.3)	32.2 (11.8)	†	30.9 (12.0)	30.0 (10.0)	-
Trail Making B (sec)	↓	71.0 (23.4)	79.4 (31.3)	-	74.4 (25.1)	76.4 (28.0)	-
Stroop Color-Word Test (word trial)	↑	101 (13)	96 (4)	†	101 (13)	97 (15)	-
Stroop Color-Word Test (color trial)	↑	74 (11)	71 (11)	-	69 (10)	70 (11)	-
Stroop Color-Word Test (color-word trial)	↑	40 (8)	37 (7)	*	37 (9)	35 (8)	-
Symbol Digit Modalities Test (number)	↑	49.0 (9.4)	46.8 (8.1)	-	46.9 (9.9)	45.0 (7.4)	-

(*table continuing next page*)

Profile of Mood States							
Tension-Anxiety	↓	42.3 (6.7)	43.7 (8.1)	-	34.7 (4.7)	35.3 (5.2)	-
Depression-Dejection	↓	42.0 (4.0)	44.6 (7.6)	*	37.4 (2.7)	37.9 (3.4)	-
Anger-Hostility	↓	46.0 (6.6)	48.9 (10.0)	†	41.0 (4.1)	42.6 (5.6)	†
Vigor-Activity	↑	53.1 (7.8)	51.3 (7.6)	-	58.2 (7.4)	57.1 (7.3)	-
Fatigue-Inertia	↓	42.0 (7.2)	44.5 (7.6)	†	42.0 (4.4)	43.1 (5.0)	-
Confusion-Bewilderment	↓	39.0 (5.9)	40.9 (7.6)	-	36.6 (3.8)	38.1 (4.7)	*

[1] GLM controlling for age, education, alcohol and smoking
*** P < 0.001; * $P < 0.05$; † $P < 0.1$; - $P \geq 0.1$

Although scores of Mn-workers were poorer than referents on Digit Span and Cancellation H at the initial examination, this was not observed at follow-up. Most of the test scores that were similar between exposure groups at the initial examination were likewise similar at follow-up: Trail Making A, Fingertapping, Hand Steadiness Test, Dynamometer, Delayed Word Recall, and the word trial and color trial of the Stroop Color-Word Test. Stratifying with respect to mean age at cessation of exposure (45 years) showed that for the color-word trial of the Stroop Color-Word Test, the poorer performance observed at the initial examination remained significant for former Mn-workers older than 45 years at cessation of exposure (scores: 33 ± 6 for Mn-workers and 39 ± 9 for referents; $p < 0.05$). At follow-up, these older Mn-workers likewise had significantly poorer scores compared to referents of similar age on Trail Making B (84 sec ± 27 for Mn-workers and 65 sec ± 21 for referents; $p < 0.01$) and Symbol Digit Modalities Test (score: 44 ± 6 for Mn-workers and 49 ± 9 for referents; $p < 0.05$). At the initial examination, the older Mn-workers had taken significantly more time on Trail Making B than referents (83 sec ± 21 for Mn-workers and 63 sec ± 25 for referents; $p < 0.05$), but scores on the Symbol Digit Modalities Test were not different.

At the initial examination, the Mn-workers reported significantly higher scores on the POMS Depression-Dejection scale than referents ($p < 0.05$), indicating more adverse mood states, but this difference was no longer present at follow-up. Mn-workers tended to obtain higher scores on the Anger-Hostility scale at both examinations ($p = 0.07$), again indicating more adverse mood states. While there was no group difference at baseline on the Confusion-Bewilderment scale, former Mn-workers had significantly higher scores than referents at follow-up ($p < 0.05$). It should be noted that, in contrast to the neuromotor and cognitive tests, the POMS' scores were lower at follow-up than at initial examination, indicating a general improvement of mood states for both exposure groups.

4.3.3 Changes in Neurobehavioral Test Scores between Examinations

In order to assess the influence of exposure status on the changes in performances between examinations, analyses were performed on differences in test scores ($score_{1990}$-$score_{2004}$) for each neurobehavioral test. Age, education, smoking and alcohol consumption were used as covariates in these analyses; the change in alcohol consumption between examinations was entered into the models but since it did not account for a significant proportion of variance it was removed. The former Mn-workers had significantly smaller declines in performances ($p < 0.05$) than referents for several tests: total score of the Luria Motor Scale, Hand Steadiness Test (number of contacts), Cancellation H, color trial of the Stroop Color-Word Test and Trail Making A.

As expected, age was a significant predictor of decline in neurobehavioral test scores ($p < 0.05$), particularly for Fingertapping, Digit Span, color trial of the Stroop Color-Word Test, and there was a tendency for the Dynamometer ($p = 0.07$). Age also had a significant effect on score changes for several POMS' scales, but here, younger age was associated with greater improvement in reported mood states on the scales of Tension-Anxiety, Anger-Hostility and Confusion-Bewilderment ($p < 0.05$). The effect of age on score changes between examinations was similar in both exposure groups.

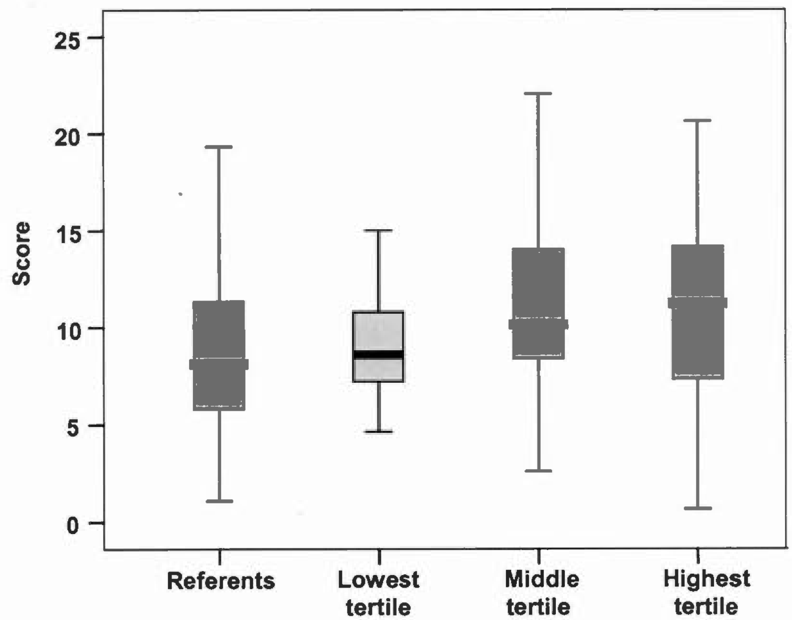
4.3.4 Relations with Cumulative Exposure

The association between the level of cumulated exposure and neurobehavioral test scores at follow-up was assessed using tertiles of CEI as an ordinal variable in regression models (0 = referents, 1 = lowest tertile, 2 = middle tertile, 3 = highest tertile). It should be noted that age was not significantly associated with CEI (Pearson correlation; $R = 0.15$, $p > 0.1$). Nonetheless, age, as well as education, alcohol and smoking were included as covariates in these analyses. Increasing levels of exposure was significantly associated with poorer scores on the Luria Motor Scale ($p < 0.01$) (Figure 4.2). Significant relations were also observed for scores on the Hand Steadiness Test (time of contact), and color-word trial of the Stroop Color-Word Test ($p < 0.05$). Likewise, increasing level of cumulated exposure was positively associated ($p < 0.01$) with scores on the Confusion-Bewilderment POMS' scale. There was also a trend for higher scores on Anger-Hostility ($p = 0.06$) and Fatigue-Inertia ($p = 0.07$) scales.

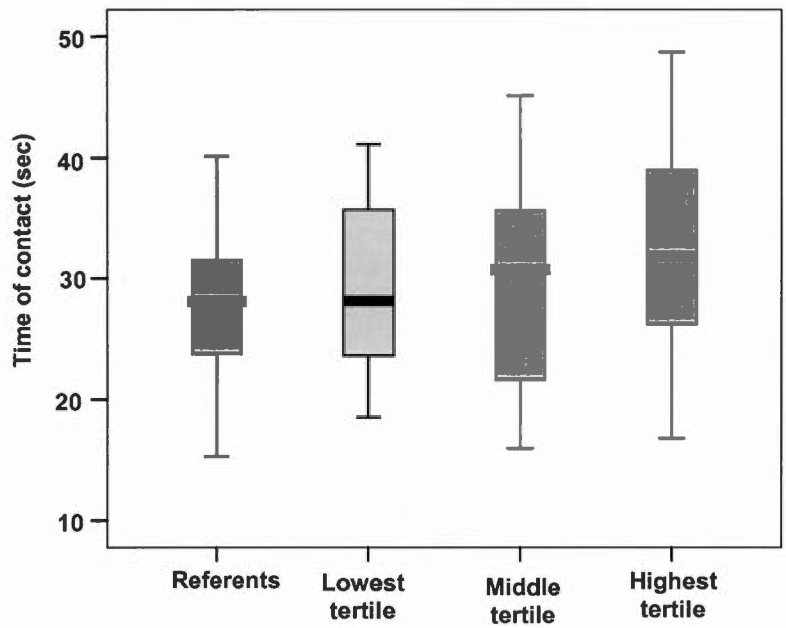
Changes in test score with respect to CEI tertiles was assessed using GLM, testing whether one tertile differed significantly from the referents' mean change in test scores, while including the same set of covariates as before. For the Luria Motor

Scale, the change in score of Mn-workers in the lowest CEI tertile differed significantly from the referents's ($p < 0.000$), showing improvement in performance. For the other tests, no clear pattern emerged.

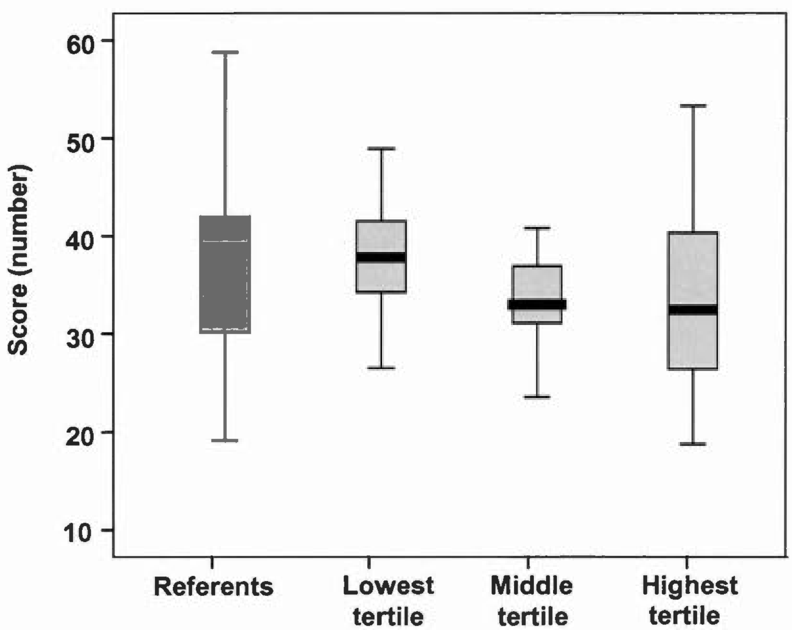
4.2a) Total score of the Luria Motor Scale



4.2b) Hand Steadiness Test



4.2c) Color-word trial of the Stroop Color-Word Test



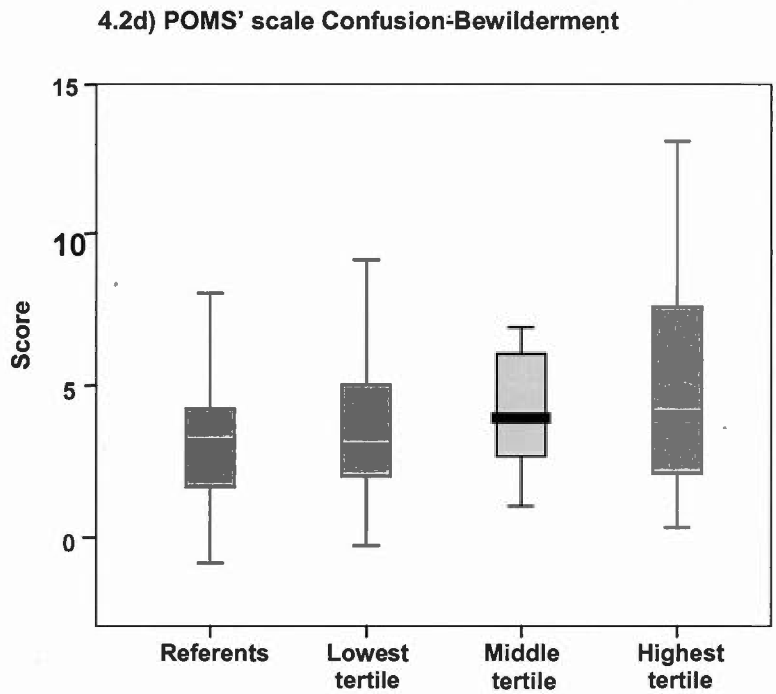


Figure 4.2: Scores at the follow-up examination (adjusted for age, education, alcohol consumption and smoking) with respect to tertiles of Cumulated Exposure Indices (referents: n = 68; lowest tertile: n = 23; middle tertile: n = 23; highest tertile: n = 23) and *p* values of the regression analyses. a) Total score of Luria Motor Scale (higher score indicate lower performance), $p < 0.01$; b) Hand Steadiness Test (time of contact) (higher score indicate lower performance), $p < 0.05$; c) Stroop Test (color-word trial) (higher score indicate higher performance), $p < 0.05$; and d) POMS' scale Confusion-Bewilderment (higher score indicate higher adverse mood states), $p < 0.01$.

4.4 Discussion

The follow-up examination of this cohort of Mn-alloy production workers revealed that, after 14 years without exposure, they performed similarly to the referents on a number of tests for which differences had been present at the initial examination, suggesting recovery of certain functions. However, a profile of deficits involving neuromotor and cognitive functions, as well as alterations of mood states remained. The formerly-Mn exposed workers still displayed significant deficits on the Motor Scale of the Luria, higher scores on the POMS Confusion-Bewilderment scale, and the older Mn-workers performed more poorly than referents of the same age on tests of cognitive flexibility, namely Symbol Digit Modalities Test, Trail Making B, and color-word trial of the Stroop Color-Word Test. Increasing cumulated exposure was significantly associated with poorer performances on several of these tests.

The deficits that persisted on the Luria Motor Scale included slowing of simple and alternate movements, and poorer quality of form drawing. However, differences were less pronounced than at the initial examination when workers were currently exposed, possibly indicating partial recovery. At follow-up, the deficits of former Mn-workers on hand stability seen at the initial examination had diminished, but decreasing hand stability was significantly associated with increasing level of cumulated exposure, suggesting that there were still discernible effects of past higher Mn exposure. Hochberg (1996) reported diminished coordinated movements in Chilean miners compared to a reference group, several years after cessation of exposure, but there was no data on status during exposure. Motor deficits, involving alternate movements and hand stability, have been repeatedly reported in cross-sectional studies of Mn-exposed workers [Iregren 1999] and are consistent with the involvement of the extrapyramidal system, which is affected by Mn [Pal et al., 1999].

Cognitive effects associated with low to moderate Mn exposure have been reported less consistently than neuromotor effects [Iregren 1999]. At the initial examination, deficits of cognitive flexibility deficits in Mn-workers were more marked with increasing age (Bouchard et al, 2005). Persistent deficits in cognitive flexibility, observed at follow-up, were only present for Mn-workers whose age was at least 45 years when exposure ceased. It is known that complex tasks requiring cognitive flexibility, with multiple steps processing are most sensitive to erosion with normal aging [Kray and Lindenberger 2000]. Mn-exposed workers' increasing deficits with older age might be explained by dwindling compensatory capacity [Weiss 2000].

In the present study, there was no evidence of delayed effects of Mn exposure on neurobehavioral functions, but this may be due to the fact that this cohort was not at a very advanced age. At the time of follow-up only 7 former Mn-workers were over 65 years, and of these, only 2 were over 70 years. As expected for persons in the age range of the study-cohort (mean age increased from 44 to 57 years), a decline in most of the neurobehavioral performances was observed between examinations. For most tests, former Mn-workers' decline in performance paralleled that of referents, but they had less of a decline for certain neuromotor and cognitive functions. Former Mn-workers had significantly reduced their alcohol consumption at follow-up compared with the initial examination, but this did not seem to explain their lesser decline in neurobehavioral performances.

The present study is the first to report changes in performances for an extensive set of neuromotor and cognitive functions and mood states among Mn-workers several years after cessation of Mn exposure. Two longitudinal studies reported changes in early neurobehavioral alterations after reduction in airborne exposure to Mn in the workplace [Lucchini et al., 1999; Roels et al., 1999], but none extended the follow-up period after complete cessation of exposure for a substantial number of individuals. Roels (1999) followed-up 92 workers from a dry alkaline battery plant during eight years following reduction in exposure to Mn dioxide. They observed no improvement of hand steadiness and reaction time deficits with exposure abatement or cessation, while the deficits of hand-forearm movement were partially reversible in workers with cumulated high exposure, and completely reversible in those with low exposure. Lucchini (1999) examined 30 workers from a ferro-alloy plant where exposure to Mn was reduced; they observed similar performances across the two examinations conducted five years apart for fingertapping, short term memory and cognitive flexibility. Unlike these studies in which exposure was reduced but not eliminated, in the present study, workers had been removed from Mn exposure for a long period, which seemed to allow for partial recuperation.

In the present study, recuperation of certain functions was manifested both by the absence of differences at follow-up for tests that were different at the initial examination, and a smaller difference between exposure groups for neuromotor tasks of the Luria Motor Scale at follow-up. Recovery to levels of functioning that are comparable to non-exposed referents of a similar age group, especially for those with the least cumulative exposure, suggests that there maybe be dose-related recuperation. It may be that continued exposure was responsible for the deficits observed at the initial examination, but once these workers were no longer exposed, the effects faded. This may be a similar phenomenon to recuperation from disturbances of dopaminergic activity induced by excessive alcohol consumption, following abstinence [Heinz et al., 2004].

The change in reported mood states between the initial and follow-up examinations showed a general improvement in affect in both exposure groups. This result may be explained by several factors related to changes in way of life with age (e.g. retirement, children left home, etc.). Nonetheless, as they did at the initial examination, former Mn-workers still reported more feelings of anger and hostility than referents at follow-up. Feelings of confusion, which were similar in both exposure groups at the initial examination, were reported more often by former Mn-workers at follow-up than by referents. The POMS' scale for confusion refers to cognitive ailments (i.e. difficulty concentrating), which are consistent with the deficits measured on complex cognitive tasks. Reported states of confusion, anger, and fatigue were associated with increasing levels of cumulative Mn exposure. The effect of cumulative exposure was also apparent on scores for neuropsychiatric symptoms, assessed with the Brief Symptom Inventory only at the follow-up study (Bouchard et al, in press). Mental health status may be particularly sensitive to long lasting effects of Mn exposure.

In addition to the effects on the nervous system, inhalation of Mn particulates may lead to an inflammatory response in the lungs [Saric and Piasek 2000]; higher rates of respiratory symptoms and pulmonary function impairment have been observed among workers exposed to airborne Mn [Boojar and Goodarzi 2002; Roels, et al. 1987]. The ten-fold increase in risk of respiratory problems among former Mn-workers observed in the present study suggests that adverse effects persist long after cessation of exposure, a finding that might warrant further investigations.

One limit of the present study is the possible bias of healthy workers effect, which has been demonstrated in many studies on various occupational exposures [Li and Sung 1999]. It is likely that those who were unable to adapt to the plant environment or those suffering from Mn-related neurotoxic effects may have left the workplace (either voluntarily of involuntarily) before the initial study; their inclusion could have influenced results for both initial and follow-up studies. Here, an additional concern is the implications of loss to follow-up. Selective attrition could bias the results, especially if the reasons for refusal are linked to Mn-related health effects. This might not be the case here, since Mn-workers who refused to participate did not report health problems. However, there was a difference between participants in follow-up and refusals, the latter had performed more poorly at the initial examination on motor tasks. Volunteers for epidemiological research are typically healthier than non-participants ("healthy volunteer effect") [Froom et al., 1999]. The selective attrition of poorer achievers was found in both exposure groups, thus, we believe this is not likely to result in a bias. Finally, another potential source bias might arise from differences in mortality. Although, the proportion of death and age at death were similar in both exposure groups, survival analyses should be conducted to ascertain any differences in the occurrence of mortality.

The strengths of the present study include a high follow-up rate, a follow-up period of long duration, a sufficient sample size, and carefully matched samples on age, education, lifestyle habits, geographic region and type of occupation. An extensive screening neurobehavioral test battery was used in order to accurately portray the neurofunctional profile of deficits associated with long-term exposure to Mn. Only three significant differences for the follow-up neurobehavioral tests scores should have been expected by chance at an alpha error of 5%, but eight such differences were found. Moreover, for most of the functions found to be affected among former Mn-exposed workers, poorer performances were associated with increasing cumulated exposure to Mn, a finding that would be highly unlikely in the case of a spurious association.

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CHAPITRE V

NEUROPSYCHIATRIC SYMPTOMS AND PAST MANGANESE EXPOSURE IN A FERRO-ALLOY PLANT

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Abstract

Introduction: Psychiatric manifestations have been reported in cases of manganism, and mood disorders are often observed in manganese (Mn) exposed workers. We examined neuropsychiatric symptoms among formerly Mn-exposed workers 14 years after cessation of exposure. Material and methods: A study was conducted in 1990 among workers from a ferro- and silico-Mn plant and unexposed working men referents from the same region in South-West Quebec. At follow-up in 2004, 77 former Mn-workers and 81 referents agreed to participate and responded to a neuropsychiatric symptom checklist, the Brief Symptom Inventory (BSI); scores were transformed into T-scores based on a normative population. Cumulated Exposure Indices (CEI) were computed for each former Mn-worker. Linear and logistic regression analyses were used. Results: Mean T-scores were significantly higher among former Mn-workers than referents on scales of Depression and Anxiety. Mean T-scores of psychological distress increased with the CEI tertiles, with significant associations for the scales Somatization, Depression, Anxiety and Hostility. Former Mn-workers in the two highest tertiles of CEI showed a higher risk for T-scores ≥ 63 for Hostility (OR, 7.5; 95%CI, 1.5 – 38.9), Depression (OR, 2.6; 95%CI, 1.1 – 8.4) and Anxiety (OR, 3.0; 95%CI, 1.1 – 8.4). Conclusion: These results suggest that past exposure to Mn may have lasting consequences on neuropsychiatric symptoms.

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Résumé

Introduction : Des manifestations psychiatriques ont été rapportés dans des cas de manganisme, et des changements de l'humeur sont souvent observés chez des travailleurs exposés au manganèse (Mn). Nous avons examiné les symptômes neuropsychiatriques chez des travailleurs, 14 ans après la fin de l'exposition au Mn. Matériel et Méthodes : Une étude a été conduite en 1990 chez des travailleurs d'une usine de production d'alliages ferro-Mn et silico-Mn et des hommes référents non-exposés de la même région du sud-ouest du Québec. Au suivi en 2004, 77 travailleurs ayant été exposé au Mn et 81 référents ont accepté de participer et n répondu à une liste de symptômes neuropsychiatriques, l'inventaire bref des Symptômes (IBS); les scores ont été transformés en scores-T sur la base d'une population de référence. Des indices cumulés d'exposition (ICE) ont été estimés pour chaque travailleur exposé. Des régressions linéaires et logistiques ont été utilisées. Résultats : Les scores-T moyens chez les travailleurs ayant été exposés au Mn étaient plus hauts que ceux des référents pour les échelles Dépression et Anxiété. Le score moyen de détresse psychologique augmentait avec les tertiles de ICE, et les associations étaient significatives pour les échelles Somatisation, Dépression, Anxiété et Hostilité. Les travailleurs ayant été exposés au Mn dans les deux tertiles supérieurs de ICE avaient un risque de scores-T $\geq$ 63 pour Hostilité (RC, 7.5; 95 % IC, 1.5 – 38.9), Dépression (RC, 2.6; 95 % IC, 1.1 – 8.4) et Anxiété (RC, 3.0; 95 % IC, 1.1 – 8.4). Conclusion : Ces résultats suggèrent que l'exposition passée au Mn puisse avoir des conséquences persistantes sur les symptômes neuropsychiatriques.

Mots clé: manganèse, exposition, symptômes neuropsychiatrique, Inventaire bref des symptômes

5.1 Introduction

Manganese is an essential element, but exposure to high levels of airborne Mn in industries, such as mining, welding, Mn alloy, and dry battery production, can produce manganism, a neurodegenerative extrapyramidal syndrome with psychiatric features (Levy and Nassetta, 2003). The first symptoms of overexposure to Mn are irritability, fatigue, headache, muscle cramps, loss of appetite, apathy, and disorders of sleep and libido (Rodier, 1955). In the early reports on Chilean miners, a peculiar acute psychiatric syndrome, termed "locura manganica" or "manganese madness", was described (Donaldson, 1987; Schuler et al., 1957). Characterized by compulsive or violent behaviours, emotional instability, disorientation, and hallucinations, this acute phase of intoxication could last for about a month and was clinically distinct from the chronic effects, which form a parkinsonian syndrome typified by muscular hypertonia, gait dysfunction with a propensity to fall backward, postural instability, bradykinesia, rigidity, micrographia, masked facies, speech disturbances, and muscle tremors (Abdel-Naby and Hassanein, 1965; Chandra et al., 1974; Huang et al., 1989; Penalver, 1955; Rodier, 1955; Schuler et al., 1957).

Lower levels of Mn exposure among active workers are associated with neurofunctional alterations characterised by neuromotor and cognitive deficits, as well as mood changes (Iregren, 1990; Lucchini et al., 1995; Mergler and Baldwin, 1997; Mergler et al., 1994; Roels et al., 1985; Roels et al., 1992; Roels et al., 1987). In a review of studies of occupational exposure to Mn, Bowler et al. (1999) identified six dimensions of psychological effects: 1) anxiety, nervousness, irritability; 2) psychotic experience; 3) emotional disturbance; 4) fatigue, lack of vigor, sleep disturbance; 5) impulsive-compulsive behaviour; and 6) aggression, hostility. Despite the importance of neuropsychiatric symptoms in mild and severe Mn intoxication, follow-up studies (Huang et al., 1998; Lucchini et al., 1999; Roels et al., 1999) have focused on the progression of neuromotor and/or cognitive deficits with little attention to mental health status.

Extensive human and animal data have shown high T1-weighted signal intensity on MRI, reflecting Mn accumulation in the basal ganglia, especially in the globus pallidus, among intoxicated humans and non-humans primates (Pal et al., 1999). Chronic exposure to Mn leads to selective dopaminergic dysfunction, neuronal loss, and gliosis in basal ganglia structures, with concurrent changes in astrocytes functioning. Alterations of content and metabolism of glutamate and γ-aminobutyric acid (GABA) activities have also been noted (Pal et al., 1999). A reduction in the content of serotonin in the putamen and globus pallidus was observed in non-human primates with chronic exposure to Mn (Eriksson et al., 1987). Deregulation of these neurotransmitter systems is known to play a role in several psychiatric diseases, including schizophrenia (Wassef et al., 2003), and in psychiatric symptoms of neurological diseases, such as Parkinson disease (Schrag, 2004). Although the mechanism of Mn toxicity and the resulting neurobiochemical changes are not completely understood, oxidative stress generated through mitochondrial perturbation is likely to be involved (Dobson et al., 2004). Unlike Parkinson's disease, Mn-induced damage probably occurs downstream of the nigrostriatal dopaminergic pathway (Shinotoh et al., 1997).

In 1990, our research team conducted a matched-pair, cross-sectional study on nervous system functions of workers from a Mn-alloy production plant (Mergler et al., 1994). The plant closed shortly after completion of the study, and in 2004, we conducted a follow-up the same cohort of formerly Mn-exposed workers and referents. The findings on neurobehavioral functions are reported elsewhere (Bouchard et al., accepted for publication). Here, we examine current neuropsychiatric symptoms in relation to past exposure to Mn.

5.2 Materials and Methods

5.2.1 Recruitment and Study Participants

The initial 1990 study was performed with exposed workers, who had been employed in a ferro- and silico-Mn production plant located in South-West Quebec; unexposed actively working men from the same region, with similar age and education level, served as referents. Details of the selection and recruitment of baseline-study participants is reported elsewhere (Mergler et al., 1994). Efforts were made to recruit as many as possible of the former Mn-exposed workers and referents into the present follow-up. Addresses of all baseline-study participants were verified in different telephone and address directories, and various strategies were used to locate those who had since moved. Table 5.1 presents the outline of participant response to recruitment. Of the 115 formerly Mn-exposed workers and 145 referents, tested at baseline, 11 (10%) workers and 9 referents (6%) had since died. Of the remaining men, contact addresses were obtained for 99 (95%) workers and 121 referents (92%) and letters were sent inviting them to participate in the follow-up study. A monetary compensation of $75 was offered for participation. Of these, 77 formerly Mn-exposed workers and 81 referents agreed to participate, resulting in a participation rate of 78% and 67% respectively (deceased and individuals not found are not included in the denominator). Informed consent was obtained from each participant and the Institutional Ethics Board (Université du Québec à Montréal) approved the study protocol.

Table 5.1: Participant recruitment

	Frequency (%)	
	Mn-exposed	Referents
Participated	77 (67 %)	81 (56 %)
Refused	22 (19 %)	40 (28 %)
Deceased	11 (10 %)	9 (6 %)
Not found	5 (4 %)	15 (10 %)
Total	115 (100 %)	145 (100 %)

Of the 22 former Mn-exposed workers who refused to participate, we were able to reach 18 to investigate their reasons for refusal and medical diagnoses; none reported nervous system ailments. The following reasons were given for refusal: seven were not interested, four had no time, two were living too far to come, and the five remaining stated that they were not available at that time.

5.2.2 Exclusions

Two former Mn-workers with less than two years of employment in the plant were excluded from the present analyses. Individuals who reported a diagnosed medical condition or accident that could have affected the nervous system, and was unlikely to be related to past Mn exposure, were excluded: 7 referents (2 strokes, 2 severe concussions, 1 senility, 1 multiple sclerosis) and 4 exposed (3 stokes, 1 multiple sclerosis). In addition, 4 referents who had been highly exposed to neurotoxicants after the baseline study were also excluded from the analyses. After exclusion, 71 referents and 71 former Mn-exposed workers were included in the analysis. Five referents and four former Mn-workers reported taking anti-depressive or anti-anxiety medication; these individuals were not excluded *a priori*, but their influence on the results was verified.

5.2.3 Data Collection

Information on work and medical history, medication, alcohol consumption and smoking habits, and exposure to neurotoxins was obtained using a questionnaire-based interview. Neuropsychiatric symptoms were assessed using a self-administered questionnaire, the Brief Symptom Inventory (BSI) (Derogatis, 1993). The BSI is a widely used instrument to assess the psychological profile of symptoms and is considered a useful screening tool in several contexts, both with clinical and general populations (Harper et al., 1990).

The French Canadian version (Fortin et al., 1989) of the BSI requires 8-10 minutes for completion; it is a multidimensional instrument composed of 53-item self-reported symptoms, experienced during the last seven days, rated on a five-point scale ranging from 0 ("Not at all") to 4 ("Extremely"). The BSI was designed to examine psychological symptom patterns of psychiatric and medical patients, but has also been validated on a stratified, randomly sampled group of 949 community non-patients. It provides a current point-in-time assessment of psychological distress. Scores are plotted on nine primary symptom dimension constructs:

- Somatization (SOM): Psychological distress arising from the perception of bodily dysfunction (e.g., cardiovascular, gastrointestinal, respiratory, and discomfort in gross musculature).
- Obsessive-Compulsive (OC): Thoughts and actions that are experienced as unremitting and irresistible by the patient but are unwanted (e.g., checking

and double checking actions, difficulty making decision, and trouble concentrating).

- Interpersonal Sensitivity (IS): Feelings of personal inadequacy and inferiority (e.g. self-deprecation, uneasiness, and discomfort during interpersonal interactions).

- Depression (DEP): Signs and symptoms of clinical depressive syndromes (e.g. dysphoric affect and mood, withdrawal of interest in life activities, and loss of energy).

- Anxiety (ANX): Symptoms associated with clinical manifestations of anxiety (e.g. restlessness, nervousness, and tension).

- Hostility (HOS): Hostile behavior including thoughts, feelings, and actions (e.g. annoyance, irritability, urges to break things and frequent arguments).

- Phobic Anxiety (PHO): Symptoms consistent with phobic anxiety states or agoraphobia (e.g. phobic fears of travel, open spaces, crowds, and public places).

- Paranoid Ideation (PAR): Paranoid behavior that is syndromal in nature (e.g. thoughts that are hostile, suspicious, and central).

- Psychoticism (PSY): Symptoms of psychoticism in mild forms (e.g. alien life style) to extreme forms (e.g. floridly psychotic states).

Three Global Indices of Distress are also computed to measure the level or depth of distress currently being experienced by the respondent:

- The General Severity Index (GSI): combines measures on the number of symptoms and the intensity of perceived distress. It is considered the single best indicator of current distress level.

- The Positive Symptom Total (PST): is a count of the symptoms that the patient reports.

- The Positive Symptom Distress Index (PSDI): is a pure intensity measure that does not include the number of symptoms.

The crude BSI scores were transformed into relative scores (T-scores, M = 50 and SD = 10) using the score distribution of a normative nonpsychiatric male population, according to the manual (Derogatis, 1993). The BSI manual suggests using T-score $\geq$ 63, which corresponds to the 90^{th} percentile of the normative population, as a definition of psychiatric "caseness". Thus, we classified individuals on each primary scale using this cut-off ($< 63/\geq 63$).

5.2.4 Exposure to Manganese

The Mn-alloy plant began its operations in 1973 and produced silico- and ferro-Mn alloys, containing 67% and up to 79% Mn, respectively. The process used a sealed submerged electric arc furnace, and workers were exposed to airborne Mn particulates (mixed oxides and salts) from the crushing and screening of the raw materials (pyrolusite ore with a content of 32-50% Mn) and from the alloy products, and to Mn fume from furnace tapping and cooling of the product in open bays. Exposure measurements were carried out in 1991 during silico-Mn production. The sampling included full-shift personal monitoring of the various job groups for total dust and Mn content in the dust. Co-located full-shift environmental samples of total dust and respirable dust were also collected at representative locations across the plant and analysed for Mn content. The eight-hour time-weighted average environmental measures of total Mn levels in dust ranged from 0.01 to 11.48 mg/m^3 (geometric mean: 0.23 mg/m^3) while the respirable Mn fraction ranged from 12-35% of total Mn (Baldwin et al., 1993).

The exposure history of each worker was reconstructed using several sources of information: personal and environmental sampling data from the 1990 survey, historical dust sampling data, company records relating to payroll and production, work history questionnaires completed at baseline study, and interviews with long-term employees (Baldwin et al., 1993). Exposure to Mn in total dust was estimated for each worker in the plant and for each year of employment, and the sum resulted in the Cumulated Exposure Indices (CEI) on the total work history in the plant. The CEI for each year of employment was divided by the number of days worked in the corresponding year to produce the average exposure intensity. Table 5.2 shows descriptive statistics on average exposure intensity and CEI for the total work history. Mean years of exposure to Mn was 15.7 years (7.4 to 17.3 years), and only one worker had less than 10 years of exposure to Mn.

Table 5.2: Tertile Cumulated Exposure Indices (CEI) and average exposure intensity

	Cumulated Exposure Indices ($mg\ Mn\ /m^3 .years$)		Average exposure intensity ($mg\ Mn\ /m^3$)	
	Mean (± SD) Median	Range	Mean (± SD) Median	Range
Lowest CEI tertile (n = 23)	6.0 (± 4.3) 6.2	0.3 – 11.8	0.39 (± 0.30) 0.35	0.02 – 0.91
Middle CEI tertile (n = 24)	19.5 (± 5.0) 19.0	12.0 – 30.4	1.29 (± 0.46) 1.17	0.71 – 2.65
Highest CEI tertile (n = 24)	58.2 (± 21.7) 55.0	31.2 – 100.2	3.84 (± 1.56) 3.48	1.98 – 6.20
All (n = 71)	27.4 (± 24.7) 19.0	0.3 – 100.2	1.79 (± 1.72) 1.14	0.02 – 6.20

5.2.5 Statistical Analysis

The statistical analyses were performed with SPSS 13.0 (SPSS, Inc., Chicago, IL), and the limit for statistical significance was set at $p < 0.05$, while p values between 0.05 and 0.1 are reported as trends. Statistical comparisons of general characteristics between formerly Mn-exposed workers and referents were performed with unpaired t test, or Pearson's *chi*-square test, for continuous and categorical data respectively.

The T-scores for each BSI scales were compared between exposure group (referents *versus* former Mn-workers) with General Linear Model (GLM) analysis, while controlling for age (years), education (years) and alcohol consumption grouped into six categories (0 g/week, 1 to 70 g/week, 71 to 140 g/week, 141 to 280 g/week, 281 to 420 g/week, and 421 to 500 g/week). Exposure-effect relations were assessed with multiple regression analyses. In order to include referents in this analysis, former Mn-exposed workers were grouped into three categories based on CEI tertiles and a dummy variable was entered in the model as continuous variable (0 = referents, 1 = lowest tertile, 2 = middle tertile, 3 = highest tertile) and the same covariates as above were entered in the model. Referents' estimated mean T-scores were compared to those of workers in each CEI tertile, using the method of *planned contrasts* that breaks down the variances accounted for by the model into component parts to avoid inflating Type I error (Field, 2000, p.325). Logistic regressions were performed to analyse the risk of caseness (T-score ≥ 63) on each BSI primary scale with regard to exposure to Mn. The same covariates as above were included in the models. The results were expressed as adjusted odds ratios (ORs), with 95% confidence intervals (CI).

5.3 Results

Table 5.3 presents the socio-demographic characteristics and alcohol consumption and smoking habits of the study population, and comparisons with respect to exposure status. There was no difference between former Mn-exposed workers and referents for age, education, marital status, alcohol and tobacco use. There was a trend for differences in occupational status, with a higher proportion of former Mn-workers unemployed or receiving Worker Compensation for disability (for reasons unrelated to Mn exposure), and a lower proportion working or retired.

Table 5.3: Characteristics of the study population and comparisons with respect to manganese exposure group

Characteristics	Referents (n = 71)	Mn-workers (n = 71)	p
Age (years)[1]	56.9 (± 7.2)	57.9 (± 5.4)	NS
Range	43.5 – 74.1	48.6 – 71.4	
Education (years)[1]	11.1 (± 2.4)	11.0 (± 1.7)	NS
Range	6.0 – 17.5	6.0 – 15.5	
Alcohol consumption (g/wk)[1]	129 (± 145)	149 (± 131)	NS
Range	0 – 594	0 – 1152	
Tobacco[2]			
Current	17 (24)	17 (24)	NS
Former	36 (51)	41 (58)	
Never	18 (25)	13 (18)	
Marital status[2]			
Married	54 (76)	47 (66)	
Divorced	10 (14)	16 (23)	NS
Widow	1 (1)	2 (3)	
Single	6 (9)	6 (9)	
Occupation[2]			
Working	47 (66.2)	33 (60.5)	
Unemployed	1 (1.4)	6 (8.5)	< 0.1
Disabled	0 (0)	4 (5.6)	
Retired	23 (32.4)	18 (25.4)	

[1] Mean (± SD); comparison with *t* test
[2] Frequency (%); comparison with chi-square
NS: not significant ($p > 0.1$)

Former Mn-workers had higher mean T-scores than referents on most BSI scales, indicating a higher degree of neuropsychiatric symptomatology, after controlling for covariates (age, education, and alcohol). The differences were significant for Depression and Anxiety scales ($p < 0.05$), while the differences for Somatisation and PSDI showed a tendency ($p < 0.1$). Table 5.4 presents the adjusted mean T-score for each BSI scale for the former Mn-exposed workers and referents.

Table 5.4: Comparison of Brief Symptom Inventory (BSI) adjusted T-scores with respect to manganese exposure group.

Primary scales	Mean (± SD) Referents (n = 71)		Mn-workers (n = 71)		p[1]
Somatization	54.3	(± 9.0)	57.2	(± 9.6)	0.07
Obsessive-Compulsive	54.0	(± 10.1)	56.4	(± 11.2)	NS
Interpersonal Sensitivity	50.1	(± 8.3)	51.6	(± 8.6)	NS
Depression	50.7	(± 8.0)	54.4	(± 9.7)	< 0.05
Anxiety	49.1	(± 8.9)	52.6	(± 10.6)	< 0.05
Hostility	49.3	(± 9.0)	51.4	(± 9.8)	NS
Phobic Anxiety	51.3	(± 7.1)	51.5	(± 7.3)	NS
Paranoid Ideation	52.4	(± 9.1)	53.9	(± 8.9)	NS
Psychotism	51.8	(± 7.8)	54.0	(± 9.0)	NS
Global Indices of Distress					
GSI[2]	51.4	(± 10.8)	54.4	(± 11.5)	NS
PST[3]	53.4	(± 11.1)	56.4	(± 11.3)	NS
PSDI[4]	47.4	(± 7.3)	49.6	(± 7.6)	0.08

[1] General Linear Model, while controlling for age, education, and alcohol consumption
[2] General Severity Index
[3] Positive Symptom Total
[4] Positive Symptom Distress Index

Since unemployment and disability can affect mental health and it was primarily the former Mn workers who were unemployed or disabled, BSI scale scores were examined for the former Mn-workers with respect to the occupational categories: working, unemployed or disabled and retired. Compared to currently working or retired and taking into account age, educational level and alcohol consumption, the unemployed and disabled had significantly higher scores on the scales of Obsession-Compulsion ($p < 0.01$), Depression ($p < 0.05$), Psychotism ($p < 0.05$) and a tendency for PST ($p < 0.1$). Inclusion of occupational category in the scale score comparison of former Mn-workers and referents did not change the outcomes.

Figure 5.1 shows the profile of the mean adjusted T-scores for each BSI scale with respect to the level of exposure expressed as tertiles of CEI. Overall, increasing CEI was associated with higher T-scores for most BSI scales. Cumulated Exposure Indices were significantly and positively associated with T-scores of Somatization ($p < 0.05$), Depression ($p < 0.01$), Anxiety ($p < 0.05$), and Hostility ($p < 0.05$). The T-scores of the three Global Indices of Distress were also positively associated with CEI, although the relations did not reach significance (GSI: $p = 0.07$; PST: $p = 0.08$, IDSP: $p = 0.06$). These relations were not affected by exclusion of individuals taking anti-depressive or anti-anxiety medication. Age, education and alcohol consumption were not significantly different between tertiles of exposure. The average intensity of exposure to Mn was not associated with adjusted BSI T-scores.

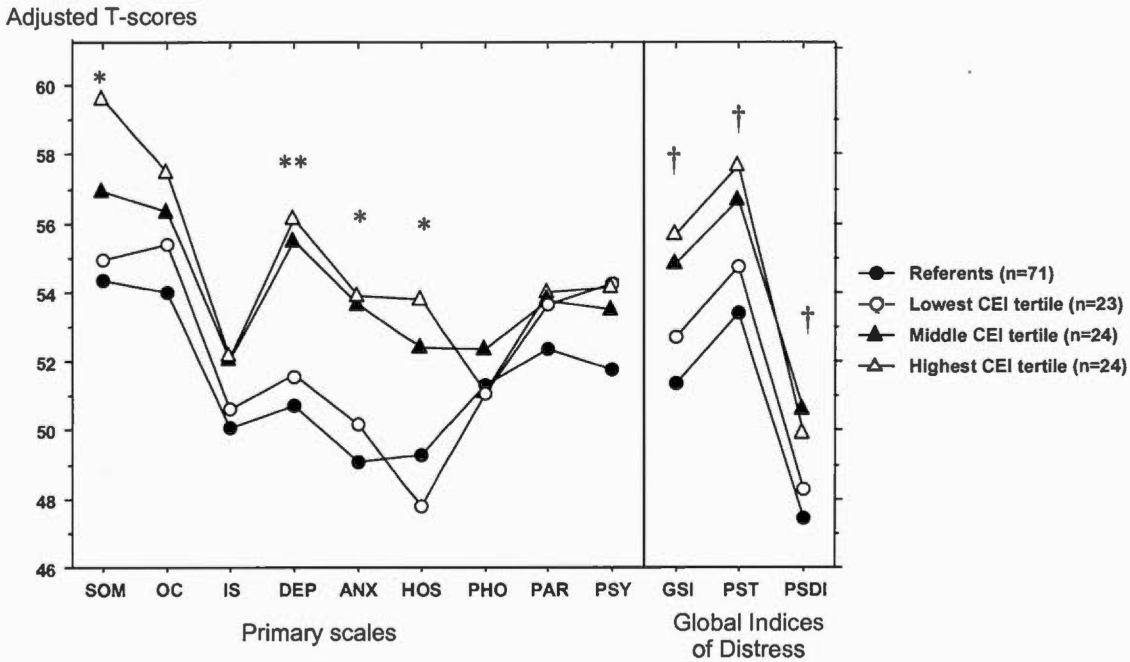

Figure 5.1: Profile of BSI scale T-scores adjusted for age, education, and alcohol, stratified by tertiles of cumulated exposure indices. Somatisation (SOM); Obsessive-Compulsive (OC); Interpersonal Sensitivity (IS); Depression (DEP); Anxiety (ANX); Hostility (HOS); Phobic Anxiety (PHO); Paranoid Ideation (PAR); Psychoticism (PSY); Global Severity Index (GSI); Positive Symptom Total (PST); and Positive Symptom Distress Index (PSDI). †: $p < 0.1$; *: $p < 0.05$; **: $p < 0.01$.

Exposure-effect relations were further explored by contrasting the means of T-scores of the BSI scales for each level of exposure with those of the referents, while controlling for covariates. Former Mn-workers in the highest CEI tertile had significantly higher T-scores than referents for the scales of Somatisation (B = 4.97, $p < 0.05$), Depression (B = 5.42, $p < 0.05$), Anxiety (B = 4.64, $p < 0.05$) and Hostility (B = 4.58, $p < 0.05$). Those in the middle tertile also had significantly higher T-scores than referents for Depression (B = 4.81, $p < 0.05$), and Anxiety (B = 4.68, $p < 0.05$). T-scores of former Mn-workers in the lowest tertile were not significantly different from those of referents.

Former Mn-exposed workers had a significantly increased risk of presenting an elevated T-score ($\geq$ 63) on the Hostility scale (OR, 6.2; 95%CI, 1.2 – 30.6) compared to referents. The middle and highest tertiles were combined, because of low number of elevated T-scores. The risk of presenting an elevated T-score for those in the two highest CEI tertiles was significantly increased for the Hostility scale (OR, 7.5; 95%CI, 1.5 – 38.9) compared to referents. Former Mn-workers in the two highest CEI tertiles also had significantly increased risk for elevated T-scores on the Depression (OR, 2.6; 95%CI, 1.1 – 6.4), and Anxiety scales (OR, 3.0; 95%CI, 1.1 – 8.4).

5.4 Discussion

Former Mn-alloy production workers displayed a higher degree of neuropsychiatric symptoms compared to a closely matched group of referents, 14 years after the end of occupational exposure. Overall, the degree of symptomatology increased with the level of exposure cumulated over the total length of employment in the Mn-alloy plant, with the strongest associations for the symptom scales of Somatization, Depression, Anxiety and Hostility. Furthermore, the former Mn-exposed workers with the highest levels of cumulated exposure had significantly increased risk of presenting elevated psychological distress for symptoms of Depression, Anxiety and Hostility. These findings suggest that, even after a long period of time without exposure, there are dose-dependant adverse effects on neuropsychiatric symptom reporting.

Because of their subjective nature, symptoms are often regarded as less reliable than other signs of health deterioration (e.g. neurological signs). In the present study, the profile of neuropsychiatric symptoms showed a consistent relationship to the magnitude of cumulated exposure. This type of relation refers to the existence of structure beyond simple differences between unexposed referents and exposed individuals, with increasing exposure reflected in increasing symptoms (Thompson and Myers, 2005), and is consensually regarded as a strong support for the hypothesis of a causality. It should be noted that the former Mn-exposed workers were not aware of their CEI and CEI was not related to seniority in the plant or age. Previous studies have shown that cumulative exposure indices constitute the exposure metric that has most been associated with Mn effects in working populations (Lucchini et al., 1999; Lucchini et al., 1995; Park et al., 2005; Roels et al., 1992).

The BSI is a widely used instrument for screening for neuropsychiatric symptoms. Although it focuses on symptoms felt during the past seven days, it provides an indication of overall mental status (Derogatis, 1993). It has been shown to be sensitive to the effects of Mn (Bowler et al., 1999) and the combined effects of alcohol consumption and Mn (Bouchard et al., 2003; Sassine et al., 2002), as well as to exposure to lead (Rhodes et al., 2003), solvents (Bowler et al., 2001) and pesticides (van Wendel de Joode et al., 2001; Wesseling et al., 2002). Similar to the present study, van Wendel de Joode and collaborators (2001) observed dose-related differences, assessed in terms of years of spraying, on BSI scores among retired DDT sprayers, at least ten years after cessation of exposure.

In the present study, twenty-two former Mn-exposed workers and forty referents refused to participate, resulting in a satisfactory participation rate (78% and 67% for former Mn-workers and referents, respectively). Nonetheless, cohort attrition is a factor that might limit the validity of our results; a bias would be expected if the reasons for refusal to participate were linked to Mn-related health effects. The consequences of cohort attrition in the present study are unknown, although former Mn-workers' reasons for refusal were not related to health concerns. In general, when no benefit is expected from participation in a study, participants are healthier than drop-outs (Froom et al., 1999).

The referents and former Mn workers were similar in age, educational level, alcohol consumption and marital status, however, the former Mn workers had undergone the stress of losing their job and, compared to the referents, there were proportionally more who were currently unemployed or disabled. Unemployment has often been associated with psychological distress (Brown et al., 2003; Liem, 1988; Murray et al., 2003; Thomas et al., 2005). Indeed, in the present study, those who were currently unemployed or disabled had significantly higher scores on several scales. It could be postulated that the stress of plant closure 14 years previously and the ensuing job instability might explain the differences in BSI profiles between the former Mn-workers and referents, but it would not explain why those in the highest CEI tertiles presented higher scores on certain scales, since there were no inter-tertile differences for socio-demographic variables, including current unemployment and disability. A possible limitation of the present study is that we did not assess common life stressors. However, there is no reason to believe that these would vary with respect to CEI. An alternative hypothesis is that high exposure to Mn was associated with unmeasured health problems, which would then increase the psychological distress.

The profile of neuropsychiatric symptoms observed in the present investigation is consistent with previous reports on occupationally and non-occupationally Mn-exposed populations. The expression of symptoms of irritability, depression and anxiety have been repeatedly reported in the literature on Mn-exposed workers without diagnosed manganism (Barrington et al., 1998; Bowler et al., 2003; Lucchini et al., 1999; Roels et al., 1987; Sinczuk-Walczak et al., 2001; Wennberg et al., 1991). In the present study, former Mn workers reported significantly more symptoms of hostility, including annoyance, irritability, urges to break things and frequent arguments. The association of Mn with hostile behaviours has been reported in several investigations carried out in different settings. In the early studies of Mn intoxicated miners, the perpetration of "stupid crimes" was noted (Penalver, 1955).

Likewise, decreased critical judgement was reported among workers from a Mn-alloy plant, with a concurrent five-fold increase in accident rate among exposed workers compared to referents (Jonderko, 1979). Finally, in a group of inmates, violent individuals were found have a significantly higher Mn concentration in hair compared to non-violent inmates (Gottschalk et al., 1991).

There is a growing literature on long term effects of neurotoxicants on nervous system functioning. Many years after cessation of occupational exposure, neurofunctional deficits have been associated with indicators of cumulative exposure for lead (Schwartz et al., 2000), mercury (Letz et al., 2000), solvents (Nilson et al., 2002), and DDT (van Wendel de Joode et al., 2001). The exposure-effect relations found in this study suggest that the neuropsychiatric symptoms assessed with the BSI might well represent a good indicator of long term effects of Mn exposure on certain neurotransmitter systems. Although the neurochemical regulation of emotions is far too complex to allow for simple one-to-one mappings between a particular neurotransmitter and specific psychiatric symptoms, certain associations have been made, e.g. dopaminergic activity and depressive feelings, serotoninergic activity and aggressive behaviour, GABA and anxiety (Smith et al., 2003). Very limited human data are available on neurochemical changes in Mn exposure, but the above mentioned neurotransmitter systems have been shown to be perturbed in several animal models (Dobson et al., 2004).

5.5 Conclusion

The findings of this study suggest that cumulative past exposure to Mn may be associated with mental health status later in life. Follow-up studies of long term effects of exposure to neurotoxic substances should include assessment of mental health, which may prove to be a very sensitive, although non-specific, reflection of persistent nervous system damage.

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CHAPITRE VI

DISCUSSION

Nos travaux ont permis de mettre en évidence un profil d'altérations neurofonctionnelles chez des travailleurs exposés au Mn pendant une durée moyenne de 15 ans dans une usine de production d'alliages de Mn. Au moment de la première étude, la concentration de Mn dans l'air de cette usine respectait la norme d'exposition, norme toujours en vigueur aujourd'hui. L'analyse des données a montré que les altérations neurofonctionnelles augmentent avec l'âge, et que ceci ne peut pas être expliqué par une exposition cumulée plus grande chez les plus âgés. Lors de l'étude de suivi menée 14 ans après la fin de l'exposition, les travailleurs plus âgés que 60 ans présentaient également des déficits significatifs aux tests de flexibilité cognitive par rapport aux référents alors que ceci n'était pas retrouvé chez les plus jeunes, ce qui suggère que l'âge confère une vulnérabilité aux effets neurotoxiques du Mn. Cela a également été observé chez la population résidant à proximité de l'usine employant les travailleurs exposés; seules les personnes âgées de plus de 50 ans présentaient des altérations neurofonctionnelles significatives associées à l'exposition au Mn (Beuter *et al.*, 1999; Bowler *et al.*, 1999; Mergler *et al.*, 1999). Les études expérimentales ont montré que la pharmacocinétique du Mn n'était pas sensiblement affectée par l'âge des animaux. La vulnérabilité pourrait plutôt s'expliquer par la diminution de la capacité de compensation du système nerveux avec l'âge.

Le suivi des travailleurs après la fin de l'exposition au Mn a permis d'enquêter sur l'importante question de la réversibilité de ses effets neurotoxiques. L'étude de suivi menée plusieurs années après la fin de l'exposition révèle deux trajectoires possibles : i) une conservation des déficits, laissant supposer que le déclin graduel de performance au cours des 14 années a été parallèle à celui des référents, et ii) une récupération pour les moins exposés, qui offrent une performance similaire aux référents plusieurs années après la fin de l'exposition. Notons cependant que l'absence de mesures des performances neurocomportementales entre 1990 et 2004 nous empêche de nous prononcer avec certitude sur les changements graduels de performance.

Au cours des dernières années, plusieurs études longitudinales ont été menées sur l'intégrité du système nerveux chez des travailleurs ayant été exposés à des substances neurotoxiques au cours de leur vie professionnelle. Plusieurs de ces études (Schwartz *et al.*, 2000; Nilson *et al.*, 2002; Stewart *et al.*, 2006) ont retenu l'hypothèse d'effet décalé (*delayed effect*), une hypothèse qui propose que l'exposition à des substances neurotoxiques puisse causer des dommages au système nerveux dont les effets passent inaperçus tant que la capacité de réserve (en neurones) est suffisante pour compenser ces dommages (Calne *et al.*, 1986; Grandjean, 1991, 1992; Weiss, 2006). Selon ce modèle théorique, les déficits subcliniques apparus durant la vie professionnelle active deviendraient davantage prononcés plus tard, alors que la capacité de réserve est réduite par l'âge (voir figure 6.1).

Sur la base de l'hypothèse d'effet décalé, ainsi que des altérations neurofonctionnelles prononcées observées chez les travailleurs plus âgés, nous avions émis l'hypothèse que les déficits seraient aggravés à l'étude de suivi par rapport à l'étude initiale. En effet, si on admet que l'exposition au Mn a causé des dommages structuraux au système nerveux central et que ces dommages sont reflétés par les performances aux tests neurocomportementaux, alors ces déficits devraient avoir été conservés plusieurs années même après la fin de l'exposition, et la perte neuronale normale associée au vieillissement s'y serait surimposée. Une autre observation soutenant l'hypothèse d'effet décalé serait l'aggravation graduelle des signes neurologiques chez des travailleurs atteints de manganisme, même après la fin de l'exposition (par exemple Huang *et al.*, 1998).

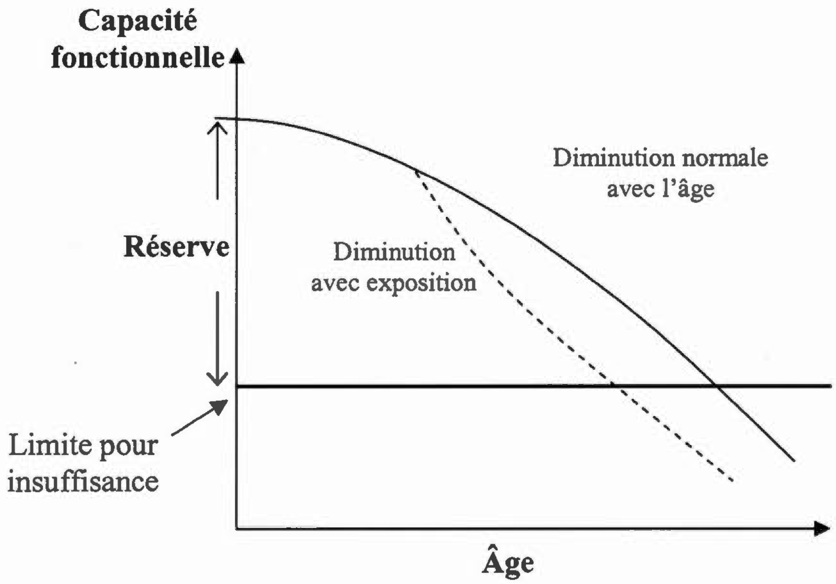

Figure 6.1 : Modèle hypothétique illustrant la perte normale de neurones et la capacité fonctionnelle en fonction de l'âge. Grâce à la capacité de réserve, cette attrition résulte normalement en des dysfonctions seulement à un âge avancé. Une exposition toxique peut causer un taux accéléré de perte cellulaire, mais après l'arrêt de l'exposition, un taux normal se rétablit. L'effet combiné de ces deux éléments résulte en l'apparition de dysfonctions à un âge moins avancé qu'il serait attendu normalement, quoique possiblement longtemps après la fin de l'exposition (tiré de Grandjean, 1991).

L'hypothèse d'effet décalé n'a pas pu être vérifiée dans notre cohorte, puisque les déficits apparus au moment de l'exposition se sont au pire maintenus, et qu'ils se sont même atténués pour certaines fonctions neurocomportementales. Nous proposons deux explications pour réconcilier les déficits plus prononcés chez les personnes plus âgées (soit les résultats de l'étude 1) avec l'absence d'effet décalé (soit les résultats de l'étude 2). Premièrement, il est possible que l'exposition au Mn ait causé des déséquilibres réversibles du fonctionnement cérébral, plutôt que des dommages structuraux irréversibles comme dans le modèle de Grandjean (1991). En effet, les changements neurobiochimiques précéderaient les changements structuraux dans l'intoxication au Mn (Dobson, Erikson et Aschner, 2004). Deuxièmement, il est possible qu'une proportion insuffisante de la cohorte ait atteint un âge assez avancé pour que la capacité de réserve soit épuisée et que les déficits se manifestent. En effet, la moyenne d'âge des travailleurs n'était pas très élevée même au moment du suivi (57 ans). Un autre suivi de la cohorte à un âge plus avancé, par exemple 65 ans, serait souhaitable pour connaître l'évolution des fonctions neurocomportementales au cours du vieillissement.

Les hommes ayant servi de référents aux travailleurs exposés au Mn résidaient à proximité de l'usine afin que l'exposition aux contaminants environnementaux autres que ceux de l'usine soit comparable dans les deux groupes. Une étude récente a montré que le sol aux alentours du site de l'usine aujourd'hui fermée est encore très contaminé, et l'air présente également une concentration de Mn anormalement élevée (Boudissa *et al.*, 2006). Ceci suggère que le groupe référent a lui aussi été exposé au Mn, quoique dans une mesure beaucoup moindre que les travailleurs de l'usine. Une association entre des tremblements et une concentration élevée de Mn sanguin chez les référents a d'ailleurs été montrée, ce qui semble corroborer l'hypothèse d'effets neurotoxiques chez les référents (Mergler et Baldwin, 1997). Cela aurait pour conséquence de diminuer la probabilité de trouver des effets chez les travailleurs exposés.

De nombreuses questions restent à être explorées afin de mieux comprendre la neurotoxicité du Mn et ainsi identifier les meilleures stratégies de prévention. Récemment, des effets neurotoxiques ont été observés chez des soudeurs à des niveaux d'exposition plus bas que ceux habituellement jugés néfastes (Park *et al.*, 2005). Ceci illustre la difficulté d'établir clairement un niveau d'exposition sécuritaire pour les travailleurs, en l'absence d'informations sur le potentiel toxique des différentes formes chimiques, états de valence et taille des particules de Mn. Dans le cas de la soudure, la composition des fumées émises lors de ce procédé inclut beaucoup de particules ultrafines de Mn, lesquelles peuvent être transportées le long des axones des neurones olfactifs et ainsi entrer directement dans le système nerveux central sans être régulées par les mécanismes homéostatiques (Elder *et al.*, 2006). Ceci pourrait expliquer l'apparition d'effets neurotoxiques à une concentration moindre que dans les autres contextes d'exposition où les caractéristiques des particules sont différentes. Finalement, bien que de nombreuses hypothèses aient été avancées quant aux mécanismes de toxicité du Mn (Verity, 1999; Crossgrove et Zheng, 2004; Taylor *et al.*, 2006), aucune de celles-ci n'a encore pu expliquer les dommages cérébraux observés chez les organismes intoxiqués (Taylor *et al.*, 2006). Il apparaît clairement que des efforts de recherche soutenus seront nécessaires afin d'élucider les nombreuses questions encore en suspens.

CONCLUSION

Nos travaux constituent une contribution originale aux connaissances sur les effets à long terme de l'exposition chronique au Mn par inhalation. Il apparaît que l'âge est une variable importante dans l'étude de la neurotoxicité du Mn qui devrait être prise en compte. Le suivi des travailleurs ayant été exposés au Mn a montré que même 14 ans après la fin de l'exposition, ceux-ci présentaient des performances neuromotrices et cognitives inférieures à celles de référents et rapportaient davantage de symptômes neuropsychiatriques. Les performances et les symptômes étaient associés au niveau d'exposition cumulée au Mn. Les déficits observés alors que les travailleurs étaient encore exposés au Mn ne se sont pas aggravés comme nous en avions émis l'hypothèse. En fait, la réversibilité de certains des effets, particulièrement chez les moins exposés, indique un potentiel d'amélioration de la santé des travailleurs suivant la diminution de l'exposition.

Au Québec, la norme maximale d'exposition (valeur d'exposition moyenne pondérée sur une période de 8 heures-VEMP) est de 5 mg/m^3 pour les poussières de Mn (RSST, 2003). Peu de données d'hygiène industrielle sont disponibles concernant l'exposition au Mn dans les différents milieux de travail à risque au Québec, mais les soudeurs et les travailleurs de fonderies sont probablement les groupes professionnels les plus concernés par ce problème (Ostiguy, Asselin et Malo, 2006). Un recensement partiel des milieux de travail à risque d'exposition au Mn a été effectué au Québec, et environ 5% des travailleurs seraient exposés à une concentration de Mn dans l'air surpassant la VEMP actuellement en vigueur au Québec (Ostiguy, Asselin et Malo, 2006). Cependant, cette proportion serait de 40% si on adoptait la recommandation de l'American Conference of Governmental Industrial Hygienists qui est de 0,2 mg/m^3 (ACGIH, 2004).

La concentration de Mn dans l'air de l'usine où étaient employés les travailleurs exposés au Mn a beaucoup varié au cours des années, mais nos résultats, et ceux d'autres chercheurs (Roels *et al.*, 1992; Lucchini *et al.*, 1995; Lucchini *et al.*, 1999; Sinczuk-Walczak, Jakubowski et Matczak, 2001; Park *et al.*, 2005), suggèrent que l'exposition cumulée constitue l'estimé d'exposition le plus déterminant pour les atteintes neurofonctionnelles. Conséquemment, les stratégies de prévention de ces atteintes, qui consistent surtout en l'établissement de normes maximales d'exposition, devraient tenir compte du potentiel d'exposition au Mn total cumulé au cours de la vie professionnelle. Dans la présente étude, l'exposition cumulée au Mn jusqu'à une valeur de 100 mg/m^3×année a été associée à des effets néfastes sur certaines fonctions neurocomportementales. Or, une personne exposée à la VEMP pendant 35 ans aurait un indice d'exposition cumulée de 175 mg/m^3×année, ce qui suggère que la norme actuelle d'exposition ne protège probablement pas la santé de tous les travailleurs. La persistance de certains des effets associés à l'exposition au Mn, même plusieurs années après la fin de l'exposition, devrait encourager l'établissement de mesures de prévention des effets.

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APPENDICE A

ÉTUDE BALDWIN ET AL.

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OCCUPATIONAL EXPOSURE TO AIRBORNE MANGANESE IN A MANGANESE ALLOY PLANT (1973-1991)

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ABSTRACT

A follow-up study of a group of manganese (Mn) alloy workers was conducted in 2004,14 years after exposure ceased, to evaluate the long-term effects of occupational Mn exposure on neuropsychological functions. The ferro- and silico-Mn alloy plant opened in 1973, and was closed in 1991. The airborne Mn exposure profile of this cohort of workers has been characterized, and cumulative exposure estimates developed. Mn exposures for various job groupings were established using personal and environmental sampling data from a 1991 industrial hygiene survey. Short-term dust data from historic hygiene reports were used to develop estimates of past dust exposure for the job groupings. Relationships between Mn content and total dust from the 1991 survey, together with sparse historic data on Mn content in dust measures were used to estimate Mn content (TMn) in the historic dust data.
Work histories for 112 workers were developed from payroll records, questionnaire data and interviews with plant personnel. These histories showed that many workers had complex exposure profiles; there was frequent job rotation among production workers, many work-related accidents, and a large number of workers interspersed periods of work with periods of lay-off depending on production levels. Although in 1991 the geometric means (GM) of Mn levels for all job groupings fell below the Quebec TLV-TWA for Mn at that time (Mn fume 1 mg/m^3, Mn in dust 5 mg/m^3), in earlier years exposure levels were extremely high. Typical results for jobs in crushing and furnace operations showed dust levels in the period prior to 1984 higher by a factor of five than those found in 1991. Changes in dust levels and corresponding TMn levels were a function of changes in ventilation, work practices and production

levels, not of product (ferro-or silico-Mn). Exposure data for the various job groupings and work histories were combined to develop various indices of exposure over time. The average occupational tenure in the Mn alloy plant for these 112 workers was 15.4 years (SD 2.17, range: 3.56 – 17.30). Their TMn CEI ranged from 0.27 mg/m^3 .years to 100.24 mg/m^3 .years, with AM 24.40 mg/m^3.years and GM 14.06 mg/m^3 .years. The fraction of TMn which was respirable (RMn) in a given area, determined from 1991 co-located environmental sampling data, was applied to the TMn estimates over time for all job groups within that area, and summed over the work history to provide a CEI of RMn in mg/m^3.years. The RMn CEI had AM of 2.95 mg/m^3 .years and GM 1.78 mg/m^3 .years with a range of 0.05 -12.03 mg/m^3.years. The exposure reconstruction for this cohort of workers was carried out for use with the follow-up study in 2004, which showed several concentration-response relationships between CEI and neuro-outcomes, thus suggesting that increase in cumulative exposure level has long-term consequences on the nervous system.

INTRODUCTION

Great variability has been observed in the occurrence and gravity of signs and symptoms of manganese (Mn) intoxication in relation to exposure; some workers who have been heavily exposed over a long period show no apparent signs of intoxication, while others manifest signs and symptoms following a moderate and short exposure[Mergler and Baldwin 1997]. While concentrations as low as 1 mg/m^3 have been reported to cause neurological signs of intoxication [for a review, see Iregren 1999], only a few studies have observed a dose-effect relationship [Lucchini et al., 1999; Lucchini et al., 1995; Roels et al., 1992]. A number of factors have been related to susceptibility to Mn neurotoxicity, including iron deficiency, alcohol intake, sex, respiratory and hepatic diseases or dysfunctions [Hudnell 1999]. These individual factors could help to explain the lack of significant exposure-response relationships in epidemiological studies of Mn-exposed workers, but the lack of adequate assessment of exposure over the total work history is likely to have obscured such relationships.

Mergler *et al.* [1994] conducted a study which documented early nervous system dysfunction associated with long-term Mn exposure in a group of workers employed in a Mn alloy production facility in south-western Quebec. A matched pair design was used with referents recruited from the same region, among actively working men with no history of exposure to neurotoxins. Results from that study showed that the pairs differed on symptom reporting, emotional state, motor functions, cognitive flexibility and olfactory perception threshold. Recent further analysis of the 1990-91 data-set [Bouchard et al., 2003] has shown that inter-pair differences between the Mn exposed workers and referents increased significantly with age for several responses, suggesting a possible age×Mn interaction.

Based on animal studies, Donaldson [1987] has suggested that Mn might accelerate aging. Kondakis et al. [1989] studied a group of persons over 50 years of age, exposed to Mn through drinking water and found a positive relationship between results from a standardized neurological examination using a rating scale and the Mn content of drinking water. In a population based study on environmental Mn exposure, Mergler et al. [1999] found an interaction between age and Mn for certain tests of cognitive and neuromotor performance, with poorest performance among those over 50 years of age and in the higher blood Mn category.

In 2004 a follow-up study of the Quebec Mn alloy workers and their referents was carried out to further explore the neurobehavioral and neurological effects of Mn after cessation of exposure as the alloy plant closed in mid -1991. The objective of the retrospective exposure assessment reported here was to characterize the Mn exposure profiles of the alloy workers during their years of employment, and to develop exposure indices for use in the 2004 follow-up study.

Background description of plant, process

The target population for the original study was a group of workers who were employed in a large ferro- and silico-Mn alloy facility situated in Southwestern Quebec [Mergler, et al. 1994]. The alloys were produced by a reduction process, using metallurgical coke and pyrolusite ore, carried out in a sealed submerged electric arc reduction furnace, with two level tap holes, and direct layer casting of the metal and slag. High carbon ferro-Mn alloy containing up to 79% Mn was the major product, while silico-Mn alloy, containing about 67% Mn was produced in smaller quantities. The furnace operated continuously, and the production process exposed workers to both Mn containing dust from the raw material handling and product crushing operations, and Mn fume during tapping and casting of the molten metal product. Due to the production process at this particular facility, work operations took place in several distinct areas, each characterised by different exposures: the raw materials handling yard, the furnace floor, the crushing and end product handling area, and a maintenance workshop located in a separate building some distance from the plant. Some maintenance operations were carried out in this workshop, while some tradesmen (electricians, welders and fitters) worked on roving teams making on the spot repairs as required.

The plant was built in 1972-1973 and began operations in the fall of 1973. Very little changed in the main organisation of work activities during the first nine years of operation according to plant sources. There were two strikes, one of 6-months in 1976, and one of 9-months in 1978-79. A major accident caused the shutdown of the furnace for nine months in 1979-80, but rebuilding was to the same specifications and production levels before and after the shutdown were similar. In 1982, an adjacent silicon plant belonging to the same company was closed down, which lead to personnel reorganization and displacement. In 1988 the sinter operation, a step in the raw materials preparation for the furnace, reported to have been a major dust contributor, was shut down. During the period 1988-90 a number of ventilation modifications were made in the furnace area to improve working conditions. Thus, all evidence suggested that Mn and dust exposure in earlier years was substantially higher than at the time of the 1990-91 study. Several months following this study, the facility was closed down permanently.

METHODS

Resources for reconstruction of Mn exposure

Reconstruction of Mn exposure levels for the cohort was based on the industrial hygiene survey carried out in conjunction with the 1990-91 study. This survey provided both full-shift personal and area samples, which were analyzed for both total dust (TDust) and Mn content in the dust (TMn). These data were used to characterize job exposure groups and plant areas at that time. To reconstruct past Mn exposure at this plant, in addition to the data gathered in the 1991 survey, the following data were available: payroll records dating back to 1973 when operations commenced; work history questionnaires completed by the cohort; some production records; historic industrial hygiene reports; and plant information from interviews with long-term workers, many of whom had been working since the commencement of operations. The historical hygiene reports located contained mostly short-term TDust measurements, with a few more recent full-shift surveys of the furnace area.

A total of 114 employed in the plant agreed to participate and informed consent was obtained from each participant and the Institutional Ethics Board from the Université du Québec à Montréal approved the study protocol.

The following strategy was used. Individual work histories for 112 workers who had been with the company for two or more years were developed from the payroll records, supplemented by the work history questionnaires of the 1990-91 study and interviews. Past dust level data from the hygiene reports and plant history were used to develop a profile of past dust exposure levels relative to the 1991 survey. Information on the relationship between dust levels and Mn content in the dust from the 1991 survey was used to interpret historic dust levels together with the sparse data on past Mn levels in the dust, and obtain an estimate of past Mn exposure for each of the job groupings over time.

The 1991 area sampling data examined the relationship between respirable Mn (RMn) and TMn in various sectors of the plant. Since there was no personal sampling for RMn, these data were used to estimate the RMn fraction for the job groupings by their work location.

Sampling Strategy of the 1991 Survey

At the time of the 1990-91 study, both full-shift personal samples and environmental area samples were collected over a 27-day period in March and April of 1991, during silico-Mn production. The initial plan had been to sample during both SiMn and FeMn production, but after the study had commenced a decision was made by the company to cease production after the silico-Mn run, and close down the plant. The furnace remained in operation until two months after the sampling was completed, and during the sampling period production levels were consistent with recent years, so that the sampling was representative of active production.

Personal Sampling

The personal sampling strategy sought to characterize Mn exposure levels of the various jobs in the plant into groupings of similar levels of exposure within areas with similar dust characteristics. The development of the initial job groupings for sampling followed plant visits, discussions with the workers, and with representatives of the parity health and safety committee, and took into account their perceptions of the relative dustiness of jobs and activities. The job groupings for sampling were defined by a combination of process operations, task description, job location, and shift length and were agreed to by all participants. Sampling was random within the defined groups, within the constraints of the study and the schedule of the workers. Priority was given to characterising the production job groupings identified by the workers as of concern because of high exposure. The number of samples collected for maintenance workers was smaller than had been originally planned, as in those groups where normal activities were reduced because of the projected plant closure, fewer representative samples could be collected. On completion of the sampling, more precise regrouping was made, based on the data and observation of tasks.

Area Sampling

Preliminary information had indicated the existence of sampling data from past years, mostly TDust measures, where the majority of samples gathered were area samples. To use this historic TDust data and to relate the personal job exposures to the areas where the jobs were located, area environmental samples at fixed locations were also collected. These data were used to establish the relationship between TDust and Mn content in the dust in the four distinct areas: the raw materials yard, the furnace floor, the crushing operation, and the maintenance building.

The area sampling was also used to investigate the "respirable" fraction of the dust and its Mn content. Co-located samples were collected simultaneously, one using a regular filter cassette, the other equipped with a 10mm Dorr-Oliver nylon cyclone placed in series with the filter cassette to selectively collect smaller particulates and provide an estimate of the "respirable" component of the dust, and its Mn content (RMn).

1991 Sampling and analysis procedures

Personal Sampling

Full shift dust samples were collected in the breathing zone using Gillian portable pumps, equipped with 38mm cassettes, with matched weight 0.08 µm cellulose acetate filters. In a few instances in heavy dust areas, two consecutive samples were collected to cover the shift. Air was sampled at a rate of 2.0 L/minute and pumps were calibrated before and after each sampling period, temperature and barometric pressure were measured. Data sheets of observations on the activities of the sampled workers were maintained.

Area sampling

Full-shift co-located dust samples were collected at three fixed locations on three days in each of the four areas previously designated, and in the furnace team room. The first sampler collected TDust, while a second co-located sampler used a 10mm Dorr-Oliver cyclone placed in series to selectively collect smaller particulates; these cyclones are 90% efficient for particles ≤10µm diameter. The samplers were suspended at breathing zone height. The three paired samples for a given area on a given day were collected during the same shift, while the three sampling shifts were randomly chosen within the sampling period.

Sample Analysis

All analyses were carried out at the Scientific and Industrial Research Unit Laboratory, Concordia University. Dust was determined gravimetrically, using matched weight filters. The mass was determined using an AUD balance model ER-180A; the detection limit was 0.001 mg. Following acid digestion of the filter, Mn content was determined by atomic absorption spectrometry (Varian AA875) using NIOSH Method 173 for Manganese, with a detection limit of 0.002 µg/mL for Mn. The precision of NIOSH Method 173 is approximately 10%. In instances where the result was a non-detectable value, one-half the limit of detection was used as a nominal value.

Description of Historical Industrial Hygiene Database

Industrial hygiene reports at this plant only commenced in the period following the introduction of health and safety legislation in Quebec in 1978; although Mn operations began in 1973, the earliest report located was from 1978. The historical industrial hygiene reports fell into two groups: those containing short-term TDust sampling data, both personal and area, and several more recent studies of the furnace team with full-shift personal sampling.

Short-term total dust sampling data

This group of reports contained both personal and area samples. These reports were all in-house work, the earliest from 1978, the purpose of which was to characterise jobs and operations, and to respond to complaints regarding dust levels raised by the union in the health and safety committee. The practice was to take short-term 15-20 minute dust samples, determined gravimetrically, both personal, characterising particular worker operations, and environmental at defined locations. More measures were taken of what were perceived as the more dusty operations. Estimation of Mn in these reports was mainly by default using the value in the ore and product samples, with very occasional laboratory determinations of Mn in the measured dust.

From each report the following information was extracted and entered into a computerized database: sample result, report number (which documented the reason for the sampling), sample description (job/location), sample type (area or personal) date of sampling, sample duration, sampling rate, production product at the time of sampling (FeMn or SiMn). Samples were excluded if they were insufficiently documented, or if they had been collected on visitors, as opposed to regular workers and tasks. From these reports a database was created for the furnace area, raw materials area, crushing department, the sinter plant and maintenance operations. A total of 969 dust measurements were included. Some samples were short-term measurements taken on a single worker or at a single location on the same day. Repeat sampling was frequently used to characterize the exposure of a worker during specific and representative activities, or at specific environmental locations. Measurements taken on the same day, which represented the exposure of an individual worker or at a given location were averaged so that only a single exposure estimate was included for any individual worker or area location on a given day. The final dataset used for the short-term dust measures consisted of 248 individual exposure estimates (54.6%) and 206 area estimates (45.4%).

Full-Shift Furnace Studies

Three earlier industrial hygiene surveys which measured both TDust and TMn contained full-shift personal sampling data on the furnace team: a survey in May 1989 during SiMn production (n = 24), one in July 1988 during FeMn production (n=18) and a third very small survey in 1987 (n = 3) during FeMn production. In 1991 the full shift personal sampling was carried out during the production of SiMn, and included 14 samples of furnace workers occupying the same positions as the earlier surveys. These data were examined to investigate changes in the exposure levels related to documented ventilation changes made following the 1988 survey, and to examine exposure differences for the furnace team between FeMn and SiMn production.

Statistical Analysis

An examination of data plots and calculation of the Shapiro Wilk's statistic showed that all sets of dust sampling data were lognormally distributed, and statistical testing was carried out on the logtransformed data. Statistical analyses on the measurement data were performed using the SAS statistical package. The limit for statistical significance was set at $p < 0.05$.

Data for job groups from the 1991 survey were combined under the following conditions:
1. No significant difference between the means of the log-transformed data and equal variance for the job groups being combined.
2. The criteria above held for the results of both TDust and TMn content in the dust. TMn was predicted to be different in different locations of the plant while the TDust could be similar.
3. Shift length was comparable for the job groupings being combined.

Analysis of variance models (ANOVA) were used to evaluate the effects of various factors on dust concentrations. Personal TDust levels were examined for the various job groupings over time; jobs which had been abolished were characterized in relation to those which had continued, and various factors which could influence past dust levels were evaluated: production product, changes between reporting periods which might relate to documented plant changes, and job changes. Tukey's multiple comparison *t* tests were used to examine differences between factor levels. The environmental TDust data were used to confirm the background level in the work areas in relation to job exposures, and to evaluate changes over time in relation to plant history.

Construction of work histories

The payroll records from which the work histories were obtained did not describe the jobs in the same terms as the industrial hygiene records. Departments were clearly designated, and corresponded to defined work areas and process activities, and remained well defined over the total time period. The payroll records also indicated changes in status - illness, work accidents and periods absent on workers compensation, layoffs and rehires, regression, and transfers between departments. Some distinctions as to job activities were not apparent from these payroll records, although they were identified in the hygiene reports.
Interviews with company personnel and older employees provided valuable information and background for the interpretation of these records, together with the

work history questionnaire data supplied by all the participants in the 1990-91 study. A concordance was developed between the payroll records and the hygiene reports to describe the work history in terms of job groupings developed from the 1991 survey data, which were used to code the historic reports for work history, adding codes for additional defined positions and groupings which no longer existed in 1991.

Development of Exposure Indices

The arithmetic mean (AM) of measurements rather than the geometric mean (GM) was selected as the exposure measure for each job group in developing exposure indices. The AM is generally considered a more adequate measure of chronic exposure than the GM, as it is more sensitive to contributions of the heavily exposed workers in the right tail of the distribution [Crump 1998]. Given that the earliest reports located were from 1978, the assumption was made, based on discussion with older employees, that conditions were similar in the preceding period 1973-78, when the plant was operating, and the earliest values for job groupings were back extrapolated to 1973.

Work histories were combined with Mn exposure estimates over defined time periods for jobs held during that time period, and summed over the entire work history to estimate a cumulative exposure index (CEI) of TMn [units of (mg/m^3) x years or mg/m^3 .years] for each subject.

An average Mn exposure estimate (AvTMn, mg/m^3) was calculated from the TMn CEI for each worker divided by time in years actively worked to provide a measure of mean exposure intensity across all jobs held. The average Mn exposure intensity was also computed for all workers for each year of operation.

The fraction of TMn which was respirable (RMn) in a given area, determined from the 1991 co-located environmental sampling, was applied to the TMn estimates over time for all job groups within that area, and summed over the work history to provide a CEI of RMn in mg/m^3.years.

RESULTS

1991 Personal Sampling and Job Groups

Table 1 shows the time-weighted average shift measures of TDust TMn results for the various job groups defined through the personal sampling and observations of tasks. Some maintenance workers who worked across the plant were the most highly exposed; data were subdivided by trade, separating electricians, fitters and welders. Fewer samples were collected for these trades, as sampling took place only on days when regular maintenance tasks were being carried out. As anticipated, the furnace team and crushing workers had higher levels of Mn exposure than other production job groups. The combined group of control room and equipment operators working in air-conditioned cabins had the lowest Mn exposure. Outside workers in the raw materials area had higher TDust exposure levels, although their TMn level was similar.

Table 1: Airborne Dust (TDust) and Mn concentrations in the dust (TMn) for 1991 job exposure groups

Job Exposure group	No. of samples	Sample type	AM (mg/m^3)	GM (mg/m^3)	GSD	Range (mg/m^3)
Furnace team	20	TMn	0.268	0.236	1.69	0.098 - 0.664
		TDust	0.923	0.856	1.47	0.378 –1.791
Crushing group	14	TMn	0.384	0.319	1.85	0.125 - 1.044
		TDust	0.942	0.792	1.86	0.227 – 2.669
Maintenance						
Electricians	4	TMn	1.005	0.617	3.28	0.143 - 2.640
		TDust	3.652	2.497	2.76	0.770 – 9.052
Fitters	3	TMn	0.516	0.366	2.65	0.197 - 1.130
		TDust	1.903	1.708	1.74	1.098 – 3.189
Welders	5	TMn	0.196	0.182	1.53	0.102 - 0.324
		TDust	1.278	1.119	1.90	0.304 – 1.838
Raw materials group	6	TMn	0.039	0.035	1.69	0.016 - 0.063
		TDust	0.418	0.339	2.06	0.147 – 0.960
Workshop group	5	TMn	0.097	0.074	2.42	0.019 - 0.217
		TDust	0.432	0.279	2.85	0.099 – 1.151
Control room operators	11	TMn	0.026	0.024	1.58	0.011 - 0.039
		TDust	0.176	0.117	2.78	0.035 - 0.373

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation

1991 Area Environmental Sampling

Table 2 shows the results of the co-located area sampling. The 8-hour time-weighted average environmental measures of TMn levels in dust ranged from 0.014 to 11.48 mg/m^3 (geometric mean: 0.225 mg/m^3, n =38) while RMn levels ranged from 0.001 to 1.273 mg/m^3 (GM 0.035 mg/m^3, n =37).

Table 2: 1991 Environmental levels of dust and manganese (total and respirable fraction).

Plant Location	n	AM mg/m3	GM mg/m3	GSD	Range mg/m3
Crushing					
TDust	9	6.728	4.123	2.77	0.790 – 25.348
RDust	9	0.694	0.397	3.14	0.065 – 2.733
TMn	9	2.861	1.592	3.11	0.250 – 11.482
RMn	9	0.304	0.184	2.75	0.048 – 1.273
Furnace					
TDust	9	5.163	3.167	2.37	1.565 – 25.386
RDust	8	0.474	0.267	3.48	0.064 – 1.489
TMn	9	1.923	0.926	3.10	0.171 – 10.520
RMn	8	0.172	0.117	2.52	0.032 – 0.568
Furnace team room					
TDust	3	0.293	0.239	2.17	0.120 – 0.554
RDust	3	0.090	0.083	1.58	0.063 – 0.142
TMn	3	0.054	0.050	1.66	0.028 – 0.070
RMn	3	0.009	0.008	1.34	0.006 – 0.010
Raw materials					
TDust	8	1.913	0.370	7.91	0.056 – 10.675
RDust	8	0.323	0.130	3.57	0.065 – 1.615
TMn	8	0.193	0.089	2.97	0.037 – 1.067
RMn	8	0.037	0.020	2.83	0.007 – 0.175
Workshop					
TDust	9	0.236	0.182	2.22	0.056 – 0.650
RDust	9	0.132	0.094	2.14	0.062 – 0.483
TMn	9	0.032	0.029	1.54	0.014 – 0.056
RMn	9	0.009	0.006	2.58	0.001 – 0.030

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation
TDust: total dust; RDust: respirable dust; TMn: total manganese; RMn: respirable manganese

Although the RMn level in the crushing area was higher than in the furnace area, there was no significant difference between the furnace and the crushing areas (Tukey tests), but these two areas differed significantly ($p < 0.05$) from the raw materials area and the workshop. The ratio of the GM of RMn to TMn indicates that in the furnace and crushing areas 10 – 13 % of the total Mn is from particulates with diameter ≤ 10 μm. In the raw materials yard and workshop area, the TMn exposure level is much lower, but RMn represents a higher fraction, 22% in the raw materials yard.

The area sampling results are of interest in relation to the personal sampling results of the job groups in Table 1. At the time of the 1991 study, the furnace team had intermittent high exposure during furnace tapping, followed by rest periods in an adjacent air-conditioned room on the furnace floor. From observations during sampling, about a quarter of the furnace team's time was spent on operations on the furnace floor, and their personal sampling Mn level (GM 0.236 mg/m^3) reflected this time distribution between locations. The workers in the crushing and product department spent the major portion of their time in closed cabins of the heavy equipment and their personal exposure levels are substantially lower than the area results. In contrast, the personal exposure measures of workers in the raw materials area and the maintenance workshop resemble more closely the environmental data. The elevated levels of dust and Mn as well as the wide range of values in the furnace and crushing areas account for the high levels of personal exposure found for some of the skilled trade maintenance workers.

Historic short-term data

For all departments where short-term dust data had been gathered during production of both Fe-Mn and Si-Mn, product was not a significant contributor to variance in TDust levels, for either the personal or the environmental samples.

Within departments, for the years covered by short-term dust sampling reports, conditions remained fairly stable over time. Dust levels across the plant were very high until 1988 when the sinter operation was closed, and a series of modifications were made to the furnace ventilation system. Job description was the most important factor in determining dust level variability. Where time period changes for specific groups of workers occurred, these coincided with documented process modifications and/or work practice changes.

Sinter Operation:

A computer controlled sinter operation had been used to agglomerate the fines from crushing and screening for use as feed in the smelting process, providing maximum usage of the raw material. A series of well-documented studies conducted between 1978 and 1984 supported that this had been a very dusty operation. Extensive environmental sampling in all sectors of the sinter operation had been carried out to characterise operations under varying conditions. Dust results for the sinter operation, and raw materials department, and crushing department are shown in **Table 3**.

Table 3. Dust levels (TDust) in the sinter operation, raw materials department and crushing department

Sample type	n	AM (mg/m^3)	GM (mg/m^3)	GSD	Range (mg/m^3)
Sinter operation					
Personal					
Operator	8	3.30	1.815	4.01	0.10 – 11.6
Asst. operator & worker	24	27.64	18.36	1.77	1.77 – 109.6
Environmental	109	21.14	8.76	3.284	0.10 - 750
Raw materials department $					
Personal					
Equipment operator	8	2.92	1.24	3.53	0.37 – 15.30
General worker	6	11.13	9.15	1.97	3.52 – 26.28
Environmental	11	8.27	7.16	1.77	2.71 – 16.11
Crushing department					
Personal					
Crushing group	99	3.291	1.511	3.72	0.04 – 37.80
Hopper worker	9	6.743	3.887	3.79	0.40 – 17.50
Environmental	36	5.686	2.691	3.97	0.11 – 38.76

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation
$ Measures1980-82

Despite recommendations for changes in the hygiene reports, none appear to have been made as there were no significant changes in dust levels over time. Three jobs were defined in the reports, operator, assistant operator and general worker. Job was a highly significant contributor to measurement variance, ($R^2 = 0.50$, $p < 0.005$); the job of operator differed from those of assistant operator and general worker, but these two did not differ from each other (Tukey tests). The main tasks of these latter two positions were to unblock the conveyors, trouble-shoot malfunctions and clean-up using compressed air. An extensive report in 1982, which gave good descriptions of the job tasks and conditions of work, supported these overall results. The assistant operator's job was described thus: *"By the nature of his work, this employee is almost always over exposed to dust, since his tasks are carried out directly at the sources,* *either in taking tests of the weight, unblocking chutes, releasing the conveyors or emptying the cyclones, etc."* while for the general worker *"This employee is principally occupied with cleaning. He is often over exposed, this being tied to the tools used and the manner of carrying out the cleaning (brusque, fast). The highest value samples were caused by blowing compressed air."* The results of the environmental sampling supported the very high personal levels found.

Raw materials Department

Prior to closure of the sinter operation dust levels in the raw materials yard were very high – see Table 3.

Crushing Department

The historic job position hopper attendant had been abolished by 1990. Preliminary analysis of the crushing department data showed that this job had the highest dust exposure level and the dustiness of this job was confirmed by discussion with the Safety Officer. Since this job did not contribute to the 1991 exposure data, and was clearly defined in both the work histories and the hygiene reports, data for this position were considered separately in order to compare the changes over time for the continuing jobs within the group. Analysis by job group for all other positions in the Crushing Department showed that there was no significant contribution to measurement variance from different job positions within that department so data for all other positions were combined. Dust levels showed no time trend over the earlier reporting periods; substantive changes in work practice were introduced in 1988. While personal sampling values for TDust levels were substantially higher in the past than in the 1991 survey for this area, it should be noted that environmental TDust levels are similar see **Table 2**. These results supported that the more recent changes in equipment and work practices had been effective in substantially reducing personal dust exposure levels.

The 1991 personal sampling data for the crushing group showed a strong linear relationship between the TMn level and the TDust level; Mn represented 41.3% of the TDust, ($F = 182.74$, $p = 0.0001$, $R^2 = 0.938$, $n = 14$). Very few of the historic dust samples were analysed for Mn content, but the few measured values of Mn in the crushing area ranged from 38% to 45% of the TDust, which is in reasonable agreement, supporting that the relationship holds for the historic data in this area.

Furnace studies

The full-shift personal sampling data from the furnace floor surveys were examined to investigate whether there had been changes in the exposure levels during the Si-Mn process since 1988 following modifications to the ventilation, and also the exposure differences between Fe-Mn and Si-Mn production. The summary statistics for the three major furnace studies are shown in **Table 4**. The levels had decreased significantly in 1991 from those in 1988, corresponding to documented ventilation modifications and work practice changes in the furnace area. The 1991 survey results fell in a narrower range, and were significantly lower ($p < 0.05$) than the results found in the 1989 study during Si-Mn production, confirming that the modifications to the workplace, and to work practices following the 1989 survey had brought the process into even tighter control. The relationship between TDust and TMn was similar for both Fe-Mn and Si-Mn production, and may be described:

$$\text{Ln TMn} = -1.24 + 1.13 \text{ Ln TDust } (n = 56, R^2 = 0.847, p < 0.001)$$

Addition of the three high full-shift personal sampling values from the less well-documented 1987 study during Fe-Mn production lay on the same line, supporting information about when modifications came into effect. This relationship was used to establish Mn content for earlier dust surveys of furnace team workers.

Table 4: Total manganese and total dust for furnace workers

			Total manganese			**Total dust**		
Survey year	Product	n	AM mg/m^3	GM mg/m^3	GSD	AM mg/m^3	GM mg/m^3	GSD
1991	SiMn	14	0.263	0.225	1.76	0.869	0.797	1.53
1989	SiMn	24	0.61	0.54	1.68	1.97	1.86	1.46
1988	FeMn	8	0.91	0.90	1.22	2.52	2.46	1.28

AM : Arithmetic mean; GM : Geometric mean ; GSD: Geometric standard deviation

Work histories

Several features characteristic of work in this plant emerged from the work history database. Some workers, particularly maintenance and furnace workers, had had many work-related accidents, of a sufficiently serious nature that they could be off work for many months. Consequently, for two workers with the same length of tenure in these positions, one may have actually worked a fraction of the time of the other, due to a sequence of work-related accidents. Of the 114 workers, 86 had spent time-off compensated for work-related accidents, with a mean time-off of 5.4 months, and a maximum of 5 years. Absent workers were replaced by seniority within the union amongst those qualified for a position, thus many regular workers rotated between the furnace team, the crushing department, and raw materials department, and some furnace workers became fitters. At the time the alloy plant was opened in 1973 the company also operated a silicon plant located across the main highway; this plant closed in 1982. Prior to 1982 some workers moved between the two operations, following the union hierarchy in determination of jobs. When the silicon plant closed in 1982 there was considerable displacement and regression, again depending on union seniority. For the 114 workers, the mean number of distinct jobs held was 5.4, while the mean number of job changes within the plant was 17.2, range 0-77. A sub-group of workers at this plant were "permanently temporary" with work histories covering many years, interspersing work with short periods of lay-off, depending on production demands, and with job rotations every few weeks. These workers were "qualified/trained" for a wide range of jobs in the plant; at one point in their pay-roll records there was a systematic listing of all the positions for which they were considered "qualified/trained", which served as confirmation of the jobs listed in their work history.

Cumulative Exposure Estimates

For the 2004 follow-up study, Mn exposed workers were drawn from the 112 workers with a minimum of two years employment. The average occupational tenure in the Mn alloy plant for these 112 workers was 15.4 years (SD 2.17, range: 3.56 – 17.30). Their TMn CEI ranged from 0.27 mg/m^3 .years to 100.24 mg/m^3 .years, with AM 24.40 mg/m^3.years and GM 14.06 mg/m^3 .years. The RMn CEI had AM of 2.95 mg/m^3 .years and GM 1.78 mg/m^3 .years with a range of 0.05 -12.03 mg/m^3.years.

Overall average exposure intensity, the TMn CEI divided by time worked in years had AM of 1.64 mg/m^3 (SD 1.58), range 0.02 – 6.23 mg/m^3 (GM 0.96 mg/m^3). However, this was not evenly distributed over time. **Figure 1** shows the yearly average personal exposure intensity. The lower values in 1976 and 1979 reflect the two strikes and rebuilding of the furnace. Yearly average exposure intensity was highest during the period 1983-1987, a period of high production prior to the closure of the sinter operation. Documented ventilation improvements and changes to work practices followed the closure of the sinter operation, leading to the substantial reduction in personal exposure levels found in the 1991 survey.

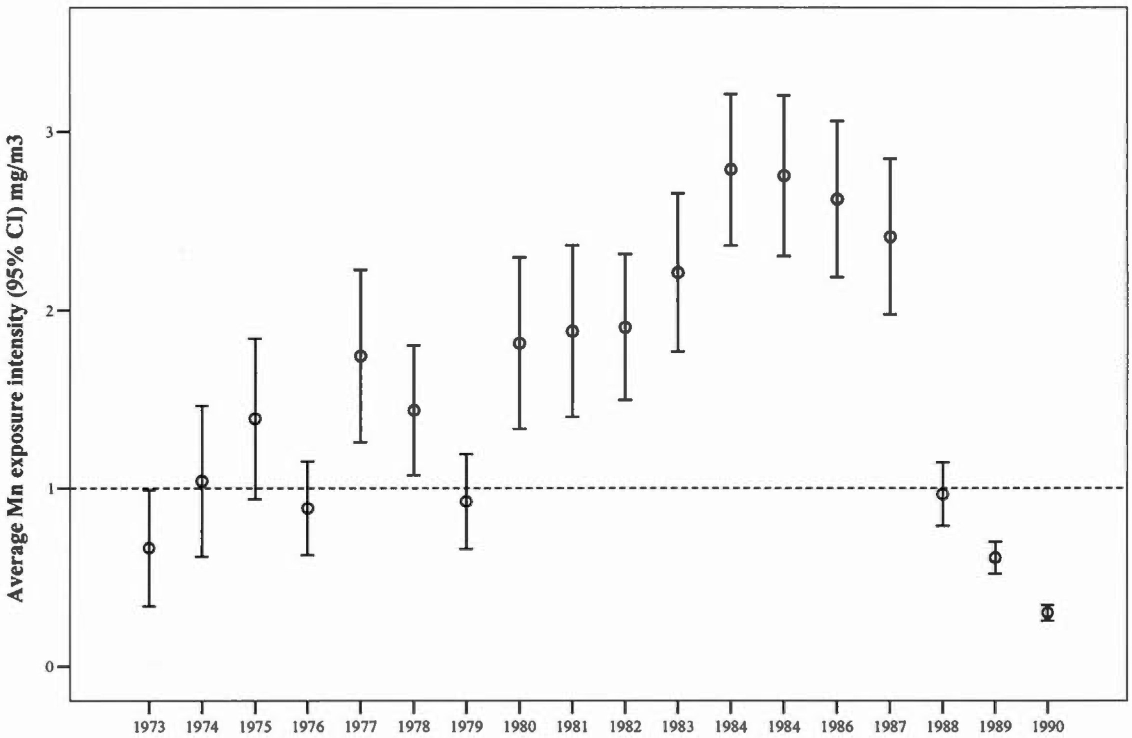

Figure 1: Average total manganese exposure intensity by year

DISCUSSION

Each retrospective exposure study is a unique investigation, tailored to the available information. In this case, in the absence of direct Mn measures for the past, the data regarding the proportion of Mn in the dust for the various job groupings and sectors from the 1991 study were used to interpret past dust levels. Sparse data from the crushing area reports and furnace area reports supported this approach.

Prior to 1987, all dust sampling was short-term. Given that in many cases multiple determinations on an individual worker or at a specific location were made on a given day, the average for the day was considered representative for the shift. Environmental as well as personal dust samples were measured, and the high environmental TDust levels supported the high dust levels found in the personal sampling for some job groups. Reduction in job group levels were associated with specific changes in work practice, e.g. no longer using compressed air to clean the bays after tapping the furnace, and some changes in operations, such as the closure of the sinter operation, and changes in ventilation on the furnace floor. For the crushing department, the environmental TDust short-term data is the same order of magnitude as that found in the 1991 full-shift survey, which is consistent with the absence of change of the basic process in that area.

In Quebec, at the time of the 1991 survey, the permissible TWA for Mn fume was 1 mg/m^3, with a ceiling value of 3 mg/m^3, while the ceiling value for Mn and its compounds in dust was 5 mg/m^3 (Québec 1987). For all of the job groups in the 1991 survey, both AM and GM for TMn in TDust were below 1 mg/m^3. Even in the most highly exposed group, the electricians, the GM for TDust was 2.64 mg/m^3, well below the TWA value for nuisance dust of 5 mg/m^3.

The results show that exposure levels in the 1970's and 1980's were very much higher than in 1991. The historic dust data clearly indicate that dust exposure in the past was consistently high. Closure of the sinter operation in 1988 substantially reduced dust levels across the plant. Changes in work practices and improved ventilation decreased the exposure for the furnace team starting in 1988, with a three-fold decrease by 1991 from the TMn level in 1989. The work histories showed that individual exposure profiles of many workers were very complex due to job rotations with differing exposure levels, periods of lay-off, and absences for work-related injuries. Frequent change of jobs has the effect of averaging out the differences between individual cumulated exposure indices. Overall the evidence supports that past personal exposure was very much higher across the plant than the levels measured in the 1991 survey.

Corrections for use of respirators were not applied in this reconstruction. If respirators had been routinely and effectively used in the dustier jobs, then the exposures may have been overestimated. However, we found that although respirator use was recommended in some of the industrial hygiene reports, there was little change in practice; workers confirmed that respirators were rarely used, and personal dust measures remained high between surveys.

The environmental levels found in 1991 were substantially higher than those found in an Italian ferroalloy plant studied by Lucchini et al.[1999], where furnace area Mn levels in 1995 ranged from 0.085 - 1.490 mg/m^3, GM 0.256 mg/m^3, AM 0.453 mg/m^3. In contrast, in this study, the1991 furnace area Mn levels were approximately four times higher. Given that exposure levels in past years were much higher than those of 1991, it is not surprising that the TMn CEI for the Quebec workers, GM 13.97 mg/m^3 .years is approximately ten-fold higher than the CEI of the Italian alloy workers (GM 1.205 mg/m^3 x years) although exposure length is similar.

In a recent study of Mn exposure in several similar Norwegian alloy plants [Ellingsen et al., 2003], overall inhalable Mn, as measured by the IOM sampler, had AM 0.769

mg/m^3 , GM 0.254 mg/m^3 (n = 265) with a maximum value of 27.20 mg/m^3. These values are of similar magnitude to those found for the more highly exposed job groups in the 1991 Quebec survey although the sampling method was different. Using parallel sampling in the Mn-alloy plants, the Norwegian authors have found that that the traditional closed-face 37 mm total dust samplers, which were used in the 1991 Quebec survey, underestimate exposure to inhalable aerosol by a factor of approximately two compared with the IOM sampler[Bast-Pettersen et al., 2004]. In the Norwegian study, the overall proportion of respirable Mn in the inhalable Mn was 10.6% with a higher value of 15.6% in the furnace workers, based on personal sampling. The stationary paired sampling in the Quebec plant showed similar proportions of respirable Mn in the total Mn in the furnace and crushing areas

Myers et al.[2003] conducted an extensive cross-sectional survey of subjects drawn from eight production environments in a South African Mn smelting complex. The mean length of service of their workers was also high, 17.2 years. Within their study, the overall mean personal Mn level determined using IOM inhalable dust samplers, found in the lowest exposed (GM 0.69 mg/m^3) of three smelters and in the raw materials plant (GM 0.26 mg/m^3) are comparable with data in the Quebec 1991 survey. However no details of job groups are given, and maintenance workers were excluded. A cumulative exposure index across all jobs within the study (n = 508), which included very low exposure workers from administrative jobs and a chemical plant within the complex, had mean 16.0 mg/m^3.years (SD 22.4) Geometric mean 5.1 (GSD 6.7) minimum 0 max 137.6 mg/m^3 .years. These authors concluded that there was no convincing evidence of effects of exposure to inhalable Mn at, or above, the current ACGIH TLV [Myers, et al. 2003].

The United Kingdom Institute for Environment and Health has proposed the respirable fraction as the most biologically appropriate measure of exposure to airborne Mn for evaluating health effects. [Institute for Environment and Health/Institute of Occupational Medicine 2004]. Young *et al.* [2005] reanalyzed data from Myers' study, using RMn as the exposure metric, to verify whether respirable Mn is a more sensitive predictor than inhalable Mn of neurobehavioral effects where such effects exist. Their estimates for RMn were based on personal sampling using a 10 mm Dorr Oliver nylon cyclone together with an IOM sampler, and used attribution and interpolation of relationships between RMn and TMn applied to various job groupings. Their cumulative RMn exposure index for 509 smelter workers had a median of 0.92 $mg.year/m^3$ (range 0.015 – 13.26 $mg.year/m^3$), with a median average intensity of Mn exposure of 0.058 mg/m^3 (range 0.003 – 0.51 mg/m^3). The median values are lower than those found for the Quebec smelter workers, although the maximum CEI is similar. However, the Quebec sample contained a larger proportion of production workers, and included maintenance workers, while the South African sample contains no maintenance workers, and a substantial group of low exposure workers. Young (2005) concluded that respirable dust is neither more nor less useful as an exposure metric in the identification of a protective OEL for Mn.

Occupational exposure studies are frequently limited by the problems and lack of data for historic exposure reconstruction, and this has been true for studies of exposure to Mn. While early neurological effects have been demonstrated, dose-response information has been shown in only a few studies. A subset of this Quebec cohort evaluated in 1990 showed early neurological effects consistent with Mn exposure relative to a matched pair cohort of blue collar workers from the same region. (Mergler *et al.* 1994). However, no relationship was found between their neurofunctional test results and either length of occupational tenure, or their personal exposure levels in the plant at the time of testing. The exposure reconstruction for this cohort of 112 workers was carried out for use with the follow-up study in 2004, 14 years after cessation of exposure, in which 77 of the former Mn workers and 81 members of the referent group participated. Several neuro-outcomes assessed at this follow-up were significantly associated with increasing tertiles of CEI to Mn, including neuropsychiatric symptoms [Bouchard et al., in press], cognitive flexibility and neuromotor speed [Bouchard et al., in press]. These results suggest that cumulated past exposure to Mn may have long-term consequences on the nervous system.

CONCLUSION

This exposure reconstruction has provided many insights into the complex exposure history of the workers who were occupationally exposed to airborne Mn in a Quebec alloy plant from its inception in 1973 until its closure in 1991. The historic dust data showed that past levels of personal exposure were very much higher than those found in 1991. The results from the follow-up of this cohort of Mn-workers suggest that cumulative exposure to Mn provides a useful metric for assessing long-term neurobehavioral effects of Mn.

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