

Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study

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Abstract

Background—A patient with unexplained minor behavioural changes associated with an axonal sensorimotor polyneuropathy had a history of chronic occupational exposure to cadmium (Cd). Although animal studies have shown that Cd is a potent neurotoxicant, little is known about its toxicity for the human central nervous system. The aim of this study was to investigate the toxic potential of chronic occupational exposure to Cd on neurobehavioural functions.

Methods—A cross sectional epidemiological study was conducted in a group of Cd workers and an age matched control group. Eighty nine adult men (42 exposed to Cd and 47 control workers) were given a blinded standardised examination that consisted of computer assisted neurobehavioural tests (neurobehavioural examination system), a validated questionnaire to assess neurotoxic complaints (neurotoxicity symptom checklist-60, NSC-60), and a standardised self administered questionnaire to detect complaints consistent with peripheral neuropathy and dysfunction of the autonomic nervous system. Historical and current data on biomonitoring of exposure to Cd, either the highest value of Cd in urine (CdU_{max} in µg Cd/g creatinine) of each Cd worker during work (CdU_{max}) or the current value (CdU_{current}) of each control, were available as well as data on microproteinuria.

Results—Cd workers (CdU_{max}: mean (range), 12.6 (0.4–38.4)) performed worse than the controls (CdU_{current}: mean (range), 0.7 (0.1–2.0)) on visuomotor tasks, symbol digit substitution ($p=0.008$), and simple reaction time to direction ($p=0.058$) or location ($p=0.042$) of a stimulus. In multiple linear regression analysis, symbol digit substitution, simple direction reaction time test, and simple location reaction time test were significantly related to CdU_{max}, ($\beta=0.35$ ($p<0.001$), $\beta=0.25$ ($p=0.012$), and $\beta=0.23$ ($p=0.021$) respectively). More complaints consistent with peripheral neuropathy ($p=0.004$), complaints about equilibrium ($p=0.015$), and complaints about concentration ability ($p=0.053$) were found in the group exposed to Cd than in the control group, and these variables correlated positively with CdU_{max} (peripheral neuropathy: $\beta=0.38$, $p<0.001$; equilibrium: $\beta=0.22$,

$p=0.057$; concentration ability: $\beta=0.27$, $p=0.020$).

Conclusion—Slowing of visuomotor functioning on neurobehavioural testing and increase in complaints consistent with peripheral neuropathy, complaints about equilibrium, and complaints about concentration ability were dose dependently associated with CdU. Age, exposure to other neurotoxicants, or status of renal function could not explain these findings. The present study also indicates that an excess of complaints may be detected in Cd workers before signs of microproteinuria induced by Cd occur.

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Keywords: cadmium; neurotoxicity; occupational exposure

Cadmium (Cd) is a major toxic metal, well known for its occupational health risks. It is also of increasing concern as a pollutant of the general environment with implications for public health. Cadmium accumulates in the human body (half life 15–20 years) and may particularly affect the kidney, which is considered the critical target organ in cases of chronic exposure.^{1,2} Inhalation of Cd fumes or dust is the main route for occupational exposure to Cd, whereas cigarette smoke and food and water contaminated by Cd are the predominant sources of environmental exposure to Cd in the general population.^{1,2} The National Institute of Occupational Safety and Health (NIOSH) estimated that in the United States 1 500 000 workers may be exposed to this metal.

Cadmium is a putative neurotoxicant; however, little attention has been paid to its neurotoxic risk for humans in the occupational setting. To our knowledge, there is until now only one epidemiological study in workers exposed to Cd (15 years of brazing operations in the manufacture of refrigerator coils) which described neurobehavioural effects involving attention, psychomotor speed, and memory.³ Unfortunately, this study did not include a control group. Also, case reports described symptoms of peripheral neuropathy in patients exposed to Cd.^{4,5} Recently, we found an increased risk of clinical signs of dysfunction of the peripheral nervous system and polyneuropathy in retired Cd workers.⁶ Diminished intelligence was described in children with increased Cd concentration in hair.^{7–9}

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Although evidence for neurotoxicity of chronic exposure to Cd is rather limited from human studies, it is fairly strong from animal studies especially those involving the immature brain.¹⁰⁻¹¹ Studies in rodents exposed to Cd showed behavioural changes and damage to the nervous system usually affecting only regions without the blood-brain barrier.¹²⁻¹⁶ In vitro cytotoxicity tests with a human neuroblastoma cell line showed Cd to be more neurotoxic than lead or aluminium.¹⁷ The molecular mechanisms of toxic action underlying the findings of these in vitro and in vivo studies are not yet well known.¹⁰⁻¹¹

Before our previous study,⁶ one of us (JL) diagnosed a case of peripheral polyneuropathy which was found in association with an unexplained psycho-organic syndrome (increased irritability, increased suspicion, concentration ability deficits, and memory disturbances). This patient was previously exposed to cadmium oxide dust and fumes in a Cd producing plant. To find out whether occupational exposure to Cd may entail signs of central nervous system dysfunction in exposed workers, we conducted a blinded epidemiological study with questionnaires and computerised neurobehavioural tests in workers previously or still exposed to Cd.

Subjects and methods

STUDY POPULATION

The investigation was incorporated in follow up studies that have been going on since 1972 and which focused on pulmonary and renal effects of exposure to Cd in employees of a Belgian company involved with the production of refined Cd.¹⁸⁻²¹ This had major advantages: (a) all workers, including the retired workers exposed to Cd were invited every time to participate in the studies, (b) historical data on biomonitoring of exposure to Cd and microproteinuria—for example, β_2 -microglobulin (β_2m) or retinol-binding protein—were available, (c) the medical histories were known so that interfering diseases could be controlled for, and (d) the participants were not particularly alarmed about the possible neurotoxic risk of their occupational exposure to Cd, because it had never been an issue in the health surveillance of the workers. At the time of the study (May-June 1995), the active workforce involved in the Cd production in this plant amounted to 39 men. Three workers were on sick leave, two were excluded for severe craniocerebral trauma (more than 10 minutes of unconsciousness), and five did not participate because of nightshift work. The number of retired Cd workers still alive was 18 of whom five did not participate in the study (one refused and four were severely ill) (participation rate was 74%). A group of 59 eligible controls was recruited from the same company among active and retired workers who had never been assigned to jobs involving Cd. Two controls refused to participate, seven were excluded because of interfering diseases (one diabetes mellitus, one grand mal epilepsy, five severe craniocerebral trauma), and three were on sick leave (participation rate 80%). The

study population retained for the statistical analysis thus comprised 89 participants, 42 subjects in the group exposed to Cd (29 active and 13 retired), and 47 in the control group (28 active and 19 retired). It should be pointed out that microproteinuria induced by Cd (urinary β_2m or retinol-binding protein $>300 \mu\text{g/g}$ creatinine²¹) was often found among the retired Cd workers as the reason for their removal from exposure to Cd. Nine subjects in this subgroup had persistently increased β_2m in urine, and of these seven also had abnormal retinol-binding protein. No signs of microproteinuria were found in the active Cd workers and the control group.

STUDY DESIGN

The participants were instructed not to tell or give any hints about their occupational history to the blinded examiner (MKV). A questionnaire inquired about possible influencing factors: schooling, lifestyle, medical and occupational histories, possible leisure time exposures to neurotoxicants, usual amount of alcohol intake on working days and at weekends, and previous misuse of alcohol ($>90 \text{ g alcohol/day}$ for >2 years). Anamnestic alcohol intake was checked by the measurement of serum γ -glutamyltranspeptidase activity. Medical history and routine blood analyses (glycaemia, liver enzymes, Na^+ , K^+ , Cl^- , HCO_3^- , Fe^{2+} , anion gap, ferritine), and urinary dipstick did not show any interfering disease. Serum γ -glutamyltranspeptidase activity and creatinine in serum were measured with an automated analyser Hitachi 917 (Boehringer, Mannheim, Germany). The concentration of creatinine in urine was determined with a Technicon RA-1000.

As the working conditions improved after 1975 and exposure markedly decreased from 1980 (especially in the present group of active Cd workers), the highest Cd concentration measured in urine during the working period (CdU_{max}) was considered as the measure of the maximal internal exposure to Cd. At the time of the clinical examination Cd in urine ($\text{CdU}_{\text{current}}$) and Pb in blood (PbB) were also measured with electrothermal atomic absorption spectrometry as was used before.²⁰⁻²¹

Self administered questionnaires were given to detect neurotoxic complaints, (a) the validated screening questionnaire (in Dutch), neurotoxicity symptom checklist-60 (NSC-60),²² and (b) a questionnaire on polyneuropathy, and dysfunction of the autonomic nervous system. A standardised clinical neurological examination was performed in the group of retired workers.⁶ A computerised neurological test battery, the neurobehavioural evaluation system (version 4.38),²³ was applied to measure neurobehavioural outcomes.

QUESTIONNAIRES

The NSC-60 questionnaire consisted of 10 categories of questions about personality, concentration ability, equilibrium, sensorimotor functioning, general somatic complaints, sleeping problems, mood, chest symptoms, fatigue, and a general category of neurotoxicity symptoms.

The questionnaire about neurotoxicity symptoms has 32 questions which also belong to other categories.²² Each individual question of the NSC-60 was scored from 1 to 4: 1 for never or almost never having the complaint, 2 for seldom, 3 for more often, and 4 for always or almost always. The mean score of the different questions in each category is thus a value between 1 and 4, and is called the category score. The personality questions were regarded as holding questions—that is, questions assumed to be relatively resistant to neurotoxic effects and reflect the attitude of answering.

Also, 14 supplementary questions on symptoms of polyneuropathy were given, about difficulties performing fine movements with the hands, difficulties performing movements requiring strength, muscle cramps, difficulties climbing stairs, unsteady gait, increased incidence of staggering, tingling sensations in the fingers, tingling sensations in the feet, burning pain in the hands, burning pain in the feet, hypoaesthesia in the hands, hypoaesthesia in the feet, feelings of heaviness in the legs, not feeling minor injuries in the feet or hands. Furthermore, five questions on dysfunction of the autonomic nervous system were also given, about abnormal sweating, dizziness when getting up, palpitations, increased incidence of diarrhoea or constipation. Symptoms were scored on a four point scale as for the NSC-60 questionnaire. To obtain the scores for complaints about peripheral neuropathy and complaints about the autonomic nervous system, the sum of scores for either the peripheral neuropathy or the autonomic nervous system questions was calculated for each person and divided by the number of questions.

CLINICAL NEUROLOGICAL EXAMINATION

In the retired workers, a standardised neurological clinical examination was performed, comprising general inspection at rest, inspection of gait, tonus in upper and lower extremities (abnormal if increased rigidity), Romberg's test (abnormal if body sway), finger to nose test (abnormal if intentional tremor), and glabella reflex (positive if not extinct after four times tapping). Tendon reflexes, and motor and sensory testing are described elsewhere.⁶

NEUROBEHAVIOURAL TESTS

Computerised neurobehavioural test systems have several advantages, for example, the game character of the tests usually stimulates motivation. The systems also minimise the observer bias and are insensitive to fluctuations of the observer's attention. The neurobehavioural evaluation system battery was applied to assess visuomotor performance by testing hand-eye coordination (root mean squared error; best of seven trials), finger tapping speed (1 minute with the right hand, 1 minute with the left hand, and 1 minute alternating right and left hand; sum of taps on the three trials), and symbol digit substitution speed (symbol digit substitution; average performance time of the two best out of five trials). To test memory and concentration the following tests were applied: digit span forward and backward (mean of

numbers recalled on 20 trials, EURONEST-version²⁴), and switching attention test (mean reaction time to four different stimuli, (a) as to the location of a square on the screen (simple reaction time test_{loc}), (b) as to the direction of an arrow (simple reaction time test_{dir}), (c) as to the direction of an arrow projected in a square on the right or the left side of the screen (switching attention test_{dir}), (d) as to the location of an arrow projected in a square on the right or the left side of the screen (switching attention test_{loc})).

STATISTICAL ANALYSIS

The statistical package for social sciences (SPSS), version 7.0 for Windows, was used. Unpaired data were compared between groups with Student's *t* test (two tailed). When needed log normal transformation was performed (simple reaction time test_{dir}, switching attention test_{dir}, switching attention test_{loc}, symbol digit substitution, peripheral neuropathy score, and chest, equilibrium, sleeping difficulties, and general somatic complaint categories). Fisher's exact test (two tailed) was used to assess differences in proportions and the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated to assess differences between control and exposed workers in the scored results of the clinical neurological examination, peripheral neuropathy, and complaints about the autonomic nervous system.

Forced entry multiple linear regression analysis with CdU_{max} as independent exposure variable (predictor) was applied on the combined control and exposed groups for outcome variables which showed significant differences between both groups. For the NSC-60 questionnaire results on polyneuropathy and complaints about the autonomic nervous system the following independent variables were considered as covariates: age, alcohol consumption (g alcohol/day), smoking habits (cigarettes/day), exposure to other neurotoxicants (0 or 1), and hypnotics (0 or 1). For the neurobehavioural evaluation system test results, the covariates were age, alcohol, exposure to other neurotoxicants (0 or 1), hypnotic medication (0 or 1), and years of schooling. The distributions of the residuals of the regression analysis were checked, no log normal transformation of the independent variables was needed. For results which were significantly related to CdU_{max}, the statistical analysis was repeated in the active subgroup of the combined controls and Cd workers, who had historically lower exposure to Cd. The CdU_{current} of the controls was considered as the maximum value in these subjects because Cd is cumulative in humans.

For the results of the clinical neurological examination multiple logistic regression was applied with age and CdU_{max} as independent variables in the analysis.

A *p* value ≤ 0.05 was taken as the level of significance.

Results

CHARACTERISTICS OF THE STUDY POPULATION

The relevant characteristics of the control and group exposed to Cd at the time of the

Table 1 Characteristics of the workers exposed to Cd and controls at the time of the study

	Exposed to Cd (n=42)		Controls (n=47)	
	Mean (SD)	Range	Mean (SD)	Range
Age	46.9 (16.4)	23–81	46.7 (14.2)	24–77
Exposure to Cd (y)	12.6 (10.0)	0.5–36		
Cadmium in urine ($\mu\text{g Cd/g creatinine}$):				
Highest value ever (CdU_{max})	12.6 (10.2)	0.4–38.4		
Current value ($\text{CdU}_{\text{current}}$)	4.6 (4.1)	0.1–16.6	0.7 (0.5)	0.1–2.0
Lead in blood ($\mu\text{g Pb/dl}$)	9.7 (4.2)	3.6–19.2	8.7 (4.2)	1.7–20.4
Serum creatinine (mg/dl)	1.13 (0.40)	0.76–2.95	1.12 (0.14)	0.90–1.53
Sleeping (h)	7.1 (0.9)	4.5–9.0	7.0 (1.0)	5.0–9.5
Education (y)	10.1 (2.5)	6–15	12.1 (3.4)	6–22
Personality score*	1.9 (0.7)	1–4	1.8 (0.5)	1–3.4
Current smokers (n (%))	10	23.8	13	27.7
Cigarettes/day	14.2 (7.2)	5–25	14.0 (8.3)	3–25
Alcohol consumers (n (%))	33	78.6	39	83
Alcohol (g/day)	0.9 (0.7)	0.1–3.1	0.9 (0.8)	0.1–3.2
Exposure to neurotoxicants other than Cd (n (%))†	15	35.7	12	25.5
Hypnotic medication (n (%))	13	31.0	8	17.0

*Questions of the neurotoxicity symptom checklist-60 (NSC-60).

†Alcohol misuse (> 90 g/day for > 2 y): eight exposed and six control subjects. Organic solvents or other neurotoxicants (> 2 y occupational exposure): eight exposed and six control subjects.

neurological examination are summarised in table 1. Both groups were well matched for age and sleeping hours, personality score, smoking habits, alcohol consumption and history of alcohol misuse, hypnotic medication, and exposure to solvents and other neurotoxicants. The mean (range) duration of exposure to Cd was 12.6 (0.5–36) years. Serum creatinine concentration was <1.4 mg/dl in most of the participants except in three retired Cd workers (1.53, 2.51, and 2.95 mg/dl) and three controls (one active, 1.46; two retired, 1.51 and 1.53 mg/dl). Serum γ -glutamyltranspeptidase concentrations were all normal (≤ 45 IU/l), although a history of alcohol misuse was reported in eight exposed and six control subjects. The current exposure to Pb in both groups as reflected by PbB was low, and moreover, during the past 10 years PbB was never >25 $\mu\text{g Pb/dl}$. As well as a slight occupational Pb exposure in the past for some retired Cd workers and a low environmental Pb exposure in the controls, the questionnaire showed a history of moderate occupational or leisure time exposure to organic solvents in eight exposed and six control workers.

In the total group exposed to Cd, CdU_{max} and $\text{CdU}_{\text{current}}$ averaged 12.6 and 4.6 $\mu\text{g Cd/g creatinine}$ respectively. In the subgroup of 29 active Cd workers, however, the mean (SD, range) values were lower, (CdU_{max} 8.4 (7.9,

0.4–37.7) and $\text{CdU}_{\text{current}}$ 2.6 (2.6, 0.1–9.4) $\mu\text{g Cd/g creatinine}$). In this subgroup the individual CdU_{max} exceeded 10 $\mu\text{g Cd/g creatinine}$ only five times (11.0, 11.1, 22.8, 24.6, and 37.7), whereas in 12 of the retired Cd workers this CdU value was exceeded over several years and was still high in four at the time of the present study (7–24 years after the end of exposure to Cd). The active workers exposed to Cd had a mean (range) duration of exposure of about 8 (0.5–26) years and the mean (range) age was 37 (23–59) years. In the 28 active controls, the mean (SD, range) value of $\text{CdU}_{\text{current}}$ amounted to 0.5 (0.4, 0.1–1.7) $\mu\text{g Cd/g creatinine}$ and the mean (range) age was also 37 (24–49) years. Besides age and CdU, none of the other variables listed in table 1 differed significantly between the active and retired workers, either exposed or controls (results not shown).

QUESTIONNAIRES

The questionnaire results are summarised in table 2. The exposed workers had a significantly higher peripheral neuropathy complaint score than the controls, whereas the score on complaints about the autonomic nervous system did not differ between both groups. As to the NSC-60 category scores, significant changes were found in the group exposed to Cd when compared with the controls for the sensorimotor, equilibrium, and neurotoxicity categories. Borderline significance was shown for concentration ability and fatigue. The other complaint category scores did not differ between the two groups.

For the exposed and control groups combined, multiple linear regression analysis of the questionnaire results showed that CdU_{max} explained a significant part of the peripheral neuropathy complaint score ($\beta=0.38$, $p<0.001$, fig 1) with hypnotic medication ($\beta=0.30$, $p=0.009$) explaining an additional significant part of the peripheral neuropathy complaint score. The relation between the CdU_{max} and the peripheral neuropathy complaint score did not change when extreme values were excluded ($\beta=0.34$, $p=0.006$). A similar result was found on separate analysis of

Table 2 Questionnaire scores in the exposed and control groups

	Exposed to Cd (n=42)	Controls (n=47)	p Values t test
	Mean (SD)	Mean (SD)	
Polyneuropathy complaint score*†	1.43 (1.32)	1.23 (1.20)	0.004
Autonomic nervous system complaint score*	1.59 (0.37)	1.50 (0.36)	>0.10
NSC-60 category scores*:			
Sensorimotor	1.61 (0.59)	1.37 (0.35)	0.025
Equilibrium†	1.27 (1.39)	1.09 (1.23)	0.015
Concentration ability	2.17 (0.57)	1.95 (0.50)	0.053
Neurotoxicity	1.80 (0.44)	1.59 (0.34)	0.016
Somatic complaints†	1.57 (1.38)	1.43 (1.32)	>0.10
Mood	1.79 (0.57)	1.60 (0.50)	>0.10
Fatigue	2.02 (0.64)	1.81 (0.47)	0.079
Sleeping difficulties†	1.74 (1.46)	1.60 (1.43)	>0.10
Chest complaints†	1.36 (1.42)	1.28 (1.33)	>0.10

*Scores from 1 (no complaints) to 4 (always complaints).

†Statistical analysis after log normal transformation of the data: geometric means (GSDs).

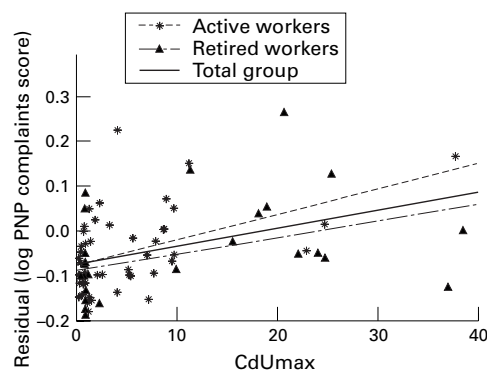


Figure 1 Linear regression between CdU_{max} (µg/g creatinine) and the peripheral neuropathy complaint score adjusted (residual (log peripheral neuropathy score)) for covariates (age, smoking, alcohol consumption, hypnotic medication, and exposure to other neurotoxicants) in the whole group exposed to Cd and controls.

the peripheral neuropathy scores in the active subgroups combined (CdU_{max}: β=0.42, p<0.002). In multiple linear regression analysis, four NSC-60 category scores were significantly and positively associated with CdU_{max} in both the total group and the active subgroup: sensorimotor score, β=0.38 (p=0.001) and 0.37 (p=0.007); equilibrium score, β=0.22 (p=0.057) and 0.35 (p=0.014); concentration ability score, β=0.27 (p=0.02) and 0.40 (p<0.001); and neurotoxicity score, β=0.30 (p=0.009) and 0.33 (p=0.020) respectively. In the whole exposed group, additional slight effects of other covariates were found for some of these associations—namely, the use of hypnotic medication which marginally affected the concentration ability (β=0.21, p=0.096) and neurotoxicity (β=0.24, p=0.050) category scores.

CLINICAL NEUROLOGICAL EXAMINATION

In the subgroup of retired subjects nine (69.2%) ex-workers exposed to Cd were found with a positive glabella reflex versus three (15.8%) in the retired controls (p=0.003, OR 12.0 (95% CI 2.4 to 60.2)). Four retired workers exposed to Cd presented with signs of parkinsonism (three with mask face; one with both mask face and cogwheel rigidity) versus none in the retired controls (p=0.058). In each group one person had an unstable Romberg's test. Clinical signs of resting or intentional tremor, or abnormal gait were not detected. In multiple logistic regression, CdU_{max} was signifi-

cantly related to the presence of a positive glabella reflex (p=0.011), whereas age was not (p>0.10). The presence of extrapyramidal signs was not related to age or exposure to Cd (p>0.10) in this group of retired subjects.

NEUROBEHAVIOURAL TESTS

The neurobehavioural test results of both groups are summarised in table 3. Both simple reaction times (simple reaction time test_{dir} and simple reaction time test_{loc}) were slowed in the workers exposed to Cd compared with the controls. The results of the more complex tasks of switching attention (switching attention test_{dir} and switching attention test_{loc}) were worse in the group exposed to Cd than in the control group, but the difference did not reach significance. Of the visuomotor tests, the symbol digit substitution results were significantly worse in the workers exposed to Cd, whereas the results of the hand-eye coordination and finger tapping did not significantly differ between the control and exposed groups. In the attention and memory tests, the digit span forward results tended to be lower in the Cd group, although they did not reach significance, whereas the digit span backward results did not differ between the exposed and control workers.

With extreme values included, multiple linear regression analysis in the exposed and control groups combined showed that simple reaction times significantly increased with increasing CdU_{max} (simple reaction time test_{dir}, β=0.25, p=0.012; simple reaction time test_{loc}, β=0.23, p=0.021) and age (simple reaction time test_{dir}, β=0.31, p=0.007; simple reaction time test_{loc}, β=0.31, p=0.007). Symbol digit substitution time increased significantly with increasing CdU_{max} (β=0.35, p<0.001) and age (β=0.41, p<0.001), and was inversely related to the number of schooling years (β=-0.20, p=0.017, fig 2). Exclusion of extreme values did not change substantially the results of symbol-digit substitution (β=0.34, p=0.002), simple reaction time_{dir} (β=0.25, p=0.021), and simple reaction time_{loc} (β=0.32, p=0.003). A separate analysis did not show significant dose-effect relations with CdU_{max} in the combined active subgroups, although there was a weak

Table 3 Neurobehavioural test results in the exposed and control groups

	Exposed to Cd	Controls (n=47)	p Values t test
	(n=42)		
	Mean (SD)	Mean (SD)	
Switching attention time (ms):			
Simple reaction time direction*	498 (217)	441 (135)	0.058
Simple reaction time location	413 (227)	330 (130)	0.042
Switching reaction time direction*	684 (282)	663 (160)	>0.10
Switching reaction time location*	580 (323)	564 (183)	>0.10
Symbol-digit substitution speed*	2.8 (1.41)	2.4 (1.19)	0.008
Hand-eye coordination	2.0 (0.4)	1.9 (0.4)	>0.10
Finger tapping	425 (108)	435 (98)	>0.10
Digit span:			
Forward	5.5 (1.3)	6.0 (1.1)	0.064
Backward	5.0 (1.5)	5.3 (1.3)	>0.10

*Statistical analysis after log normal transformation of the data: geometric means (GSDs).

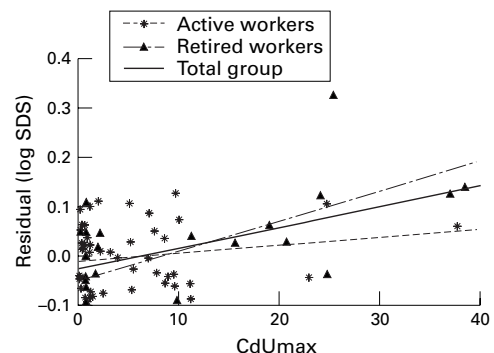


Figure 2 Linear regression between CdU_{max} (µg/g creatinine) and symbol digit substitution adjusted (residual (log symbol digit substitution)) for covariates (age, schooling years, alcohol consumption, hypnotic medication, and exposure to other neurotoxicants) in the exposed and control groups combined.

Table 4 Literature review of Cd neurotoxicity in humans

Authors	Study design	E/C (n)	Exposure to Cd	Other exposures and observations	Neurotoxic effects	Dose-effect relation
Adults:						
Prodan, 1932 ²⁶	Review of case reports	9	2 Chronic, 7 acute	Zn	2/2: Weakness; 5/7: headache	
Baader, 1952 ²⁷	Case report	1	ExpY: 16	?	Anosmia; intramural plexus lesions (bronchial-abdominal)	
Cotter, 1958 ²⁸	Case report	3	2-6 months, >1 y	?	2/3: Mental irritability	
Dunphy, 1967 ³⁰	Case reports	50	4-36 h	?	Headache (34%), weakness (12%), dizziness (34%)	
Blum <i>et al</i> , 1989 ⁴	Case report	1	CdH: 17.1 µg/g CdU: 5 µg/l	Pb, pipefitter.	Axonal sensorimotor PNP	-
Kilburn and McKinley, 1996 ⁵	Case report	2	30 min (fumes); CdU: 25 and 485 µg/day	Ni, CO, PVC fumes	Severe (485 µg); moderate (25 µg) psycho-organic syndrome	
Struempfer <i>et al</i> , 1985 ³⁵	Case-control (behavioural difficulties)	40	CdH: 0.2-2 µg/g	Proteinuria	Demerits (behavioural difficulty)	r=0.55
Adams and Crabtree, 1961 ²⁹	Cross sectional	106E/84C	Airdust: 350 µg Cd/m ³ (range: 340-27600)	Ni dust, proteinuria	Anosmia: 15% in E vs 5% in C	+
Musiol <i>et al</i> , 1981 ³²	Cross sectional	49E	ExpY: 13 (1-33) CdU: <10 >10 µg/l	?	Polyneuropathy: <10 µg Cd/l: 17% >10 µg Cd/l: 55%	+
Hart <i>et al</i> , 1989 ³	Cross sectional	31E	CdU: 25.9 µg/l (1-110)	PbB: 9.3 µg/dl renal function: normal	↓ Attention, memory, and psychomotor speed	+
Bar-Sela <i>et al</i> , 1992 ³¹	Cross sectional	38E	ExpY 8.4 (range: 1-14) CdU: 67 µg/l (8-306)	Ni, Mn, Mg, Zn	90% Headache; 42% dizzy spells 21% weakness; 16% brain atrophy	(+)
Rose <i>et al</i> , 1992 ²⁵	Cross sectional	55E/16C	ExpY: 9.2 airdust: 300 µg Cd/m ³	Proteinuria	Hyposmia: E: 13%; C: 0%	+
Viaene <i>et al</i> , 1998 ⁶	Cross sectional	13E/19C	ExpY: 23; mean CdU _{max} : 22 µg/g creatinine	PbB: 9 µg/dl proteinuria	7E/2C: Polyneuropathy OR: 9.92 (95% CI 1.60-61.6)	+
Viaene <i>et al</i> , (present study)	Cross sectional	42E/47C	ExpY: 13; mean CdU _{max} : 12.6 µg/g creatinine	PbB: 9.7 µg/dl retired: proteinuria active: no proteinuria	↓ Motor speed, attention, memory ↑ equilibrium, PNP, and concentration complaints	+
Children:						
Stellern <i>et al</i> , 1983 ⁸	Case-control (learning difficulties)	25	CdH: 0.94 µg/g	Hair: 8.96 µg Pb/g	↓ Visuomotor skills Pb: r=-0.43	+
Thatcher <i>et al</i> , 1982 ⁷	Cross sectional	149	CdH: 1.65 ppm (0-8.9)	Hair: 7.5 ppm Pb (range: 0-178)	Cd: ↓ verbal IQ Pb: ↓ performance IQ	+
Marlowe <i>et al</i> , 1985 ⁹	Cross sectional	69	CdH: 0.7 µg/g (0.2-2.0)	Pb, As, Hg, Al	Combined exposure index Pb-Cd ↓ visuomotor skills	+
Bonithon <i>et al</i> , 1986 ³³	Cross sectional	26 Babies	CdH: 0.6 µg/g (0.2-1.9)	Hair: 19.3 µg Pb/g (range: 4.6-104.7)	Cd: ↓ perceptual, cognitive, motor Pb: ↓ perceptual, motor	+
Lewis <i>et al</i> , 1992 ³⁴	Cross sectional	92	Amniotic fluid: 1.0 µg Cd/dl	Cr, Pb, Co, Ag, Ni, Hg	↓ Verbal, perceptual, cognitive, and motor abilities	+

E=exposed subjects, C=control subjects; CdU=urinary cadmium concentration; CdH=concentration of cadmium in hair; ExpY=exposure-years; PbB=blood lead concentration; PNP= polyneuropathy.

trend for symbol digit substitution ($\beta=0.22$, $p=0.092$).

Discussion

This study on neurobehavioural effects of occupational exposure to Cd suggested dose (CdU_{max}) dependent increases in subjective symptoms consistent with peripheral neuropathy, complaints about equilibrium, and complaints about ability to concentrate, as well as decreases in visuomotor performance (simple reaction time_{dir}, simple reaction time_{oc}, symbol-digit substitution). It should be pointed out that among the 13 retired Cd workers (mean $CdU_{max}=21.9$ µg Cd/g creatinine) clinical polyneuropathy was diagnosed in seven of them (OR 9.92, 95% CI 1.60 to 61.6, $p=0.01$).⁶ In multiple linear regression analysis, the active subgroup exposed to Cd with a much lower internal exposure to Cd (mean $CdU_{max}=8.4$ µg Cd/g creatinine) still showed a dose (CdU_{max}) dependent increase in complaints consistent with peripheral neuropathy, complaints about ability to concentrate, and complaints about equilibrium, and also a slight but non-significant worsening of symbol digit substitution time was found. Lack of power of the study might be a reason why these changes did not reach significance. More studies with objective measurements (nerve conduction velocities, needle EMG studies, neurobehav-

oural testing) are needed to further confirm these findings.

Putting our findings in the context of the existing publications is not easy because data on human Cd neurotoxicity are sparse, but they suggest possible involvement of the peripheral and central nervous systems. Symptoms of fatigue, mental irritability, headache, muscle weakness, syncope, and hyposmia or anosmia were reported in patients or workers acutely or chronically exposed to Cd.²⁵⁻³¹ There is a case report of a 54 year old pipefitter with a demyelinating polyneuropathy which might be related to occupational exposure to Cd.⁴ A case report on acute exposure of two train conductors due to fighting a fire in a nickel-cadmium battery box on a passenger train, reported persistent neurophysiological and neurobehavioural abnormalities 6 and 12 months after the accident.⁵ Another case report described symptoms of polyneuropathy in patients exposed to Cd,²⁶ and two cross sectional studies in active workers exposed to Cd showed evidence of a dose-dependent increase in risk of polyneuropathy.^{6, 32} Diminished intelligence, especially of the verbal, psychomotor, cognitive, and perceptual skills were described in children with increased Cd concentration in amniotic fluid and hair.^{7-9, 33, 34} In young adults behavioural changes were also reported.³⁵ In several studies the possible influence of other

factors, such as exposure to Pb or reduced renal function, cannot totally be excluded (review: table 4).

Evidence for neurotoxicity of chronic exposure to Cd is fairly strong from several studies on exposure to Cd during gestation, or in neonatal or weanling animals. They reported behavioural changes at a later stage in life—for example, hyperactivity,¹² increased locomotor and rearing activities,¹³ or deficits in visual discrimination or learning.¹⁴ After long term repeated exposure to Cd, the neurotoxic effects in adolescent or adult rats, however, may be different. On inhalation of CdCl₂ aerosols (5 days/week, 6 h/day, for 12 weeks) rats showed no change at 0.33 mg Cd/m³, but there was a significant increase of relative brain weight at 1.034 mg Cd/m³.³⁶ Oral or intraperitoneal Cd dosing induced aggressive behaviour,¹⁵ increased failure to respond to a conditioned or unconditioned stimulus,¹⁶ decreased locomotor, exploratory, and rearing activities.^{13, 16} Tissue culture studies on rat dorsal root ganglia showed degenerative changes in the neurons at low doses of Cd,³⁷ and peripheral neuropathy (a slight reduction in nerve cells of dorsal root ganglia) developed after 31 months of treatment in rats receiving drinking water with low Cd concentrations.³⁸ After intravenous injection of a bolus of labelled Cd in adult rats a preferential accumulation of Cd was found in the peripheral ganglia versus a low uptake of the metal in the brain parenchyma.³⁹ On the contrary, adult rats fed a diet containing 100 ppm Cd showed after 2 months a generalised penetration of Cd into all brain regions with an elective accumulation within the olfactory bulbs, whereas no increase was found at 20 ppm Cd, except in the olfactory bulbs.⁴⁰ These two findings suggest thus that in the case of an acute rise of Cd in the blood circulation³⁹ most of the central nervous system is protected from rapid entry of Cd by the blood-brain barrier. On repeated administration of lower Cd concentrations over a prolonged period,⁴⁰ however, the toxicokinetics of Cd probably present distribution or accumulation features which may affect the integrity or permeability of the barriers.¹¹ In this context, it is conceivable that chronically raised blood Cd may induce changes in cerebral blood vessels, glial cells, or the choroid plexus, and hence, compromising their barrier or sequestering functions.^{41, 42} Several lines of evidence indicated also that Cd may reach brain structures through axonal transport in cranial nerves.^{43, 44}

Several hypotheses have been suggested to explain the molecular mechanisms of the neurotoxic action of the Cd²⁺ ion, competition with Zn²⁺ or Ca²⁺, interaction with nucleic acids, second messenger systems, enzymes, receptors or translocating proteins, effects on neurotransmitter concentrations and reuptake, disruption of membrane dynamics including the function of Ca²⁺ channels, and damage to glial cells resulting in accelerated lipoperoxidation that ultimately influences neuronal function.^{10, 11}

Our study corroborates the dose dependent slowing of reaction times reported in the

exposed group studied by Hart *et al.*³ However, the CdU_{max} values in the present Cd group suggest that the exposure was most likely half that reported in the Hart *et al* study (mean CdU=25.9 µg Cd/l). This may well explain why the learning and memory tests results (digit span forward and backward) in our exposed group did not reach significance. One might argue that our findings in adult workers are not related to exposure to Cd because the metal does not easily cross the blood-brain barrier or blood-nerve barrier. It should be borne in mind that after systemic absorption blood Cd is rapidly processed in the liver resulting in the induction of metallothionein proteins (6–7 kDa) which in turn appear as a main Cd carrier (Cd metallothionein) in the blood circulation. As well as its role in detoxification of Cd, the biosynthesis, homeostasis, and storage of metallothioneins (metallothionein-I, II, III) are usually controlled by zinc and copper, which like Cd, may dramatically up regulate metallothionein biosynthesis.^{45–47} However, compared with the liver, kidney, pancreas, and intestine, the brain has a much lower capacity to produce metallothioneins.⁴⁸ In short term acute or subacute exposures, animal studies have shown that Cd metallothioneins hardly cross the blood-brain barrier and blood-nerve barrier,¹⁰ but the metabolism of Cd and its distribution or accumulation may be different when dealing with chronic low level exposure, especially when combined with the consumption of alcohol.⁴⁹ In view of the potential toxicity of Cd to the central nervous system in long term low level exposures, it is interesting to note that brain specific metallothionein-III^{50, 51} is predominantly expressed in neurons which sequester Zn in synaptic vesicles and it is thought that metallothionein-III acts as a neuromodulator of the glutamate response in the brain through changes in cellular Zn deposition.^{52–54} Also, it has been suggested that the neurotoxic properties of Cd might to some extent also result from disruptions of vascular membranes⁵⁵ and that, at least in rats, Cd induced alterations in the permeability of the blood-brain barrier may be related to the depletion of microvessel antioxidant substances along with an increase in lipid peroxidation.⁵⁶ Moreover, the effectiveness of the blood-brain barrier and blood-nerve barrier can decline with aging,⁵⁷ and hence, the effect of Cd on the nervous system could well be exacerbated in older people exposed to Cd. In ex-workers exposed to Cd a long time after removal from exposure or retirement there are markedly increased concentrations of blood Cd still circulating because of the substantial Cd pool in the liver.^{2, 58}

It must be pointed out that neurobehavioural test outcome is related to premorbid intelligence. Therefore, it might be argued that the difference between the control and exposed group is due to the slight difference in educational level between the groups (table 1). However, even after allowing for this covariate in multiple regression analysis symbol digit substitution remained associated with CdU_{max}. Also in the subgroup of active workers exposed

to Cd schooling (average 11.2 years) was similar to that of the active controls (average 12.2 years) and in this subgroup the trend for relation between symbol digit substitution and CdU_{max} remained.

Occupational exposure to Cd in most job functions is usually accompanied by a slight exposure to Pb. In some retired exposed workers, a slight coexposure of Pb may have occurred in the past. Neurobehavioural defects and peripheral neuropathy are well documented effects of high protracted exposure to Pb. Lower exposures (PbB 25–40 µg Pb/dl) may produce subclinical changes in nerve conduction velocities⁵⁹ and a decrement of visuospatial and visuomotor function.⁶⁰ However, several authors have found more variety in associations between neurological or neurobehavioural deficits and the intensity of internal exposure to Cd than with concomitant markers of exposure to Pb.^{6–8, 33} In the present study, the PbB of retired exposed and control workers was not >25 µg Pb/dl in the past 10 years⁶ and the highest PbB ever measured in the active group exposed to Cd was >25 µg Pb/dl only once in one subject (37.8 µg Pb/dl). Also, after adjustment for exposure to Pb and other neurotoxicants no change was found in the dose effect relation between CdU_{max} and scores of central nervous system (decreased concentration ability) and complaints of the peripheral nervous system (peripheral neuropathy and sensorimotor categories) or the slowed visuomotor performances (simple reaction time test_{dir}, simple reaction time test_{loc}, symbol digit substitution).

As the kidney represents the critical organ in cases of chronic exposure to Cd, it may be argued that neurological effects are secondary to a reduced renal function. This seems unlikely because in the study of Hart *et al*³ none of the subjects had abnormal serum creatinine concentrations and in the present study serum creatinine was <1.4 mg/dl in all the subjects, except in three retired Cd workers and three controls who showed some increase in serum creatinine (1.46 to 2.95 mg/dl). Furthermore, in multiple linear regression analysis serum creatinine did not enter the models. Increased microproteinuria is considered to be an early adverse health effect of chronic exposure to Cd and occurs usually in workers whose CdU was repeatedly >10 µg Cd/g creatinine.²¹ In the study of Viaene *et al*,⁶ the subgroup of retired Cd workers did not show any association (Fisher's exact test, p=0.56) between the presence of microproteinuria and the diagnosis of polyneuropathy, suggesting that both phenomena occur independently. This suggests that neurological effects of chronic exposure to Cd might occur before renal tubular function is compromised. In our group the presence of microproteinuria was not related to peripheral neuropathy complaint scores or other questionnaire results, and also not to simple reaction time_{dir}, simple reaction time_{loc}, or extrapyramidal signs. Only the results of symbol digit substitution and positive glabella reflex were to some extent significantly related to microproteinuria (p=0.068 and p=0.006).

In this context, a major finding of the present study is that the active workers exposed to Cd reported an excess of complaints in the absence of microproteinuria. Therefore, it would be of value to measure central and peripheral nerve conduction velocities in an appropriate group exposed to Cd and controls.

Our study provides evidence for supplementary central nervous system toxicity as well as peripheral nervous system damage as already described.⁶ Indeed, strictly speaking, complaints of diminished equilibrium and slowed reaction times can still be associated with peripheral neuronal dysfunction, but difficulties in ability to concentrate cannot. This together with the finding of excess frontal desinhibition signs—for example, positive glabella reflex—and extrapyramidal clinical signs in the subgroup of retired exposed workers is indicative of incipient central neurodegeneration most likely associated with the longstanding high exposure to Cd in the past. Especially, the finding of signs of parkinsonism in retired Cd workers might be interesting, because Cd has been thought to induce an excess of oxidative stress.⁴⁹ Other explanations—such as an increase in cerebrovascular disease—could not be detected in a cohort mortality study in workers exposed to Cd.⁶¹ In view of the increasing belief that parkinsonism might be associated with environmental influences earlier in life or with an interaction between these influences and genetic susceptibility,⁶² these findings in the retired exposed workers merit further attention.

Conclusion

Chronic occupational exposure to Cd (as reflected by urinary Cd) was found to influence in a dose-dependent manner the slowing of psychomotor functions and the increase of complaints consistent with peripheral neuropathy, and complaints about equilibrium, and ability to concentrate. These neurotoxic effects are likely to occur in the absence of microproteinuria induced by Cd. Age, exposure to other neurotoxicants, or renal function could not be held responsible for these findings. The presence of extrapyramidal clinical signs merits further investigation of the toxicity of Cd on the central nervous system. The question remains open about the impact of long term low level exposure to Cd on vulnerable subgroups of the general population—for example, foetuses, pregnant women, young children, and elderly people—living in historically polluted areas where non-ferrous industries are or were in operation.

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