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Are Current Biomarkers Suitable for the Assessment of Manganese Exposure in Individual Workers?

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Background *Whole blood and urinary manganese have been measured in occupational and environmental studies for the assessment of exposure. The aim of this study was to assess the relationship between the airborne concentrations of manganese and these biological indicators.*

Methods *Environmental and biological monitoring was performed in a group of 94 employees in a ferroalloy production, who were exposed to manganese (Mn) oxides (MnO_2 and Mn_3O_4). The results were compared with those from a control group of 87 subjects not exposed to Mn.*

Results *Mn exposure levels ranged between 5 and 740 $\mu g/m^3$, with arithmetic and geometric mean and median values being 202.6, 97.6, and 150 $\mu g/m^3$, respectively. Arithmetic and geometric means for Mn in total blood (MnB) were, respectively, 10.3 ± 3.8 and 9.7 $\mu g/L$ in the exposed and 5.9 ± 1.7 and 5.7 $\mu g/L$ in the controls. For urinary Mn (MnU), arithmetic and geometric means were, respectively, 4.9 ± 3.6 and 3.8 $\mu g/L$ in the exposed and 1.2 ± 1.4 and 0.7 $\mu g/L$ in the controls. On a group comparison, a significant relationship was found between high and low exposed subgroups, identified according to Mn atmospheric concentrations (MnA), for both MnB (F value $= 38.0$, $P > 0.0001$) and MnU (F value $= 36.1$, $P > 0.0001$). On a linear relationship, a correlation was observed between MnA and MnB ($r = 0.34$; $r^2 = 0.112$; $P = 0.001$), whereas no association was found between MnA and MnU. A significant relationship emerged also between MnB and MnU ($r = 0.48$, $r^2 = 0.23$, $P < 0.0001$). No association was observed between an index of cumulative exposure and the biological indicators of exposure.*

Conclusion *These results confirm that MnB and MnU can discriminate groups of occupationally exposed workers from groups of nonexposed subjects. MnB is also related to the intensity of external exposure on a linear relationship, but given a high variability, it is not suitable for individual biological monitoring. Therefore, further research should focus on more accurate biomarkers of Mn exposure.* Am. J. Ind. Med. 37:283–290, 2000. © *2000 Wiley-Liss, Inc.*

KEY WORDS: occupational exposure; manganese oxides; biological indicators; biological monitoring

INTRODUCTION

Manganese (Mn) is an essential element that can cause health problems either below or above a certain level in the organism. Mn is physiologically absorbed through the digestive tract, and the absorbed dose is kept within a certain

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range by homeostatic mechanisms that prevent large fluctuations of Mn concentrations in blood through changes in absorption and excretion rates [Rehnberg et al., 1982].

Deficiency situations, although rare, are well known in the literature as well as the clinical intoxication (called manganism), that can occur when high amounts of Mn are absorbed via the respiratory tract. Common clinical findings are represented by a Parkinsonian syndrome associated with psychiatric disturbances. These effects have been described in subjects exposed to high air concentrations of Mn, generally above 1 mg/m^3 [WHO, 1981].

In addition, early signs of extrapyramidal neurotoxicity and behavioral disorders have been demonstrated for lower exposures to Mn (between 1 and 0.230 mg/m^3) in a number of field occupational and environmental studies [Roels et al., 1987a, 1992, 1999; Iregren, 1990; Mergler et al., 1994, 1999; Lucchini et al., 1995, 1999].

Interest in biological and toxicological aspects of Mn has grown in the past few years for many reasons: the industrial use of the metal is expanding in many fields, from ferroalloy to the iron industry and the use of Mn based alloys during welding. In agriculture Mn based pesticides are widely used, especially in developing countries. Environmental exposure to Mn can also occur when the organic compound of Mn known as MMT (Methylcyclopentadienyl Mn Tricarbonyl, $C_9H_7MnO_3$) is used in gasoline as lead substitute. This was the case in Canada, where MMT was introduced in 1976 and completely replaced tetraethyl lead in gasoline by 1990 [Hurley et al., 1992]. In the United States, the Environmental Protection Agency (EPA) prohibited the use of MMT until 1995 when a court decision allowed the sale of this chemical to refiners for use as an addition in unleaded gasoline [Wallace and Slonecker, 1997]. MMT has also been approved for use in Argentina, Australia, Bulgaria, Russia, and New Zealand [Zayed et al., 1999]. Therefore, the extensive exposure to this metal and the demonstration of early effects after prolonged occupational exposure to low concentrations are causing wide spread concern about the possible health implications in the general population worldwide [Alessio and Lucchini, 1996].

For the assessment of health effects, a precise methodology for the quantification of Mn exposure is needed. In this regard, noninvasive methods for a direct in vivo quantification of the critical dose of Mn in the critical organ (i.e., the central nervous system) are still lacking. Hence, it is necessary to rely on estimates of external dose, such as Mn airborne concentrations, and internal dose, such as Mn concentrations in biological media. For several years, whole blood (MnB) and urinary (MnU) Mn and less frequently serum and hair Mn have been suggested in occupational and environmental studies as biomarkers. However, both MnB and MnU are generally considered as unsuitable to assess Mn exposure on an individual basis, although they can discriminate between groups of occupationally exposed and control subjects [Roels et al., 1987b].

Based on a relatively scarce number of studies in the literature on the biological monitoring of manganese exposure, the objective of this research was to assess the relationship between airborne Mn levels, MnB, and MnU among a group of ferroalloy workers, in order to verify previous observations [Roels et al., 1987b].

MATERIALS AND METHODS

Plant Description and Target Populations

Two ferroalloy plants located in Northern Italy were studied. The production process and the raw materials used are the same in the two factories: Mn ore is charged and melted in electric furnaces to obtain ferro-Mn and silico-Mn. The molten material is poured in casting areas, where after cooling and solidification, it is crushed and prepared for shipping. The maintenance operation including welding also takes place in a separate shop.

Among the total groups of employees from the two companies, we considered the workers at different Mn exposures, thus including the production staff and personnel for maintenance, transport, and various handling activities. Due to similarities in work processes, job rotation, environmental concentrations of Mn, age, and work seniority, the workers of the two companies were merged into a group to improve the power of statistical elaboration.

Out of 101 workers enrolled in the study, 94 subjects accepted to participate, with a participation rate of 93%. They had a mean age of 40.2 ± 8.3 (range 24–56) years. The average duration of Mn exposure was 15.8 ± 7.0 (range 3–38) years. The job rotation is rather frequent, with about the 40% of the subjects having worked at more than one worksite on a daily or weekly basis.

As control group, 87 maintenance workers with comparable age and life habits (smoke, alcohol, tea, and coffee consumption) accepted to participate from a total group of 95 hospital workers (participation rate 92%). These subjects were not occupationally exposed to manganese and were residents of urban areas with MnA levels ranging from 0.2 to 0.9 μg/m^3 [Apostoli, unpublished]. The mean age was 42.56 ± 8.81 (range 27–62) years and length of employment was 18.62 ± 9.81 (range 2–39).

Environmental Monitoring

The exposure levels of airborne dust and fume of Mn were determined by personal sampling. Samples were collected for each worker in the breathing zone by Zambelli

pumps model Chronos, 2.5 L/min flow rate, equipped with cellulose acetate membrane filters with a pore size of 0.8 μm. The sampling duration was equal to half the workshift, on a working day representative of the usual production rate. The workers wore masks quite regularly, but not constantly.

To assess the ratio between the total particulate and the respirable fraction (particles with >5 μm of diameter), parallel sampling was conducted in 10 representative areas and working conditions. The respirable fraction was measured by using Lippmann cyclones. Since the ratio was found to be relatively constant (between 2.8–2.0) in these samples, it was used to estimate the percentage of respirable fraction from total dust in the different plant areas.

A cumulative exposure index (CEI) was calculated for each subject by multiplying the average annual airborne Mn concentration in respirable dust characteristic of each job, by the number of years in which this activity was performed. The following formula was used, where MnA is the average annual concentration of airborne Mn for a specific job, at the time when the job was performed, and YRS is the number of years spent on that job:

$$\mathrm{CEI} = \{[\mathrm{MnA}_1 \times \mathrm{YRS}_1] + [\mathrm{MnA}_2 \times \mathrm{YRS}_2] + \ldots + [\mathrm{MnA}_n \times \mathrm{YRS}_n]\}.$$

CEI was expressed in μg Mn/m^3 × years.

The analysis was performed by graphite furnace atomization atomic absorption spectrometry and Zeeman background correction (Varian SpectrAA 400 Zeeman). The accuracy was evaluated by using the Standard Reference Material 2676c and 1643c of National Institute of Standard and Technology, Gaithersburg, MD, USA.

Biological Monitoring

A venous blood sample of 5 mL and a spot urine sample were collected at the end of the shift, kept at 4°C, and analyzed within 24 h. All urine samples had a specific gravity ranging between 1018 and 1032, therefore, urinary concentrations were expressed in μg/L, without creatinine correction. All workers had to take a shower before the biological samples were taken.

MnB, MnU were determined by electrothermal atomic absorption spectrometry with Zeeman effect background correction system (Varian SpectrAA400) according to the method by Apostoli et al. [1992]. For biological samples the accuracy was assessed by using control urine samples with assigned values of 4.6 and 6.9 μg/L for 1st level and 19.6 and 29.1 μg/L for 2nd level (Lyphocheck, BioRad). The relative standard deviation for biological determinations ranged between 5% and 7%. The laboratory involved in analytical determinations adheres to the current quality assurance procedures and participates in the external quality program organized by the Institute of Occupational, Social and Environmental Medicine, University of Erlangen, Nuremberg.

Statistical Analysis

The normality of variables distribution was checked with the Kolmogorov–Smirnov test and skewed variables were log-transformed. Unpaired Student's t test was used to assess the intergroup differences. The relationship between the parameters of external and internal exposure was first assessed by dividing the exposed group according to MnA levels, choosing the cut-off points in order to obtain three homogeneous subgroups. ANOVA analysis with Scheffe's test were used for subgroups comparisons. Simple regression analyses were then used to study the relationship between exposure parameters.

RESULTS

Environmental Exposure

Figure 1 shows the frequency distribution of airborne Mn in total dust (time-weighted average concentrations) in the two ferroalloy plants. The air concentrations of Mn in a total number of 94 samples ranged from 5 to 740 μg/m^3 with an overall arithmetic mean ± SD, geometric mean, and median value of 202.7 ± 184.7 μg/m^3, 97.6 μg/m^3, 150 μg/m^3, respectively.

Biological Indicators of Exposure

Figures 2 and 3 compare the percentile distribution, respectively, of MnB and MnU for Mn-exposed workers and

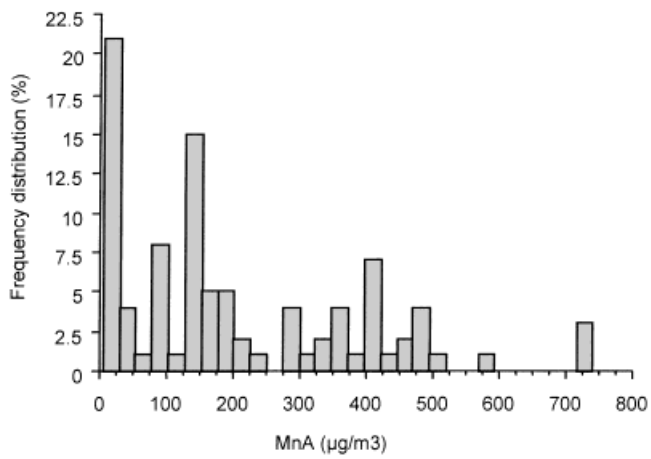

FIGURE 1. Frequency distribution of TWA concentration of Mn in total dust measured at two ferroalloy plants.

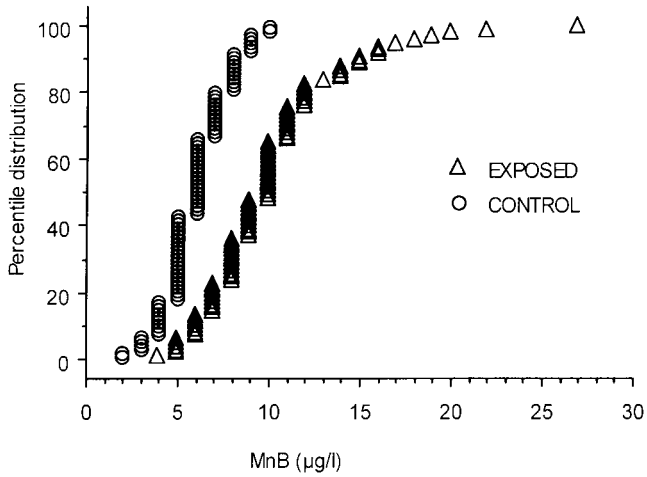

FIGURE 2. Percentile distribution of MnB of exposed and controls.

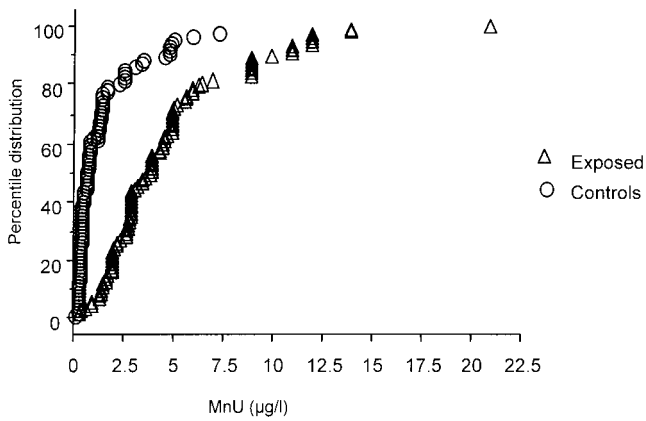

FIGURE 3. Percentile distribution of MnU of exposed and controls.

controls and shows significantly ($P < 0.0001$) higher values for both indicators in the Mn group.

MnB levels ranged from 4 to 27 µg/L in the exposed subjects and from 2 to 10 µg/L in the controls. Arithmetic and geometric mean values for MnB were, respectively, 10.3 ± 3.8 and 9.7 µg/L in the exposed and 5.9 ± 1.7 and 5.7 µg/L in the controls. About 44% of the Mn exposed workers exceeded the highest MnB value of the controls.

MnU levels ranged from 0.4 to 21 µg/L in the exposed subjects and from 0.1 to 7 µg/L in the controls. Arithmetic and geometric mean values for MnU were, respectively, 4.9 ± 3.7 and 3.8 µg/L in the exposed, and 1.2 ± 1.42 and 0.7 µg/L in the controls.

Overall, average MnB levels were found to be about twice as high in the exposed than in controls, whereas the MnU concentration exhibited a fivefold increase over the controls. Moreover, age, smoking habits, and alcohol consumption did not show any association with MnB and MnU.

Relationship Between Environmental and Biological Indicators

Three subgroups were selected based on MnA levels: low exposure (< 90 µg/m^3), mid exposure (between 90 and 250 µg/m^3), and high exposure levels (> 250 µg/m^3).

Tables I and II show the results of the ANOVA test that indicates significant differences between all three subgroups and the controls. In addition, the Scheffe's test indicated significant differences between all the three subgroups considered.

In the second step, the relationship between external and internal parameters of exposure was assessed on a linear relationship. In this case, the simple regression between log values of MnA and MnB ($\text{MnB} = 8.829 + 0.007 \times \text{MnA}$; $r = 0.34$; $r^2 = 0.112$) was statistically significant ($P = 0.001$) (Figure 4) although with a limited explanation of variance. A similar result was observed between MnA and MnU ($r = 0.35$, $P = 0.0009$, $r^2 = 0.12$). A significant relationship was observed between the two biological indicators MnB and MnU, in the exposed group ($\text{MnU} = 0.379 + 0.438 \times \text{MnB}$; $r = 0.47$, $r^2 = 0.22$, $P < 0.0001$), whereas it was not significant in the control group (Figure 5).

Instead, the biological parameters of exposure were not associated with the CEI, nor with the duration of exposure, either on a group comparison and in the linear relationship.

DISCUSSION AND CONCLUSIONS

Biological monitoring is a useful methodology for the risk assessment in occupational and environmental settings. For industrial hygienists, it is complementary to environ-

TABLE I. Mean MnB Values (in µg/L) in the Different Subgroups Based on MnA Levels: F-value: 37.94, $P < 0.0001$

Subgroup	N	Mean MnB	S.D.
Low exposure	34	8.65	3.18
Mid exposure	29	10.93	3.25
High exposure	31	11.39	4.61
Controls	89	5.99	1.73

TABLE II. Mean MnU Values (in µg/L) in the Different Subgroups Based on MnA Levels. F-value: 46.26, $P < 0.0001$

Subgroup	N	Mean MnB	S.D.
Low exposure	34	3.12	1.48
Mid exposure	29	4.79	2.87
High exposure	31	7.04	4.72
Controls	89	1.19	1.41

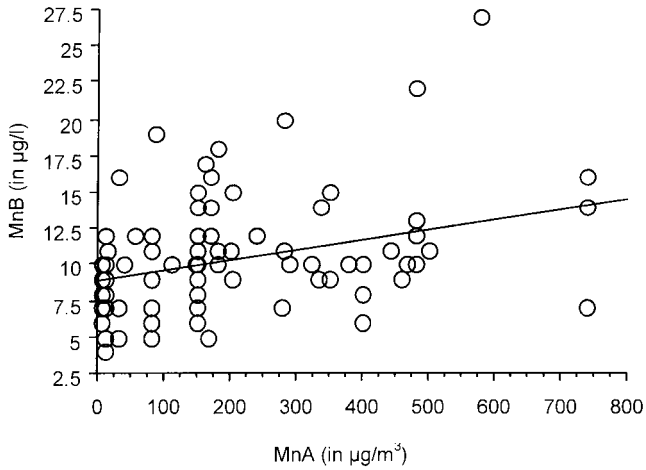

FIGURE 4. Relationship between MnA and MnB.

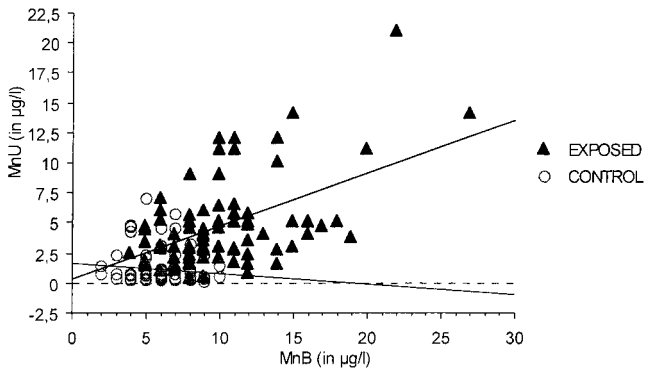

FIGURE 5. Relationship between MnB and MnU in the exposed and control subjects.

mental monitoring [ACGIH, 1998] but in many cases is preferable since it provides an integrated evaluation of individual absorption of toxic substances in the human body from all routes of entry. Specific conditions are required for the correct use of biological indicators of exposure on a routine basis: scientific validation is needed, specific, sensitive and accurate analytical methods must be available, proper knowledge about toxicokinetics and correlation with airborne concentrations must be demonstrated.

The use of biomarkers in exposure assessment should be aimed at: (i) unequivocally establishing the exposure in population studies, (ii) reducing misclassification in epidemiological studies, (iii) modeling internal dose, i.e., the concentration in the critical organ, cell, or molecule. Whatever the aim, biomarkers of exposure should focus on the body burden or on the total dose absorbed, integrating multiple sources of exposure and routes of intake, the pattern of exposure over time, and inter-individual differences in history, habits, and behaviors [Mutti, 1995].

Biomarkers of exposure may have different meanings depending on the kinetics parameters [Bernard, 1995], and this is crucial when interpreting Mn biomonitoring. In occupational settings, Mn is mainly absorbed through inhalation and, to a lesser extent, through the gastrointestinal tract (via contamination). The olfactory pathways provide an alternative route of passage of Mn into the brain, that can bypass the blood-brain barrier system [Tjalve et al., 1996]. After absorption Mn travels into the blood bound to transferrin in the trivalent state and to an α_2-macroglobulin in the divalent state [Gibbons et al., 1976]. Once in the bloodstream, Mn is rapidly distributed and excreted.

Being an essential element, this metal is under the constant control of very efficient homeostatic mechanisms, that by regulating absorption and excretion rates, are able to maintain Mn internal doses within a specific range of

TABLE III. MnA (in mg/m^3) and MnB (in µg/L) Concentrations in Occupationally Exposed Populations

Industry/Mn form	*N*	MnA (Geometric mean)	MnB (Geometric mean)	References
Ferroalloy/Mn oxides	33	0.19	9.8	Lucchini et al., 1997
Ferroalloy/Mn oxides	19	0.27	11.9	Lucchini et al., 1995
Ferroalloy/Mn oxides	19	0.12	8.6	Lucchini et al., 1995
Ferroalloy/Mn oxides	20	0.02	6.0	Lucchini et al., 1995
Ferroalloy/Mn oxides	69	0.23	10.3	Mergler et al., 1994
Mn ore mill/Mn oxides	17	1.59[a]	25.3[a]	Chia et al., 1993
Batteries/MnO_2	92	0.95	8.1	Roels et al., 1992
Welders	73	0.078	7.5	Gobba et al., 1998
Welders	26	0.04	7.2	Gobba et al., 1988
Chemical/Mn salts and oxides	141	1.0	12.2	Roels et al., 1987b
Chemical/Mn salts and oxides	85	0.94	12.9	Lauwerys et al., 1985

[a]Arithmetic mean.

normality. In particular, dose-dependent biliary excretion of divalent Mn serves to regulate the percentage of absorbed Mn in systemic tissues [Andersen et al., 1999]. As a result, the MnB half-life in blood becomes very short and the range of variation of Mn in whole blood is very narrow. In non-occupationally exposed populations, average MnB levels are generally between 6 and 9 μg/L [Alessio and Lucchini, 1996] and average concentrations in plasma less than 0.5 μg/L [Versieck and Cornelis, 1989]. A major route of Mn excretion is in the gastrointestinal tract via bile [Papavasilion et al., 1966], and in overloading conditions, more gastrointestinal routes participate in the excretion.

Although the urinary excretion is low and about 0.01–1% of the absorbed dose [Stokinger, 1981], Mn can be measured in urine. Lauwerys and Hoet [1993] indicated urinary values in the general population of less than 3 μg/L, and Minoia et al. [1990] suggested an average value of 1.02 μg/L.

Since MnB and MnU have a short half-life (likewise the concentration of organic solvents in blood), the internal dose may represent the amount of chemical absorbed during the sampling time or shortly before [Bernard, 1995].

MnB and MnU have been measured in a number of occupational studies. Considering the data from various studies presented in Table III, it is possible to observe that MnB tends to increase as a function of MnA with a linear relationship ($MnB = 6.644 + 0.008 \times MnA$; $r = 0.81$; $r^2 = 0.66$; $P = 0.002$) (Figure 6). Based on this equation, an approximate value of 8.2 μg/L of MnB corresponds to the ACGIH's TLV for MnA of 200 $\mu g/m^3$. The value of 10.2 μg/L of MnB can be calculated with the equation derived from our data, that should be considered as more precise, as being measured with the same methodology.

In conclusion, according to the most recent literature, our results confirm that MnB and MnU represent indicators of exposure on a group basis. Given the high variability of the results, they cannot be considered as suitable biomarkers of exposure. This is in agreement with Droz [1993] who on the basis of pharmacokinetic modeling, stated that for half-lives below 10 h, there is no statistical advantage in using biological monitoring. In addition, for half-lives greater than 10 h, and with elevated environmental variability, there is a definite advantage for biological monitoring no matter what biological variability is considered.

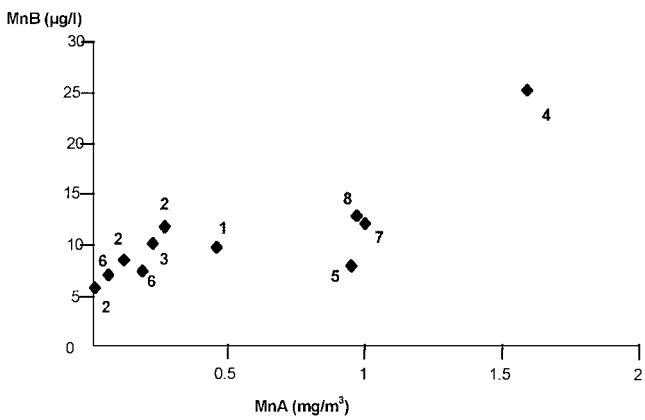

FIGURE 6. Relationship between MnA and MnB concentrations from occupational field studies (1 = Lucchini et al., 1997; 2 = Lucchini et al., 1995; 3 = Mergler et al., 1994; 4 = Chia et al., 1993; 5 = Roels et al., 1992; 6 = Gobba et al., 1988; 7 = Roels et al., 1987b; 8 = Lauwerys et al., 1985).

The CEI parameter has not shown any relationship with MnB and MnU. In our experience, a positive correlation was seen between MnB and the CEI in a previous study where the workers were examined from one to two weeks after the cessation of exposure [Lucchini et al., 1995]. On that occasion, the correlation coefficients were found to increase as a function of the latency after the exposure's cessation. A possible explanation of this phenomenon was that the Mn percent present in the bloodstream represented a measure of the body burden, whereas the MnB percent related to current exposure was already cleared out.

In the present study, the relationship between MnB and CEI is probably disturbed by the fact that the workers were tested during exposure, so that MnB does not reflect only the Mn body burden, but is also related to the current MnA levels. In fact, in our previous study there was no relationship between MnB and MnA measured immediately before the cessation of exposure, whereas in the present study the same relationship is more evident. The term ''biological indicator of internal dose'' has essentially the same meaning of ''biomarker of exposure,'' but with a closer relationship with adverse effects on the critical organ or tissues. It is represented by a xenobiotic substance or its metabolite(s) that can be measured within a compartment of the organism and can reflect the external exposure to the same substance [NAS/NRC, 1989]. In fact, in the case of Mn, we were able to demonstrate in previous studies a dose–effect and dose–response relationships by using MnB and not the measure of Mn airborne concentrations. This was the case for both neurobehavioral and neuroendocrinal effects [Lucchini et al., 1995; Mutti et al., 1996]. Therefore, if the currently available tests (MnB and MnU) are not suitable for biological monitoring, attention should be focused on other indicators, such as Mn in plasma and serum, to measure the biologically active route of free Mn.

Speciation may play an important role when using both environmental and biological data [Apostoli, 1997]. In fact, knowledge of chemical composition, particle size distribution, and the bio-availability of Mn aerosol in industry is still limited. The speciation of Mn may be of interest not only for the oxidation state (among the 11 theoretical oxidation states, only the $+2$ and the $+3$ are of biological interest), but also for metal–protein complexes. There is a general agreement that toxic effects become evident when free ions of toxic metals reach the critical site. The protein–

metal complexes are important in transport and distribution mechanism. For Mn, several protein complexes have been suggested: metallothionein, albumin, transferrin, monoglobulin. The measure of different fractions of Mn may consequently become an important tool for understanding the element's toxicity.

Due to the low concentrations of Mn in serum and its fractions, the accuracy and sensitivity of analytical technique are crucial. They underline the importance of analytical methods that represent prerequisites for biological monitoring, together with a thorough knowledge of toxicokinetics and toxicodynamic properties.

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